# Resilient Crops for Water Limited Environments:

## Proceedings of a Workshop Held at Cuernavaca Mexico

24 – 28, May 2004

D. Poland, M. Sawkins, J.-M. Ribaut, and D. Hoisington
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THE WELLBEING OF MANKIND THROUGHOUT THE WORLD
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CIMMYT

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CIMMYT MR

CIMMYT® (www.cimmyt.org) is an internationally funded, not-for-profit organization that conducts research and training related to maize and wheat throughout the developing world. Drawing on strong science and effective partnerships, CIMMYT works to create, share, and use knowledge and technology to increase food security, improve the productivity and profitability of farming systems, and sustain natural resources. Financial support for CIMMYT's work comes from many sources, including the members of the Consultative Group on International Agricultural Research (CGIAR) (www.cgiar.org), national governments, foundations, development banks, and other public and private agencies.

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# Foreword

In May of 2004, more than 150 scientists from around the world—mostly from Asia and Africa—met in Cuernavaca, Mexico to present and discuss their research on one of the world's most intractable agricultural problems: drought tolerance in crop plants. The meeting, entitled the "Resilient Crops for Water-Limited Environments Program Workshop," was supported by The Rockefeller Foundation and the International Maize and Wheat Improvement Center (CIMMYT).

A truly global critical mass of expertise participated in the workshop, originating from more than 20 countries and three of the world's international agricultural research centers; CIMMYT, IRRI, and WARDA. Of particular interest were maize, rice, and wheat, which account for more than half of the calories consumed by people in the developing world, and are the basis for their food security and livelihoods. Scientists comprehensively addressed the physiology, biochemistry, and genetics of plant response to water stress. In addition, they looked at drought tolerance from the ground-level perspective of incorporating farmer participation into varietal development, to the heights of molecular genetics / genomics / bioinformatics, and even how plant gene regulation pathways interact and respond to water deficits in a growing developing crop plant. Indeed our information and hence knowledge base in this subject is growing at a rapid rate.

Specific goals of the workshop were information sharing among Rockefeller Foundation grantees from Africa and Asia; updating participants on knowledge generation, breeding technologies and capacity building, and seed delivery systems; and finally planning of collaborative research among the participants. These objectives were achieved beyond the expectations of the organizers and all are to be congratulated for their outstanding progress since our previous workshop held May 2002 at IRRI, Philippines.

The ultimate aim of this program's outputs is to stabilize food crop production through genetic improvement of cereals, primarily maize and rice, for drought tolerance, thereby decreasing the shock of drought on the livelihoods of poor farm households in Africa and Asia. This program's vision of success—the creation and delivery of new drought tolerant crop varieties to farmers—in turn is closely tied to parallel efforts to stem the decline of soil fertility in much of the developing world, and build up input and output markets in rural areas. Taken together, these broad initiatives offer a strong and viable approach to lifting millions from crushing poverty through creation of more sustainable rural and agricultural development.

These proceedings contain, in condensed form, hundreds of thousands of research hours dedicated to developing crops that can help farmers withstand the destabilizing impact of drought in some of the most needy farm communities of Africa and Asia. Although we have not yet reached our full potential in this effort, exceptional progress is clearly being made, indeed it seems to be accelerating, and the pipeline of research and collaboration is just beginning to deliver new varieties to the people who need them most. Specific examples of new varieties actually being delivered and utilized on large areas were reported from both southern Africa and eastern India, providing realistic "proof of concept" and motivating all concerned to redouble their efforts. The conference participants are to be commended for their exceptional work and information sharing at the workshop, as is the CIMMYT organizing committee, for the superb preparations and support for the meeting and this publication.

**John O'Toole**
**Associate Director, Food Security**
**The Rockefeller Foundation**

# Opening Address

## The War on Drought: Technologies, Markets, and Partnerships

**Gordon Conway**
**President**
**The Rockefeller Foundation**

I am very happy to be here today with all of you, having just completed my first-ever visit to CIMMYT. The visit helped set in my mind the context for our current emphasis on drought and agriculture, and the objectives of the scientists attending this workshop.

Our story begins with Norman Borlaug and the Green Revolution. His work and vision, supported by The Rockefeller Foundation at that time, continues to inspire us. Yield ceilings of staple crops increased dramatically, especially in well-favored, well-irrigated lands. Production grew faster than population, and the real price of staple foods decreased. In short, the Green Revolution fed the world. Wheat productivity rose 200 percent from 0.9 tons per hectare in 1962, to 2.7 tons in 2002. Rice productivity went from 1.8 tons per hectare to 3.9 tons, an increase of 117 percent. And maize went from 1.2 tons per hectare in the early 1960s to 3 tons at the turn of the millennium.

The Green Revolution also fed the world by bringing down the price of staple grains. The real price for rice decreased from $800 per metric ton in 1975 to around $200 in 1995. Less dramatic but still highly significant, wheat went from a little over $300 per metric ton in 1975 to $200 in 1995. Without a Green Revolution, the price of cereals would have increased year by year to meet demand in Japan and Europe, mainly for livestock feed.

That said, today 1.2 billion people remain in poverty, with 90 percent of them in Africa and Asia, and 70 percent in rural areas. Critics of the Green Revolution say that we need to tackle poverty first. But the reality is that only through agriculture will those people work their way out of poverty, a daunting task. The best lands have been fully utilized and the poor are often forced to farm on less-favored lands. Furthermore, the soils have low native fertility and water resources are frequently inadequate at best.

In Africa, large areas are at risk for drought. There are two ways that drought impacts on farmers' livelihoods. It can affect everyday stability, as with fluctuations in production. Drought also threatens sustainability, through "drought shock," when production collapses.

We are making some progress addressing water stress and its impact on stability, as will be reported at this meeting. In response to drought shock, we will have to reach even further, perhaps into genes of plants like the so-called "resurrection plant."

Drought's impact cannot be overstated: it reduces tropical maize yields 17 percent annually, or by $2.2 billion. In two regions where Foundation grantees are concentrated, China and eastern India, annual losses to drought total 4.4 million tons and 2.9 million tons, respectively. Globally, $3.6 billion of rice production is lost each year to drought. Annual losses for these two crops approach $6 billion. But to really put these losses in perspective, we must recall that these impacts are on the economies of mainly agriculture-based developing countries of Africa and Asia. If researchers like you are successful, you will save some of the world's *neediest* people $6 billion a year.

But drought and responses to it must also consider a host of non-technical issues. Farmers have a range of coping mechanisms, which are often effective, but place a heavy, long-lasting burden on the family. For instance, in Africa families frequently sell their livestock, or sell or mortgage their land. In the shorter term, farmers purchase less nutritious foods, delay needed health care, and curtail their children's education for lack of school fees. Migration, and all that it entails, is another common response to drought. Taken individually, or one by one, these coping mechanisms impose a significant burden on farm families.

Given the complex nature of the problem, there is a diversity of strategic responses we can pursue, both technical and social. On the social side, we can develop roads, irrigation systems, provide crop insurance, or create opportunities for non-agricultural employment. The enormity of that type of infrastructure and civic development renders it impractical for the Foundation's grantmaking.

On the technical side, we can opt for genetic improvement or breeding for drought tolerance, an approach that builds on the progress we've made to date and is compatible with our mission in that it addresses a global concern. Forecasts are that global warming's impact on Africa's maize production will be especially severe, making the situation that much more dire. These needs are consistent with The Rockefeller Foundation's history and reputation. We accept risk, we lead the way, and we stay the course. Given the competence of the Foundation's staff and their experience and familiarity with new scientific advances, we have confidence that we will continue to move forward.

To move forward, I believe that what Africa and to a lesser extent Asia need is a Doubly Green Revolution—one that is equally successful to the first Green Revolution, but that is more equitable, sustainable, and environmentally friendly than its predecessor. And it is critical that the poor literally reap its benefits. This is not simply an altruistic wish—it is emphasized in the Foundation's mission statement that declares: "a commitment to enrich and sustain the lives and livelihoods of poor and excluded people." We must meet these criteria to have projects funded.

The Rockefeller Foundation's food security goal is simple: to promote sustainable livelihoods in areas bypassed by the Green Revolution. How and where are we investing? In Asia and Africa, we are investing in more resilient crop varieties. But given the severity and nature of the agricultural problems in Africa, we are going further and supporting efforts to enhance soil productivity and develop markets to improve incomes of farmers. Globally we are supporting the development of international public goods for poor farmers.

Improved crop varieties and better soil fertility lead to higher productivity. When coupled with better markets, this results in increased incomes for farmers and improved livelihoods for their families. It's a neat, concise model, but The Rockefeller Foundation only provides the funding. Researchers like you are doing the work.

Now let me say a few words about nutrients and markets. Africa is losing soil nutrients at an alarming rate. In western Kenya, for instance, farmers are losing 125 kg of nitrogen per hectare per year. This is what Europeans add to their land annually. A quick look at the figures reveals the dire need for fertilizer and the cost constraints that limit its use in Africa in particular. Average fertilizer application in Africa is 3.3 kg per hectare as compared to 42.9 kg per hectare in Asia. The yields reflect this inequity; the average yield in Africa is 1.1 tons per hectare, while in Asia it is nearly 3 tons. We quickly see that cost is the major determinant in use or lack of use. In Europe you can buy a ton of urea fertilizer for about $90. In Mombassa that same quantity of fertilizer will cost about $400; in western Kenya, $500; and in Malawi, an astronomical $770. This is an enormous barrier to improved agriculture in Africa.

It is absolutely critical to provide affordable nutrients to farmers, which requires efficient input markets. I have some direct experiences with this end of the farm business. My grandfather was an input vendor for agricultural products. He would go door-to-door selling seed and other inputs and tools to farmers. We need something like that in Africa and Asia; small vendors selling small amounts of fertilizer to small-scale farmers.

The other side of the equation is output markets. In western Kenya, I recently came across a very large grain silo, similar to the type seen throughout the Midwest of the United States. This one, however, had been sitting empty for nearly five years. Clearly, the market is not working.

The Rockefeller Foundation is experimenting with small-scale cereal banks as a way to create market opportunities for resource-poor farmers. Now we even see farmers using mobile phones to access regional markets in order to get better prices for their maize. In East Africa, over a four-month period, a group of local cereal banks sold 393 tons of maize to the largest millers in the region at a premium price. Compared to the previous year, the villagers have doubled the income from their grain sale. And for the first time, villagers have sufficient cash to provide for their food and medical needs, as well as the children's school fees.

However, none of this works without partnerships! If the scientist can't access proprietary technologies, and in turn, can't develop them and get them to the farmers, progress breaks down. Furthermore, the technologies must be affordable when they arrive at the farmgate. In particular, the key to food security in Africa is the creation of genuine partnerships, starting and ending with the farmers and involving the public and private sectors, the National Agricultural Research Systems (NARS), the International Agricultural Research Centers (IARCs), such as WARDA, CIMMYT, and IRRI, and the advanced labs of Japan, China, Europe, and the USA.

Partnerships can be seen as a three-legged stool—the public sector, private sector, and the communities, all brought together by civil society. The public sector—and to a lesser degree the private sector—provide money and scale. The private sector provides entrepreneurship, and the community provides participation and sustainability. We've seen this work in pharmaceuticals as well as crop varieties**.** This system is what will bring greater prosperity to those in need. Key to the success of African breeders and public research is access to patents.

The Rockefeller Foundation has initiated two programs in an effort to get these technologies to African breeders and farmers. The first, the African Agricultural Technology Foundation (AATF), is an African-led and based, freestanding, not-for-profit organization focused on bringing proprietary technologies from both public and private sectors to bear on African agricultural development, while remaining responsive to smallholder needs. We are looking to the AATF to procure licensing agreements for existing technologies, underwrite adaptive research and development, promote regulatory consent, and stimulate new technologies and delivery to the farmers. One reason this approach is needed is that it can tailor solutions to specific situations required to win the war on drought. Because, as we all know, one size does ***not*** fit all. Local adaptation is imperative—different varieties will be needed for numerous and diverse target populations.

Second is the Public Intellectual Property Resource for Agriculture (PIPRA) program, which will help American universities profit from their patented discoveries while making those technologies available for humanitarian goals.

Both of these new programs focus on the farmers' needs as a starting point; for the old model of the scientist telling the farmer what to do and how to do it is finished. Today, the farmer increasingly tells the scientist what to do. We have reached the stage where we are at least consulting with farmers over characteristics like taste and acceptability. We need to further shift the emphasis to having farmers tell us what they want us to do.

So in the battle against drought, what would success look like? First, we would see that small-scale maize and rice farmers have access to drought-tolerant varieties. These would provide the buffer against the shocks of drought years. This sustainability would lead to increased productivity and stability of crop yields, which in turn would generate more income for farmers and all that entails, including better nutrition and health care, and money for their children's education. All this is incumbent on participation in serviceable input and output markets.

And what would failure look like? Above the Grand Canyon there is an abandoned Puebla village that was inhabited around the 12th century AD for about 30 years. We don't know what occurred there to drive the people out, but most likely it was drought—which leads me to a recent exchange I had with a Hopi Indian in America's dry Southwest. He told me that according to Hopi tradition, one day their culture, along with their maize, will save the world. It reminds me that in this war on drought and water stress, we must look to indigenous people—not just as those we must help, but also to see how we can learn from them.

Thank you.

# Welcoming Statement

**Masa Iwanaga**
**Director General**
**International Maize and Wheat Improvement Center (CIMMYT)**

Bienvenidos, or for those unfamiliar with Spanish, welcome, to Mexico and thanks to you all for coming such great distances to be here today with us. I'd also like to extend a special greeting to our guests from The Rockefeller Foundation, notably Gordon Conway, the president of the Foundation, John O'Toole, and Gary Toenniesen. It is an honor to have you in attendance.

This is not the first time CIMMYT has shared the stage or an important mission with The Rockefeller Foundation. We share a long and productive history of collaboration going back to the mid-1940s when The Rockefeller Foundation and the governments of the United States and Mexico formed the Office of Special Studies—CIMMYT's predecessor—to address food shortage issues in Mexico. CIMMYT was officially established in 1966 with support from The Rockefeller Foundation and the Mexican government, but Rockefeller's involvement did not end there. The Foundation has continued to initiate and promote international agriculture research collaboration worldwide. Closer to home, today the Foundation supports numerous CIMMYT strategic projects.

Two recent and important examples of Rockefeller / CIMMYT collaboration concern soil fertility and Quality Protein Maize (QPM). Recognizing the worrisome and continuing decline in soil fertility in the developing world, CIMMYT and Rockefeller have made long-term efforts to promote sustainable soil management and identify "best bet" technologies for resource-poor farmers. Extensive partnering through efforts such as the Southern Africa Drought and Low Soil Fertility project (SADLF) and the Soil Fertility Network (SoilFertNet) have lent focus to research and the diffusion of knowledge in this critical area. The success of SoilFertNet will serve as the foundation for the new Consortium for Soil Fertility, a Rockefeller-supported network with 200 members, that will extend partnering and ground level impact in the region.

The diffusion of QPM is another important project on which we collaborate. QPM promotes better livelihoods in two ways: directly through human consumption and via better feed for poultry and pigs. The Rockefeller Foundation currently funds a CIMMYT project to develop and disseminate locally adapted QPM varieties in sub-Saharan Africa, which possess a range of biotic and abiotic stress tolerances and are well liked by local consumers. Putting QPM into locally adapted backgrounds is accomplished with the help of National Agricultural Research Systems (NARS), which the project also supports through capacity-building initiatives. Socioeconomic impacts assessment are integrated into the project to assure that the target farmers are being well served.

These successful examples of partnering make me very optimistic about the endeavor that those of us here are now engaged in. Drought presents probably the most important agricultural problem for small-scale farmers. The complex challenge of drought tolerance is a high priority for both The Rockefeller Foundation and CIMMYT because growing demand for food, climate change, competition for fresh water, and expansion to marginal lands have intensified the need for drought tolerant plants in many parts of the world. CIMMYT is already fully engaged in this effort through breeding for water-stress resistant wheat and maize and by screening the extensive genetic resources in our genebank collections. With our extensive global network of NARS and advanced research institute partners, we continue to work on developing new breeding strategies for drought, including molecular techniques, and study ways to ensure that drought tolerant varieties get out to farmers and meet their criteria.

As I mentioned earlier, in eastern and southern Africa, we have strengthened research capacity to help NARS breed drought tolerant varieties. Through smallholder involvement, the process of participatory maize variety selection, and "Mother / Baby" trials, we are fostering community-based seed production and delivery systems in those regions.

To understand drought tolerance in staple food crops we must first look at genetic bases. CIMMYT is examining these bases in 30 different environments, all under water-stress conditions. This background information provides the data needed to effectively use marker-assisted selection for drought resistance in maize.

Turning to wheat, CIMMYT is now testing transgenic wheat we produced that carries the dehydration responsive element-binding protein (DREB) gene, which comes from the *Arabidopsis thaliana,* and was provided by the Japan International Research Center for Agricultural Sciences (JIRCAS). These trials provide a basis for comparing genetic reactions to water stress and have shown very encouraging results. The trials also represent the first transgenic wheat field trials in Mexico, and are the most advanced level of testing for food crops containing DREB to date. The trials will be evaluated regarding their performance in both water-stress and normal conditions.

Another encouraging avenue of CIMMYT wheat research involves crossing wheat with its wild relatives to incorporate various resistance traits, including drought tolerance. We are also developing wheat varieties adapted to bed planting and resource conservation technologies and selecting for improved drought resistance traits, taking germplasm x environment (G x E) interactions into account in the experimental strategies and data analysis.

In addition, CIMMYT is undertaking a massive study of wheat landraces at our research station in Obregon, Mexico. Researchers have identified about 50 varieties showing superior drought responses. The desired drought tolerance traits will be moved into modern wheats during pre-breeding projects. One can only imagine the good we could do if we combined three sources of drought tolerance for wheat—the DREB gene, wild relative crosses, and preferred alleles culled from landraces!

We at CIMMYT, and many of you here at this conference, recognize that since drought is one of agriculture's most intractable problems, it will take innovation and an integration of many diverse approaches to overcome it—at the breeding level as well as in farmers' fields where the ultimate impact will be measured. The new CIMMYT strategy is designed in such a way that we can do just that. Our new strategic plan focuses on improving livelihoods—a feat that could be achieved with the help of drought tolerant crops. Through new partnerships and revamped programs, CIMMYT plans to make a difference in the lives of those who need us most. We are happy to have The Rockefeller Foundation and all of you as partners in this effort.

Thank you all very much for your attention. I'm sure this week will prove to be an important and productive event, and I look forward to meeting with all of you and benefiting from our shared knowledge. Again, welcome to Mexico. I hope you enjoy your time here.

# Breeding for improved drought tolerance in maize adapted to southern Africa

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In 1997, CIMMYT initiated a product-oriented breeding program targeted at improving maize for the drought-prone midaltitudes of southern Africa. Maize varieties were selected in Zimbabwe using simultaneous selection in three types of environments, recommended agronomic management / high rainfall conditions, low-N stress and managed drought. Between 2000 and 2002, 41 hybrids from this approach were compared with 42 released and prereleased private seed company hybrids in 36-65 trials across eastern and southern Africa. Average trial yields ranged from less than 1 t / ha to above 10 t / ha. Hybrids from CIMMYT's stress breeding program showed a consistent advantage over private company check hybrids at all yield levels. Selection differentials were largest between 2 to 5 t / ha and they became less significant at higher yield levels. An Eberhart-Russell stability analysis estimated a 40% yield advantage at the 1 ton yield level, which decreased to 2.5% at the 10 ton yield level. We conclude that including selection under carefully managed high priority abiotic stresses, including drought, in a breeding program and with adequate weighing can significantly increase maize yields in a highly variable drought-prone environment and particularly at lower yield levels.

# Rice seed systems in southern China: Views from institutions and farmers

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## Introduction

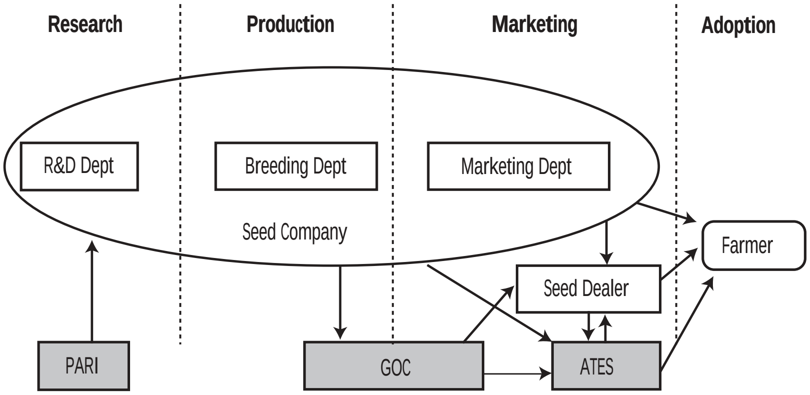
Agents involved in rice seed systems in China include agricultural administrations, research institutes, universities and colleges, seed companies, and farmers. Although farmers may request locally adapted seed and direct delivery channels, the other agents, who have more power over the seed systems, have different views about the rice seed systems (Lin, 1992; Huang et al., 2000). The farmers do not have much say on the seed systems, which adversely affects rice farmers in southern China, especially marginalized (or subsistence) farmers. Farmers and other agents are required to work together towards establishment of comprehensive rice seed systems. By doing so, the understanding and views of farmers and other agents, and the differences between them, can be investigated.

## Methods

Institutional interviews and a rice farm household survey were carried out. Institutional interviews from the supply side of the rice seed systems were mainly carried out with officials and scientists at various agricultural administrative offices (AAO), public agricultural research institutes and universities (PARI), and agricultural technology extension stations (ATES). The rice farm household survey from the demand side was then implemented. Huopai village from Xiangyang county of Hubei province in south central China, which represents the main rice production area, and Yueli village from Nandan county of Guangxi province in southwest China, which represents marginalized rice production areas, were selected as fieldwork sites. Officials and scientists from AAOs, PARIs, ATESs, and seed companies in two provinces, were interviewed; 61 rice farm households from Yueli, Guangxi, and Huopai, Hubei were surveyed from March to May 2002.

## Results

A rice seed system based on data from interviews in two provinces was identified (Figure 1). “Rice yield” was rates as the highest priority breeding target by staff from four PARIs in two provinces; “rice quality” followed. From farmers’ perspectives (Table1), the largest proportion of rice farmers (56.5 %) in Hubei chose “rice quality” as the first criterion when



PARI: Public Agricultural Research Institutes / Universities.
GOC: Companies organized by former staff from agricultural administrative office.
ATES: Governmental Agricultural Technologies Extension Stations at county and township.

**Figure 1. Rice seed systems identified based on data from institutional interview in provinces of Hubei and Guangxi.**

**Table 1. Percentage distribution of farmers' answers to "why do you adopt the rice varieties currently grew in the field" by village**

| | Huopai | Yueli |
|---|---|---|
| Good quality | 56.5 | 27.8 |
| High yield | 30.5 | 16.7 |
| High ratio of milled rice | 8.7 | 2.8 |
| Local adaptability | 4.3 | 33.3 |
| No other choices | 0 | 19.4 |
| No. of answers | 23 | 36 |

purchasing seed, while the largest proportion of rice farmers (33.3%) in Guangxi chose "local adaptability" as the first criterion. Farmers in Huopai have a wide range of seed dealers available for seed purchases, while seed can only be purchased in township ATES as dealers, in Yueli. Furthermore, 19.4% of farmers in Yueli reported that the rice they grew in the field was the only variety that could be found in the market.

## Conclusions

Seed systems can be horizontally divided into four processes: research, production, marketing, and adoption. The systems can also be vertically separated into several participating agents: public agricultural research institutes, seed companies, agricultural technologies extension stations, seed dealers, and rice farmers. Seed companies play an increasingly important role in providing rice seed to farmers, while government seed provision, such as the agricultural technology extension station's network, still work. Private companies closely cooperated with public agricultural research institutes and government agricultural administrative offices, although some government officials and scientists from research institutes were "jumping into the sea" to start their own seed business. Different views of farmers and other agents involved in seed systems obviously could be found (Chen et al., 2004). While farmers in Huopai village requested rice seed with "good quality" and farmers in Yueli village looked for more "local adaptability," research institutes and companies took "high yield" as the primary target for their breeding strategies, with "good quality" next. Locally adapted rice seed breeding was not yet considered important by breeders, implying that the marginalized rice farmers'needs were neglected. Public and private research institutes and companies should listen to farmers while preparing their seed development strategies.

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# Adaptability of 16 upland rice varieties to two moisture regimes

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## Introduction

Despite its history in the region dating back to 1904 (Jameson, 1970), rice did not become a major crop until the early 1990s (Agricultural Secretariat Report, 1992). Its importance is significantly increasing due to urbanization, changing eating habits of the new generation, and increasing number of public institutions; as such, per capita consumption quickly jumped from 300 grams in 1980 to the present 10.5 kg in 2002. The increase in demand has stimulated production, especially in the in the wetlands, and increased imports from the Far East. Rice imports stand at US$120 million annually. Production of rice in the wetlands is raising environmental concerns among the government and other organizations (State of the Environment Report for Uganda, 1994). A project on upland rice was initiated with the aim of increasing rice production in general and productivity of rice under upland conditions in particular. This would attract both rice farmers in lowlands and non-rice growers to upland rice that is easy to produce, thus increasing rice production. Consequently, this would reduce the amount of imported rice and protect wetlands.

## Methods

Sixteen upland rice varieties selected in preliminary yield trial were evaluated in separate trials under optimum and drought conditions. Both trials were conducted at Namulonge Agricultural and Animal Research Institute (NAARI). Treatments were arranged in a randomized block design with four replicates. Individual plots consisted of 8 rows, 5 m long at spacing of 30 cm between rows and 5 cm between plants. The trial under optimum conditions was planted at the onset of rains and received 678 mm of well -distributed rainfall. The drought stressed trial received one month's rain and an irrigation 40 days after planting and another at milk stage. Both trials received N45P30 applied at planting.

From the above experiments, ten varieties were selected for participatory selection on-farmers' fields. The selection was based on maturity period, resistance to rice blast, yield, and drought tolerance. The ten varieties were planted in five districts, and the top five varieties subsequently selected for further evaluation on-farm. The five were then reduced to three and these were released for commercial production in Uganda. Farmers' selection criteria included grain yield and maturity period, followed by resistance to lodging and diseases, grain size, and culm length.

## Results and discussion

All the varieties tested flowered in a narrow range of nine days (71 and 80 days) under adequate moisture, but under moisture stress, the range increased to 19 days (76 and 96.3 days). WAB 189 out-yielded all the other varieties under optimum conditions, but was observed to be the most sensitive to drought. Its percent productive tillers was reduced by 96.5%; panicle length was reduced by 40.7%. Days to flower increased from 79 to 96.3 days under drought compared to optimum conditions (Table 1). Varieties WAB 450, ITA 325, ITA 257, and Sikamo had significantly higher yields than the local check and were also observed to be least affected by drought on the traits studied.

## Conclusions

Varieties that performed well under drought stress at Namulonge continued to perform well on farmers' fields and three of them—ITA 257, ITA 325, and WAB 450—were finally selected by farmers in all the districts they were evaluated. They have been released under the names: NARIC 1, NARIC 2, AND NARIC 3, respectively. NARIC 3 has become so popular that many seed companies, NGOs, and government institutions are trying to produce more of its seed to meet the demand. This suggests that the farmers have discovered that upland rice can be produced more easily than the tedious swamp rice

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**Table 1. Performance of 16 upland rice varieties under drought stress and normal conditions**

| | DAY TO FLOWER | | CULM LENGTH | | PANICLE LENGTH | | YIELD | |
|---|---|---|---|---|---|---|---|---|
| VARIETY | UNDER STRESS | NORMAL | UNDER STRESS | NORMAL | UNDER STRESS | NORMAL | UNDER STRESS | NORMAL |
| IRAT 233 | 89.3* | 79* | 51.1* | 79.6 | 21.1 | 24.5* | 756 | 4831 |
| IDSA-17 | 80.5 | 75 | 39.4 | 71.9 | 19.3 | 22.3a | 592 | 3935 |
| BR-1890-1-1-2 | 90.3* | 80-* | 49.3-* | 86* | 19.2 | 23.6* | 467 | 4530 |
| WAB 3299 | 91.7* | 78* | 44.2 | 85.1* | 19.5 | 24.1* | 147 | 4879 |
| IRAT 240 | 89.2* | 77 | 39.5 | 86.1* | 18 | 23.5* | 1010 | 5109 |
| WAB 332 | 89.2* | 78* | 46.1 | 89.7* | 18.2 | 23.1* | 263 | 4321 |
| SIKAMO | 77.3 | 73 | 41.2 | 86.2* | 18.3 | 25 | 1765* | 4882 |
| IITA 257 | 76 | 71 | 44.7 | 71.6 | 20.2 | 23 | 1789 | 4420 |
| WAB 189 | 96.3* | 79* | 36.2* | 81.5 | 16.2 | 27.3* | 275 | 5215 |
| WAB 450 | 81 | 77 | 46.6 | 76.3 | 23 | 23 | 1915* | 4536 |
| Check | 80.7 | 74 | 44.3 | 79.3 | 22 | 23 | 882 | 3868 |
| ITA 325 | 79.3 | 74 | 53.2* | 94.3* | 20.1 | 22.1a | 1802* | 4392 |
| UK 2 | 78.7 | 74 | 43 | 72.3 | 19.1 | 22.5 | 952 | 3192 |
| NP 4 | 87.4* | 74 | 39.5 | 82.1 | 17.2 | 24.5* | 427 | 4935 |
| NP2 | 80.3 | 73 | 42.1 | 79.6 | 19.5 | 23.6* | 1030 | 3775 |
| NP3 | 78.3 | 74 | 41.3 | 75.2 | 18 | 24.1* | 79.9 | 3370 |
| MINIMUm | 77.3 | 71 | 36.2 | 71.6 | 16.2 | 22.1 | 147 | 3192 |
| MMAXMUM | 96.3 | 80 | 53.2 | 94.3 | 23 | 27.3 | 1915 | 5215 |
| MEAN | 84.1 | 75.6 | 43.6 | 81 | | | 878.6 | 1473.6 |
| LSD (0.05) | 4.1 | 3.8 | 3.1 | 4.2 | | | 929.4 | 4386.9 |

a = Significantly below the Check
* = Significantly above the Check

# Farmers' participatory varietal selection at target drought prone area of Tamil Nadu

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## Introduction

Success of new crop varieties depends upon quick adoption. Often it is rather slow in marginal environments. Many times the improved varieties from the researcher fail due to the reason that farmers' perceptions change over time. Lines selected from such networked Participatory Varietal Selection (PVS) trials are likely to be broadly adapted, stress tolerant, and acceptable to farmers (Atlin et al., 2002).To determine the acceptability of and the preferences for the newly developed rice cultivars, a PVS was carried out.

## Methods

PVS by the local farmers was conducted on-station during the 2002-2003 cropping season at the agricultural research station, Paramakudi. A total of 61 progressive farmers were asked to select the genotypes by scoring for selected traits. Scoring was between "1" and "10" based on their preferences. Highly preferred was scored "10" while the a very low preference was scored "1." Plant height, duration, grain quality, drought tolerance, and grain yield were surveyed apart from overall acceptability of the genotypes. Thirty rice genotypes comprising 19 rice cultures in advanced stage and 11 released varieties were presented for selection. The identity of the cultures/varieties was hidden and a random code number was assigned for each genotype. Each farmer was provided with a spreadsheet, with numbers of genotypes, to give scores based on their phenotypic evaluation for the five traits and for overall acceptability. The farmers scored for the traits and the scores were consolidated by summing up.

## Results

Farmers enjoyed scoring the genotypes and the response was good. This being their furst such experience, they showed great interest in scoring. The total scores for individual traits are presented in table 1. Among the genotypes, the culture IET 17458 and PM 9106 scored high in terms of overall acceptability. Besides overall acceptability, the culture PM 9106 bagged up top score for duration, plant height, grain quality, and grain yield. The rice variety TRY (R) 2 scored the highest rank for drought tolerance.

## Conclusion

The culture IET 17458 and PM 9106 were highly preferred by the farmers, indicating that sthe advanced stage rice cultures are highly acceptable and have potential for introduction as new varieties in farmers' fields. PVS paved the way for need-based selection by the farmers and thereby helps promote quicker adoption of useful varieties in the farming community.

**Table 1. Overall acceptability by the farmers on the major traits in participatory evaluation**

| Lines | PAS | Plant height | Duration | Grain quality | Drought tolerance | Grain yield |
|---|---|---|---|---|---|---|
| IET-17458 | **1** | 5 | 6 | 5 | 8 | 3 |
| PM 9106 | **2** | 2 | 1 | 1 | 3 | 1 |
| TRY-2 | **3** | 12 | 11 | 4 | 1 | 4 |
| PM 2 K 022 | **4** | 6 | 8 | 7 | 4 | 5 |
| TKM-11 | **5** | 4 | 15 | 2 | 2 | 2 |
| PMK-2 | **6** | 1 | 4 | 8 | 5 | 7 |
| TM 97032 | **7** | 7 | 5 | 6 | 6 | 6 |
| PM 2K 019 | **8** | 3 | 3 | 9 | 7 | 10 |
| ASD-20 | **9** | 8 | 9 | 3 | 12 | 9 |
| ADT-36 | **10** | 11 | 2 | 10 | 9 | 8 |
| IET-17450 | **11** | 5 | 17 | 12 | 10 | 11 |
| IR-36 | **12** | 15 | 13 | 13 | 17 | 16 |
| PM 2K 020 | **13** | 9 | 12 | 11 | 11 | 13 |
| IR-20 | **14** | 19 | 10 | 14 | 22 | 15 |
| IET-17455 | **15** | 20 | 24 | 17 | 16 | 19 |
| IET-17448 | **16** | 13 | 18 | 16 | 20 | 17 |
| IET-16704 | **17** | 26 | 21 | 18 | 18 | 12 |
| IET-17441 | **18** | 21 | 19 | 15 | 14 | 14 |
| IET-14567 | **19** | 17 | 23 | 21 | 13 | 21 |
| RM 96019 | **20** | 24 | 16 | 20 | 23 | 18 |
| IET-17446 | **21** | 22 | 27 | 26 | 28 | 25 |
| IET-17434 | **22** | 27 | 22 | 23 | 25 | 20 |
| CO-45 | **23** | 16 | 25 | 25 | 19 | 28 |
| TKM-10 | **24** | 14 | 24 | 22 | 21 | 22 |
| TM -97198 | **25** | 28 | 26 | 28 | 24 | 26 |
| IET-16820 | **26** | 25 | 14 | 24 | 26 | 24 |
| PM 2K 017 | **27** | 23 | 28 | 27 | 27 | 27 |
| TKM-12 | **28** | 10 | 15 | 19 | 15 | 23 |
| CO-47 | **29** | 29 | 30 | 29 | 30 | 29 |
| IET-17433 | **30** | 30 | 29 | 30 | 29 | 30 |

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# Farmers' participatory plant breeding technique: An effective tool for the early selection and adoption of rice varieties in rainfed rice ecosystems

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## Introduction

The shadow of drought loomed heavily on this year's harvest of *kharif* crops. Rice production in the current year is likely to be drastically reduced and there is a large decline in the production of coarse cereals (Economic Survey, 2003-04). Ramanathapuram district situated in the southeast coast of Tamil Nadu, India falls in the rain shadow region and therefore is a highly drought prone and most backward in development. The district's average rainfall is 827 mm, and of this, 60% is received during the North East Monsoon (October to December). Rice is the predominant crop cultivated in an area of 104,223 ha under semi-dry and rainfed conditions. The crop suffers either early drought or late drought as a consequence of erratic and uneven distribution of rainfall during the cropping season. This often resulted in low crop yields or at times complete crop failure. For instance, during 2003-04 season, due to the deficit of rainfall in December, complete crop failure has been recorded in an area of 100,017 ha, accounting for losses of 2.51 lakh tons rice production and 90% of the total rice production in Ramanathapuram district. Thus, drought is threatening food security in this region of Tamil Nadu. To sustain the rice production and to achieve higher yield, it is vital to develop drought tolerant varieties in addition to other moisture stress management techniques. Crop improvement for drought resistance must be considered within the broader context of a total agricultural research and extension strategy (Nix, 1982). Breeders instinctively look for new sources of variation when attempting to improve plants, but such empirical selection in the case of drought resistance has been difficult. Also, selection under controlled environments rarely correlates with performance in the field and the consistency of response to drought also varyies year after year due to variability in the environment. Embarking on a participatory plant breeding (PPB) approach may have many motivations, among them, increased and more stable productivity, faster release and adoption of varieties, better understanding of farmers' varietal criteria, enhanced biodiversity, increased cost effectiveness, facilitated farmer bearing, and empowerment of farmers (Sperling et al., 2001).

## Methods

Four blocks of Ramanathapuram district were selected for this programme based on their representation of the environments targeted in the breeding work, diversity and range of ecological conditions, involvement of women in farm activities, availability of previous survey data, and easy access to the site. As a part of this study, a participatory rural appraisal was also conducted with the objective to characterise the farmers' crop management practices, gender roles, and farmers' preferences for traits in improved varieties to suit the local needs. Farmers were selected based on their primary occupation and degree of literacy. Five rice varieties were selected and raised in an area of 50% of the farmers' fields and managed by the farmers under the supervision of our scientists. In addition, farmers were involved in the participatory varietal selection process at our research farm.

## Results

The participatory rural appraisal results revealed that the awareness about high yielding varieties was as high as 93%. However, the farmers preferred low yielding landraces to high yielding varieties for their tolerance to drought, low input cost, and readily available seed materials. Men (74%) decide upon the variety to be cultivated, despite the fact that the women spent more (4 times more) time than men in the field. Among the five varieties tested in the participatory varietal selection (PVS) programme in the farmers fields, three varieties (Ashoka 228, Ashoka 200F, and RM96019) provided yields and were liked by farmers due to the short duration of the variety, which is a drought escape mechanism. Thus, yield and duration were important traits considered by farmers. The on-farm participatory breeding programme (PBP) (Table 1) revealed that farmers' preference rankings were not always correlated with scientists' ranking. However, the lines selected by farmers were almost tallies at 75% with scientist selection, indicating that farmers too are capable of identifying varieties by considering important plant growth characteristics like duration and plant stature and grain quality of their choice. This reflects their great experience, which also mattered when selecting rice varieties at PBP. This shows that there is a strong agreement among farmers and between farmers and scientists' in selecting best varieties suited to their environment.

**Table 1. On-farm farmers participatory varietal selection**

| Parentage | Lines selected by scientist in order | Grain yield (kg/ha) | Rank given by farmers |
|---|---|---|---|
| IR 64 x Azucena | 17 and 23 | 4266 | 8 and 5 |
| IR 64 x Azucena | 18 | 4178 | - |
| IR 64 x Azucena | 15 | 4089 | 6 |
| IR 64 x Azucena | 21 and 19 | 4000 | 1 and 7 |

## Conclusions

A participatory plant breeding programme and participatory varietal selection programme are essential in unfavorable rainfed environments and with diverse socioeconomic groups that depend on rice for their livelihoods, such as Ramanathapuram district of Tamil Nadu. Although it is too early to show the impact of PBP and PVS through the spread of materials generated, farmers' participation in the coming years will become vital in the selection of suitable varieties of their choice for the complex rainfed environment. This is because farmers will be in a better position to screen new varieties at their own levels of management with the assistance of scientists involved in this programme. This approach will not only promote the identification of suitable varieties for this environment, but will also create confidence among the farming community, which will result in early and easy spread of seed materials in the shortest time possible.

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# Participatory maize variety evaluation for increased adoption

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## Introduction

Development and transfer of improved maize varieties has often been found to take too much time, resulting in little impact on the farmers. National maize programs in the Southern Africa Development Community (SADC), in collaboration with CIMMYT sub-regional office in Zimbabwe, adopted a participatory variety evaluation method, the Mother / Baby scheme, through which evaluation of the varieties was done under conditions representing those of the farmers. The scheme allows for the selection of appropriate varieties for the target environments.

## Methods

The scheme uses a network of partners within each countr, through which environments, representative of a range of biophysical and socioeconomic aspects of the target environments, are sampled (Bänziger and de Meyer, 2000). Local counterparts—who could be an extension officer, a local researcher, an agricultural teacher or an NGO staff—supervises the trials while farmers evaluate the varieties in their fields, providing feedback on what they perceive as important characteristics for their conditions.

## Results

The result has been that selected varieties possess important characteristics that conform to farmers' production and utilization expectations. Farmers, as beneficiaries, have a major say in the development of the varieties, thus uptake and use of these new varieties is quicker. Other benefits include cost-effectiveness, i.e., time from evaluation to utilization as partners disseminate the varieties, and comprehensiveness resulting from partners contributing their expertise during implementation.

During the last four years, the Mother / Baby scheme has contributed to the rapid release of ZM 421 and ZM 521 in RSA, Malawi, Tanzania, and Zimbabwe, and ZM 621 in Malawi. Seed production of these varieties is currently in excess of 100,000 tons, a significant input of improved technology for farmers.

## Conclusions

The evaluation of maize varieties in the target environments, which involved the beneficiaries, has provided an opportunity for the identification and selection of appropriate varieties for the small-scale farmers. Quicker use of improved technology could have a direct and positive impact on the poverty and food security coping strategies of the farmers

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# Economic costs of drought and rice farmers' coping mechanisms: A synthesis of cross-country comparative analysis

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Drought is a major problem affecting agricultural production in the humid / subhumid South and Southeast Asia, where rice is a major crop. The economic costs of drought include not only the production loss of rice, but also the loss in production of post-rice crops that are grown on residual soil moisture. Additional economic and social costs result from the choice of production practices that are designed to reduce the losses from drought and from a longer-term decline in production capacity resulting from the depletion of productive assets due to distress sale during drought years. A good understanding of these coping mechanisms are essential for designing technological and policy interventions for a more effective drought mitigation and drought relief.

For this paper, estimates of economic losses resulting from drought in rainfed and partially irrigated areas of southern China, eastern India, and northeastern Thailand were obtained and farmers´ drought coping mechanisms were analyzed. These three regions capture a range of climatic conditions, income levels, and institutional set-ups. Through a comparative analysis, deeper insights on factors that moderate or amplify the effect of drought on the welfare of farmers differentiated by socioeconomic strata are obtained. Previous contributions to this literature are based on arid and semi-arid zones and have seldom used the analytical framework that involves a cross-country comparative analysis.

Production losses due to drought were estimated using provincial and district level time series data. Production, area, and yield were regressed on time trend and drought dummy variables representing different intensities of drought. The coefficients associated with the drought dummy variables provide estimates of the effect of drought on production, area and yield. These were suitably weighted by the probability of drought estimated from the long-term rainfall data to obtain the expected loss. In addition, the elasticities of production, area, and yield with respect to rainfall were estimated using the district-level data. The variations in elasticities across districts were explained using a set of independent variables that describe the district characteristics.

The average value of production loss resulting from drought was estimated to be 8-10% of the value of agricultural output, although the production loss during the drought years was found to be as high as 40%. The loss in output was not just due to yield loss but also due to a contraction in the area planted and harvested. The effect of this production loss on the employment and income of the poor were also estimated. This second round effect of drought was found to be substantial in India, where production losses were also high. In southern China and northeastern Thailand, the production losses were found to be relatively lower.

Farmers´ drought coping mechanisms and the impact of drought at the household level were analyzed using a survey of more than 1,500 farmers from these three regions. Information on both ex-ante (strategies that reduce the losses from drought) and ex-post (strategies that are designed to maintain consumption in the face of production losses) strategies were collected. Farm households were found to employ elaborate strategies that involve careful choice of rice varieties, planting date, planting method, and weeding and fertilization practices to minimize the effect of drought. In addition to these adaptations in rice production, farmers were also found to make temporal adjustments in cropping patterns based on drought incidence. These adjustments were analyzed through a comparison of production practices of households during drought and normal years. The economic costs of these ex-ante adjustment mechanisms were measured by comparing the farm incomes of households experiencing different intensities of drought. The results indicate that farmers with smaller farm size and poorer resource base bear a higher cost relative to farmers with larger farm size and better resource base.

The survey data were also used to analyze the ex-post coping mechanisms. Increased dependence on wage income, asset depletion, and public relief were found to be the major mechanisms used to meet the shortfall in income. The relative importance of these strategies was found to vary across the region with asset depletion and public relief being more important in India than in China and Thailand. Despite these mechanisms, most farmers were unable to maintain their pre-drought level of consumption, especially in India. Women and children were found to bear the burden of drought disproportionately. This indicates that the coping strategies currently employed by farmers are not fully effective in protecting their consumption. A better market integration and increased opportunities for off-farm employment resulted in a lower consumption loss in China and Thailand.

The overall implications of the findings of the study for technology design and for policy improvements for drought mitigation and drought relief are derived. Attention is drawn to improve drought forecasting, encourage income diversification, increase moisture availability to crops through irrigation and moisture conservation, develop drought-tolerant crop varieties, and improve the targeting of drought relief programs.

# Development and dissemination of drought tolerant rice varieties through on-farm, farmer-oriented approaches

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## Introduction

The Gramin Vikas Trust (GVT) manages two rainfed farming projects, funded by DFID, UK, for improving the food security and livelihoods of poor men and women in nearly 1,500 villages in six states in India. The GVT uses a participatory development approach facilitated by village *jankars* (skilled, trained village motivators) and self help groups (SHGs).

Poor farmers in the GVT villages cultivate marginal lands much of which (about 40%) is rainfed upland. Farmers have traditionally grown low-yielding landraces in the upland because of a lack of suitable improved cultivars. A participatory varietal selection (PVS) programme was started in 1995 to allow farmers to test and select suitable varieties and this was followed by a participatory plant breeding (PPB) programme.

## Methods

In PVS, Kalinga III was identified as the most preferred variety. However, it is prone to lodging and has a poor root system. It was used as a parent in a PPB programme started in 1997 as a collaboration between GVT, BAU, and CAZS with funding from the Plant Science Research Programme of DFID. This used collaborative methods (farmers selected in $F_4$ bulks on their farms) and consultative methods (farmers selected among $F_4$ bulks on research station) (Virk et al., 2003).

Attempts were made to encourage private seed producers, but the low profit margin from the sale of seed to poor upland farmers was unattractive. Therefore, community-based seed production was initiated by GVT in the winter season of 2001-02 in Orissa, through SHGs that were motivated to undertake seed production. GVT procured and distributed this seed to other SHGs, NGOs, and GOs.

## Results

The PPB resulted in the release of two drought tolerant varieties, Ashoka 200F and Ashoka 228, in 2003 in Jharkhand. In surveys in December 2002, most farmers perceived that the new varieties were superior for many traits including drought tolerance (Figure 1). Farmers were increasing the area under the Ashoka varieties and spreading them through the informal seed sector.

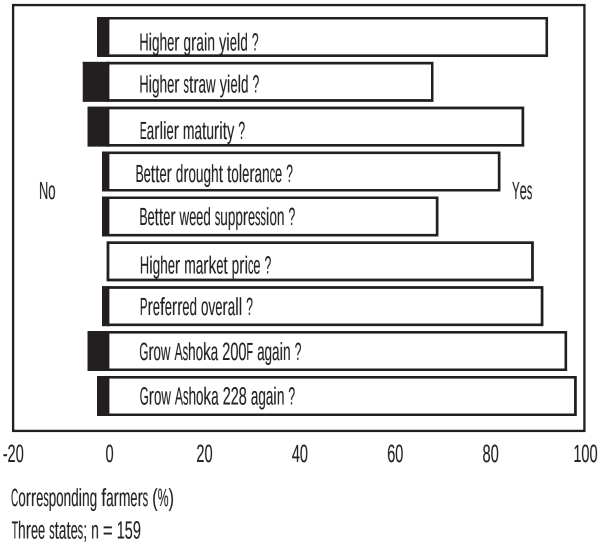

**Figure 1. Farmers' perception (expressed as % of farmers) for Ashoka 228 and Ashoka 200F rice varieties in comparison to the local cultivars. Based on a survey of 159 households sampled over all three states (Orissa, Jarkhand, and West Bengal) in December 2002.**

Farmers sold between 2 and 50 kg of seed to farmers within the village and outside the villages up to a distance of 300 km. The number of SHGs and villages and the quantity of seed produced in the winter seasons has increased over years (Table 1, Figure 2).

**Table 1. Distribution of Ashoka 200F and Ashoka 228 varieties in the rainy season of 2003**

| Agency (number) | No. of farmers | Quantity (t) |
|---|---|---|
| GVT East | 2465 | 30.2 |
| GOs (7) | 129§ | 19.5 |
| NGOs (13) | 312 | 18.8 |
| GVT West | - | 32.0 |

§ Based of 5 GOs in Orissa as the number of farmers from 2 GOs in Jharkjand is not avaialable.
- Not avaialable.

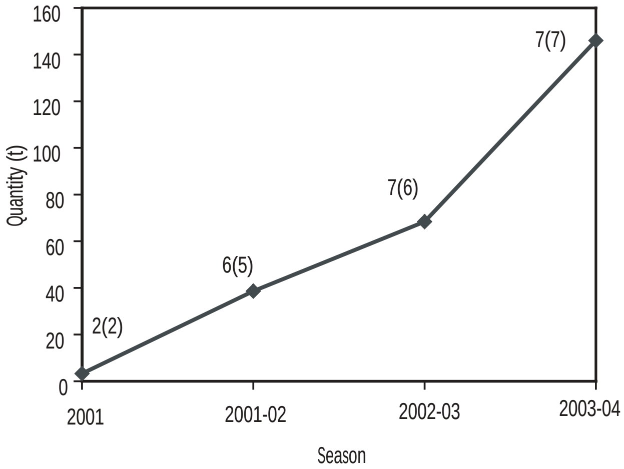

**Figure 2. Quantity of seed produced (t) by GVT from 2001 main season to projected production in 2003-04 winter season. The number of self help groups with number of villages in brackets involved in seed production are above the line.**

## Conclusions

The community-based seed production has removed the conventional five-year gap between release and dissemination. Although informal spread from farmer to farmer is an effective means of spread, seed production and distribution by NGOs and GOs will greatly accelerate the spread. To gain maximum benefit from the new varieties, the effectiveness of informal spread increases with the number and wider distribution of primary adopters. Hence seed production and distribution needs to be continued for some years, and the seed needs to be widely distributed outside GVT villages, particularly in states not served by GVT (i.e., Chhattisgarh and Bihar). This will be done through internationally funded projects (e.g., DFID-funded Western Orissa Livelihood Project), private seed agencies, and other NGOs.

PPB is a continuous process and efforts are in place to replace Ashoka varieties with superior alternatives. A number of potential varieties with better drought tolerance developed at CAZS, through marker-assisted selection for root traits are in the advanced stages of testing (Prasad et al., 2003). Efforts on PPB continue, with success most likely to come from crosses involving Ashoka 200F and Ashoka 228.

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# Drought and cropping pattern change in Tamil Nadu, India: Needed technological transformation in rice farming

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## Introduction

Demand for rice in India is projected at 128 million tons for 2012 and will require a production level of 3,000 kg / ha, significantly greater than the present average yield. After a long period of technological breakthroughs and adoption, the yield gap still exists (Siddiq, 2000). Of the total area under rice in Tamil Nadu State, nearly 7% of the area is under dry and semi-dry conditions. Dry and semi-dry types of cultivation are predominant in the districts such as Ramnad, Sivagangai, and Thiruvallur. Studies identified several production constraints including insects / pests, diseases, adverse soils, genetic / physiological and adverse climatic and environmental factors, which contribute to major yield losses in rice and indicated the possibility of biotechnological approaches to solve such problems (Ramasamy et.al., 1996; Ramasamy and Jatileksono, 1996). Studies revealed that the average yield was about 30-35% higher in the irrigated ecosystem than in the rainfed ecosystem (Janaiah et al., 2000). This paper highlights the impact of drought on cropping pattern change and income distribution in Tamil Nadu. The paper emphasizes the importance of crop breeding for water limiting environments in order to cope with drought. While attempting to develop such varieties, it is crucial to consider the traits of the varieties that are predominantly grown by the farmers.

## Methods

Effect of drought on cropping pattern change was assessed by construction of the Herfindahl index; the factors affecting the diversification were analyzed using the log linear equations. Factors such as rainfall, irrigation intensity, fertilizer consumption per hectare, wholesale price index, and productivity index were included in the model to examine their influence on acreage diversification. Influence of rainfall and productivity of rice on area allocation decisions and influence of rainfall distribution on paddy productivity was also assessed. Weakening of the trickle down effect of agriculture on poverty reduction due to drought was estimated using a system of equations. An inequality index was employed to analyze impact of drought on income distribution.

## Results

Rice yield recorded a compound growth rate of 2.13% during 1965-66 and 2001-02 in Tamil Nadu. Sub-period growth rates indicate that rice productivity witnessed a high growth rate of 4.69% during the 1980s. However, productivity of rice registered a negative growth rate of 0.38% in 1990s. Growth of rice in terms of area, production, productivity varied among the various rice production environments such as rainfed tank, tank, tank cum well, canal (river), and canal (reservoir). Productivity growth in the rainfed tank environment was stagnant, only 0.12% from 1984-85 to 2001-02. This included the large tract of dryland regions with less dependable water resources. In the state, nearly 50% of the districts are drought prone. The average rainfall in the state decreased by more than 40% during the drought period and the cultivated area has not been extended due to lack of water. There was a decline in gross sown area due to drought and the estimated reduction was 4 lakh hectares (7% decline as compared to normal period). The effect of drought was also reflected in the expansion of area under current fallow and other fallow lands. Current fallow increased to 15.6 lakh hectares during the drought period from 12.2 lakh hectares in the normal period. Net area irrigated in the state was 28.01 lakh hectares and gross area irrigated was 34.12 lakh hectares, which constitute almost 55% of the net and gross cropped area in the state during 2001-02. There was a decline in area under irrigation in the drought period; the extent decline was 8% due to drought. Failure of the southwest monsoon affected the storage capacity of Tamil Nadu's major reservoirs and water supplies from surface and groundwater sources for irrigation during the first half of the monsoon was a major problem. Area in Tamil Nadu irrigated by canals declined by 9.62%, tanks by 10.38%, ordinary wells by 4.19%, and tube wells by 7.55% due to drought.

Based on the rainfall distribution and availability of groundwater, farmers' change their cropping pattern and mitigate the drought impact. In Tamil Nadu, shortfalls in rainfall occurred during 1974-75, 1980-81, 1986-87, 1988-89, 1990-91, 1995-96, and 2002-03. There were seven drought years during the last three decades. Diversification of cropping patterns, particularly from high water consuming crops like paddy to other lower water consuming crops, is not reflected strongly, as the Herfindahl index registered almost equal figures during both the drought and normal periods. However, to some extent, crop diversification was noticed in the Ramnad district, as the index worked out to 0.24 and 0.37 during the drought and normal periods, respectively (Table 1). Reduction in area under paddy was observed due to drought and crops like groundnut, gingelly, caster, tobacco, blackgram, greengram, and redgram gained area during the drought period. Area under paddy declined, while area under blackgram, greengram, and gingelly increased in the Thiruvallur district during the drought period. Similarly, in Coimbatore district, the area under paddy, maize, sorghum, pearl millet, and vegetable crops declined during the drought period, while groundnut, cotton, bengalgram, redgram, horsegram, finger millet, and kodo millet increased, implying that there was a shift from water intensive crops to rainfed crops due to rainfall failure. Reduction in paddy area and shift to other rainfed crops was also exhibited in Ramanad district.

**Table 1. Drought and crop diversification (Herfindahl Index)**

| Particulars | Drought period | Normal Period |
|---|---|---|
| Tamil Nadu State | 0.14 | 0.15 |
| Ramad | 0.24 | 0.37 |
| Thiruvallur | 0.40 | 0.41 |
| Coimbatore | 0.14 | 0.15 |

The econometric results showed that rainfall had a negative effect on crop acreage, revealing that good rainfall is expected to discourage diversification in the state and in most of the selected districts. Irrigation intensity (ratio of gross irrigated area to net irrigated area) had a positive and significant effect on acreage diversification, suggesting that availability of irrigation water year round is expected to promote crop acreage diversification. Coefficients of the whole price index and productivity index reveal that farmers prefer cultivating the same crops if they fetch higher income either through increases in productivity or product price. Rice-yield boosting technology for water limited environments is construed as more of an instrument promoting the risk-taking function of the farmers than anything else. It is imperative to maximize risk-taking ability of the farmers and several alternative solutions are proposed in this regard by several studies. There is a clamor for reducing input prices and an equal urge for raising output prices. Fixing higher prices for output will lead to the large farmers getting an extra ordinary level of profit and a further perpetuation of income inequalities. Real price of paddy was similar both during the drought and normal periods (Rs 39.27 and 38. 67 per quintal of paddy - 1970-71 base) indicating that yield risk has not been compensated by product price.

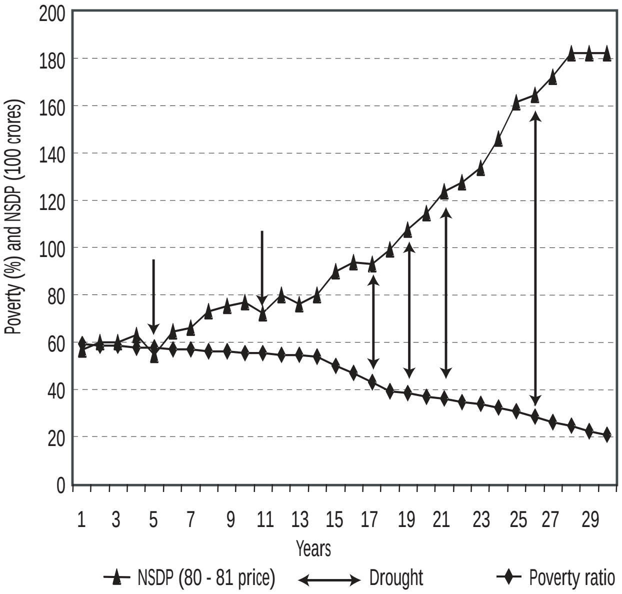

**Figure 1. Poverty and NSDP.**

It was estimated that an increase in productivity of paddy by one ton would replace paddy area by 1.89 lakh hectare in Tamil Nadu. Similarly, area expansion under paddy was also noticed in the selected districts, revealing that infusing high productivity traits in the drought tolerant rice varieties will enable the farmers to allocate some amount of land to other crops. Such diversification can generate adequate income to alleviate poverty in the rainfed areas. Rainfall influences the risk-taking function of the farmers to a very large extent in drought prone and dryland areas, as evident from the estimated results. Rainfall has the positive influence on area expansion of paddy and it is found that for every additional increase in rainfall by one mm, about 208 hectares of additional area would be brought under paddy. The risk due to drought is reflected in the level of investment made in modern inputs such as fertilizers and pesticides. The current level of fertilizer consumption in the state in terms of NPK was 51.36, 20.71 and 24.76 kg / ha. Further, it was noticed that there was a marginal decline in per hectare fertilizer consumption in the state during the drought period. Nitrogenous fertilizer consumption in the state decreased to 4.09 lakh tons in the drought period from 4.68 lakh tons in the normal period. Similarly, there was a reduction in consumption of phosphatic and potash fertilizers during the drought period. It is also evident from the econometric results that agricultural growth has a trickle down effect and such effect weakened during the drought period (Table 2). Income inequality (paddy) was found less in the drought period statewide and in selected districts as drought affected paddy cultivation in the state irrespective of the size groups and regions (Table 3). The government launched the Calamity Relief Fund Scheme, which provides relief for crops based on the extent of damage. Current area under rice in the state is 20.60 lakh hectares, of which ADT 43 constitutes nearly 21% followed by Improved White Ponni (16%), ADT39 (14%), ADT36 (8%), CO43 (7.5%), ADT38 (6.73%) and IR20 (6%). It is imperative that the traits (genetic and marketability) of these varieties are considered in breeding varieties for water limiting environment, thereby income realization from paddy can be sustained.

**Table 2. Effect of drought on poverty reduction – log linear estimates**

| Variables | Equation 1 | Equation 2 | Equation 3 | Equation 4 |
|---|---|---|---|---|
| Net State Domestic Product | -0.811*** | -0.204 | -0.200 | -0.460 |
| | (-20.914) | (-0.813) | (-0.7621) | (-1.588) |
| Net State Domestic Product (dummy) | 0.002 | -0.001 | -0.001 | -0.001 |
| | (0.488) | (-0.304) | (-0.3019) | (-0.208) |
| Net State Domestic Product (lagged one year) | - | -0.634*** | -0.629*** | -0.644*** |
| | | (-2.506) | (-2.301) | (-2.462) |
| Consumer Price Index | - | - | -0.005 | -0.162 |
| | | | (-0.055) | (-1.308) |
| Time | - | - | - | -0.024* |
| | | | | (1.804) |
| Intercept | 11.179*** | 11.426*** | 11.383)*** | 14.532*** |
| | (31.503) | (34.197) | (13.236) | (7.531) |
| $R^2$ | 0.94 | 0.95 | 0.96 | 0.96 |

(Figures in parentheses denote t ratios)
*** Significant at one per cent level of probability and * significant at 10 per cent level of probability

**Table 3. Inequality of income distribution (Gini Index - rice)**

| Period | Chengalpet | Coimbatore | Ramnad | Tamil Nadu |
|---|---|---|---|---|
| Normal | 0.3712 | 0.3539 | 0.4037 | 0.3639 |
| Drought | 0.4824 | 0.3063 | 0.3439 | 0.2621 |

Period: 1970-71 to 2000-01

## Conclusion

Productivity growth in the rainfed tank environment, which includes the large tract of dryland regions with less dependable water resources, was stagnant. There was a decline in gross sown area due to drought. The effect of drought was also reflected in the expansion of area under current fallow and other fallow lands. Diversification of cropping pattern, particularly from high water consuming crops like paddy to other lower water consuming crops, was not strongly reflected. However, to some extent, crop diversification is noticed in Ramnad district. Rainfall had a negative effect on crop acreage revealing that good rainfall is expected to discourage the diversification in state and almost in all the selected districts. Irrigation intensity had a positive and significant effect on acreage diversification, suggesting that availability of irrigation water on a year round basis may be expected to promote crop acreage diversification. Rice-yield boosting technology for water limiting environments is construed as more of an instrument for promoting risk-taking functions of the farmers than anything else. Real price of paddy was similar during both the drought and normal periods, indicating that yield risk has not been compensated by product price. Infusing high productivity traits in the drought tolerant rice varieties will enable farmers to allocate some amount of land to other crops; such diversification can generate adequate income to alleviate poverty in the rainfed areas.

Agricultural growth has a trickle down effect in reducing poverty and such effects weakened during the drought period. Varieties like ADT 43, Improved White Ponni, ADT39, ADT36, CO43, ADT38, and IR20 are the predominant varieties grown in the state. It is imperative that the traits (genetic and marketability) of these varieties should be considered in breeding varieties for water limited environments, thereby income realization from paddy can be sustained.

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# Varietal adoption and farmers' coping strategies in rainfed rice ecosystems of Tamil Nadu

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## Introduction

Drought is one of the most important constraints in rainfed rice production. Lack of high yielding varieties and low rates of their adoption are the major reasons for low productivity in water limiting environments. Therefore, raising productivity in these areas is crucial for alleviating poverty and ensuring food security of the rural poor (Hossain, 1990). Although productivity of food grains grew faster in the rainfed areas (1.66% between 1985-86 and 1998-99 in Tamil Nadu), the average productivity of food grains in irrigated areas is higher by 50% than rainfed areas (Selvaraj et.al., 2002)). Rainfed areas are poor in resource endowments and infrastructure; the marginal impact of HYVs on production is much higher in high and low potential rainfed areas than irrigated areas (Fan and Hazell, 2000). Various safety nets are employed by the farmers during the stress years in order to cushion the adverse impacts of stress. The effectiveness and economic cost of these coping mechanisms vary depending upon the intensity of drought and the nature of production system (Pandey et al., 2000)

## Methods

Secondary data on rainfall, production, area under HYVs and landraces were used to estimate probability of occurrence of drought in the rainfed districts and the effect of drought on area and paddy production. Estimates were derived from the field survey conducted in selected districts of Tamil Nadu covering 200 sample farms. Yield loss due to drought and curtailment in use of modern inputs was analyzed using the regression decomposition framework. Land, labour, fertilizer, and plant protection chemicals were considered in the decomposition framework in order to estimate the yield loss due to these factors apart from drought. Impact of risk of drought on yield of HYVs and landraces was assessed by log linear equation. The risk was measured in terms of deviation between the recommended and actual use of fertilizers. Transcendental production was used to estimate the optimal level of yield and cost. Factor shares (land, fertilizer, labour, and plant protection chemicals) under different technologies (HYVs and landraces) and proportionate change in the factor shares were estimated based on the elasticity coefficients following Hicks analytical tool.

## Results

There were six, seven, and eight drought years in Ramnad, Tiruvallur, and Coimbatore districts, respectively, over the last three decades. In Tiruvallur district, the shortfall in rainfall was 60%, while it was 53%, and 47% in Coimbatore and Ramnad districts, respectively, during the drought years as compared to the normal years. Some of the landraces are still popular due to their tolerance and resistance to biotic and abiotic stresses. Area under high yielding varieties during the drought years decreased by 2.47 lakh hectares. Area under landraces in Ramnad and Tiruvallur district increased during the drought period. Increase in area under landraces was 0.4 lakh hectares in Ramnad, while the increase was minimal in Tiruvallur district. Due to severity of drought, there was a reduction in production of about 39% in the Ramnad district, 32% in Tiruvallur, and more than 50% in Coimbatore district. Actual yield reduction was much higher in Ramnad district 618 kg/ha) than in Thiruvallur district (338 kg/ha).

Most farmers (40%) cultivated landraces (Mattai) in Ramnad district, followed by HYV like MDU 5 and IR36. High yielding varieties like TRY2, PMK2, ASHOKA2007, ASHOKA228, MDU5, and RM96019 were distributed to the farmers of Ramnad district by TNAU on trail cultivation in the farmers field for subjective assessment of the varieties by the farmers.

Varieties like ADT36, Chellaponni, Jothi, Culture Ponni, IR20, and MDU5 are predominantly grown in the Sivagangai district. In Coimbatore district, the majority of the farmers cultivate ADT39, ADT36, IR20, CO43, and CO42. Short duration varieties ADT39 and CO43 are the drought tolerant rice varieties with a yield potential ranging from 3,000 to 3,500 kg / ha. ADT 43 and Bapatla are the most popular varieties in Thiruvallur district. Average marketable surplus was 70% for HYVs, while it was 56% for landraces in Ramnad, implying that farmers, for consumption purposes, mostly prefer local races, particularly in Ramnad district. According to the majority of the farmers, the probability of occurrence of drought was more than 30% in Ramnad district and it varied among the districts. Probability of occurrence of drought is highest in Coimbatore district (more than 50%).

Farmers in the rainfed production environment are operating at a sub-optimal level of production. Further, comparison of actual and optimal cost of cultivation reveals that for the production level realized by the farmers, they incurred higher cost due to risk and adoption of varieties with less response to technological inputs. Farmers incurred an additional cost of Rs 899 for the realized production, whereas the scope exists to increase yield by 228 kg / ha with the available technology and resources. About 90% of the farmers in the rainfed environment were found to be inefficient since actual yields were lower than the optimal yield on those farms. Econometric results indicate that yield loss due to risk of rainfall failure was higher in the case of HYVs as compared to landraces. Even a 10% increase in risk resulted in 5.4% decline in yield of HYVs in Ramnad district. However, yield reduction in landraces was minimal and it was found that a 10% increase in risk could cause yield to decline by only 0.2% (Table 1). In order to cope with the risk, farmers cultivated alternate crops like sorghum, blackgram, groundnut, finger millet, and vegetables like ladies finger. Similarly, the farmers sold livestock and borrowed loans from both the formal and informal sources as drought mitigating measures. Farmers also reduced consumption expenditures during the drought period. Share of income from paddy, other crops, other sources (labour income, both off-farm and non-farm) and livestock were estimated during the normal and drought period. Extent of reduction in consumption expenditure was also estimated to understand farmers' coping behaviour and the cumulative effect of drought on livelihood of the farmers.

On average, farmers realized 4.2 t / ha of (paddy) from the high yielding varieties in Ramnad district during the normal period, while they realized 3.3 t / ha from the local varieties. Although yield realization from the local varieties was less during the normal period (0.9 t / ha), farmers cultivated landraces due to assurance of a minimum level of yield during the drought period. It was estimated that the farmers realized an incremental benefit of Rs 5,783 per hectare by cultivating landraces during the drought period as compared to HYVs. (Based on the difference in the yield reduction of high yielding varieties and landraces due to drought. Although yield reduction is lower in landraces, productivity of high yielding varieties is higher in a drought period. However, due to reduction in cost, landraces fetch marginally higher return.) Cultivation of HYVs during the normal period fetches an incremental benefit of Rs. 2,165 per hectare as compared to landraces (Table 2). Non-system tank forms the major

**Table 1. Impact of risk (drought) on yield of paddy - log linear estimates**

| | Co-efficients | t-value |
|---|---|---|
| Ramnad | | |
| HYVs | -0.540** | -2.746 |
| Land Races | -0.016 | -0.110 |
| Sivagangai | | |
| HYV | -0.190* | -1.736 |
| Thiruvallur | | |
| HYV | -0.062** | -2.442 |

** significant at 5 per cent level, * significant at 10 per cent level

**Table 2. Comparative analysis of high yielding and local varieties (Rs / ha)**

| | Land Races– Drought years | | High yielding varieties – Normal years | |
|---|---|---|---|---|
| Cost | Debit Added Cost | Credit Reduced Cost | Debit Added Cost | Credit Reduced Cost |
| | - | 2563.21 | 2563.21 | - |
| Return | Reduced Return | Added Return | Reduced Return | Added Return |
| | - | 3220.00* | - | 4727.89 |
| Total | | 5783.21 | 2563.21 | 4727.89 |
| Incremental benefit (Rs) | | 5783.21 | | 2164.68 |

* Based on the difference in the yield reduction of high yielding varieties and land races due to drought. Though yield reduction is lower in land races, productivity of high yielding varieties is higher in drought period. However, due to reduction in cost, landraces fetch marginally higher return.

source of irrigation in Ramnad district, which depends on rainfall. Therefore, it is imperative that the varieties meant for water limiting environment should ensure a minimal level of yield during the stress period and that could induce the farmers to go for higher levels of adoption.

However, yield reduction of HYVs in other selected districts, namely Sivagangai and Thiruvallur districts, was found to be less, despite the occurence of variability in rainfall, because of supplementary sources of irrigation. Yield reduction of HYVs would be 2.0–0.6% in Sivagangai and Thiruvallur districts, if risk of drought were to increase by 10%. Technological change in rice cultivation is land saving but fertilizers, labour, and plant protection chemicals are also factors (Table 3). Farmers are of the opinion that HYVs need high quantities of fertilizers due their responsiveness and due to weeds growing easily and profusely in the wake of fertilizer application. This, in turn, requires more labour for weeding as compared to landraces. Further, labour requirement for other operations like planting, harvesting, and threshing is higher in the case of HYVs as compared to local varieties. Use of capital for chemical fertilizers and insecticides was also higher in the cultivation of HYVs, while local varieties have much more resistance to pests and diseases. However, the production elasticity of fertilizer (0.319) and labour (1.051) implies that marginal return from application of fertilizer and labour is higher in HYVs as compared to landraces.

Use of higher doses of fertilizers in the cultivation of land races affects the standing crop. The benefits of the new technology can be derived if it assure minimal level of yield during the drought period because the farmers curtail the use of modern inputs during the drought period, which may result in further decline in productivity. Nitrogen consumption per hectare of gross cropped area in the state was 3.248 kg in irrigated area, while it was 0.998 kg in rainfed area during the period between 1985-86 and 1998-99. Similarly, phosphorus consumption was 1.186 kg per hectare of gross sown area in irrigated areas, while it was 0.377 kg per hectare in rainfed areas. In the case of potash, per hectare consumption in irrigated area was 1.43, whereas it was 0.517 per hectare in the rainfed areas during the same period (Selvaraj et al. 2002). Since high yielding varieties require higher doses of fertilizers to realize their yield potential, fertilizer consumption is higher in the irrigated areas. Fertilizer consumption in rainfed areas is still less than half the rate used in irrigated areas. Results of decomposition analysis reveal that reduction in yield due to curtailment of input usage accounts for only 3-10 percent and this could be attained through creation of awareness among the farmers and more than 90 percent of yield reduction is due to water stress. Estimated yield loss due to drought is 1400 kg per hectare in the case of HYVs, while it was 840 kg per hectare in the case of landraces. Therefore, breeding varieties with drought tolerance is crucial. Varieties should possess the characteristics of assuring a minimum level of yield and thus enable the farmers to realize additional yield by 1,260 kg of paddy per hectare from the present level of yield obtained during the drought period.

**Table 3. Estimates of factor share under different technologies and proportionate change in the estimated factor shares**

| | Factor Share | | |
|---|---|---|---|
| Factor Inputs | Land Races | HYVs | Proportionate Change |
| Land | 1.498 | 0.567 | -0.621 |
| Fertilizer | 0.096 | 0.484 | 0.802 |
| Labour | -0.172 | 0.943 | 6.468 |
| Plant Protection Chemical | -0.606 | -0.810 | 0.252 |

## Conclusion

Average marketable surplus was 70% for HYVs, while it was 56% for landraces, implying that farmers, for consumption purposes, mostly prefer local races, particularly in Ramnad district. According to the majority of the farmers, the probability of occurrence of drought was more than 30% in Ramnad district and it varied among the districts. Probability of occurrence of drought is highest in Coimbatore district (more than 50%). Farmers in the rainfed production environment are operating at sub-optimal levels of production. Furthermore, comparison of actual and optimal cost of cultivation reveals that for the production level realized by the farmers, they incurred higher cost due to risk and adoption of varieties with less response to technological inputs. Econometric results indicate that yield loss due to risk of rainfall failure was higher in the case of HYVs as compared to landraces. Technological change in rice cultivation is land saving, but fertilizers, labour and plant protection chemicals are also factors. Estimated yield loss due to drought is 1,400 kg / ha for HYVs, and 840 kg / ha for landraces. It was observed that the cumulative effect of drought on the livelihoods of the farmers was high if the ex-ante and ex-post coping mechanisms are taken into account. Therefore, breeding varieties with drought tolerance is crucial. Varieties meant for rainfed areas should possess the characteristics of assuring a minimum level of yield, thus enabling the farmers to realize additional yield of 1,260 kg of paddy per hectare above the present level of yield obtained during the drought period.

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# Application of maize mega-environments in seed systems in the Southern African Development Community

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## Introduction

New open pollinated maize varieties that mature earlier, are stress tolerant, and higher yielding are now available. With the development of new varieties farmers and relief organizations still find it difficult to select an appropriate variety for their environment. Variety selections are usually based on availability of seed and price, ignoring adaptation factors such as rainfall, minimum and maximum temperatures, and the length of growing season. Identification of maize mega-environments offers farmers, relief organizations, and others an opportunity to select appropriate varieties for their environments. Based on maize regional yield trial data and geographical information system (GIS) parameters, maize mega-environments were identified.

## Methods

Using the revised mega-environments (Setimela et al., 2002), a basis for choosing the right open pollinated maize variety for a given environment was developed. Similar areas across the SADC region were given the same color code based on classification of the mega-environment. By determining one's location on the map, color code (yellow, orange, red, and green), and maturity group (early, intermediate, and late maturing variety) one selects an appropriate group. Once an appropriate group has been chosen, varieties within the group are then assessed for their appropriateness in the given environment. A description of important characteristics for each variety is also given. Based on these combinations of factors, farmers are guided to the optimum variety.

## Results

A clear and easy to follow system for choosing appropriate varieties has been developed. A booklet to guide farmers on choosing appropriate varieties for their environments has been published and is now available and widely distributed in the SADC region.

## Conclusions

The use of mega-environments in combination with descriptions of varieties has provided a quick and transparent basis of choosing maize varieties in the SADC region. When appropriate varieties are clearly identified their seed production and provision is facilitated..

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# Breeding drought tolerant varieties of rice through participatory plant breeding for the rainfed uplands

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## Introduction

Drought is the most important abiotic stress that inhibits plant growth and reduces productivity. Varieties developed through the conventional plant breeding approach have not been adopted by the farmers of rainfed uplands because they not only lack in drought resistance but also in many of the farmer preferred traits such as high fodder yield and cooking qualities. Farmers of rainfed uplands thus cultivate old and unimproved cultivars and obtain low yields. Of the 141 varieties released for the uplands in India, only a few have become popular with farmers. In Jharkhand, most of farmers either grow the Brown Gora landrace, or its derivative, Birsa Gora 102, that have low yield and poor grain quality.

## Materials and methods

A collaborative project on rice improvement was undertaken between BAU, Gramin Vikas Trust (an NGO), and the Centre for Arid Zone Studies, University of Wales, Bangor, UK with funding from the DFID bilateral project managed by GVT and Plant Sciences Research Programme of DFID in 1997 to develop farmer preferred drought tolerant varieties through participatory plant breeding (PPB). GVT has a network of farmers in Jharkhand, Orissa and West Bengal and thus provided means of farmer field testing and selection.

As a first step, a participatory varietal selection (PVS) was undertaken with GVT adopted farmers who identified Kalinga III. However, it lacked in lodging resistance. Its improvement was initiated by crossing it with a diverse variety IR64. Collaborative PPB (farmers selected in the $F_4$ bulk in their own field) and consultative PPB (farmers selected among $F_4$ progeny bulks on station) resulted in the development of Ashoka 200F and Ashoka 228 that were released in 2003 by BAU as BVD 109 and BVD 110. Since these varieties were developed with the participation of farmers who widely experimented with them, they excel in farmer preferred traits. Similar methods will be followed in development of drought tolerant varieties of rice for the rainfed upland in The Rockefeller Foundation supported project to BAU. Formal seed production of these varieties has not been possible because of lack of their notification by the government of India. However, GVT has undertaken their dissemination through community based seed production and these varieties are becoming very popular.

## Results

Screening of local germplasm and other pure-breeding lines among a large collection of upland germplams at BAU is being undertaken for drought tolerance and characterization for yield and root traits. The other important traits of interest are: early maturity, more productive tillers, longer panicles with more grains or grain weight. Among the root traits attention is being given to longer roots, more roots, higher root volume and weight and a higher root-shoot ratio. Lines with superior traits for various quantitative traits will be identified for use as donors in the improvement of upland varieties for drought tolerance and higher yielding ability. Varieties Ashoka 200F and Ashoka 228 that have high drought resistance will also be used as parents.

## Development of backcross populations

Backcrosses of Vandana x IR72 and Vandana x IR72975 crosses will be made with Vandana as a recurrent parent. Effort will be made to incorporate the superior grain quality traits of IR72 and IR72975 into Vandana's background since Vandana has been reported to be a drought tolerant variety with longer roots but inferior grain quality.

The research aims at putting farmer preferred traits into a drought tolerant variety for the rainfed uplands; work includes

- identification of drought tolerant genotypes;
- development of genotypes with early vigour for better weed suppression in the direct seeded rainfed uplands;
- breeding for early maturity to impart this drought escape mechanism;.
- higher yielding ability under direct seeding (2.5 to 3.0 t / ha);
- breeding tall varieties (95 to 100 cm) for higher straw yield;
- breeding varieties with straw colour husk and white kernels; and
- breeding varieties with superior cooking quality.

## Conclusions

Keeping farmers' preferences in mind, a number of crosses for developing drought tolerant varieties are in the advanced stages of breeding ($F_6$, $F_7$). These are Vandana x IR72, Vandana x IR72975, WARDA 45 x A 157, Sathi 85-3 x A 162, PBRC78 x Komal 13, PBRC x A 228, CH 45 x MT1, and bulks derived from marker assisted selection programme for drought tolerance from Kalinga III x Azucena cross.

Five to 10 lines from each cross have been selected by scientists and farmers for drought tolerance and higher yield (Figure 1). These will be tested in All India Coordinated Trials and State Trials along with on-farm testing. Simultaneous seed production and dissemination will be undertaken for the farmer-preferred varieties through GO-NGO collaboration to bridge the gap between release and dissemination. Advanced generation four bulks developed through marker assisted selection for root traits to impart drought resistance were tested in on-farm trials in West Bengal in *kharif* 2003. Differences between bulks were significant for grain yield (Table 1, Figure 2); they yielded 11 to 56% more than the check variety Kalinga III. Bulk 5 also has an aroma QTL and was the most preferred. This bulk will be proposed for release and wider dissemination.

**Table 1. Analysis of variance for grain yield (kg / ha) of marker assisted selected bulks for root traits from Kalinga III x Azucena cross tested in on-farm mother trials in West Bengal in *kharif* 2003**

| Source | df | MS | F | P |
|---|---|---|---|---|
| Between varieties | 5 | 227731 | 4.94 | 0.01 |
| Between villages | 7 | 507592 | 11.02 | 0.00 |
| Between farmers/ villages | 11 | 59910 | 1.30 | 0.32 |
| Error | 14 | 46053 | | |

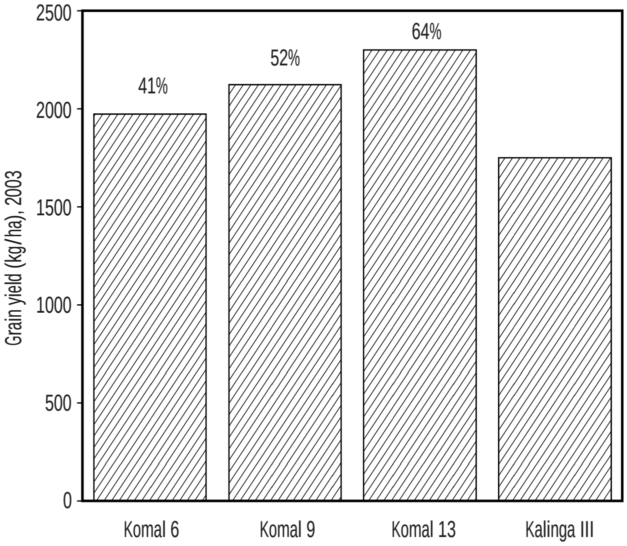

**Figure 1. Grain yield superiority of pipeline varieties (Komal series) developed from Kalinga III x Vandana cross in BAU trilas in *kharif* 2003.**

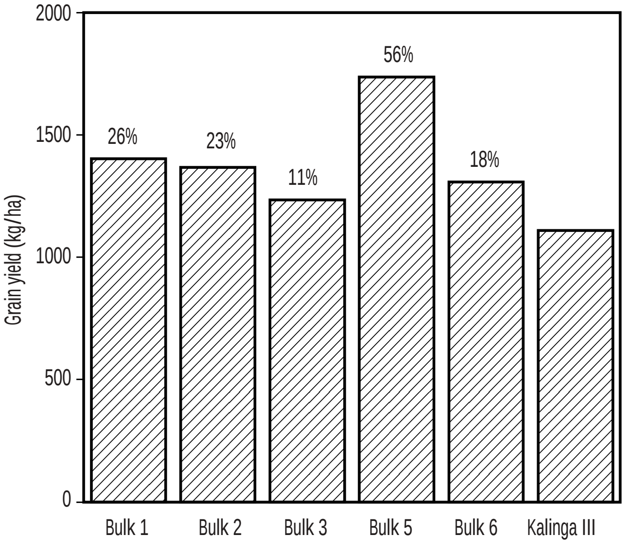

**Figure 2. Grain yield of four marker assisted selected bulks of Kalinga III x Azucena cross in on-farm trials in West Bengal in *kharif* 2003.**

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# Reaching the poor maize farmers in the hills of Nepal: Experiences and achievements of the Hill Maize Research Project (HMRP)

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## Background

Maize in the hills of Nepal is cultivated in a wide range of environmental regimes. The variations in altitude, aspects, uncertainty of pre-monsoon and / or monsoon rainfall leading to yearly variations in planting time and maize growth, cropping systems, management practices, indigenous knowledge systems, traditions, input regimes, socioeconomic ground realities, farmers specific preferences and needs influence what is currently practiced and what will likely be adopted by farmers. The above variations across the mid-hills of Nepal are so overwhelming that it is a tremendous challenge to focus meaningful maize research so as to have any bearing on technology generation that has potential for large-scale extrapolation and impact. About 95% of the farmers have less than one hectare of land and 72% of households have food sufficiency for less than six months (Gurung, 1999). Maize is staple food and very little goes to market. Most maize farmers in the remote areas, inaccessible by roads and devoid of any development infrastructures, are so poor that they cannot afford to buy external inputs. Under such circumstances, it is a daunting task to reach those farmers with maize production technology and to make an impact. The Hill Maize Research Project (HMRP) is trying to do just that and has made progress by developing technology for and with poor farmers. This paper highlights the experiences and achievements of the project and methodologies followed.

## The project

The HMRP, a collaborative project between CIMMYT and the Nepal Agriculture Research Council (NARC), funded by the Swiss Agency for Development and Cooperation (SDC), started in 1999 with the overall goal to improve food security and livelihoods of farming families through the increased productivity and sustainability of maize-based cropping systems in the hilly areas of Nepal. In these chronically food-deficit hills, maize is the most important food crop, grown under rainfed conditions, mainly on a small scale by resource-poor farmers in an area covering approximately 1.8 million hectares. It is reasonable to infer that food security will reduce conflict in the society and thus enhance harmony in the households and thereby in the community. The majority of the hill population, especially children and women, suffer from malnutrition because their diets lack protein. Because of this, HMRP is working on the development and promotion of Quality Protein Maize (QPM). To reach to the resource-poor farmers in the remote areas, HMRP has been expanding its research and development activities through its partners. Participatory variety selection (PVS) and community based seed production (CBSP) through various partners such as NARS and non-government organizations (NGOs) is making the difference. This has helped in disseminating results generated by the project.

## Approach

The project strategy is based on working and supporting research, training, and dissemination in a holistic and concerted way through effective partners. The strategy deals aims to promote an efficient research system and the development and dissemination of research products, through increasing capacity building for maize researchers under NARC and extension workers under the Department of Agriculture (DoA). Linking research and dissemination institutions and monitoring their activities at outreach research sites with key players like NGOs and community based organizations (CBOs) for the client-centered programs are key elements of the project.

To achieve the goals of the project, several approaches are employed: enhancement of institutional effectiveness of NARC to develop and deliver maize technology; organization of short- and long-term training; incorporation of gender and equity awareness and analysis into the research process; strengthening of the capacity of the National Maize Research Program (NMRP) to conduct participatory research; and improvement of planning, monitoring, evaluation, and reporting in NMRP. Expanded collaboration with partners; implementation of a small grants scheme; support partnerships at the agriculture research station level and annual partnership meetings; development of improved maize technologies and assessment of the adoption of technologies are the major areas of focus.

## Varietal development and testing

Developing and maintaining open pollinated varieties (normal and QPM) with stress tolerance (biotic and abiotic stresses) is continuing. Screening for turcicum resistance and drought tolerance is also continuing.

Testing of germplasm is undertaken in four steps: the Observation Nurseries / Trials (ON), the Intermediate Yield Trial (IYT), the Coordinated Variety Trials (CVT), and the Farmer's Field Trial (FFT). The project has continued to support the improvement, maintenance and multiplication of currently released hill maize varieties as well as the development and testing of new cultivars. As a result, Hill Pool Yellow, ZM-621, Pop 44 c10, Pop 45 c10 and Hill Pool White as a full season; and Arun-4, Pool 15E, Pool 17E and Pool 16 as early and extra early genotypes, have been identified as promising genotypes.

## Participatory approach

Participatory methods in varietal testing and dissemination of maize production technology have been initiated with partners in outreach research for technology verification through agriculture research stations, DoA I / NGOs and through small grant schemes. PVS has been used as an effective tool to work for and with the farmers to verify and disseminate promising technologies.

The project has expanded considerably on-farm testing with relatively good stocks of seed of the newest varieties that includes coordinate farmers field trials, front line demonstration, mother / baby trials and farmers acceptance test (FAT). Thirty-five mother trials were established following PVS methodology along with 625 baby trials involving 670 farmers throughout the country in 2003. In addition, 4,500 sets of FAT consisting of released and pipeline varieties were distributed to farmers through the agriculture research stations, Agriculture Development Office (ADOs) and NGOs. Similarly about 230 front line demonstrations were distributed in areas where there is no OR activities.

## CBSP

Once a suitable maize variety has been identified, HMRP encourages its partners to multiply it locally as a community based seed production (CBSP) effort. It has shown a positive impact in adoption of improved maize in the hills of Nepal and has been successful in empowering poor and women farmers in organizing them as maize seed producing groups. The impact of these programs has started to show sustainable positive feedback in other communities also. The maize seed marketing system, however, remains weak and needs to be strengthened. In order to strengthen innovative partnerships that will enable NARC scientists to organize seed production in remote areas, partnership with CBOs at grassroots level are encouraged. About 259 tons of improved maize seeds of different varieties were produced in 2003 in collaboration with NARC ARSs, DoA, and CBOs (Figure 1).

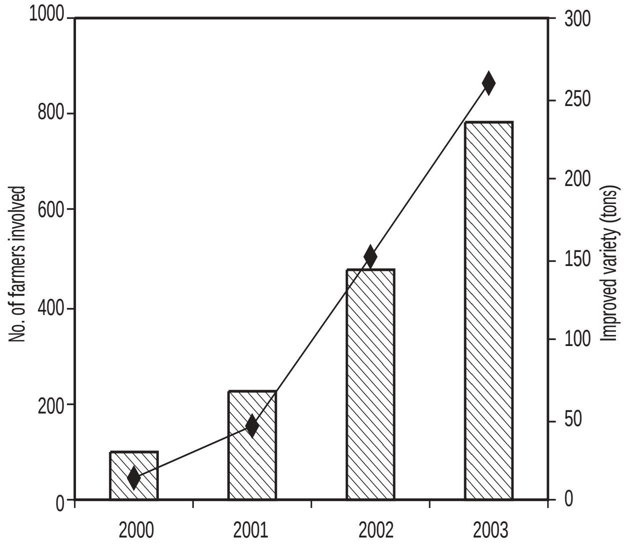

**Figure 1. Community based seed production under HMRP from 2000-2003.**

## Agronomic and other studies

To develop crop management practices, research has continued on the effect of organic inputs and lime on maize productivity; seed priming; nitrogen efficiency and soil limiting factor verification; minimum tillage; fertilizer management in maize based cropping system; identification of legumes for suitable intercropping; long-term soil fertility management in maize / millet systems, and soybean intercropping in a double plant per hill system. Given our preliminary work, we envision this research having a significant impact in the hills.

Research focused on reducing losses due to insect pests is continuing in 2004. Monitoring postharvest losses of maize, and developing strategies to control white grubs is being supported under a small grant scheme wherein Institute of Agriculture and Animal Sciences and NARC scientists are isolating and culturing indigenous pathogens for the control of white grub in maize. Gender, equity, and poverty awareness are seriously considered in all maize improvement activities.

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# An international partnership for the breeding and delivery of drought-tolerant rice varieties by market-oriented plant breeding and marker-assisted selection

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## Introduction

Farmers of the Chhotanagpur plateau of eastern India predominantly grow rice landraces in the rainfed uplands. To better address the needs of the market, on-farm and farmer-oriented approaches were used in a collaborative programme between GVT (NGO), BAU (GO), and CAZS, an advanced research institute (ARI), using funds from a DFID bilateral project managed by GVT and from the DFID Plant Sciences Research Programme. This collaborative research has led to the development and delivery of farmer-accepted varieties in less than six years.

## Methods

In participatory varietal selection (PVS), farmers preferred Kalinga III for higher yield, earlier maturity, and good grain quality. However, it has weak straw (giving lodging susceptibility) and poor roots (giving poor drought resistance). The improvement of Kalinga III was undertaken by crossing it with IR64, and by marker-assisted backcrossing (MABC) for root traits from a Philippines variety Azucena (Price et al., 2002) to transfer improved root growth that imparts drought resistance (Fukai and Cooper, 1995). We used two methods of involving farmers more closely in breeding, called participatory plant breeding (PPB). Either farmers selected in heterogeneous bulk (a form of collaborative participation; Biggs, 1989 ) or farmers made selections among materials grown on-station by researchers (a form of consultative PPB; Biggs, 1989). In the MABC progenies, six bulks were selected from the BC2 generation for selection in 1999, having various target root QTL. In addition, pure-breeding lines have been developed with individual and pyramided QTLs. These bulks / lines have been provided to farmers for testing and selection in on-farm trials since 2001. The PPB and MABC varieties were evaluated at GVT and BAU farms in 2002 and 2003 in rainfed trials.

Adoption and impact of two varieties from the PPB programme (Ashoka 200F and Ashoka 228) in Jharkhand, Orissa, and West Bengal was studied through surveys in 2002 and 2004. Bioeconomic modelling was done to assess the longer-term impact of these varieties on poverty alleviation.

## Results

PPB resulted in the rapid release of two varieties (Ashoka 200F and Ashoka 228) that were preferred by farmers in Jharkhand in 2003 (Virk et al., 2003). In rainfed trials, coarse-grained varieties Vandana and BG 102 (both released for uplands) yielded significantly less and matured later than Kalinga III. New varieties, Ashoka 200F, Ashoka 228, Bulk 4, Bulk 6, P 82 showed significantly higher yield (7 to 17% more than Kalinga III) and were either earlier or similar to Kalinga III in days to flowering. Bulk 5 from the MABC had 25% higher grain yield but was not later flowering (Table 1) than BG 102 or Vandana. It also has superior grain quality and aroma.

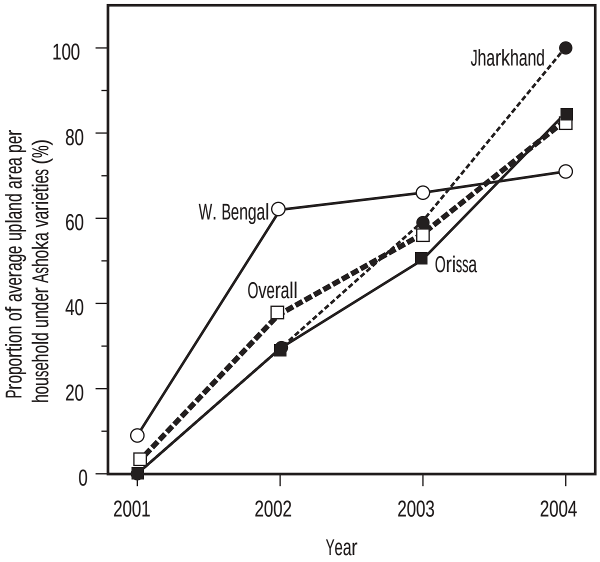
The adoption and testing of these varieties was simultaneous and their scaling up with community seed production has resulted in a wider dissemination (Prasad et al., 2004). The share of upland rice area allocated by farmers to the Ashoka varieties has shown a remarkable increase (Figure 1).

**Table 1. Mean grain yield (t ha$^{-1}$) and days to 50% flowering for some selected PPB varieties and marker assisted backcross bulks having root growth QTL in rainfed trials conducted at two sites in Jharkhand in 2002 and 2003. * P<0.05; **P<0.01.**

| Variety | Grain yield (t ha$^{-1}$) | Significance from Kalinga III | Days to 50% flowering | Significantly earlier than Kalinga III | Significantly later than Kalinga III |
|---|---|---|---|---|---|
| A 200F | 1.92 | * | 62.0 | * | |
| A 228 | 2.02 | ** | 62.8 | | |
| Bulk 3 | 1.85 | | 62.1 | * | |
| Bulk 4 | 1.91 | * | 60.4 | ** | |
| Bulk 5 | 2.24 | ** | 69.7 | | ** |
| Bulk 6 | 2.09 | ** | 62.9 | | |
| P 81 | 1.94 | ** | 64.6 | | ** |
| P 82 | 2.04 | ** | 63.1 | | |
| Kalinga III | 1.79 | | 63.3 | | |
| Vandana | 1.68 | | 67.8 | | ** |
| BG 102 | 1.65 | | 67.6 | | |
| *Lsd* 5% (1%) | 0.10 | | 1.1 | | |
| *Lsd* 1% | 0.14 | | 1.5 | | |

## Conclusions

Varieties developed through PPB and MABC have better drought resistance as they yield more than BG 102 and Vandana (both coarse grained), and Kalinga III (slender grained) varieties with earlier or similar maturity. New material is still being produced both from MABC progenies, and from new crosses involving Ashoka 200F and Ashoka 228 as parents.



**Average area of upland rice per household (ha)**

| State | N | 2001 | 2002 | 2003 | 2004 |
|---|---|---|---|---|---|
| Orissa | 14 | 0.72 | 0.80 | 0.81 | 0.87 |
| WB | 15 | 0.28 | 0.28 | 0.30 | 0.33 |
| Jhar | 7 | 0.26 | 0.36 | 0.21 | 0.29 |
| Overall | 36 | 0.70 | 0.78 | 0.79 | 0.85 |

**Figure 1. Adoption patterns of Ashoka varieties in the rice uplands per household in three states. Survey of February 2004.**

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# II. Gene Discovery and Novel Approaches

## Heritability of rice yield under reproductive-stage drought stress, correlations across stress levels, and effects of selection: Implications for drought tolerance breeding

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To be useful in variety development, screening methods for tolerance to reproductive-stage drought stress need to be repeatable, applicable at a reasonable cost to large breeding populations, and predictive of grain yield under stress. Secondary anatomical and physiological parameters have generally not fulfilled these criteria in rice. As a result, there has been increasing interest in screening for yield under stress, either in easily-drained fields in the wet season or in managed-stress nurseries in the dry season. However, little guidance is available regarding how to incorporate such screens into cultivar development programs, and there are few reports on the effectiveness of direct selection for yield under stress. We present experimental evidence that direct selection for improved yield under severe reproductive stage stress is likely to be effective, based on information from the evaluation of populations of selected and unselected lines at IRRI and elsewhere. The results of a selection experiment conducted under severe upland stress are also presented, and the integration of drought tolerance screening in IRRI's aerobic rice breeding program is described.

### Heritability within and correlations across stress levels in unselected breeding populations

If selection for yield under drought stress is to be effective, yield in the stress treatment must be repeatably measurable. The stress treatment should also provide information about cultivar differences that is not available from screening under non-stress conditions. Information on this question has been assembled for 6 populations of unselected lines evaluated in a total of 10 experiments at locations in India, Thailand, and the Philippines under both well-watered conditions and severe stress. Broad-sense heritabilities (H) within water regimes and genetic correlations across them are presented in table 1. Yield under stress averaged 35% relative to the well-watered controls across these experiments. H estimates for grain yield in the stress environments were similar to those for yield in the non-stressed controls, indicating that selection for yield under stress is likely to be as effective as selection for yield under favorable conditions.

**Table 1. Repeatability (*H*) of grain yield estimates in well-watered and moisture-stressed treatments , and genetic correlations across stress levels ($r_G$), in trials evaluating unselected populations**

| | | | | | | H | | | |
|---|---|---|---|---|---|---|---|---|---|
| Location | Year | Stress environ-ment | Non-stress environ-ment | Population | Relative yield under stress | Stress | Non-stress | $r_G$ | Data provided by: |
| Bet Dagan, Israel | 1997 | Upland | Upland | CT9993/IR62266 | 0.26 | 0.81 | 0.63 | 0.35 | A. Blum |
| Coimbatore, Tamil Nadu | 1999 | Upland | Upland | CT9993/IR62266 | 0.31 | 0.60 | 0.56 | 0.86 | R. Chandra Babu |
| Paramakudi,Tamil Nadu | 2000 | Upland | Upland | CT9993/IR62266 | 0.41 | 0.76 | 0.23 | 0.91 | R. Chandra Babu |
| Ubon, Thailand | 2000 | Upland | Upland | CT9993/ IR62266 | 0.30 | 0.50 | 0.54 | 0.71 | G. Pantuwan |
| Raipur, India | 2000-02 | Lowland | Lowland | CT9993/IR62266 | 0.21 | 0.37 | 0.45 | 0.80 | R. Kumar |
| Los Banos,Philippines | 2003 | Upland | Lowland | Vandana/IR64 | 0.67 | 0.42 | 0.27 | 0.69 | R. Venuprasad |
| Los Banos,Philippines | 2003 | Upland | Lowland | Apo/IR64 | 0.13 | 0.24 | 0.45 | 0.35 | R. Venuprasad |
| Los Banos, Philippines | 2003 | Upland | Lowland | Apo/IR72 | 0.29 | 0.67 | 0.30 | 0.64 | R. Venuprasad |
| Los Banos, Philippines | 2003 | Upland | Lowland | Vandana/IR72 | 0.31 | 0.07 | 0.42 | 0.78 | R. Venuprasad |
| Los Banos, Philippines | 1998-9 | Upland | Upland | IR64/Azucena | 0.56 | 0.68 | 0.74 | 0.62 | B. Courtois |
| **Mean** | | | | | **0.35** | **0.51** | **0.46** | **0.67** | |

Genetic correlations across water regimes were consistently positive, averaging 0.67. This indicates that, within segregating populations of lines generated from a single cross, nearly half the genetic variation for yield under severe stress is accounted for by factors that also affect yield potential. The relatively high correlations observed across establishment methods and stress levels indicate that plant type and partitioning differences that are also expressed in non-stress environments are causing much of the yield variation observed under stress in unselected breeding materials. These results show that direct selection for yield under stress is likely to result in yield gains under stress, and that improvement in yield under stress can be combined with improvement in yield potential.

## Heritability within and correlations across stress levels in advanced cultivar testing

The results presented above refer to unselected breeding lines from single crosses. In more diverse cultivar sets exhibiting a wide range of maturity, the correlation of genotype means across stress levels is affected by the magnitude of the relative yield in the stress and non-stress environments. This was observed in an experiment conducted at IRRI, wherein a diverse set of upland and lowland cultivars, breeding lines, and landraces was evaluated for two years under 4 water regimes: (1) full lowland irrigation, with transplanted management; (2) favorable upland conditions in the wet season; (3) moderate dry-season continuous stress; (4) severe dry-season continuous stress. Means in the four environments were 2.7, 2.2, 1.1, and 0.5 t ha$^{-1}$, respectively. Genetic correlations stress levels are presented in table 2. Yields were positively and highly correlated among the non-stress and moderate stress treatments, but yields under the severest stress level were not associated with yields under non-stress conditions.

## Sources of variation in trials measuring yield under stress

Although the heritability of yield is similar in stress and non-stress environments, it is rather low. To plan effective screening programs, an understanding of the sources contributing to the variance of cultivar means is needed. Major potential sources are within-trial field variability, which may be exacerbated by water stress treatments, and genotype x environment interaction. The latter source is likely to be especially important in drought screening trials conducted under natural stress in the wet season. This was illustrated in a set of 39 advanced Thai breeding lines and released varieties evaluated under stress and non-stress conditions in transplanted and direct-seeded trials at Ubon and Chumpae in 2003. Stress was applied by draining paddies 2 weeks before the onset of flowering and not re-watering. Very high genotype x environment interactions were observed, due to differences in stress timing and blast disease pressure among sites (Table 3). Residual within-trial variances were also very high resulting. This resulted in very low predicted heritabilities for both stress and non-stress trials.

By contrast, managed-stress screens for drought tolerance can have relatively high repeatabilities by controlling genotype x trial interaction. This is illustrated in the variance component and H estimates derived from the 44-entry cultivar set (described above) evaluated under upland and lowland management at IRRI. Moderate and severe stress treatments were applied in the relatively uniform dry season. In this experiment, genotype x environment interaction in the stress treatments was low relative to the genotypic variance, and relative to GxE interaction in the wet season. The main source of variation in the stress trials was within-trial residual field variation, indicating that useful levels of H can be achieved in a single managed-stress screening trial if it is well-replicated and designed (Table 4).

**Table 2. Genetic correlations across water regimes for 44 upland and lowland cultivars evaluated under 4 water regimes at IRRI, 2000-2003.**

| Environment | Moderate upland stress | Upland non-stress | Lowland non-stress |
|---|---|---|---|
| Severe upland stress | 0.51 | -0.08 | -0.20 |
| Moderate upland stress | | 0.78 | 0.85 |
| Upland non-stress | | | 0.97 |

**Table 3. Variance component and broad-sense heritability (H) estimates for the combined analysis over Ubon and Chumpae in northeast Thailand for 4 types of screening trial. (Wet season 2003)**

| | Variance components | | | H | |
|---|---|---|---|---|---|
| REGIME | Genetic | Genotype x environment | Residual | 2 sites, 3 replicates | 1 site, 3 replicates |
| Direct-seeded: non-stress | 0 | 983 | 3205 | 0.00 | 0.00 |
| Direct-seeded: stress | 32 | 393 | 966 | 0.08 | 0.04 |
| Transplanted: inon-stress | 161 | 0 | 1264 | 0.43 | 0.28 |
| Transplanted:Stress | 27 | 97 | 355 | 0.20 | 0.11 |

## Selection experiment

The results reported above indicate that direct selection for yield under stress is likely to be effective if conducted in a well-managed screening environment. To test this hypothesis, a direct selection experiment was conducted in two populations at IRRI in 2003-4. Populations were derived without selection from the crosses Apo / IR64 and Vandana / IR64. IR64 is an elite irrigated lowland variety. Apo (IR55423-01) is a high-yielding upland rice variety with moderate tolerance to continuous water stress in the field. Vandana is a highly drought-tolerant eastern Indian upland variety derived from a cross between an *aus* traditional variety and an improved Philippine tropical *japonica*. Two hundred and twenty-five (225) random $F_2$ –derived $F_3$ lines from each cross were evaluated under severe intermittent upland stress as well as under non-stress irrigated lowland management in the dry season of 2003. Evaluation was conducted in 2 replicate alpha-lattice trials in which the experimental unit was a single plot, 2 m in length in the upland and 5.25 m in length in the lowland. The 25 highest-yielding lines were selected from each screening environment (giving a selection intensity of 12.5%) and compared with 25 random lines.

**Table 4. Variance components for 44 upland and lowland cultivars evaluated under 4 water regimes at IRRI, 2000-2003**

| Environment | $_G^2$ | $_{GY}^2$ | $_e^2$ | H(one 4-rep trial) | H(two 4-rep trials) |
|---|---|---|---|---|---|
| Severe upland stress | 58 | 20 | 86 | 0.48 | 0.58 |
| Moderate upland stress | 86 | 36 | 138 | 0.46 | 0.55 |
| Upland non-stress | 337 | 168 | 184 | 0.76 | 0.61 |
| Lowland non-stress | 481 | 299 | 163 | 0.75 | 0.59 |

**Table 5. Effects of direct selection for yield under upland stress in 2 populations at IRRI: 2003-4**

| | Vandana / IR64 | | Apo / IR64 | |
|---|---|---|---|---|
| | Evaluation environment | | | |
| Entry | Upland | Lowland | Upland | Lowland |
| Upland-selected population | 68.9 | 182 | 16.7 | 191 |
| Lowland-selected population | 57.8 | 214 | 12.8 | 224 |
| Random population | 54.8 | 184 | 18.1 | 193 |
| IR64 | 3.6 | 286 | 5.8 | 293 |
| Apo | 14.8 | 240 | 17.8 | 242 |
| Vandana | 104.6 | 146 | 83.2 | 51 |
| $LSD_{.05}$ for checks | 59.0 | 49.4 | 21.4 | 91 |
| $LSD_{.05}$ for populations | 11.8 | 24.0 | 4.3 | 18 |

Results of the selection experiment are presented in Table 5. Under severe upland stress, the tolerant upland parent, Vandana, yielded 104.6 g $m^{-2}$, whereas Apo and IR64 yielded only 14.8 and 3.6 g $m^{-2}$, respectively. The selected Vandana / IR64 population outyielded the random set of lines by nearly 20% under severe stress. The selected population derived from Apo / IR64, however, exhibited no improvement over the random set under severe stress. Neither upland-selected population exhibited reduced mean yield relative to the checks under lowland conditions.

It is important to note that, on average, random lines derived from Vandana / IR64 outyielded random lines from Apo / IR64 by 300%. The effect of using a highly tolerant donor on yield under severe upland stress was greater than the effect of selection.

In general, this experiment demonstrates that direct selection for yield under severe stress in small plots can be highly effective in populations where adequate genetic variability for the trait exists. It also illustrates the importance of using highly tolerant donors when tolerance to severe stress is the breeding objective.

## Implications for drought-tolerance breeding

Yield under drought stress is a moderately heritable trait with repeatability similar to that of yield in non-stress environments. Direct selection for yield under stress is effective, if stress tolerance screening is done in replicated field trials that effectively control within-trial field heterogeneity. This means that selection for yield under stress is best initiated after pedigree breeding lines have been fixed for plant type, disease resistance, quality, and other high-heritability traits, as is the case for selection for yield potential. This was demonstrated in a selection experiment. The moderate correlation usually observed between yields in stress and non-stress environments indicates that improvements in stress tolerance can be generated while maintaining high yield potential. Highly-tolerant donors are likely to be much more efficient in generating progeny tolerant to severe stress than donors with only moderate levels of tolerance. Such donors, however, are unlikely to be agronomically acceptable in high-productivity environments. This is a strong argument for pre-breeding highly drought-tolerant donor lines with acceptable agronomic traits under non-stress conditions. Such donors could be directly used as sources of drought tolerance in cultivar development programs that must generate cultivars combining high yield potential with moderate stress tolerance.

# Empowering rice drought gene discovery activities through bioinformatics

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## RF scholars and their research

The Rockefeller Foundation funded six dissertation fellowships for rice drought bioinformatics research at the International Rice Research Institute. The scholars funded are noted in the following table:

| Scholar | Scholarship | University Affiliation | Country |
|---|---|---|---|
| Xusheng Wang | M.Sc. | Zheijiang University | China |
| Locedie Mansueto | M.Sc. | University of the Philippines, Diliman | Philippines |
| Jutharat Prayongsap | Ph.D. | Kasetsart University | Thailand |
| Naveen Sharma | Ph.D. | Kuvempu University | India |
| Muthurajan Raveendran | Postdoc-toral | Tamil Nadu Agricultural University | India |
| Ravindra Babu | Postdoc-toral | University of Hyderabad | India |

The scholars' research focuses upon the application of bioinformatics methodologies to mine rice structural and functional genomics information for candidate gene loci and associated alleles potentially conferring drought tolerance in rice. There are estimated to be more than 50,000 predicted genes in the rice genome. Identification of the candidate subset of genes involved in drought stress requires a strategy of intersecting gene position, function, expression, and allele selection evidence. Bioinformatics provide an effective means for achieving such integration. Each scholar in the project was asked to focus on subsets of drought experimental data, for integration into the International Rice Information System (IRIS; Bruskiewich et al. 2003; www.iris.irri.org).

## Integration of QTL data with rice genome sequence data

Initial project efforts focused on anchoring Quantitative Trait Locus (QTL) data on a limited set of genetic maps to publicly available rice genome sequences and expressed sequence tags (EST) from an IRRI commissioned IR64 drought stress panicle library (work undertaken by X. Wang, Wang, Zhu, Mansueto, and Bruskiewich, submitted). Further work along this theme (by J.Prayongsap) compiled additional drought QTL maps in a comparative manner, for anchoring to the rice genome, for publication in IRIS.

## The role of ABA in drought stress

The phytohormone ABA regulates many important physiological and developmental processes in plants (Leung and Giraudat, 1998). ABA regulates stomatal responses, stress tolerance responses, and growth (Zeevaart and Creelman, 1988) in addition to preparing the seed for dormancy and germination (McCarthy, 1995). It also mediates stress responses such as environmental stress adaptation to salinity, low temperature and water deficiency (Ingram and Bartels, 1996). In spite of its role in stress tolerance and dormancy, ABA appears to interfere with panicle development and emergence. For the above reasons, ABA has become a primary research target for the scholars.

## ABA and root elongation

Roots penetrating deeper soil layers contribute to maintaining plant water potential when the topsoil dries up. Drought tolerant rice genotypes grow roots deeper under low-water potential when compared to roots of susceptible plants grown in similar conditions. Abscisic acid (ABA) and ethylene play major roles as primary elicitors or secondary messengers for inducing genes during biotic and abiotic stress by plants (Bray, 1993). These phytohormones interact with each other to promote longer roots in tolerant genotypes during low-water potential (Spollen et al., 2000). One scholar (N. Sharma) is initiating gene expression studies with drought stressed root tissues probing whole genome arrays to shed light on the genes, regulatory pathways, and overall mechanisms by which ABA and ethylene interact in promoting longer roots. Allele mining of the promising candidate genes identified from the expression studies will give insight into the genetics and function in the germplasm and possible use of the candidate genes as markers (MAS) for maximum root length and drought tolerance.

## ABA and yield under stress

Although drought survival may be enhanced by deeper roots, it is yield under drought stress that is of primary agronomic concern. Another scholar (J. Prayongsap) is therefore studying the impact of ABA on the rice panicle by integrating QTL mapping data with gene expression data obtained form the IR64 drought stressed panicle EST library. In addition, IR64 mutant stocks are being screened for lesions in ABA pathway genes.

## Proteomics of ABA catabolism

The ABA level in plant is simultaneously regulated by opposing forces of catabolism and / or biosynthesis (Zeevaart, 1999). The genes involved in ABA catabolism have not been identified or characterized in rice. The identification of genes encoding for proteins in ABA catabolism may be a crucial element in a breeding program for reproductive stage drought tolerance. One RF scholar (M. Raveendran) is striving to study the effect of ABA on rice plant growth (panicle emergence, spikelet fertility, biomass, photosynthesis, transpiration, etc.) using proteomic analysis of total leaf proteins and microsomal extracts from plants subjected to exogenous application of ABA under well watered conditions. He is assaying ABA content by HPLC-based assay of ABA catabolism and ABA-8’ hydroxylase activity in the microsomal preparation.

## Bioinformatic integration of drought gene information

Integration of all the above datasets is being greatly facilitated through software engineering efforts of one of the scholars (L. Mansueto), who comes to the project with a computing science background. This latter scholar also played a major role in the analyses of the ~8,000 clone drought stress panicle EST library, in the commissioning of a microarray laboratory information management system currently used by the project, and in the integration diverse project data with publicly available pathway and network information. These activities have included Java software engineering activities as part of an ongoing effort to develop a new informatics platform for crop gene discovery.

## Project status

X. Wang obtained his M.Sc. from Zeijiang University last July 2003 and a paper is submitted for publication. L. Mansueto is expected to defend his M.Sc. in the near future and continue in the project for a short while as a non-RF computer consultant at IRRI. J. Prayongsap, N. Sharma, and Dr. Raveendran are expected to complete the RF-funded component of scholarship later this year. Ms. Prayongsap intends to return to Thailand to complete her PhD; however, as a doctoral scholar, N. Sharma is planning to continue his work at IRRI under the auspices of the new CGIAR Genetic Resources Challenge Program (www.GenerationCP.org). Dr. Babu has only recently joined the project. His research will focus upon the characterization of regulatory networks using whole genome analysis and large-scale integration of bioinformatics data generated within the project.

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# Functional analysis of plant hydrophilins

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## Introduction

The Late Embryogenesis Abundant (LEA) proteins are plant polypeptides synthesized at the onset of desiccation in maturing seeds and in vegetative organs exposed to water deficit. We have shown that most LEA proteins are comprised in a more widespread group denominated ‘hydrophilins’. The defining characteristics of ‘hydrophilins’ are high glycine content and a high hydrophilicity index. By database searching, this criterion specifically selects most known LEA proteins, as well as additional proteins from different taxons (Garay-Aroyo et al., 2000). Thus, ‘hydrophilins’ might represent an analogous adaptation to a common problem in organisms as diverse as plants, bacteria, and fungi.

To gain insight into the function of different hydrophilins, including LEA proteins, we developed an in vitro partial dehydration assay wherein the activity of malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) is measured in the presence or absence of a putative protecting protein. Since a number of LEA proteins are also accumulated upon low temperatures in vivo, we have adapted a second in vitro test where freezing is applied as a mean to limit water availability (Lin and Thomashow, 1992). Under the conditions of the partial dehydration assay, where the progressive loss of water occurs in the absence of other perturbing factors, such as heating or freezing, we show that hydrophilins are able to protect these enzymatic activities. Under these conditions, the compatible osmolyte trehalose needs to be in a $10^5$-fold excess over LDH to exhibit the same protective level as hydrophilins. Our data show that group 2 LEA proteins (dehydrins), as well as different LEA proteins from groups 3 and 4 (LEA76 and LEA D113) are capable of protecting both MDH and LDH when exposed to a controlled desiccation process, as well as during freezing conditions. ‘Hydrophilins’ from *S. cerevisiae* as well as from *E. coli* also present protective characteristics in these in vitro assays.

## Results

The ubiquity of hydrophilins, their responsiveness to water deficit, and their capacity to protect enzymatic activities from inactivation due to in vitro partial water removal have led us to pursue their function in vivo as well as the mechanisms involved in the regulation of their gene expression. One approach has been to analyze one of the smallest *Arabidopsis* LEA protein families, the LEA-IV family, which is predicted to consist of three members: *Atlea-IV-1, Atlea-IV-2,* and *Atlea-IV-5.* In addition to the transcript accumulation patterns, using a specific polyclonal antibody, we also obtained the protein accumulation patterns for AtLEA-IV-5, which showed that this protein accumulates in dry seeds and, in vegetative organs in response to drought, osmotic and ionic stress, but not in response to low temperatures. Transgenic *Arabidopsis* plants overexpressing *Atlea-IV-5* gene did not show advantage compared to the wild-type when seeds are germinated in hyperosmotic (500 mM mannitol) or high salt (250 mM NaCl) media. However, a significant resistance to dehydration / rehydration treatment was observed in adult plants, as indicated by the higher survival percentage and dry weight of overexpressing lines compared to non-transgenic plants. To address the function of these proteins during water limitation and seed development, we are silencing either *Atlea-IV-5* or the complete *Atlea-IV* gene family using RNA interference.

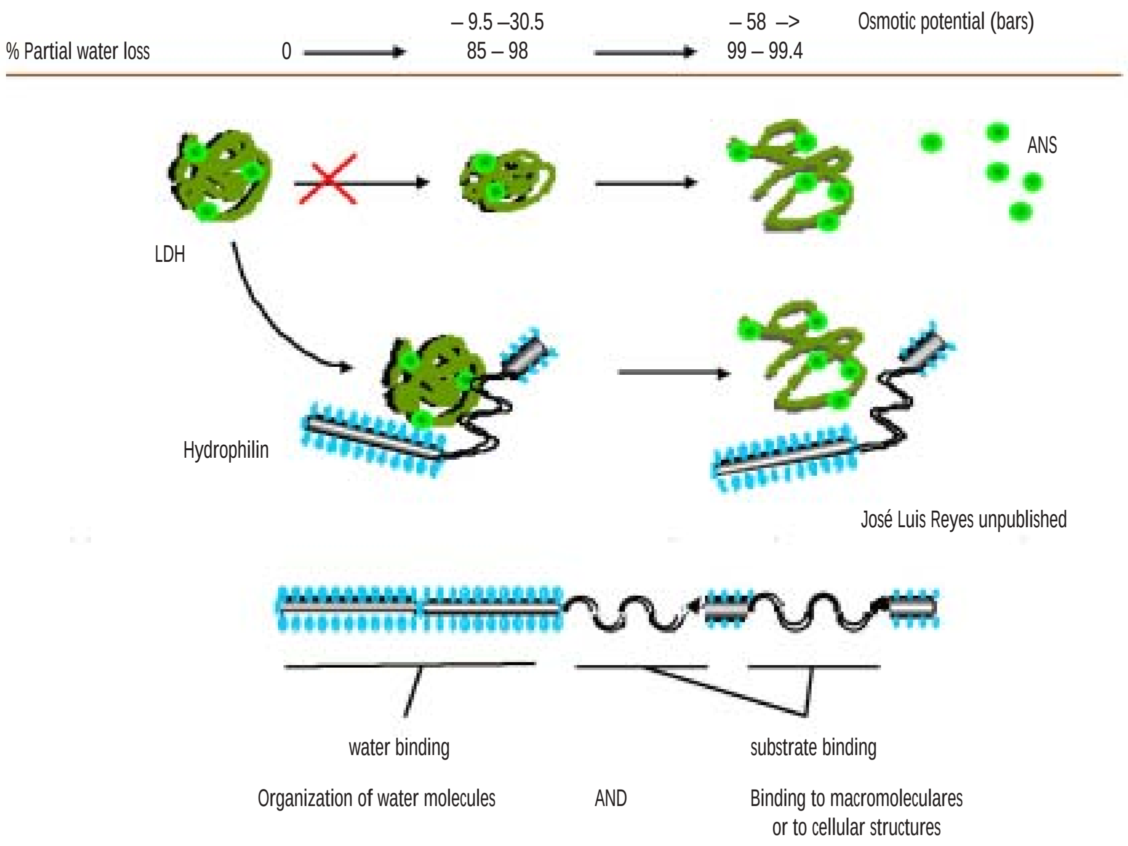

**Figure 1. A schematic model to explain the protective effect showed by hydrophilins during in vitro partial water loss. It is proposed that hydrophilins are constituted by two domains, one able to bind and / or organize water molecules and other, which identifies poarticular targets (macromolecules or cellular structures). Their flexible structure (random-coil) may be important for optimal protection of their targets, they may be unstructured in solution but structured when complexed with some macromolecule.**

## Conclusions

These findings lead us to suggest that, at least, some 'hydrophilins' preserve enzymatic activities during partial dehydration by a mechanism involving hydrophobic interactions, which would prevent enzyme denaturation. Our results provide basis to understand the role of hidrophilins in the adaptive response to water stress.

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# Rice SNP map between *indica* and *japonica* subspecies: DNA marker resolution on the kilobase scale

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## Introduction

Marker-assisted selection (MAS) will be an invaluable tool for the development of rice varieties with a higher tolerance to abiotic and biotic stresses. However, many heritable, agriculturally-relevant, stress-tolerance phenotypes are modified in a complex, polygenic manner. This is evident in the large number of stress-relevant rice QTLs in the literature, and the genetic difficulties faced by the breeder attempting stress-tolerance allele introgression. In order to design flexible and efficient MAS strategies, the rice breeder will require genetic markers that are reliable, low-cost, and localized to multiple genomic regions. However, due to coverage gaps in current rice genetic marker collections and the cost/time commitment in marker development for uncharacterized genotypes, there is an urgent need for alternative marker solutions. We have aligned the *indica* (cv. 93-11) and *japonica* (cv. Nipponbare) rice genomes for the purpose of discovering single nucleotide polymorphism (SNP)/insertion-deletion (INDEL) markers offering potentially high genetic resolution, and which should be informative in crosses involving the two rice subspecies. These SNP/INDEL markers could be used for marker-assisted selection, LD scans, and other applications in the pursuit of higher stress-tolerant rice varieties.

## Methods

The International Rice Genome Sequencing Project (IRGSP) has used a BAC based strategy to sequence the rice genome (ssp. *japonica* cv. Nipponbare). The Beijing Genomics Institute (BGI) used a shotgun strategy to sequence a separate subspecies of rice (ssp. *indica* cv. 93-11). All sequence information is publicly available. We used BLAST to align the BGI *indica* contigs (Rice GD, http://btn.genomics.org.cn:8080/rice/) with the Institute for Genomic Research (TIGR) *japonica* pseudomolecule assembly (v1.0; the TIGR Rice Genome Project, http://www.tigr.org/tdb/e2k1/osa1). Prior to alignment, all *indica* shotgun contigs were masked for repetitive DNA. SNPs and single-base INDELs were extracted from the BLAST alignments using in-house Perl scripts after filtering repetitive DNA, paralogous DNA, and polymorphisms of low quality.

## Results

After applying the stringent set of filters, the total number of polymorphisms remaining was 408,898 (94% SNPs). On average, this resulted in a polymorphism rate of 1.02 polymorphisms per kilobase based on a rice genome size of 400 megabases. The number of polymorphisms per chromosome is shown in figure 1. In order to determine the quality of

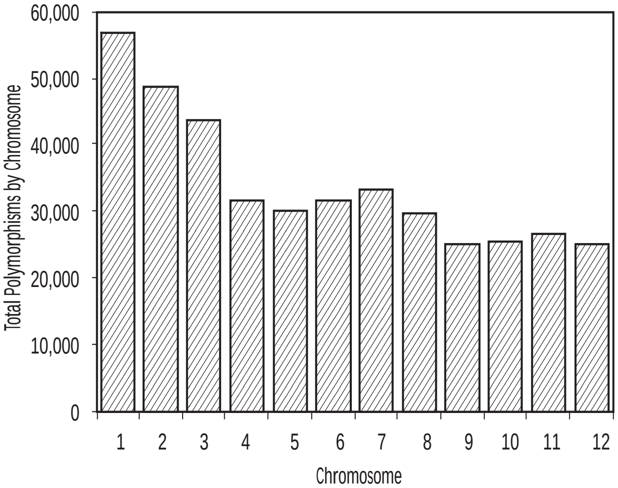

**Figure1. Total number of *indica-japonica* SNP/INDEL polymorphisms by chromosome.**

this dataset, we sequenced random loci containing SNPs from each chromosome. PCR primers were designed from the pseudomolecule and used to amplify Nipponbare and 93-11 genomic DNA. Approximately 80% (87 / 109) contained SNPs that matched our dataset. To test whether these polymorphisms would extend to other *indica-japonica* experimental crosses, we tested for overlap with the SNP dataset from Nasu et al., which is the largest collection of published rice SNPs. We examined a subset of their SNPs for overlap with our dataset in which all three *japonica* genotypes shared the same nucleotide and two *indica* genotypes shared a different nucleotide. Forty-eight percent (41 / 86) showed exact overlap with our *indica-japonica* dataset.

## Conclusions

On average, we estimate that 38% of the discovered polymorphisms will be informative in other *indica-japonica* crosses. This corresponds to approximately 152,000 SNP / INDELs or 0.38 polymorphisms / kilobase based on a genome size of 400 megabases. Therefore, the number of discovered markers should be sufficient for most genetic studies involving *indica-japonica* crosses. This work was supported by The Rockefeller Foundation initiative on 'Resilient Crops for Water-Limited Environments,' USAID Comparative Cereal Genomics Initiative, and US National Science Foundation Plant Genome Research Program.

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# XVSAP1 from *Xerophyta viscosa* improves salinity and water deficit stress tolerance in *Arabidopsis* and tobacco

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## Introduction

It has been estimated that two-thirds of the yield potential of major crops are routinely lost due to unfavourable growing environments (Bajaj et al., 1999). In recent years, genetic engineering has been used to improve stress tolerance in plants. A potential rich source of genes that could confer tolerance to abiotic stresses is a small group of angiosperms known as the resurrection plants. These plants can lose more than 90% of their relative water content, survive in their dried state for prolonged periods, and then resume active life when water becomes available again (Bartels et al., 1990; Sherwin and Farrant, 1996). Genes that could potentially improve the drought tolerance of agriculturally important plants such as maize and wheat have been isolated from *C. pantagenium* (Itturiaga et al., 1992) and *X. viscosa* (Garwe et al., 2002; Mundree et al., 2000;).

## Methods

The strategy of "complementation by functional sufficiency" was used to isolate a cDNA designated *XVSAP1* from a cDNA library constructed from *X. viscosa* leaves dehydrated to 85%, 37%, and 5% relative water content. Following molecular characterization, the induction of *XVSAP1* under water deficit conditions was analysed using semi-quantitative RT-PCR. To confirm the functional role of the gene in stress tolerance, *XVSAP1* was transformed into *Arabidopsis thaliana* and *Nicotiana tabacum* by Ti plasmid-mediated transformation under the control of a cauliflower mosaic virus 35S promoter, a *nos* terminator and *bar* gene selection.

## Results

Analysis of the cDNA sequence indicated a highly hydrophobic protein with six transmembrane regions. The deduced amino acid sequence showed 49% identity to WCOR413, a low temperature regulated protein from wheat. The protein also showed 25% to 56% identity to WCOR413-like proteins from *A. thaliana*. Southern blot analysis revealed that there are at least two copies of *XVSAP1* in *X. viscosa*. . It was shown that *XVSAP1* is induced by dehydration, salt stress (100 mM), both low (4˚C) and high temperature (42˚C) and high light treatment (1500 mmol $m^{-2}$ $s^{-1}$). Expression of *XVSAP1* in both *Arabidopsis* and tobacco plants led to constitutive accumulation of the corresponding protein in the leaves. Transgenic *Arabidopsis* grown in tissue culture and tobacco grown hydroponically were more tolerant to salt and osmotic stress. Non-transgenic plants had shorter roots, leaf expansion was inhibited, and leaves were more chlorotic than those of the transgenic plants. In addition, transgenic tobacco plants attained a higher fresh and dry weight than the untransformed controls. Transgenic tobacco showed greater tolerance to drought stress when grown in soil.

## Conclusion

The study demonstrated that *XVSAP1* is involved in the response to abiotic stress in *X. viscosa* and confers tolerance to heat, drought, salt, and osmotic stresses when expressed in heterologous plant systems. The expression of XVSAP1 in crops such as maize and wheat could lead to increased crop production in southern Africa.

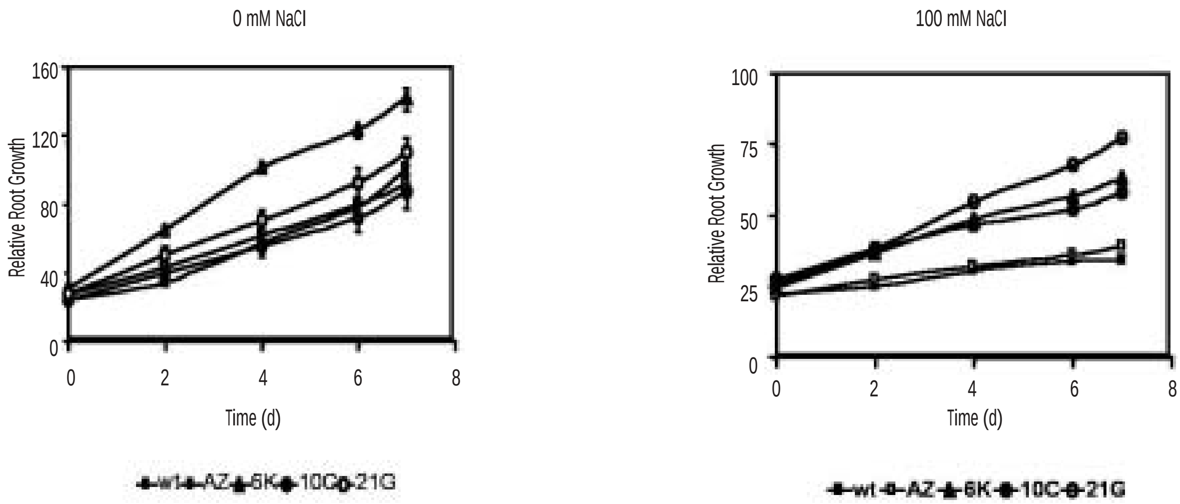

**Figure 1.** Comparison of the relative root growth of Arabidopsis plants transformed with *XVSAP1* (6K, 10C, 21G), the azygous control (AZ) and the untransformed control (WT) on salt stress media. Five-day-old seedlings were transferred to plant nutrient agar without sucrose (PNA) or to PNA supplemented with the indicated concentrations of NaCl. Root length was determined from eight plants.

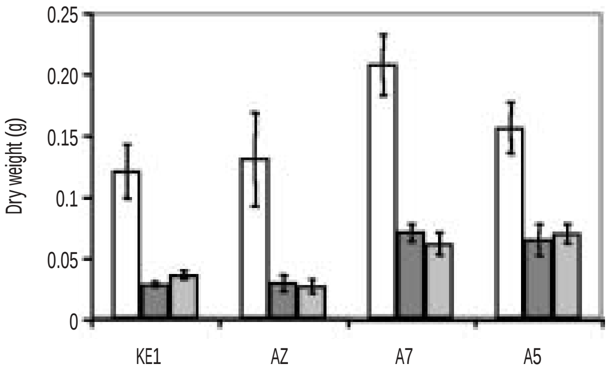

**Figure 2.** Dry weight comparison of tobacco plants transformed with *XVSAP1* (A5, A7) and untransformed controls (azygous, AZ; wild type, WT) after salt and osmotic stress. Six-week old tobacco plants grown hydroponically in one-fourth-strength MS (MS-4) solution were transferred either to fresh MS-4 medium (MS, white bars) or MS-4 supplemented with 200 mM NaCl (dark grey bars) or MS-4 supplemented with 9% polyethylene glycol (PEG, avg mol. wt. 3.350, light grey bars) for one week. Measurements were taken one week after recovery in fresh MS-4 solution. Error bars represent standard deviation based on the mass of eight plants.

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# Molecular dissection on rice photosynthesis-related traits at reproductive stage in irrigated and drought conditions

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## Introduction

Photosynthesis is an essential physiological process in plants' growth and dry matter production. There is great genetic diversity in photosynthesis among rice germplasm (Cao et al., 2001). Usually, the plant with high photosynthesis can produce more biomass and grain yield. Recent studies on paddy rice under irrigated conditions indicates that the photosynthetic rate (PR), chlorophyll content (CC), stomatal resistance (SR), and transpiration rate are quantitative traits and affected by several QTLs (Teng et al., 2004). In this paper, a RIL population from a paddy rice and upland rice cultivar were investigated for PR, CC, SR, TR, as well as water use efficiency (WUE) in both irrigated (normal) and drought (stress) conditions. The molecular dissection was also conducted to map the related QTLs. The results will help us understand the genetic bases of photosynthesis under drought occurrence in rice production.

## Methods

A set of 195 F10 recombinant inbred lines were developed from Zhanshan 97B (paddy rice with the largest growing area in China) and IRAT 109 (drought tolerant upland rice) and its parents was planted in the drought screening facility, which can create gradients of soil water content, at Shanghai Agrobiological Gene Center. Each genotype was seeded directly in two-rows plots in random block design with three replications. Each row in a plot consisting of 15 plants with a spacing of 18 cm. After stress at the reproductive stage, two representative plants in different soil water status in each plot, were selected as normal and stress treatments to measure the PR, SR, TR, WUE, and CC by using the BAU System and SPAD 502, respectively.

Standard analyses of variance were performed to check the genetic variance among the RI Lines for the investigated traits. The phenotype correlation was calculated using S-PLUS statistics software. The genotyping was conducted according the published procedures (Luo et al., 2001). An integrated genetic linkage map with 186 SSR markers was constructed using Mapmaker version 3.0 (Lincoln and Lander 1992). The putative quantitative trait loci linked to the traits were identified using Windows QTL Cartographer V2.0 (Basten et al., 2001) with a threshold LOD score of 2.0.

**Table 1. Putative QTLs for photosynthetic rate, stomatal resistance, chlorophyll content, transpiration rate and water use efficiency in a Zhenshan 97B / IRAT109 RIL population**

| Trait | QTL | Water status | Chr. | Marker interval | LOD | a | $R^2$% |
|---|---|---|---|---|---|---|---|
| Photosynthetic Rate (PR) | *QPr12* | normal | 12 | RM101-RM179 | 2.83 | 0.994 | 6.96 |
| | *QPr2* | stress | 2 | RM263-RM526 | 2.15 | 0.785 | 4.69 |
| Chlorophyll content (CC) | *QCc3a* | normal | 3 | RM22-RM231 | 3.26 | -0.896 | 7.04 |
| | *QCc3b* | | 3 | RM16-RM426 | 3.03 | -0.971 | 8.57 |
| | *QCc3c* | stress | 3 | RM203-RM520 | 2.26 | 0.784 | 5.66 |
| | *QCcc7* | normal | 7 | RM134-RM248 | 3.66 | 0.931 | 7.87 |
| | | stress | 7 | RM134-RM248 | 2.51 | 0.795 | 5.86 |
| Stomatal resistance (SR) | *QSr2a* | stress | 2 | RM110-RM211 | 2.04 | 0.247 | 5.19 |
| | *QSr2b* | | 2 | RM279-RM555 | 2.52 | 0.252 | 5.38 |
| | *QSr9* | | 9 | RM215-RM245 | 2.46 | 0.252 | 5.43 |
| | *QSsr10* | | 10 | RM311-RM467 | 2.22 | 0.358 | 6.7 |
| TranspirationRate(TR) | *QTr5* | normal | 5 | RM274-RM480 | 3.04 | -65.495 | 7.34 |
| | *QTr6* | | 6 | RM30-RM340 | 2.03 | 48.899 | 4.3 |
| | *QTr12* | stress | 12 | RM4A-RM19 | 3.9 | -75.42 | 11.85 |
| Water Use Efficiency (WUE) | *QWue1* | normal | 1 | RM472-RM104 | 2.4 | -4.095 | 8.03 |
| | *QWue11* | | 11 | RM206-RM144 | 2.31 | -3.315 | 5.15 |
| | *QWue7a* | stress | 7 | RM500-RM320 | 2.62 | -3.355 | 7.52 |
| | *QWue7b* | | 7 | RM351-RM505 | 2.67 | 2.861 | 5.73 |
| | *QWue9* | | 9 | RM434-RM410 | 2.04 | -2.519 | 4.35 |

## Results

1. There is significance difference in five photosynthesis-related traits between the parents. Under water stress conditions, IRAT109 has larger PR, TR, WUE, and CC, but less SR than Zhenshen 97B.Both parents have higher WUE and PR, but lower TR in stress. All the traits were normally distributed with transgressive segregation in the population.
2. Two QTLs related to PR were located on chromosomes 2 and 12, respectively. One (*Qpr2*) was detected under stress conditions, explaining 4.69% of total variation. The allele from Zhenshan 97B has positive effects. Another (*Qpr12*) in marker interval RM101-RM179 was detected under normal conditions.
3. Three QTLs on chromosome 3 were found to associate with CC, but only one was detected under stress conditions. A QTL in marker interval RM134-RM248 on chromosome 7 was detected under both stress and normal conditions.
4. Four QTLs influencing SR were located on chromosomes 2, 9, and 10, respectively, under stress. Together, these QTLs explained about 26% of total variation. No significant QTL was detected under normal conditions.
5. Three QTLs affecting TR were located on chromosomes 5, 6,12, respectively. The QTL on chromosome 12 (*Qtr12*) has the largest contribution to total variation. The allele from Zhanshan 97B has negative additional effect.
6. Five QTLs underlying WUE were detected. Among them, two (Qwue1, Qwue11) were detected under normal conditions and located on chromosome 1 and 11, respectively. Three (Qwue7a, Qwue7b, and Qwue9) were detected under stress and mapped on chromosomes 7 and 9, respectively.

## Conclusions

The photosynthesis-related traits performed differently under normal irrigation and drought conditions. As the water stress increased, the transpiration rate of the plant decreased significantly, but the water use efficiency as well as photosynthetic rate increased. A total of 18 putative QTLs were detected that showed association with photosynthesis-related traits; they were located on 10 chromosomes, and not found on chromosomes 4 and 8. Only one QTL, on chromosome 7, was detectable under both water condition treatments.

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# Identification of SSR markers linked to candidate genes for drought tolerance in rice

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Microarray analysis of drought-stressed rice panicles identified a number of ESTs showing significant changes during water stress in a set of three diverse rice cultivars growing in the field (Kathiresan, 2004; Lafitte et al., 2004). We identified 96 of these drought-responsive ESTs that were annotated as having drought-related functions, and were not distant from QTLs reported for growth or yield under drought. The QTLs used were primarily those reported for a rice mapping population derived from upland-adapted parents (Lafitte, Price et al., 2004), with supporting information from another population (Babu, Nguyen et al., 2003). We used 350 SSR primers located on the same BAC / PAC clones as these drought-responsive ESTs to study polymorphism in 11 rice cultivars. The markers were not evenly distributed across the genome, but were clustered. The cultivars surveyed have been used as parents in mapping populations, and they include *indica* and *japonica* subspecies as well as upland and lowland-adapted cultivars. Specific allelic patterns were generated from 325 of the markers. The pattern analysis constructed from the banding patterns clustered the cultivars by subspecies, with the exception of the *japonica* / *indica* intermediate CT9993, but not by adaptation (Figure 1). Some of the regions associated with drought QTLs may reflect allelic differences that are characteristic of *indica* vs *japonica* contrasts, but ample variation for drought-responsive EST regions exists within subspecies as well. Among the non-*japonica* cultivars, Co39 showed little similarity to other cultivars. The subtropical high-yielding line from southern China, Teqing, unexpectedly showed similarity to early-maturing Bala from eastern India.

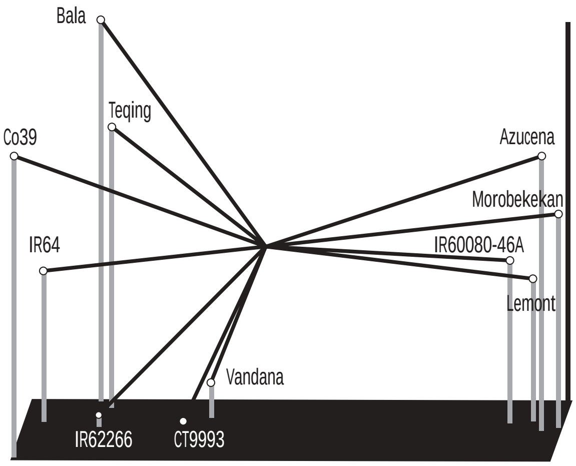

**Figure 1. Principal coordinate analysis of 11 rice cultivars on the basis of 325 SSR markers. The SSR markers were selected as being both tightly linked to drought-responsive ESTs in panicles at heading and also lying near drought-related QTLs for plant growth and yield.**

Groups of 40 to 60 markers per population were identified as being clearly polymorphic between parents of two additional mapping populations (IR64 / Azucena and Vandana / Moroberekan). Each population was genotyped, and single marker analysis was conducted for performance under managed drought stress in field experiments. Populations were screened under upland stress conditions with stress applied during the reproductive stage. A number of interesting regions contained QTLs across both populations, and the ESTs, or other genes on the BAC clone, may be considered putative candidate genes for drought tolerance (Table 1). These included protein kinases, genes associated with ubiquitin-mediated degradation of proteins, genes associated with protection from oxidative damage, signaling or defense genes, and cell cycle genes. In some cases, the EST sequence hit several locations in the genome, but significant marker effects were found only at a single location. Co-localization of the significant marker effects with the drought-responsive ESTs across diverse populations supports the hypothesis that allelic variation in these genes results in differences in crop performance under field conditions. Further steps to confirm the identity of these candidate genes will include gene expression studies in contrasting sets of lines from the mapping populations.

**Table 1. Details of drought-responsive ESTs, the number of QTLs reported in that region (QTLs), and the number of traits for which significant marker effects were observed in this study using 2 mapping populations grown with mild or severe reproductive stage drought (# traits)**

| EST: AF53-Rpf_ | Annotation | SSR Loci | CHR | cM | QTLs | # traits |
|---|---|---|---|---|---|---|
| 01B_E06_T7.ab1 | Casein kinase | RM3360 | CH01 | 27.3 | 3 | 11 |
| | | RM1183, RM7643 | CH01 | 127.3 | 0 | 8 |
| 05_K18_T7_077.ab1 | Serine/threonine protein kinase | RM6277, RM7466, RM8081 | CH01 | 28.9 | 3 | 0 |
| | | RM1220, RM6167, RM6651, RM8077 | CH01 | 37.4 | 3 | 8 |
| | | RM1356 | CH04 | 82.5 | 4 | 0 |
| 05_M18_T7_071.ab1 | Calcium-dependent protein kinase | RM1342, RM5305 | CH02 | 118 | 3 | 15 |
| | | RM4266, RM6013 | CH03 | 7.9 | 13 | 0 |
| 03_G07_T7_028.ab1 | COP9 signalosome complex sub 2 | RM572, RM8045, RM8046 | CH01 | 52.4 | 2 | 28 |
| 07_D17_T7_073.ab1 | Vacuolar membrane ATPase sub G | RM1168, RM3212, RM5833, RM8248 | CH02 | 121 | 3 | 5, 11, 24 |
| | | RM3217, RM5503 | CH04 | 100.7 | 1 | 0 |
| 10_C02_T7_009.ab1 | Ubiquitin-conjugating enzyme | RM186, RM6329 | CH03 | 127.4 | 0 | 6 |
| | | RM124, RM3332, RM3333 | CH04 | 128.8 | 0 | 8 |
| 05_K16_T7_062.ab1 | Cullin 1 | RM6864 | CH05 | 22.5 | | 23 |
| | | RM592 | CH05 | 26 | | 10 |
| 01C_J19_T7.ab1 | 26S proteasome regulatory particle | RM5454, RM6024 | CH05 | 67.5 | | 15 |
| 11_H20_T7_076.ab1 | 26S proteasome regulatory particle | RM5454, RM6024 | CH05 | 67.5 | | 15 |
| 10_K19_T7_078.ab1 | RCI2B (Low temp/salt responsive) | RM3627 | CH01 | 54.3 | 2 | 5 |
| | | RM1024, RM5374 | CH05 | 12.5 | 5 | 6, 20 |
| | | RM1209, RM5455 | CH07 | 99.3 | 3 | 0 |
| 06_B16_T7_050.ab1 | Glutathione peroxidase | RM6366 | CH02 | 110.6 | 3 | 0 |
| | | RM1367 | CH02 | 110.9 | 3 | 8 |
| | | RM124, RM3332, RM3333 | CH04 | 128.8 | 0 | 8 |
| 03_C05_T7_025.ab1 | Glycolate oxidate | RM234 | CH07 | 93.9 | 3 | 12 |
| 06_D13_T7_057.ab1 | Aldose reductase | RM3295, RM3759, RM7084 | CH05 | 103 | | 2, 5 |
| 08_D07_T7_026.ab1 | Brassinosteroid signalling positive regulator-related | RM151, RM118 | CH01 | 28.4 | 3 | 0 |
| | | RM3187 | CH06 | 73.2 | 6 | 0 |
| | | RM5508 | CH07 | 81.05 | 4 | 4 |
| 07_P19_T7_080.ab1 | Ras-related GTP binding protein | RM2857, RM5946 | CH05 | 116.2 | | 21 |
| 02_D19_T7_074.ab1 | Dihydroflavonol-4-reductase DFR1 | RM2256 | CH07 | 49.7 | 3 | 0 |
| 06_J09_T7_037.ab1 | Dihydroflavonol-4-reductase DFR1 | RM25, RM4085, RM5432 | CH08 | 35.7 | 5 | 26 |
| 02_N02_T7_007.ab1 | Diadenosine 5',5''"-P1,P4-tetraphosphate hydrolase | RM124, RM3332, RM3333 | CH04 | 128.8 | 0 | 6 |
| 09_M23_T7_088.ab1 | Senescence-related proteins | RM3917 | CH01 | 145.3 | 13 | 0 |
| | | RM409, RM5122, RM3700 | CH09 | 55.3 | 0 | 7 |
| 06_M03_T7_008.ab1 | Myotubularin | RM3120, RM3496 | CH08 | 119.6 | 1 | 4 |
| | | RM281, RM3155, RM7400 | CH08 | 119.9 | 1 | 5 |
| 05_C16_T7_058.ab1 | Ribosomal protein L10 | RM3917 | CH01 | 145.3 | 13 | 0 |
| | Ribosomal protein L10; nucleoside hydrolase | RM3120, RM3496 | CH08 | 119.6 | 1 | 4 |
| | Ribosomal protein L10; nucleoside hydrolase | RM281, RM3155, RM7400 | CH08 | 119.9 | 1 | 5 |
| | Ribosomal protein L10 | RM477, RM5545 | CH08 | 120.4 | 1 | 0 |

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# Monitoring the transcriptome changes of 14013 rice unigenes in response to drought by cDNA microarray

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## Introduction

Environmental stresses, such as drought, high salinity, and low temperature, have adverse effects on plant growth and seed production. Plants have evolved a number of mechanisms to cope with different abiotic stresses. One important step in the control of the stress responses appears to be the transcriptional activation or repression of genes. Expression of many genes has been demonstrated to be induced by these stresses, including those encoding transcription factors; some have been identified have been shown to be essential for stress tolerance. Transcriptome analyses using microarray technology is very powerful and useful tool in identifying several genes that are induced by dehydration stress and these genes can further be classified into different functional groups (Katiyar et al., 2003, 2004). We describe here the assembly of EST-based microarrays, which include transcripts from 14,103 rice unigenes, predominantly from drought and salt stressed rice plants, leaves, and roots. These microarrays were used to examine global transcript abundance changes of rice to drought stress. The results (i) provide a detailed characterization of the changes in transcript abundance following drought shock treatment, and (ii) support the concept of a succession of gene expression changes that fit into a logical framework of sensing, signaling and response networks, and identify numbers of genes that are correlated with the drought shock response. Identification of a succession of gene expression changes begins to trace the underlying regulatory networks of drought-responsive genes and should allow us to define a response hierarchy that reflects mechanisms of tolerance or avoidance of drought stress.

## Method

To identify the drought responsive transcripts during vegetative growth, expression profiles were established for different rice cultivars using microarrays containing 14,013 rice unigenes, mostly from cDNAs libraries of plants challenged by abiotic stresses, harvested at different developmental stages. The behavior of the shoot transcriptome was monitored in a time course experiment. For selected transcripts, behavior was also tested by real-time RT-PCR.

## Results

The behavior of the shoot transcriptome was monitored in a time course from 3 h to 9 h after imposition of drought stress (shock by removing water completely). The transcript abundance of hundreds of genes was changed after drought shock treatments of different durations. The analyses

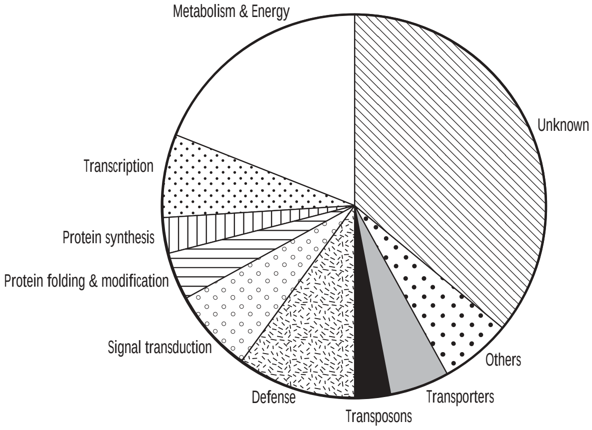

**Figure 1. Global changes in transcript expression (> 2.5 folds) in rice after drought treatment.**

identified hundreds of transcripts, significantly induced and repressed for the gene expression at various time points. For selected transcripts, behavior was also tested by real-time RT-PCR. Categorization of drought-regulated transcripts and clustering revealed a structured response of cellular and biochemical activities characterized by functions in metabolism, energy, transcription, protein synthesis, defense and cell rescue, transport facilitation, and signal transduction pathways.

## Conclusions

The massive analysis of gene expression at genome level will accelerate rice research, not only for the understanding of gene networks, but also for the development of new varieties with novel agronomic traits for water-limited environments.

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# Molecular responses to dehydration and salinity in rice: Differences and cross-talk between two stress signaling pathways

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## Introduction

Drought and salt loading are environmental conditions that cause adverse effects on the growth of rice plants and productivity. Plants respond to these stresses at molecular and cellular levels, as well as the physiological level. Expression of a variety of genes has been demonstrated to be induced by these stresses. The products of these genes are thought to function not only in stress tolerance but also in the regulation of gene expression and signal transduction in stress response. Recently, cDNA microarray analysis has been developed for quantitative global analysis of expression profiles. Microarray technology is a powerful tool for identifying genes induced by environmental stimuli or stress and for analyzing their expression profiles in response to environmental signals. This technology is also useful in identifying target genes for stress-related transcription factors, opening a way to analyze gene networks in abiotic stress responses (Shinozaki et al., 2003). Salt and drought stress signal transduction consists of ionic and osmotic homeostasis signaling pathways, detoxification response pathways and pathways for growth regulation (Zhu, 2002). Salt and drought stress signaling has largely remained a mystery until recently. Now the molecular identities of some signaling elements have been identified. The challenge in the near future remains to identify more signaling elements. Once, more components are known, signaling specificities and cross talks can be properly addressed (Zhu, 2002). We prepared a rice cDNA microarray containing 14,013 rice unigenes and analyzed the expression profiles of these genes under salt and drought stress and identified hundreds of genes responding to each stress including several transcription factors. A significant number of drought-inducible genes are also induced by high salinity treatments, indicating the existence of significant crosstalk among the drought and high salinity responses.

## Methods

To identify the stress (both salt and drought) responsive transcripts during vegetative growth, expression profiles were established for different rice cultivars using microarrays containing 14,013 rice unigenes, mostly from cDNAs libraries of plants challenged by abiotic stresses, harvested at different developmental stages. The behavior of the shoot transcriptome was monitored in a time course experiment.

## Results

The transcript abundance of hundreds of genes was changed after drought-, and salt-stress treatments. The cDNA microarray analysis showed that many genes were induced after drought- and high salinity-stress treatments, and that there is cross-talk between drought and salinity responses. The gene products are of two types: the first group includes proteins that probably function in stress tolerance, and the second group contains the protein factors involved in further regulation of signal transduction and gene expression and probably functions in stress response. Many stress-inducible genes include those that encode signaling molecules and transcription factor genes, suggesting that many transcriptional regulatory mechanisms exist in stress signal transduction pathways.

## Conclusions

Our gene expression profiling using a 14K cDNA rice microarray provides evidence of differences between dehydration-signaling and salt stress-signaling cascades, and of cross-talk between them. Functional analysis of stress-inducible transcription factors identified in this study should provide more information on the complex regulatory gene networks that are involved in response to drought and high salinity stresses.

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# The primary studies on gene expression of drought tolerance or sensitive rice cultivars in different water conditions

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## Introduction

Plants respond and adapt to abiotic stresses to acquire stress tolerance in order to survive (Bray et al., 2000). The response of plants to abiotic stress involves hundreds of genes with different functions (Crookshanks et al., 2001). In *Arabidopsis,* many stress-inducible genes have been cloned and some of them have been used to improve the stress tolerance of plants by gene transfer (Yamaguchi-Shinozaki et al., 1993; Liu et al., 1998). Drought tolerance related genes and their function in indica rice have yet to be studied thoroughly (Dubouzet et al., 2003). It is important to clone and analysis the functions of drought-inducible genes in rice, especially in drought tolerant rice, in order to understand the molecular mechanisms of drought tolerance and to further improve the drought tolerance of rice by gene manipulation. In this study, a primary effort was made to understand the expression patterns of four rice cultivars in both drought-stressed and non-stress conditions by using the mRNA differential display (mRNA DD-PCR) approach.

## Materials and methods

Two paddy field rices (*Oryza sativa* L., indica cv. Zheng Shan 97B, Zhong 413, drought sensitive) and two upland rice (*Oryza sativa* L., japonica cv. Zhong Han 3, and IRAT 109, drought tolerance) were used in this study. Plants were grown in the drought-screening house of SAGC. Drought-stress treatment began at microspore stage and lasted for four weeks, while the control plants were grown in the nearby paddy field house. Total RNA was extracted from leaves as described by Puissant and Houdeline (1991). Reverse transcription was carried out using AMV reverse transcriptase as descripted in the Promega's technical bulletin with slight modifications. Three different anchored primers $HT_{11}M$ (H means HindIII site, M may be C, A, or C), abbreviated as AP1-AP3, were applied in the reverse transcription. Eight arbitrary primers (as ABP1-ABP8) were designed as reverse primer for PCR reactions. PCR amplification of each reverse transcription products was carried out in combination with one of eight arbitrary primers. The amplified cDNA products were size fractionated by a 3.5% denaturing polyacrylamide electrophoresis (PAGE) using Bio-Rad Sequence Cell. 4 μl of each PCR sample were incubated with 2 μl of loading dye (95% formamide, 10 mM EDTA, pH 8.0, 0.01% bromophenol blue) at 95ºC for 5 min and then kept on ice for at least 2 min before immediately loading to the Sequence cell. Silver staining of the polyacrylamide gel was carried out to identify the specific expression pattern.

## Results

Amplified transcription products of RNA extracted from drought-stressed and non-stressed leaves of rice were clearly visualized from the agarose gel electrophoresis. A number of drought specific fragments that were bigger than 500bp were detected from the primer combinations of AP1/ ABP2, AP1 / ABP6 (Figure 1). Drought tolerance cv. Zhong Han 3 produced more specific bands than drought sensitive cv. Zhong 413. After gel extraction and PCR amplification, three fragments of about 500-700bp were isolated from drought-stressed leaves of cv. Zhong Han 3 through the combinations of anchored primer AP1/ ABP2, AP1/ ABP6, AP2 and ABP7. Several other drought-specific fragments were also isolated but failed to be amplified again in further PCR.

**Figure 1.** Expression patterns of rice plants cv. Zhonghan 3 and Zhong 413 by mRNA DD-PCR on agarose gel electrophoresis. M 1kb DNA ladder, lanes 1, 2 were Zhonghan 3 in drought-stressed and non-stressed condition, lanes 3, 4 were Zhong 413 in drought-stressed and non-stressed condition.

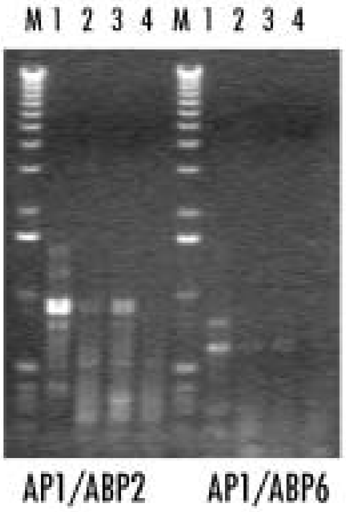

**Figure 2.** Expression patterns of rice plants cv. Zhong 413 by mRNA DD-PCR with primers AP3/ABP1-ABP8 on polyacrylamide gel electrophoresis. Lanes 1, 3, 5, 7, 9, 11, 13, 15 were patterns under drought-stress; Lanes 2, 4, 6, 8, 10, 12, 14, and 16 were patterns in normal paddy field condition. Black arrows (66) indicate cDNA expressed only in drought-stress condition, while white arrows (3) indicate cDNA disappeared under drought-stress.

Almost the same expression patterns as in agarose gel electrophoresis were clearly visualized from the result of PAGE with silver staining, except that the number of drought-specifically expressed bands from PAGE was much more abundant (Figure 2). In the combination of primer AP1 and arbitrary primer ABP1-8 from cv Zhong 413, some 31cDNA fragments were drought-specific transcription products, while two fragments disappeared in drought-stressed conditions. In the combinations of primer AP2/ABP1-8 and AP3/ABP1-8, drought-specific expression fragments were 34 and 66, respectively, while expression fragments disappeared in drought-stressed conditions were 5 and 3, respectively; the same trend as in the combination of primer AP1/ABP1-8. The expression patterns by mRNA differential display for other cultivars were similar to those of cv. Zhong 413.

## Conclusions

The abundant specific expression bands from drought-stressed rice leaves verified that rice plants respond to drought-stress by altered gene expression. There is some difference among cultivars in their response patterns to drought stress. DT rice showed more expression patterns than drought sensitive rice. These drought-stress specific cDNA fragments could be isolated and used to clone drought tolerance related genes, and then further improve the drought tolerance of rice by genetic engineering. Both agarose electrophoresiss and PAGE with silver staining could be used to investigate the gene expression patterns in mRNA differential display approach. PAGE could produce many more differntial bands.

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# Effects of the *brachytic-2* dwarfing gene on maize (*Zea mays*) root systems and grain yield under moisture stress

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## Introduction

Drought resistance is a complex of many morphological, physiological, and biochemical characteristics. Maize cultivars adapted to marginal rainfall areas of Zimbabwe should have deep and dense rooting systems in order to reduce the effects of frequent and intermittent droughts during the growing season. Deep and dense rooting systems were associated with drought resistance in wheat (Cholick et al., 1977; Hurd and Spratt, 1975) and rice (Chang et al., 1982; O'Toole, 1982; Yoshida and Hasegawa, 1982). Dwarf plants were found to have a more extensive and deeper rooting system than normal height wheat cultivars (Hurd, 1974; Lupton et al., 1974; Cholick et al., 1977). On the other hand, Mackay (1973) reported that root growth occurred in a mirror image manner of the aerial plant in spring wheat. The recessive *brachytic-2 (br-2)* gene reduces plant height without a corresponding decrease in the size of the major plant parts of maize (Anderson and Chow, 1963; Scott and Campbell, 1969). This study was conducted to investigate the effects of the br-2 gene on maize root systems and grain yield under moisture stress, in order to determine whether it can be exploited for improving drought tolerance in maize.

## Methods

Dwarf inbred lines (namely, dw2N3d, dwK64r, dwSC and dw211DR) were developed by transferring the *brachytic-2* gene to elite tall inbred lines (namely, 2N3d, K64r, SC and 211DR), by six backcrosses. The dwarf and tall inbred lines were used to develop six pairs of near-isogenic dwarf and tall single-cross hybrids. For root studies, the six pairs of near-isogenic tall and dwarf hybrids were planted in plastic pots (20 cm diameter and 1 m deep) filled with washed river sand, and arranged in a randomised complete block design with four replications. The experiment was carried out during the hot dry season, and each pot received 500 ml of nutrient solution, five times a week. Root length, volume and dry mass (DM) were measured at the mid-silking stage. For field trials, the six pairs of tall and dwarf hybrids were planted at three sites for two summer seasons (1993/94 and 1994/95). Two sites [Makoholi (MES) and Matopos (MTP) Research Stations] were located in marginal (450-650 mm) rainfall areas, characterised by periodic seasonal droughts. The third site, University of Zimbabwe farm (UZ farm), was located in a high yield potential area characterised by fairly well distributed high (750-1000 mm) rainfall. The hybrids were arranged in a randomised complete block design with three replications, at a population of 53 333 plants ha$^{-1}$. Compound D (8N: 14$P_2O_5$: 7$K_2O$) fertiliser was applied at 300 kg ha$^{-1}$ as a basal dressing at planting. Ammonium nitrate (34.5% N) was applied as a top dressing at 200 kg ha$^{-1}$. Plots were kept weed free.

## Results

There were no significant ($P>0.05$) differences in root length between near-isogenic hybrid pairs (Table 1). Two dwarf hybrids, dw211DR x dwSC and dw2N3d x dwSC, had significantly ($P< 0.05$) smaller root volume and less dry mass than their tall counterparts (Table 1). Dwarf hybrid dw211DR x dw2N3d had a significantly ($P< 0.05$) smaller root volume than its tall counterpart (Table 1). The roots of all the dwarf hybrids were thinner and more fibrous than those of the tall hybrids. There were no significant differences in grain yield between the tall and dwarf hybrid pairs at both marginal and high rainfall sites (Table 1).

**Table 1. Mean grain yield and root length, volume and dry mass of six pairs of near-isogenic tall and dwarf maize hybrids grown in Zimbabwe during the 1993/94 and 1994/95 summer seasons**

| Near-isogenic hybrid pair | Root DM (g) | Root length (cm) | Root volume (cm$^3$) | Grain yield at MES and MTP (t ha$^{-1}$) | Grain yield at UZ farm (t ha$^{-1}$) |
|---|---|---|---|---|---|
| 2N3d x SC | 275.8a[#] | 106.1a | 428.8a | 2.15a | 4.84a |
| dw2N3d x dwSC | 201.3b | 101.0a | 316.3b | 1.77a | 4.60a |
| 211DR x 2N3d | 247.0a | 103.5a | 498.3a | 2.16a | 6.93a |
| dw211DR x dw2N3d | 222.3a | 97.2a | 371.3b | 2.14a | 7.54a |
| 2N3d x K64r | 208.8a | 109.5a | 402.0a | 2.28a | 7.54a |
| dw2N3d x dwK64r | 229.5a | 104.2a | 403.8a | 2.25a | 6.69a |
| 211DR x SC | 326.8a | 101.2a | 542.5a | 1.95a | 6.08a |
| dw211DR x dwSC | 204.5b | 111.0a | 423.8b | 1.43a | 5.81a |
| SC x K64r | 242.8a | 105.8a | 428.5a | 2.05a | 6.43a |
| dwSC x dwK64r | 257.3a | 104.5a | 406.0a | 2.01a | 7.06a |
| 211DR x K64r | 163.0a | 113.0a | 392.0a | 2.53a | 7.61a |
| dw211DR x dwK64r | 180.1a | 100.2a | 321.0a | 1.99a | 6.76a |
| SEM[*] | 24.3 | 5.1 | 32.1 | 0.24 | 0.69 |
| $\text{LSD}_{(0.05)}$ | 69.8 | 15.0 | 92.3 | 0.70 | 2.02 |
| CV (%) | 21.1 | 9.8 | 15.6 | 24.00 | 18.02 |

[#] For each characteristic of a specific near-isogenic hybrid pair, means followed by the same letter are not significantly different at the 5% level.

[*] SEM = standard error of the mean.

## Conclusions

The *br-2* gene had no effect on root length but it induced the maize root system to become finer and more fibrous irrespective of the genetic background to which the gene was incorporated. A fine and fibrous rooting system is expected to be more efficient in extracting immobile nutrients and water because there will be more root surface area in contact with the soil. Root volume and dry mass were either reduced or not affected depending on the genetic background to which the gene was incorporated. The *br-2* gene did not negatively affect any root characteristics of dwarf hybrids where dwK64r was used as one of the parents. The superiority in root volume and dry mass of some tall hybrids was a result of thicker roots. The *br-2* gene did not cause the dwarf hybrids to be better or less adapted to marginal or high rainfall areas than tall hybrids. Thus, dwarf hybrids can be developed for both marginal and high rainfall areas.

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# Towards the improvement of abiotic stress tolerance in maize using genes isolated from the monocotyledonous resurrection plant *Xerophyta viscosa*

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Water is a major limiting factor in world agriculture. In general, most crop plants are highly sensitive to even a mild dehydration stress. There are, however, a few genera of plants unique to southern Africa, called “resurrection plants,” which can tolerate extreme water loss or desiccation. We have used *Xerophyta viscosa*, a representative of the monocotyledonous resurrection plants, to isolate genes that are associated with abiotic stress tolerance. Several genes that are differentially expressed, and that confer functional sufficiency to osmotically-stressed *Escherichia coli* (*srl*::Tn10) are being studied at the molecular and biochemical levels. Some of these genes include ones that code for a novel antioxidant, *XVPer1*, a subunit c’’-like protein of the vacuolar $H^+$-adenosine triphosphatase, *VcVHA-c*”, a galactinol synthase, *XVGols*, an aldose reductase, *ALDRXV4,* a cell membrane binding protein, *XVSAP1* and a transcription factor, *DREB*1A. To determine the effects of the expression of these genes in monocots, they are first introduced into our model system, *Digitaria sanguinalis,* for which we have developed an efficient transformation system. Thereafter, if results are positive, the genes are transformed into crops such as maize (*Zea mays*). To do the same for dicots we first introduce them into *Arabidopsis thaliana* and *Nicotiana tobacum*. This presentation will focus on the latest developments towards the improvement of abiotic stress tolerance using some of the above genes isolated from *X. viscosa.*

# Gene mining of African rice germplasm (*Oryza glaberrima* and *Oryza sativa*) to improve drought resistance in rainfed production systems for resource poor farmers of Africa

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Rice has been cultivated in West and Central Africa for centuries and is now considered as one of the region's staple foods. In these regions, drought is one of the major constraints as it severely depresses yield in upland and rainfed lowlands, where the majority of producers are resource-poor farmers. Drought resistance, however, is a complex trait, controlled by the interaction of many genes, as it involves several physiological, phenological, and morphological mechanisms, and because of the polygenic nature of resistance. Consequently, conventional breeding for drought resistance in Africa has had limited success. DNA markers and genetic mapping are expected to provide impetus, not only in gaining a better understanding of the traits associated with drought, but also contribute to enhanced selection efficiency.

The project seeks to (i) characterize drought in different environments and identify the most important traits associated with drought tolerance; (ii) select and characterize sources of drought resistance for genetic mapping and QTL analysis; and (iii) develop advanced lines combing drought resistance with heavy yield and agronomic and quality characteristics acceptable to farmers and consumers. To achieve these objectives, the project will exploit a core germplasm pool of (i) drought resistant O. *glaberrima* accessions, collected and screened in Mali by the Institut d'Economie Rurale (IER); (ii) drought tolerant interspecific breeding lines developed by WARDA from crosses between *O. sativa* and *O. glaberrima*; and (iii) a range of traditional *O. glaberrima* and *O. sativa* accessions from WARDA's gene bank. Confirmed sources of resistance amongst this core germplasm will be crossed with elite, but drought susceptible, *O. sativa* lines to develop interspecific and intraspecific populations segregating for drought resistance. These populations will be phenotyped in replicated field trials in different environments in Mali and Nigeria. QTLs analysis will be performed to identify across environment, drought-improving alleles for future breeding purposes. In other populations, selection will be conducted to generate agronomically superior, drought resistant lines.

# Structural-function relationships in the middle region of *S. cerevisiae* HSP104 protein

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## Introduction

High temperatures affect growth and development, causing the accumulation of aggregated proteins within the cell. Hsp100 / ClpB chaperones facilitate the resolubilization of aggregates in conjunction with Hsp70 and Hsp40 (Glover and Lindquist, 1998). Hsp100 / ClpB are found in bacteria, protozoa, fungi, and plants where their expression is inducible by heat stress (Schirmer et al., 1995). In yeast, *Arabidopsis*, and maize, Hsp100 / ClpB play major roles in the acquisition of thermotolerance, i.e., the ability to survive very high temperatures following acclimation to mild heat shock treatments (Sánchez and Lindquist, 1990; Queitsch et al., 2000; Nieto-Sotelo et al., 2002). Expression of Hsp101 in maize causes a reduction in the growth rate of primary roots (Nieto-Sotelo et al., 2002). Both the primary and secondary structure of the middle region of Hsp100 / ClpB proteins have been conserved during evolution (Nieto-Sotelo et al., 1999). The length of the middle region is quite different or absent in other members of the Clp family (ClpA, ClpC, ClpD), whose functions seem to be unrelated to those of ClpB (Schirmer et al., 1995). The middle region has the propensity to form a coiled-coil (Nieto-Sotelo et al., 1999). To understand the mechanism of action of Hsp100 / ClpB, we studied the structure-function relationship within their middle region.

## Methods

Homozygous maize mutants *hsp101-m4::Mu1* and *hsp101-m5::Mu1* were grown in a greenhouse where maximal daily temperatures reached close to 40ºC). Their growth was compared relative to their corresponding wt sibling lines (Figure 1). Analysis of cell proliferation activity was made by FACS analysis of nuclei from leaf samples. Analyses of coiled-coils were made with the COILS program. Site-directed mutagenesis of *HSP104* middle region was used to obtain amino acid substitutions that changed the hydrophobic face of the amphipatic helices 1, 2 or 3 (*hsp104-mI*, *hsp104-mII*, and *hsp104-mIII*, respectively). Both wt and *HSP104* mutants were used to transform *Dhsp104* cells to evaluate their function *in vivo* by means of induced thermotolerance assays. Partially purified HSP104 protein preparations from both wt and each mutant protein were tested for their ability to form hexamers on an ATP-dependent fashion by size exclusion chromatography. Protein conformations were evaluated by protease sensitivity assays and the western blot procedure using an anti-HSP104 specific antibody. The T-COFFE, SWISS MODEL, and Deep View Swiss-PdbViewer programs were used to model the structure of HSP104 middle region using the known structures of *T. thermophilus* ClpB and *E. coli* Hsc20 co-chaperone proteins as templates.

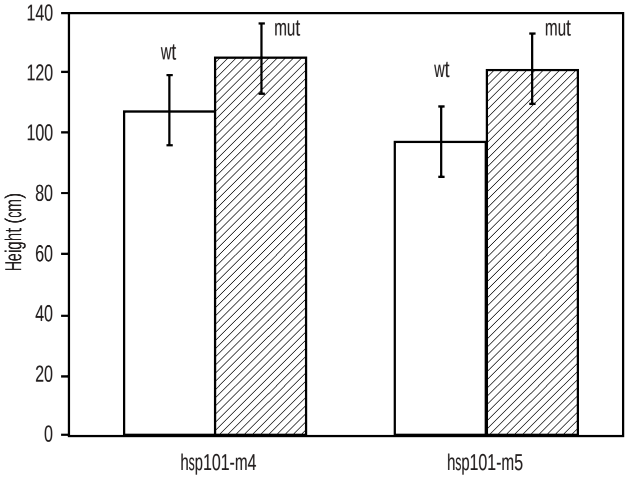

**Figure 1. Height of two-month old maize plants grown in a greenhouse where daily maximum temperatures fluctuated between 36 and 40˚C. Values between wt an mutants are statistically different at P=0.05.**

## Results and discussion

Hsp101 negatively affects growth of stems and leaves in adult plants. In leaves, this effect is at least due in part to a decrease in cell proliferation. Thus, maize Hsp101 plays an important role in restraining growth under moderately high temperatures, suggesting that slow growth rates are more convenient to achieve a stress tolerant state. Because a full-length maize cDNA clone encoding *ZmHSP101* did not complement the thermotolerant deficient phenotype of the yeast *Dhsp104* mutant, we studied HSP104 of *Saccharomices cerevisiae per se*. Both in yeast Hsp104 and plant Hsp101 the middle region seems to form 4 amphipathic a-helices. Site-directed mutagenesis of the middle region of yeast Hsp104 showed that this domain is very important for function. Mutations that changed the amphipathic character of helices 2 and 3, but not those of helix 1, caused the loss of function of Hsp104 (Figure 2) suggesting that helices 2 and 3 play an important role in the maintenance of a coiled-coil structure. Moreover, these mutations abolished hexamer formation and changed the conformation of the complex. Our experimental data are consistent with a homology-model for yeast Hsp104 middle region resembling the C-terminal domain of *E. coli* Hsc20 co-chaperone (Cupp-Vickery and Vickery, 2000) and inconsistent with a model based on the middle region of the recently published structure of *T. thermophilus* ClpB (Lee et al., 2003). Helix 2 and helix 4 seem to form a parallel coiled-coil, whereas helix 3 interacts with both helix 2 and 4 through their hydrophilic face.

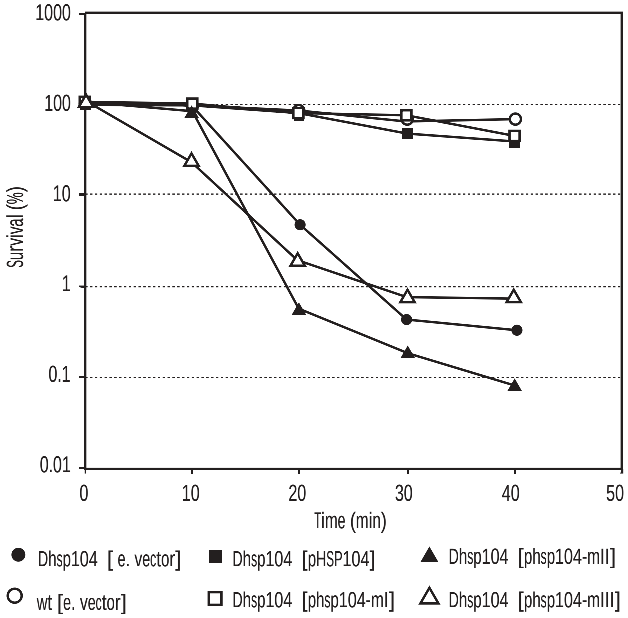

**Figure 2. Induced thermotolerance assay of *Dhsp104* mutant transformed with plasmids encoding wt or middle-region mutant versions of HSP104.**

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# Differential gene expression in cell cultures and plants of chili pepper (*Capsicum annuum* l.) under water stress

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## Introduction

Chili pepper (*Capsicum* spp.) is a very important horticultural crop in Mexico and worldwide (FAO, 2004). Chili pepper is very sensitive to water stress and different physiological, biochemical, molecular, and genetic approaches are necessary to increase its resistance to drought. In order to study drought tolerance mechanisms at the cell level, different cell lines of chili pepper resistant to drought imposed by the presence of polyethylene glycol (PEG) in the culture medium were selected (Santos-Diaz and Ochoa-Alejo, 1994). These cell lines exhibited more negative osmotic potential, and accumulated higher levels of proline, glycine betaine, and potassium than the non-selected cell cultures. Since metabolic adjustment to drought is very often accompanied by gene expression changes, a comparative gene expression study was carried out by differential display with a PEG-resistant cell line (T7) grown in the absence (P0) or in the presence of 15% PEG (P15), and with a PEG-sensitive cell line (ST). Approximately 124 cDNA fragments were differentially expressed in the cell cultures as revealed by differential display (Verástegui-Peña, 1999). Initially, eight cDNA fragments were cloned for expression studies and sequenced. Comparison of sequences of cDNA fragments with those of GenBank (NCBI) revealed that ODE1 and ODE2 (Osmotically Differentially Expressed) cDNAs exhibited high homology with an ELIP (Early Light Induced Protein) and with an ARF (Auxin Responsive Factor), respectively. Further analysis of these two cDNAs and 23 additional cloned cDNA fragments were carried out in this work.

## Methods

cDNA fragments were cloned in the PCR TOPO 2.1 vector (Invitrogen). After restriction with EcoR1, the cDNAs were radiolabeled with $^{32}$P-dCTP and used as probes for Northern analysis. Cell cultures were maintained in the MS liquid medium (Murashige and Skoog, 1962) as described by Santos-Díaz and Ochoa-Alejo (1994). Biomass of the cell cultures collected by filtration on day 7 after subculture, and tissues of chili pepper plants subjected to water withholding for 0, 3, 6, 9, and 12 days were used for total RNA extraction according to the protocol reported by Camacho-Villasana et al. (2002). Northern analysis was performed as described by Sambrook et al. (1989). In order to try to get the whole encoding regions of the differentially expressed cDNAs, the 5’ RACE system (Promega) was used.

## Results

Six of 23 cDNA cloned fragments, namely ODE11, ODE12, ODE13, ODE14, ODE15, and ODE16 showed differential expression in the cell suspension lines by Northern analysis (Figure 1). Higher expression of these cDNA fragments was observed in the PEG-resistant cell line T7 grown in 0% PEG (P0) and only ODE11 exhibited higher expression in 15% PEG (P15). Sequence analysis of the cDNA fragments revealed no homology with known sequences from the GenBank, except for ODE12 that showed 93% nucleotide homology with the mitochondria rRNA 26S of maize, sugarbeet and rice. In the case of expression analysis in tissues from water-stressed chili pepper plants, only four cDNA fragments were tested (ODE1, ODE2, ODE9, and ODE14) and a different pattern of expression was observed as compared to cell cultures. It was observed also a tissue-dependent differential

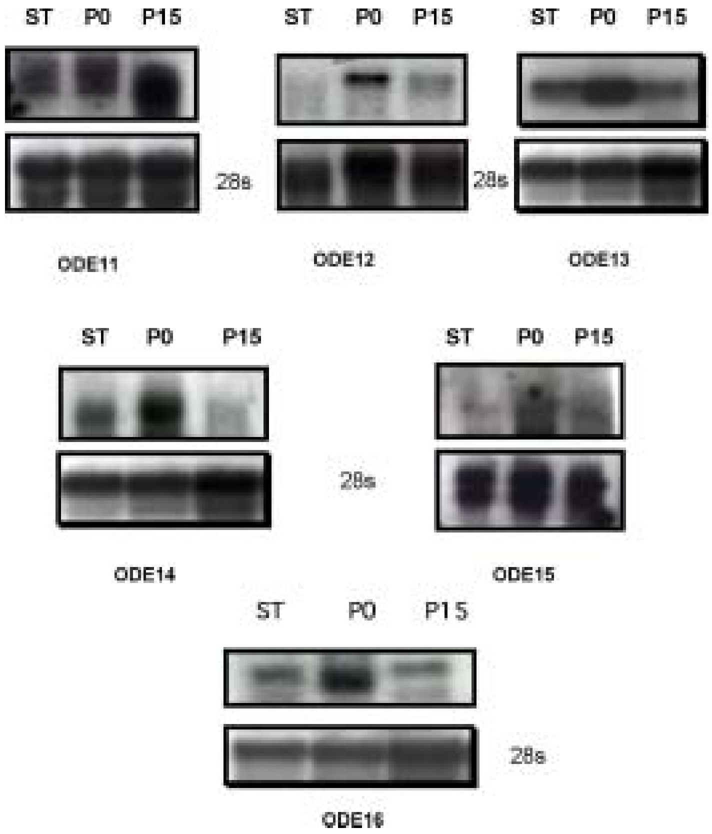

**Figure 1. Northern analysis of chili pepper cell cultures using six different cDNAs (ODE11 to ODE16) as probes. ST, PEG-sensitive cell line cultured in 0% PEG; P0, PEG-resistant cell line T7 grown in 0% PEG; P15, cell line T7 in 15% PEG: 28s, ribosomal RNA (load control).**

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expression of these fragments. Amplification of the 5' end (RACE) was tried with ODE1, ODE2, ODE9, and ODE14 and only with ODE14 a 936 bp cDNA was amplified and its nucleotide sequence showed 81 to 84% homology with a ketoacyl-CoA thiolase from five different plant species.

## Conclusions

A comparative gene expression study was carried out by differential display with a PEG-resistant cell line T7 and with a PEG-sensitive cell line. Approximately 124 cDNA fragments were differentially expressed. After cloning and northern blot analysis 6 cDNA fragments were selected and sequenced. The expression of these fragments was also studied in tissues of chili pepper plants subjected to water stress and differential expression was observed. One of the cDNAs was amplified by 5' RACE and showed high homology with a ketoacyl-CoA thiolase.

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# Development of functional markers for drought tolerance in rice: Identification and validation of candidate genes and SNPs

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## Introduction

Drought is a major constraint to rice productivity. Genetic improvement of drought tolerance by conventional breeding has been rather slow due to lack of proper phenotypic selection criteria, high genotype X environment interaction, and low heritability. Recent advances in molecular mapping and functional genomics contributed significantly to the identification of QTLs and genes associated with drought tolerance. However, lack of precise information on genes that are directly associated with drought tolerance phenomenon in rice is still a major limitation. Developing indepth coverage of rice coding sequences through large-scale EST generation is beginning to play a major role in quality annotation. These resources are of potential use in gene discovery, comparative genomic analysis, and SNP identification. Furthermore, with near completion of a finished high-quality rice genome sequence, these tools will be routinely used for dissecting complex traits such as drought tolerance. Towards this end, we have generated large scale ESTs from a cDNA library made from drought stressed indica rice (N22) seedlings and have identified a large number of putative candidate genes and mapped to genetically anchored BAC/PAC clones. A number of SSRs and QTLs associated with drought tolerance have been identified using the rice mapping population CT9993 X IR62266, DHLs. Microarray experiments and validation of candidate gene functions through transgenics are in progress.

## Materials and methods

### *ESTs and candidate gene discovery*

We generated ~6,000 Expressed Sequence Tags (ESTs) (accession Nos BI305180 to BI306756; BU 672765 to BU 673915 & CB964418 to CB967504) from a normalized cDNA library constructed from drought stressed *Oryza sativa* cv. Nagina22 (Reddy et al., 2002**.** Standard sequence processing tools PHRED, Phrap, and Crossmatch were used with Codoncode InterPhace. Homology search was done against non-redundant (nr) nucleotide and protein sequence databases using BLASTN 2.2.2 and BLASTX 2.2.2 versions of the BLAST programs (Altschul et al., 1997) through BLAST 2.0 network client software with the DNA tools interface (http://www.crc.dk/dnatools). The BLASTN program was also used to identify rice EST hits on High Throughput Genomic Sequences (HTGS) and the Chinese WGS (whole genome shotgun contigs) draft sequence of indica rice genome available in GenBank. The results of the BLAST analysis were manually checked for similarity in the aligned region. Genscan, GeneMark HMM, RiceHMM, Glimmer R, FGENESH, Rice Genome Automated Annotation System (Rice GAAS) were used for accurate gene prediction. Plant CARE and PLACE Databases were utilized for identifying *cis*-acting elements in the promoter regions.

The ESTs associated with stress response were identified from multiple sources based on the compiled list of stress regulated genes documented or presumed to be relevant to abiotic stress tolerance in more than one plant species (http://stress-genomics.org/stress.fls/expression/expression.html).

Further, it is based on the microarray expression profiles of possible candidate gene sequences, which include 650 from *Arabidopsis* (Seki et al., 2001, 2002; Kreps et al., 2002), 150 from barley (Ozturk et al., 2002) and 100 from rice (Matsumura et al., 1999; Kawasaki et al., 2001; Rabbani et al., 2003). All the stress responsive gene sequences were retrieved from the above studies and a local database was constructed and utilized for BLAST analysis using TBLASTX with E-value >1e$^{-20}$. The identified putative candidate ESTs were mapped onto genetically anchored BAC / PAC clones to identify the possible candidate genes at the QTL associated with drought tolerance (Babu et al., 2003; Price et al., 2002; Price and Courtois, 2000; Zhang et al., 2001).

Currently we are analyzing the expression profiles of these ESTs using microarrays (in collaboration with University of Georgia). Poly A$^+$ RNA from various stages of field drought stressed N22 plants has been prepared for this study using a rain out shelter with regulated water supply.

### *SNP detection and analysis*

Allelic variations in protein kinases, phosphatases, and other genes associated with stress response signal transduction pathways are identified using ESTs. BAC / PAC clones sequences of Nipponbare, *indica* WGS scaffold sequences of Guangluai 4 and the Unigene clusters of corresponding hits have been obtained using NCBI BLASTN tool. We developed a new cvCluster v1.0 script for clustering ESTs using xsact (Malde et al., 2003) engine of suffix array construction. Assembling was done based on cultivar specific data available in public domain to score for SNPs and to discern true allelic variation from that of sequencing errors, and to generate more informative SNP data. These sub clusters were evaluated using Polybayes (Marth et al., 1999) SNP scoring program.

### *SSR marker addition, map construction and QTL identification*

A set of 250 rice microsatellite primer pairs (Research Genetics Inc USA.) were used to amplify the simple sequence length polymorphic (SSLPs) DNA between parental lines according to Chen et al., (1997). Polymorphic primer pairs were used to amplify SSLPs in complete DHL population, CT9993 / IR62266. Markers were assigned to 12 rice linkage groups at LOD >3 to anchor markers (Nguyen et al., 2002) using MAPMAKER / EXE V.3 program. The assigned makers were ordered using three-point analysis at LOD 3. Ripple command was used to verify the order of markers on each chromosome. This revised genetic linkage map of was used to identify QTLs controlling plant morphological (leaf), phenological and yield related characters under control and field drought stress conditions using MAPMAKER / QTL program. The putative QTLs were declared significant when LOD score was $\geq$ 2.3.

Rice-sorghum syntenic mapping was carried out with 127 ESTs as RFLP probes. Test genotypes of rice (6), sorghum (6), pearl millet (2) finger millet (2) and maize (1) were screened for RFLPs. Population screening was done in rice mapping population (CT9993 X IR62266, DHLs) and sorghum mapping population (N13 X E36-1, RILs).

### *Construction of plant expression vectors*

The cDNA clone encoding rice anthocyanidin synthase (*Ans*) was cloned in sense and antisense, downstream to a constitutive promoter in the plant expression vector pE1806 that is known to enhance gene expression by about 150-fold. The construct was mobilized into *Agrobacterium tumifaciens* strain LBA4404 through electroporation. The scutellar callus derived from rice line Nootripathu was transformed with LBA4404 harboring pE1806+*Ans* and the transgenic plants were regenerated with hygromycin selection.

## Results

Annotation of high-quality ESTs through homology search in the NCBI nr nucleotide and protein databases, using BLASTN and B LASTX programs resulted in the identification of putative genes (78% of the ESTs) and novel genes (22%), which have no significant homology in nr sequence database and dbEST division of GenBank. Homology search of non-redundant ESTs in rice dbEST division showed 1157 hits and 912 ESTs do not have significant similarity to rice ESTs. These 912 ESTs constitute a novel 3’ sequence resource for accurate gene annotation. The functional distribution of the identified putative stress responsive genes represented those belonging to diverse pathways associated with stress adaptation process (Figure 1). Further, a number of stress responsive genes were localized to the described QTL regions

Table 1 shows that a total of 1,620 ESTs representing protein kinases, phosphatases, and other signal transduction pathways genes were grouped in to 39 clusters and 157 cultivar specific contigs using cvCluster1.0. A total of 60 SNPs were identified using Polybayes with the probability greater than 0.9. These include 29 transitions, 22 transversions, and 9 indels. Screening of non-synonymous substitution and the development of an automated pipeline for high throughput SNP detection and analysis are in progress

### *New SSR markers and QTLs*

A total of 54 new microsatellite markers and three EST-PCR markers were added to the existing genetic linkage maps and subsequently used for QTL identification. The map length has increased covering 1,978 cM in length on the basis of Kosambi function with an average distance of 5.8 cM between adjacent markers. A total of 67 QTLs with a LOD score of $\geq$ 2.3 were identified for leaf, phenological and yield related traits under control and field drought conditions (Table 2). The number of QTLs identified for each trait varied from one to nine with percent variance explaining 6.8 to 19.2. Major QTLs for leaf, phenological and yield traits under field drought stress were localized to a genetic region on Chromosome 1 and 4. Of the 127 rice ESTs screened for synteny, 41 showed polymorphism in rice and 19 in sorghum. Work is in progress to map informative EST-RFLPs in the mapping populations of rice and sorghum.

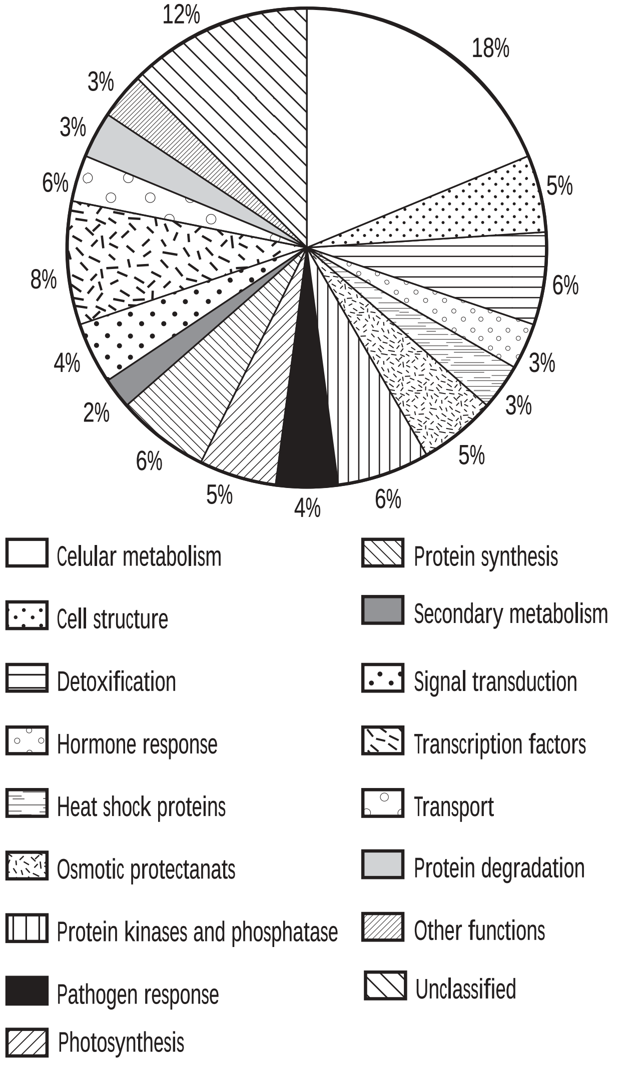

**Figure 1. Functional distribution of putative stress response genes (580).**

**Table 1. Putative candidate genes within drought tolerance QTL regions uncovered from mapping experiments**

| Putative function | Nearest marker | QTL location |
|---|---|---|
| **Signaling:** | | |
| Mitogen-activated protein kinase homolog MMK 2 | - | Chromosome 10 |
| Putative receptor-like protein kinase | - | ” |
| Small GTP-binding protein (rab5a) | R2292, S13561 | Chromosome 12 |
| 14-3-3 protein homolog GF14-12 | S4036S | Chromosome 8 |
| Signal recognition particle receptor-like protein | S4036S | ” |
| Calcium dependent protein kinase | C2161 | Chromosome 5 |
| 1-aminocyclopropane-1-carboxylate oxidase | C60626SB | Chromosome 7 |
| **Transcription Factors:** | | |
| EREBP-like protein | S2769 | Chromosome 3 |
| AP2 domain containing protein | S2769 | ” |
| Ethylene responsive protein | S2769 | ” |
| Helicase-like transcription factor | R78 | Chromosome 4 |
| OSMYB1 | C308 | Chromosome 5 |
| Homeodomain leucine zipper protein | S4036S | Chromosome 8 |
| **Metabolism:** | | |
| Sucrose-6F-phosphate phosphohydrolase SPP3 | S3382S, R1547 | Chromosome 1 |
| Beta-oxyacyl-[acyl-carrier protein] reductase | E61384S | Chromosome 3 |
| Putative anthocyanidin reductase | R78 | ” |
| HMG protein | - | Chromosome 1 |
| RNase S-like protein | S10578, S955 | Chromosome 9 |
| **Membrane proteins:** | | |
| Photosystem I chain IV precursor | R658 | Chromosome 7 |
| Mitochondria F0 ATP synthase D chain | S4036S | Chromosome 8 |
| Water channel protein | C735 | Chromosome 7 |

**Table 2. QTL's identified in CT9993 X IR62266 population in control and field stress condition**

| S.No. | Trait | No. of QTL's identified |
|---|---|---|
| 1 | Leaf characters (specific leaf area and specific leaf weight) | 22 |
| 2 | Phenological characters | 19 |
| 3 | Spikelet sterility | 5 |
| 4 | Biomass | 5 |
| 5 | Grain weight | 11 |
| 6 | Harvest index | 5 |

***Molecular analysis of transgenics***

A total of twenty-one (21) regenerants (To) were obtained on the selection medium from different independent transformation experiments. The integration of the transgene was confirmed by PCR and Southern blot analysis. Of these, five regenerated seedlings exhibited a strong purple pigmentation in the leaf sheath and internode. TLC and Proton-NMR spectroscopy confirmed the nature of pigment. Northern analysis of transgenic plants showed increased levels of *Ans* transcript. Western analysis of the transgenic leaf extracts revealed the presence of detectable levels 41KDa band while control non-transgenics did not show this detectable protein.

## Conclusions

This EST library formed a rich source of drought stress-related genes represented in GenBank for the first time from indica rice seedlings subjected to progressive drought. Annotation and mapping of the ESTs onto the genomic sequences resulted in identifying putative functions and corresponding genomic regions for large number of ESTs.

The probable candidate genes of the stress-response transcriptome in rice were uncovered by comparing with the expression profiles from cDNA microarrays of different plants subjected abiotic stress treatments. The newly developed cvCluster tool identified the true SNPs through sub clustering of cultivar specific ESTs. The inclusion of new SSRs to the existing maps allowed precision mapping of tightly linked QTLs. These functional marker resources developed through discovery of candidate genes and SNPs are useful in eventual deployment in crop breeding for genetic improvement of rice for water limited environments. These syntenic maps will be used to identify common genetic segments controlling drought tolerance in rice and sorghum. Currently, T2 *Ans* transgenic plants are being analyzed and tested for their response to biotic and abiotic stress. Further, microarray data on drought stress responsive gene expression pattern will be described.

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# A complexity of genes underlie the response to drought tolerance in maize at flowering

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## Introduction

Water stress occurring at any plant developmental stage is undesirable. However, in crop plants, and in particular maize (*Zea mays* ssp. *mays*), drought during flowering can be catastrophic in terms of productivity (grain yield). During this time, growth of both the ears and silks slows and a delay in silking in relation to pollen shed, commonly referred to as the anthesis-silking interval (ASI) (Bolaños and Edmeades, 1996; Ribaut et al., 1996) is observed. Selection for reduced ASI has been successfully used to increase yield under drought in maize (Bänzinger et al., 2000). The response of maize to drought involves several genes, involved in yield components and other morphological traits. The overall objective of the drought work at CIMMYT is to explain why some genotypes of maize are able withstand drought during anthesis, and can maintain a good yield performance under field conditions. To do so, we adopted a multidisciplinary approach to elucidate the mechanisms underlying the genetic response of maize in the field under water stress, with the hope of identifying the most important genes conferring drought tolerance during stress at anthesis. Results are presented from the application of two technologies based on the expression of genes to determine how particular genes are responding during this phase of growth and under a particular abiotic stress. These two activities are (i) the use of microarrays in collaboration with Poineer Hi-Bred Int., to provide a global picture of changes in gene expression in ears and silks under water stress during flowering, and (ii) a more focused approach to study the expression of candidate genes selected from the microarray results, literature, and other sources using quantitative RT-PCR.

## Materials and methods

### *Field design*

Plants were grown in a replicated randomized block design in the field (Tlatizapan, Mexico, 2003). Field trials consisted of genotypes of both parental lines (Ac7643 and Ac7729 / TZSRW) and contrasting families from the “tails” of the distribution of the segregating populations (the most drought tolerant and susceptible families). These were selected on the basis of contrasting ASI and grain yield and that they had a pollen shed date no greater than two or three days before or after the mean male flowering time, in order to minimize the difference in gene expression due to differences in stress intensity. Irrigation was stopped three weeks before flowering, thus ensuring that plants would be stressed during pollen shed.

### *Tissue collection*

Ear tips, bases, and silks from Ac7643, Ac7729 / TZSRW, and the eight segregating genotypes were collected over the three replicates. Ears were bagged before pollen shed to ensure that the experiments were not compounded by the effects by pollination. Length of ears were measured and 2 cm from the tip and the base removed and collected. Approximately 40 samples from all genotypes and tissues were collected. All tissues were immediately frozen in liquid nitrogen in the field.

### *Microarray experiment*

RNA extraction, hybridization to slides, and analysis of results were performed at Pioneer Hi-Bred Int. 60-mer custom in situ oligonucleotide microarrays (22K) were used (Agilent Technologies). Two slides (44K) were used in each comparison and probes selected from the gene databases at Pioneer. We first compared Ac7643

(P1) and Ac7729/TZSRW (P2) from ear tips and silks. Future experiments will compare the genotypes of the segregating population to one of the parents.

### *RT-PCR*

Although RT-PCR is a complex assay, it has the advantages of being able to detect rare transcripts, is more sensitive than other established methods (e.g., Northern blotting), and requires less tissue. RT-PCR has been used to examine the expression of candidate genes over the same tissues and genotypes, but over a greater range of times and in different environments. As considerable care needs to be taken to optimize all parameters for RT-PCR, we have recently moved to kinetic, or real-time, fluorescence RT-PCR, using the ABI PRISM ®7000 Sequence Detection System (Applied Biosystems). The results presented here will be based on quantitative RT-PCR. We initially opted to study genes in sucrose regulation as sucrose metabolism is a major component under drought.

## Results

### *Microarray*

Comparisons generated between P1 and P2 showed a large number of changes in gene expression. This number was reduced when the three replicates were considered together and when only genes common to silks and ear tips were considered. The silks showed the greatest changes in gene expression with 1,491 genes showing significant up- or down-regulation. The ears showed 1,332 genes with significant changes in expression. Combining this data reduced the dataset to 751 genes. Results were highly consistent across replicates. Pearson's product-moment and Spearman's rank-order correlation coefficients were calculated on the log (ratio) values. The results show that there is remarkable consistency among replicates for each gene (see Table 1), with all comparisons highly significant.

**Table 1. Correlations across field harvested replicates in changes in gene expression using microarrays. Correlations were calculated using the log(ratio) values.**

| Ear Tips | | | Silks | | |
|---|---|---|---|---|---|
| Comparison | Pearson's | Spearman's | Comparison | Pearson's | Spearman's |
| Rep1 vs. Rep2 | 0.981262 | 0.955404 | Rep1 vs. Rep2 | 0.971110 | 0.933336 |
| Rep1 vs. Rep3 | 0.987445 | 0.969333 | Rep1 vs. Rep3 | 0.957953 | 0.900847 |
| Rep2 vs. Rep3 | 0.967657 | 0.925731 | Rep2 vs. Rep3 | 0.973611 | 0.947272 |

In terms of the types of genes being differentially expressed, the 751 genes can be classified into many classes. Many are those that are normally found in large-scale expression studies under abiotic stresses (molecular chaperones, water and ion movement, detoxification, osmoprotection, signal perception, and transduction and transcription control). Genes involved in hormone metabolism (ABA, cytokinin, ethylene, and IAA), starch and sucrose metabolism and cell growth (expansins, cellulose synthesis) also show differences in expression between the two parental lines.

### *RT-PCR*

We studied the expression of six carbohydrate metabolism genes; Sucrose Phosphate Synthase (SPS), Sucrose Phosphate Phosphatase1 (Spp1), Sucrose Phosphate Phosphatase2 (Spp2), Cell Wall Invertase2 (Incw2), Vacuolar Invertase2 (Ivr2), and Sucrose Synthase (Sus). We found differences in the expression of these genes under drought and well watered conditions in the ears when a comparison was made between the drought tolerant and drought susceptible material. A number of these genes showed no differences in expression under drought. Although some correlation was found with the microarray results, there are some interesting differences particularly in the silks (which were not used in the RT-PCR assays), where there was a difference in the types of carbohydrate metabolism genes showing

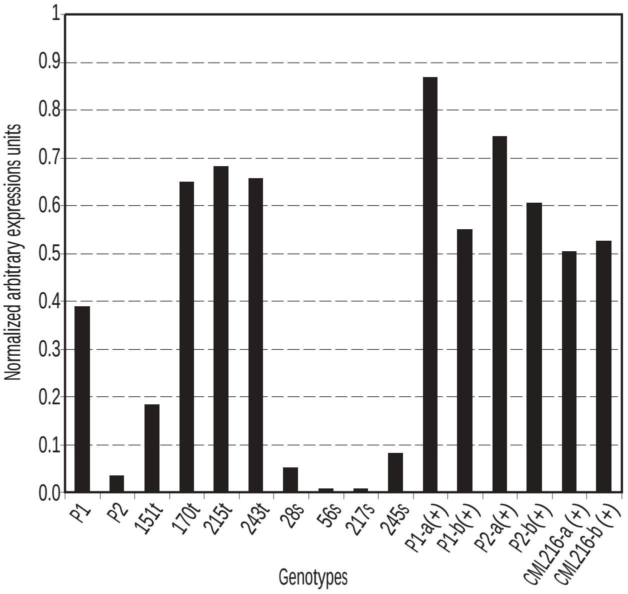

**Figure 1. Example of a gene that shows differences in gene expression during drought but not in well-watered conditions.**

differential expression. The results for one gene using RT-PCR, Cell Wall Invertase2, is shown in figure 1 under drought and well-watered conditions. We aim to study these genes in more detail over the three tissues in the near future using instead real-time RT-PCR.

## Conclusion

By combining a number of different approaches such as QTL detection with functional genomics tools will generate useful information and a way of validating those genes important to drought tolerance in maize during anthesis. This knowledge will be used to improve germplasm under drought through marker assisted selection (MAS) experiments.

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# Genetic analysis of IR64 introgression lines of rice under irrigated and water stressed field conditions

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Rice is the primary and staple food for more than 60% of the world's population. In India, rice is grown on 45 million hectares, with an annual production of 89 million tons. Large upland areas of eastern Uttar Pradesh are still sown to traditional rice varieties due to the unavailability of drought and stable yield per forming genotypes. Drought is major constraint, which limits rice production in eastern Uttar Pradesh. Major rainfed area lines are under the northern plane zone (NEPZ), where rainfall is erratic. Therefore, major rice lines encounter multiple stresses like drought, submergence at crop establishment at the vegetative stage, etc., which are major limiting factors in rice production (Fukai and Cooper, 1995).

## Methods

IR64 introgression (21 lines) received from the Central Rice Research Institute, Cuttack, India were grown in two sets of environment: irrigated and water stressed (23 days duration). The lines trial was conducted under upland conditions at the Crop Research Station, Masodha Faizabad (U.P.), during the wet season 2003. A 1 m wide buffer zone was kept between irrigated and stress plots. Water stress was created by withholding irrigation in stressed plots and plant growth morpho-physiological traits like days to 50% flowering, days to maturity, plant height, and effective tillers per plant were studied (70 to 93 days). Flag leaf length and breath, panicle length, grains / panicle, fertile and sterile grain / panicle test weight, and grain yield per plant and per plot were recorded 23 days after water stress. Drought scoring data were observed on the basis of SES scoring (IRRI, 1996). Evaluation of various quantitative traits like general mean, genotypic co-efficient of variation (GCV), phenotypic co-efficient of variation (PCV), heritability (broad sense), genetic advance as percent of mean, and correlation with grain yield were analysed under both irrigated and stress conditions.

## Results

Significant genotypic variation existed among the introgression lines for all the traits. There was delay in days to 50% flowering and maturity under stress conditions. Significant reductions in all traits were noticed under stress conditions in relation to the control plot. Yield reduction under stress conditions was mainly due to reduction in yield contributing traits. Under stress, high heritability (Table 1) was found in traits like grain yield and yield contributing traits, suggesting that importance of these traits as criteria for improving overall yield under stress. Moderate habitability was found for panicle length, and low heritability for flag leaf breadth and test weight; significant and positive correlation was observed between grain yield per plant and yield contributing traits, effective flag leaf breadth and total grains per panicle. A negative correlation in was found between grain yield and plant height (Table 1).

**Table 1. Estimates of genetic variability parameters and correlation for fourteen traits in rice, IR64 introgression line (21) under well-watered and stress (23 days) at heading stage**

| | General mean | | GCV | | PCV | | Heritability(B.S.) | | GA as % of mean | | Correlation with grain yield | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Characters | C | S | C | S | C | S | C | S | C | S | C | S |
| Days to 50% flowering (days) | 80.14 | 90.09 | 7.03 | 8.49 | 8.11 | 9.14 | 75.15 | 86.10 | 12.56 | 16.23 | 0.35 | 0.03 |
| Days to maturity (days) | 107.38 | 115.80 | 5.25 | 6.98 | 6.06 | 7.58 | 75.16 | 84.68 | 9.38 | 13.23 | 0.41 | 0.001 |
| Plant height (cm) | 90.01 | 82.60 | 13.23 | 13.93 | 13.55 | 14.30 | 95.20 | 94.89 | 26.57 | 27.96 | -0.33 | -0.24 |
| Effective tillers / plant | 8.22 | 5.86 | 16.06 | 26.17 | 16.43 | 26.62 | 95.65 | 96.74 | 32.36 | 53.07 | 0.47* | 0.45* |
| Flag leaf length (cm) | 31.60 | 25.94 | 19.18 | 15.48 | 19.71 | 16.45 | 94.74 | 88.55 | 38.92 | 29.99 | 0.27 | 0.04 |
| Flag leaf breadth (cm) | 1.30 | 1.07 | 6.07 | 7.56 | 10.65 | 12.96 | 32.51 | 34.00 | 7.07 | 9.13 | 0.47* | 0.46* |
| Panicle length (cm) | 25.09 | 21.80 | 7.45 | 10.78 | 9.74 | 12.97 | 58.63 | 69.11 | 11.75 | 18.46 | 0.25 | 0.14 |
| No. of total grains / panicle | 85.95 | 63.69 | 20.20 | 25.40 | 20.41 | 25.71 | 97.93 | 97.63 | 41.18 | 51.71 | 0.44* | 0.47* |
| No. of fertile grains / panicle | 78.58 | 49.24 | 22.08 | 35.56 | 22.28 | 35.88 | 98.23 | 98.26 | 45.07 | 72.62 | 0.34 | -0.09 |
| No. of sterile grains / panicle | 11.50 | 14.22 | 59.80 | 35.81 | 60.41 | 36.09 | 97.99 | 98.42 | 79.30 | 73.20 | -0.02 | -0.21 |
| Grain yield / plant (g) | 15.52 | 8.59 | 32.60 | 37.10 | 32.78 | 37.64 | 98.81 | 97.12 | 66.75 | 70.62 | 0.84** | 0.55** |
| Test weight (1000 grain), g. | 24.40 | 23.13 | 3.35 | 4.93 | 6.37 | 7.72 | 27.72 | 40.90 | 3.60 | 6.48 | 0.22 | 0.03 |
| Grain yield / plot (g) | 513.09 | 215.20 | 33.95 | 41.71 | 33.98 | 41.85 | 99.79 | 99.35 | 69.85 | 82.73 | - | - |

C= Irrigated, S= Stress at heading stage (23 days drought)
D/S= 26.07.2003
$h^2$ (bs) = heritability in broad sense

## Conclusion

We suggest that when selecting superior genotypes for rainfed conditions, grain yield, effective tillers, fertile grains, and yield per plant should be considered as important criteria for the breeding programme.

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# Search for molecular markers in cereals: An approach by ‘intron scanning’ and genome complexity reduction using DOP-PCR

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## Introduction

In crop plants, molecular markers are useful for the creation of genetic maps, map-based cloning, and many breeding applications including marker assisted selection, backcross conversion, and genotyping (Bhattramakki and Rafalski, 2001).There is a transition beginning in the use of DNA markers, from the types such as RFLPs, AFLPs, and SSRs, that have dominated plant genomics in the past decade, to single nucleotide polymorphism (SNP) based markers. A SNP is a single base mutation in DNA and is the most simple polymorphism and most common source of genetic polymorphism in the human genome (Kruglyak, L. 1997; Collins et al., 1998). Unlike the human genome for which information has accumulated on the frequency, nature, and distribution of SNPs, SNP discovery in cereal genomes is just beginning (Zhu et al., 2003)**.** To address this problem, the approaches of ‘intron scanning’ and DOP-PCR were adopted for the discovery of DNA polymorphisms in cereals. Intron scanning involves the design of conserved PCR primers that amplify specific intron-containing loci in order to unravel the DNA polymorphisms across the grass species while DOP-PCR involves use of a single degenerate primer to amplify multiple loci spread throughout a genome (Jordan et al., 2002). Use of polymorphism discovery methods such as these will accelerate marker development in particular and strengthen efforts toward gene discovery in general.

## Methods

The intron scanning primers sets have been designed by blasting sorghum / buffelgrass (*Pennisetum ciliare*) EST’s against the TIGR rice pseudomolecule for chromosomes 1-12 (www.tigr.org). The DOP PCR protocol was followed according to Jordan et. al.(2002). The degenerate primer used in the study was DOP 1B (5’-CTCGAGNNNNNNAAGCGATGW-3’). PCR products obtained from intron scanning were direct sequenced while DOP products were cloned and sequenced. The sequencing has been done on an ABI 3700 sequencer (Applied Biosystems, Foster City, California, USA). We have sequenced intron scan / DOP products from seven rice, three sorghum, two millets, and two bermuda grass genotypes. Sequencing data were analyzed with Phred / Phrap / Consed system (Ewing et al., 1998). The genomic loci were scanned for SNP’s using Polybayes (Marth et al., 1999).

## Results

A significant number of SNP’s were identified from intron-scan and DOP PCR techniques (Table 1). DOP-PCR resulted in a higher total number of SNPs while the success rates for sequencing were comparable for

**Table 1. The DNA polymorphism identification of grass species by intron scanning and DOP PCR approaches**

| Taxon | Monomorphic loci | Polymorphic loci | SNP’s |
|---|---|---|---|
| **Intron Scan** | | | |
| *Pennisetum glaucum* | 18 | 6 | 7 |
| *Oryza sativa* | 17 | 22 | 54 |
| *Sorghum spp.* | 4 | 23 | 69 |
| **DOP-PCR** | | | |
| *Oryza sativa* | 419 | 142 | 273 |
| *Sorghum spp.* | 360 | 28 | 164 |

both approaches (Table 2). Intron scanning was found to be significantly more cost effective than DOP PCR (Table 3), with a 33% reduction in the cost incurred per SNP identified.

**Table 2. The comparative success rates for Intron scan and DOP-PCR approaches**

| Taxon | Genotypes | Primer sets | PCR success rate | Reads | Successful reads | Sequencing success rate |
|---|---|---|---|---|---|---|
| Intron-Scan | | | | | | |
| *Oryza sativa* | 3 | 96 | 67.70% | 144 | 115 | 79.86% |
| *Sorghum spp.* | 7 | 96 | 66.70% | 336 | 230 | 68.45% |
| DOP-PCR | | | | | | |
| *Oryza sativa* | 2 | 1 | 100.00% | 1152 | 869 | 75.43% |
| *Sorghum spp.* | 7 | 1 | 100.00% | 2496 | 2060 | 82.53% |

**Table 3. Analysis of performance and cost incurred for Intron scan and DOP-PCR techniques (based on rice)**

(a)

| | Performance | | | | |
|---|---|---|---|---|---|
| Technique | Genotypes | Primer set | Reads | Loci | Polymorphisms |
| Intron_scan | 7 | 96 | 336 | 39 | 70 |
| DOP PCR | 7 | 1 | 2496 | 561 | 164 |

(b)

| | Cost incurred ($) | | | | |
|---|---|---|---|---|---|
| Technique | [a]Primers | [b]PCR Reactions | [c]Sequencing | Total | Polymorphisms |
| Intron_scan | 622.08 | 336.00 | 840.00 | 1798.08 | 25.69 |
| DOP PCR | 3.24 | 3.50 | 6240.00 | 6246.74 | 38.09 |

[a] 0.18/nt; [b] 0.5/rxn; [c]2.50/read (including cleanup and sequencing)

## Conclusions

Much effort has been made to study SNPs in the human genome, but studies on the grass genome are scarce and limited. This study has led the way to scanning polymorphisms economically and effectively. The intron-scanning approach was found to be highly acceptable and can also can be employed to identify SNPs at corresponding loci across diverse taxa, detect evidence of selection by virtue of its close relationship to genes, and discover conserved non-coding (CNC) regions in closely related genomes. The SNPs detected in this study are under validation process. After successful genotyping, these SNP's could be then used as molecular markers for molecular breeding and other purposes in diverse cereal taxa.

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# Over-expression of exogenous superoxide dismutase gene (*MnSOD*) and its effect on stress resistance in maize

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In this study, the *MnSOD* gene from wheat was designed to be promoted by maize ubiquitin promoter and the expression product to be located at mitochondrial membrane by mitochondrial transport polypeptide. Expression vector for monocotyledon was constructed with this expression structure and embryonic calli of elite maize inbred lines were transformed with microprojectile bombardment. It was attempted to improve tolerance of maize to drought and other stress by overexpression of the *MnSOD* gene and the product accumulation in mitochondrion.

## Materials and methods

### *Construction of expression vector of MnSOD gene for monocotyle*

Mitochondrial transport polypeptide gene *TP* and MnSOD gene *MnSOD* were cut off by BamHI and ClaI from pBSOD3.1 and integrated into pUGFPocs. This produced an intermediate plasmid pUSOD3.1ocs, which contained the complete expression structure '*P-Ubi-MnSOD3.1-T-ocs*'. This structure was then cut off by KpnI and integrated into pCAMBIA1300 with hygromycin phosphotransferase gene *hpt*. The structure of *MnSOD* expression vector pC1300SOD3.1 is shown in figure 1.

### *Transformation and screening*

After four hours of subculture on highly osmotic medium, the embryonic calli were transformed with the expression vector by microprojectile bombardment model PDS-1000 / He, using system pressure of 1300psi, vacuum degree of 27ˇ^28 inch mercury, and vector DNA of 1g for each bombardment. The transformed calli were cultured one week on regulatory subculture medium for resume and three weeks on each gradient of hygromycin concentration of 5, 10, and 15 mg / l for screening. Differentiation and rooting media also contained hygromycin (5 mg / l).

**Figure 1. Structure of *MnSOD* expression vector pC1300SOD3.1**

### *PCR and Southern hybridization identification*

DNA fragment of *hpt* gene was amplified as marker of exogenous gene with DNA template extracted from the leaf of the regenerated plants and specific primers (P1: 5'-TACACAGC CATCGGTCCAGA-3', P2: 5'-TAGGAGGGCGTGG ATATGTC-3') designed according to *hpt* sequence. 10 g of total DNA was digested with HindIII, separated with 0.7% agarose gel and transferred to Hybond $N^+$ membrane (Amersham, UK). Full length sequence of *hpt* gene was radiolabeled with -$^{32}$P dCT and used as probe to be hybridized with the template DNA on the membrane. The signal was detected after washing with 0.1xSSC and 0.1%SDS, and radioautography on X-ray film.

### *MnSOD activity detection*

SOD enzyme was extracted from the leaf of the regenerated plants with the method introduced by Luo Guanghua and Wang Aiguo (1983), separated with nondenatured polyacrylamide gel electrophoresis of gradient concentration, treated and stained with the method introduced by Beauchamp and Fridovich (1971).

### *Antioxidant capacity detection of transgenic plant lines*

Leaf discs of 1.0 $cm^2$ were sampled from the fourth leaf of the transgenic offspring plants, weighted (m), and treated with 3 ml methyl viologen (MV) solution of 1.0 and 1.5 mol / l under the conditions of 21ºC and darkness for 16 hours, room temperature and illumination for 2 hours, and 30ºC and darkness for 16 hours. The methyl viologen solution was collected for each treated sample, $ddH_2O$ was added to 3 ml and electrolyte seepage rate (C1) was detected with electric conductometer model DDS-11A (specific conductivity = 0.98). The solution was collected and the leaf discs were put back. Electrolyte seepage rate (C2) was detected again after boiling 15 minutes, cooling to room temperature, and adding dd$H_2O$ to 3 ml. The detection was repeated four times for each plant line. The electric conductivity of 35 mg sample wass calculated as: Electric conductivity = 35C1 / mC2. Significance test was made by variance analysis and least significant difference (LSD).

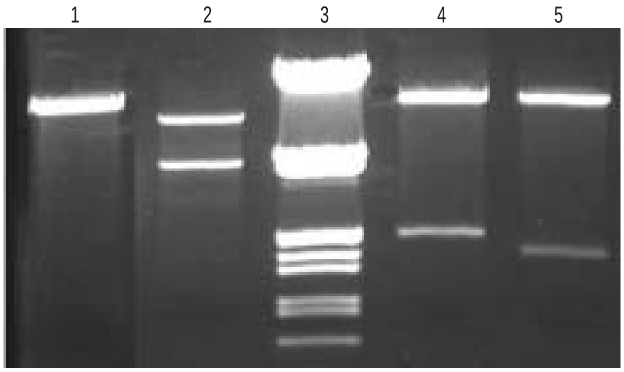
## Results and analysis

### *Restriction analysis of expression vector*

The *MnSOD* expression vector pC1300SOD3.1 was digested with SacI, KpnI, XbaI and SacI + ClaI, respectively. A 13.5 kb band of the linear vector, a 4300 bp band of the complete expression structure 'P-Ubi-*MnSOD3.1*-T-ocs', a 1800 bp band of *ubiquitin*, and a 1600 bp band of terminator fragment were identified by agarose gel electrophoresis (Figure 2). This result indicated that the expression vector followed the designed structure.

### *PCR and Southern identification of transgenic plants*

After transformation and strict screening, 49 plants were regenerated and 35 of them reproduced fertile seeds. Specific *hpt* fragment of 832 bp was amplified from nine of the fertile regenerated plants (Figure 3). HindIII was used to digest total DNA of the nine plants, because this enzyme had no recognition site during *hpt* sequence. It was shown by Southern hybridization with radio-labeled full length sequence of *hpt* gene that the exogenous gene had integrated into maize genome (Figure 4).

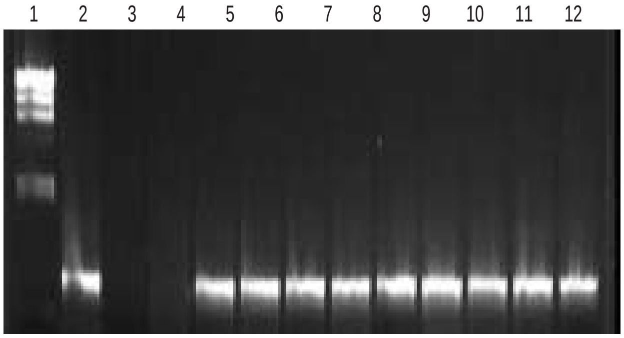


1. Sac I 2. Kpn I 3. 1 Kb marker 4. XbaI 5. Sac I+Cla I

**Figure 2. Restriction analysis of expression vector pC1300SOD3.1.**

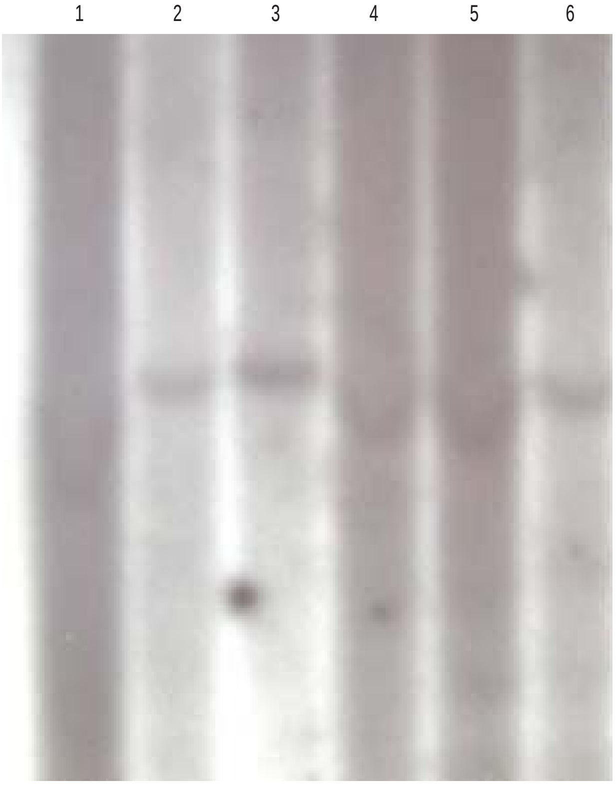
### *MnSOD activity detection*

The plants identified as positive through PCR and Southern blots were planted to reproduce plant lines of T1 generation and used for MnSOD activity detection. SOD had three isozymes: FeSOD, Cu / ZnSOD, and MnSOD. 5 mmol / l $H_2O_2$ inactivated FeSOD completely, reduced activity of Cu / ZnSOD, and had no significant influence on MnSOD. 5 mmol / L $H_2O_2$, therefore, was used to inhibit endogenous activities of FeSOD and Cu / ZnSOD in maize. Activity



M. DNA marker, 1. positive control, 2. negative control, 3. un-transgenic plant, 4-12. transgenic plants.

**Figure 3. PCR identification of transgenic plants.**



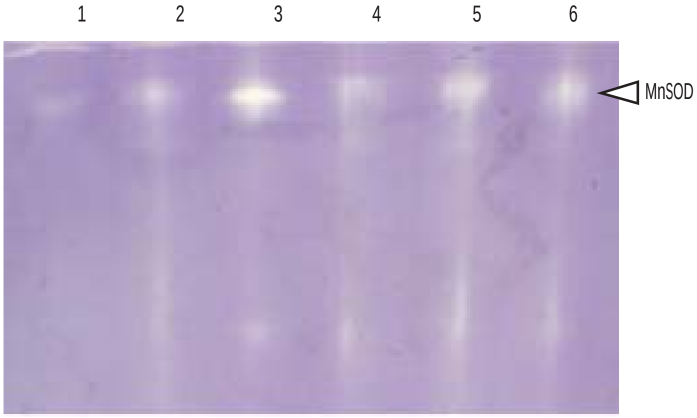
1. un-transgenic plant, 2-6. transgenic plants.

**Figure 4. Southern hybridization identification of transgenic plants.**

of MnSOD was detected after nondenatured polyacrylamide gel electrophoresis of gradient concentration. All the transgenic plant lines showed higher activity than the negative control, while plant line 3 displayed the highest activity (Figure 5).

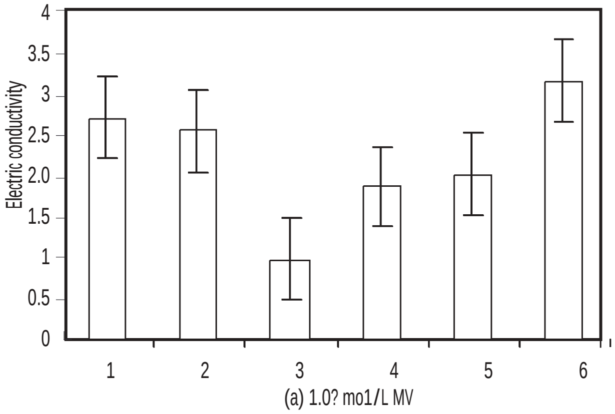
### *Antioxidant capacity of transgenic plant lines*

After treatment with 1.0 mol/l methyl viologen, electric conductivity of all the transgenic plant lines, except number 6, was less than the non-transgenic control, while plant line 3 showed greatly significant difference (Figure 6 a). After treatment with 1.5 mol/l methyl viologen (MV), electric conductivity of plant lines 2, 3, 4, and 5 was less than the control, while numbers 2, 3, and 5 showed greatly significant difference (Figure 6 b). This result indicated that antioxidant capacity of cellular membrane of the transgenic plants was increased more significantly than the control while oxidation damnification was increased.

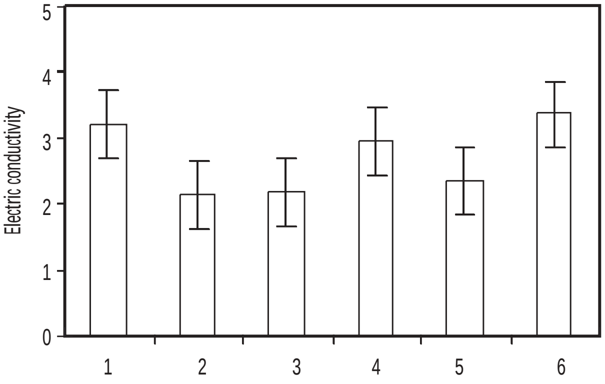


1. un-transgenic plant, 2-6. transgenic plants.

**Figure 5. Profile of MnSOD enzyme of transgenic plants.**



(a) 1.0? mo1/L MV



1.5? mo1/L MV

1. un-transgenic plant, 2-6. transgenic plant lines.

**Figure 6. Electric conductivity of transgenic plant lines.**

# EcoTILLING candidate genes for drought tolerance in rice

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## Introduction

EcoTILLING of diverse germplasm allows the discovery of SNPs and the delineation of haplotypes at loci of interest (Comai et al., 2004). This technique relies on the enzymatic cleavage of heteroduplex molecules formed between reference and query lines by an S1 type single-strand endonuclease from celery, CEL1. Dual-labeling of the PCR amplicons by different fluorescent tags allows the detection of the cleavage products on denaturing PAGE with automated genotyping. The appearance of new bands against the background products allows the detection of single nucleotide polymorphisms. Furthermore, the banding patterns across a range of diverse germplasm can be grouped into haplotypes. This combination of haplotyping and identifying SNP loci reduces the cost associated with SNP discovery so that only those germplasm carrying SNP differences need be sequenced in order to establish the identity of the nucleotide difference. As such, EcoTILLING is a powerful, inexpensive tool for the detection of natural variation.

## Materials and methods

At IRRI, we have begun employing this technique to characterize SNPs and determine haplotype structure at candidate genes for drought tolerance in diverse germplasm selected from the International Rice Genebank Collection (IRGC). This mini-core collection of 1,546 cultivated, diverse *Oryza sativa* accessions and contains diverse landrace, traditional, and advanced varieties representative of the variety groups and cultural types or non-sativa *Oryza* species, 48 accessions covering the genome types were chosen. Overall, these two sets represent about 1.5% of the genebank collection.

Candidate genes putatively involved in drought tolerance in rice were identified through converging evidence taking into account functions implicated in drought tolerance, altered expression, co-localization with drought QTLs, and / or shifts in allele frequencies under selection. The co-localization with QTLs was largely done to QTLs for yield components under field stress. Our initial target loci for implementing EcoTILLING are protein phosphatase 2a-4, DREB1, trehalose 6-phosphatase, and 9-cis epoxycarotenoid dioxygenase (Table 1). Allelic variation at pp2a4 was identified in pilot studies at the University of Washington; this locus has served as a postive control for implementing EcoTILLING in rice at IRRI. Additional candidate gene loci have already been identified, and primers to detect regions of these genes are being designed.

**Table 1. Drought candidate gene targets and primers**

| Gene | Function | Primer | Sequence (5’to 3’) Left (IRD700), Right (IRD800) | Chr. | RGP (cM) | Prod. (bp) |
|---|---|---|---|---|---|---|
| pp2a4 | protein phosphatase | pp2a4L | ggTTggggCATA TCTCCTCgTggT | CH10 | 30.2 | 928 |
| | | pp2a4R | TCCTAggAgCTggTTCAAACTgCAA | CH03 | 16.8 | 668 |
| dreb1 | drought response binding protein 1 | DREB1L | CCgTTgATTgCTgATAgCCTCCTTgA | CH01 | 16.1 | 969 |
| | | DREB1R | TgAAATATTCCTATTgACCCgCAgCA | | | |
| tps | trehalose phosphatase | TPSL | ggCACACTgTCgCCTATTgTggATg | CH02 | 109.3 | 997 |
| | | TPSR | gTTTACgAgCCgTgCgACCAgTTTC | | | |
| vp14 | viviparous14 (9-cis-epoxy-carotenoid dioxygenase) | VP14L | TggCAAgAAgAAggATgggCTgAAC | CH12 | 105.1 | 1013 |

## Results

EcoTILLING has been successfully implemented at IRRI. Figure 1 shows the results for pp2a4 locus on 22 germplasm contrasted to the reference lines, IR64 and Nipponbare. Figure 2 shows the result for this locus on 24 wild species contrasted to IR64. This may well be the first example of the use of EcoTILLINg to detect inter-specific differences. We are in the process of testing whether or not pooling levels up to 16-fold are possible. If so, then one run of the automated genotyper with 96 lanes will accommodate 1,536 samples for first pass screening. Recent results of pooling tests and EcoTILLING at other loci will be presented.

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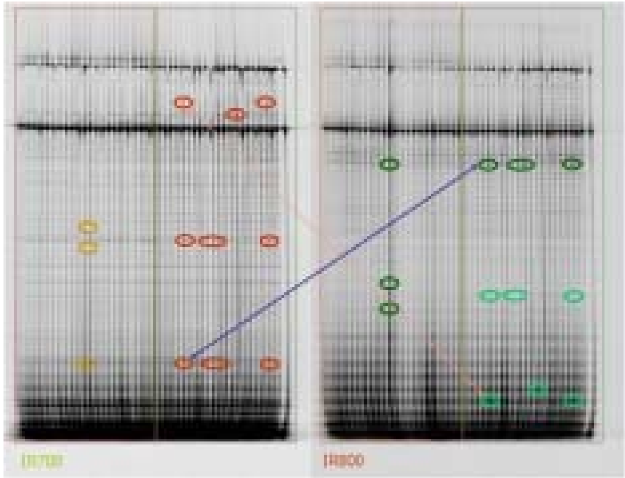

**Figure 1.** EcoTILLING of 22 accessions at the pp2a4 locus contrasted against IR64 (left) and Nippon-bare (right). Circles indicate possible polymorphisms.

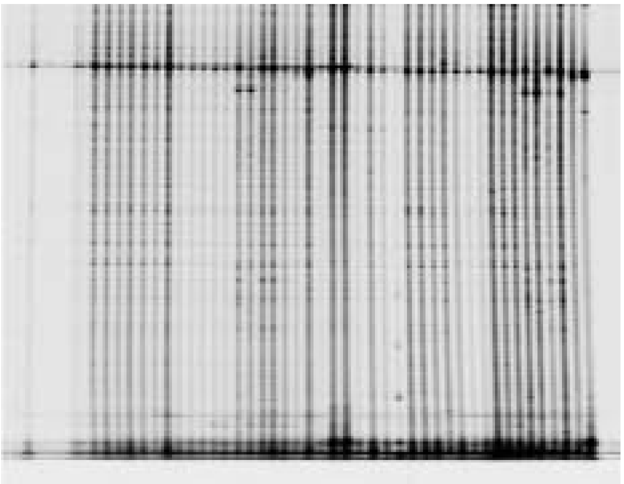

**Figure 2.** EcoTILLING of 24 wild accessions at the pp2a4 locus contrasted against IR64. These species AA, CC, CCDD, EE, and GG genome types.

# Genetic transformation and testing of stress responsible candidate genes on improving drought tolerance in rice

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## Introduction

Rice is the most important crop in China and in many developing countries. Drought stress is one of the most important constraints in rice production, mostly due to variation in the rainfall patterns from one year to another and also uneven distribution of rainfall and increasing shortage of water resources in the rice-growing areas. A project supported by The Rockefeller Foundation has been initiated to engineer rice plants with improved drought tolerance, targeting the drought stress at late stage of the growing season under field conditions in the agroecosystem of central and southern China. The long-term goal of the proposed project is to generate new rice cultivars and hybrids with significantly improved tolerance to major abiotic stresses such as drought and salinity.

## Methods

The specific objective for the current phase of this project is to evaluate the effects of 10 candidate genes from different functional categories on improving drought tolerance in rice under field conditions of central and southern China at the late stage of the growing season. These 10 genes (Table 1), mostly from Arabidopsis (*CBF3, LOS5, SOS2, HVA1, ZAT10, NCED1, NHX1, CodA*) or from other species (*NPK1, TPS*), were recommended and / or provided by a panel of worldwide leading experts in stress biology invited by The Rockefeller Foundation. All these candidate genes were constructed under the control of constitutive promoter or stress inducible promoter and transformed, mediated by *Agrobacterium*, into a drought-sensitive rice cultivar Zhonghua 11. As an important addition to the project, rice homologues to

**Table 1. Transformation progress of candidate genes and rice homologues**

| Priority[a] | Gene | Gene product or description | Transformation status |
|---|---|---|---|
| 1 | CBF3 | AP2 type transcription factor | Resistant callus selection |
| | 3 rice homologues | 85%-90% of similarity to CBF3 | $T_1$ seeds available |
| 2 | LOS5 | Enzyme in ABA biosynthesis | Resistant callus selection |
| 3 | SOS2 | Protein kinase | Resistant callus selection |
| | Rice homologue | 75% of similarity to SOS2 | Resistant callus differentiation |
| 4 | TPS | Trehalose-6-phosphate synthase | Similar work already reported |
| 5 | HVA1 (rice homologue) | LEA protein, 93% of similarity to HVA1 | $T_0$ plants (more than 100 lines each construct) |
| | HVA22 (rice homologue) | 93% of similarity to HVA22 | $T_0$ plants |
| 6 | NPK1 | MAPKKK | Resistant callus selection |
| | Rice homologue | 76% of similarity to NPK1 | Resistant callus differentiation |
| 7 | ZAT10 | Zinc finger transcription factor | Resistant callus selection |
| 8 | NCED3 | 9-cis-epoxycarotenoid dioxygenase | Resistant callus differentiation |
| 9 | NHX1 | Vacuolar $Na^+/H^+$ antiporter | Resistant callus selection |
| | Rice homologue | 78% of similarity to NPK1 | Resistant callus differentiation |
| 10 | CodA | Choline oxidase | Gene source unavailable |

[a] The priority was determined by the discussion from a panel of leading experts in stress biology invited by Rockefeller Foundation.

above candidate genes were also chosen for rice transformation and testing. For each construct, at least 30 independent single copy transgenic lines, were planned to be generated, and 20 plants each line will be tested for drought tolerance following the treatment and evaluation protocol provided by Dr A. Blum (personal communication).

## Progress and perspective

At present, all candidate genes, except *TPS* and *CodA*, and 8 rice homologous genes have been constructed in a high throughput binary vector under the control of a constitutive promoter (rice *Act1* promoter) and a stress inducible promoter (from a rice *HVA22* homologue that is strongly induced by drought), respectively. Each construct has been transformed into the rice cultivar Zhonghua 11. The current status of transformation is summarized in table 1. Transgenic plants in $T_0$ generation have been generated for five rice genes and the transformation for remaining genes is expected be finished before July, 2004. $T_1$ or $T_2$ seeds will be available in the next rice grown season for drought testing.

The completion of this work may create many new opportunities for the development of drought tolerant rice crosses or varieties and will also contribute to the elucidation of functional conservation of the candidate genes used in this project, which in turn will provide insight into the mechanisms of adaptation of plant cells to dehydration stresses.

# An *Arabidopsis* gain-of-function mutant with enhanced drought tolerance by activation tagging

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## Introduction

Drought is a worldwide problem. Although drought tolerance is widespread in nature, the underlying mechanisms are still not well understood, but significant progress is being made (Xiong et al., 2002; Xiong and Zhu, 2002; Zhu, 2002; Chinnusamy et al., 2003; Seki et al., 2003; Shinozaki et al., 2003; Xiong and Yang, 2003). To study drought tolerance mechanisms and mine drought tolerance genes, we have taken advantage of the model plant *Arabidopsis* by isolating gain-of-function mutants with enhanced stress tolerance. From an activation tagging library of 55,000 individual lines that we created, one mutant with enhanced drought tolerance was isolated. Initial characterization showed that this mutant has enhanced drought tolerance under greenhouse conditions, a well-developed root system, and enhanced tolerance to oxidative stress compared with the wildtype. In addition, this mutant was also found to have enhanced tolerance to salt stress. The results from this study demonstrate that accelerated evolution by activation tagging is a feasible approach to obtain gain-of-function mutants with enhanced stress tolerance and altered expression level and pattern can afford new functions to certain genes.

## Methods

To isolate gain-of-function mutants with enhanced stress tolerance, an *Arabidopsis* activation tagging library of 55,000 independent lines was created as described (Weigel et al., 2000). The mutant library was initially screened for individuals with growth vigor. Several such putative mutants were isolated and tested for their tolerance to drought, salt, and oxidative stresses. Drought tolerance test was done with mutant and wild-type plants grown in soil under greenhouse conditions while salt and oxidative stresses were tested on media containing NaCl or paraquat. Co-segregation analysis was carried out after stress tolerance test by examining the resistance to glufosinate herbicide of each individual plant using leaf paint method.

## Results

An *Arabidopsis* gain-of-function mutant with enhanced drought tolerance was isolated (Figure 1). The mutant phenotype co-segregates with herbicide resistance, thus confirming the drought tolerance phenotype was directly resulted from the T-DNA insertion. This was further confirmed by recapitulation study. Molecular analysis further revealed that the mutant phenotype was caused by the elevated expression level and altered expression pattern of the tagged gene. In addition to drought tolerance, this mutant also shows enhanced tolerance to salt stress and oxidative stress. Physiologically, the mutant leaves show slower rate of water loss in contrast to wildtype leaves. Morphologically, this mutant shows growth vigor and a well developed root system compared with wild-type. Apparently, the well-developed root system, slower rate of water loss from leaves, and oxidative stress tolerance collectively contribute to the enhanced drought tolerance of this mutant.

**Figure 1. Drought tolerance test. Wild-type and mutant plants were grown side by side under identical conditions in the same tray. Watering was withheld for two weeks when plants were 4 weeks old. The wilted plants were wild-type.**

## Conclusions

The results from our study with this mutant demonstrate that accelerated evolution by activation tagging is a feasible approach to obtain gain-of-function mutants with enhanced stress tolerance. Altered expression level and pattern can afford new functions to certain genes. The mutant we isolated should be a valuable resource for studying the underlying mechanisms of drought tolerance. The tagged gene has the potential of crop improvement for enhanced stress tolerance.

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# Generation Challenge Programme: “Cultivating plant diversity for the resource-poor”

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## Introduction

Rich genetic resources, whose diversity could benefit the global community, are kept in trust for humanity by CGIAR Centres. National genebanks, too, hold immense reserves of untapped riches. Advances in molecular biology can put this diversity to work in powerful and exciting ways. But at present the power of modern biology is rarely used to improve crops grown by resource-poor people in the developing world. The Generation Challenge Programme (formerly the Challenge Programme for Unlocking Genetic Diversity in Crops for the Resource-Poor) aims to create a public platform that will use molecular biology to unlock genetic diversity and put it to use in bettering crops for the world’s poorest farmers.

## Organization

The Generation Challenge Programme brings together three sets of partners. The CGIAR Centres keep vast amounts of plant diversity in trust for humanity and have expertise in molecular research and global breeding programmes. The National Agricultural Research Systems of developing countries bring expertise in the assessment and breeding of plants under specific conditions, with the participation of farmers. Advanced Research Institutes develop novel techniques and strategies to decode genetic diversity. Together, these three constituents can vastly improve the productivity of crucial crops in marginal environments. The Generation Challenge Programme is composed of five subprogrammes, detailed below. A major objective of the GCP is to apply genomics tools and technologies to a better understanding of drought tolerance mechanisms in the 22 mandate crops of the CGIAR.

## Drought tolerance: The ultimate challenge in a world of climate change

One of the most difficult challenges faced by resource-poor farmers and scientists alike is drought. The Generation Challenge Programme is focused on harnessing the genomics revolution to enhance drought tolerance in the staple crops of developing countries. Plants’ responses to drought are complex and involve interactions between many different molecular, biochemical, and physiological processes. Moreover, the nature of drought stress itself varies by crop, cropping system, region, and year. Using comparative genomics, scientists can begin to dissect the intricate relationships and pathways at work at various plant component levels and across species. Intense investigation of drought tolerance in one species or plant type may well provide the vital clues needed to unlock it in other species.

## Subprogramme 1: Genetic diversity of global genetic resources

Using a selection of known genes as probes, this subprogramme’s goal is to assay the diversity in existing collections. The assays are of two types. Structural characterization comprises the sequences, markers, and other genomic information that enables researchers to identify particular genes. Functional characterization links genetic information to the performance of the plant; for example, it can relate specific sequences to the plant’s ability to withstand low temperatures.

## Subprogramme 2: Comparative genomics for gene discovery

Given that many functional genes are found in the same order on the chromosomes of different species, the need for universal genetic maps is clear. These incorporate information from many species and will be useful for all species. Technologies such as the gene chip can provide a snapshot of genes that are active in a particular tissue under particular circumstances. This genetic portrait will generate insights into the molecular bases of complex performance characteristics such as drought tolerance. In concert with universal maps, gene chips will help plant breeders home in on candidate genes to use in crop improvement programmes.

## Subprogramme 3: Trait capture for crop improvement

The ultimate goal of the Challenge Programme is not more information and greater knowledge, but their application to the needs of farmers. Thus, the discoveries of comparative genomics must lead to improved crops. This subprogramme will enable more breeders to readily move valuable traits into targeted crops. Several different techniques will be involved, from relatively simple marker-assisted selection, which uses genetic information to speed the assessment of new varieties, to more efficiently transferring desired genes within and among selected species.

## Subprogramme 4: Genetic resources, genomic, and crop information systems

At the heart of the enterprise is information of various kinds. A key challenge is to link and integrate these disparate data into a cohesive whole: molecular data and agronomic data need to relate to one another across all species studied. The goal is to develop a platform of bioinformatics, biometrics, and data management to create a resource that will drive gene discovery and crop improvement.

## Subprogramme 5: Capacity building

The goal of the capacity building subprogramme, which bridges the four technical subprogrammes, is to ensure that scientists in developing countries can make full use of Challenge Programme products and discoveries. Each of the technical subprogrammes will conduct various activities to train scientists around the world in new techniques and approaches. There will be scientific exchanges, support for young scientists, conferences, and other initiatives to meet the needs of partners.

## Consortium members

*CGIAR Centres*: International Center for Tropical Agriculture (CIAT); International

Maize and Wheat Improvement Center (CIMMYT); International Potato Center (CIP);

International Center for Agricultural Research in the Dry Areas (ICARDA);

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT);

International Institute for Tropical Agriculture (IITA); International Plant Genetic

Resources Institute (IPGRI); International Rice Research Institute (IRRI).

*Partners*: Agropolis (France); Brazilian Agricultural Research Corporation

(EMBRAPA), Brazil; Chinese Academy of Agricultural Sciences, China; Cornell

University, USA; John Innes Centre, UK; National Institute of Agrobiological Sciences,

Japan; Wageningen University, the Netherlands.

# Evaluation of discriminant analysis as a tool for rapid identification of markers associated with drought resistance in rice

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## Introduction

Drought stress in rice (*Oryza sativa L.*) severely depresses yield in rainfed ecologies and reduces productivity in well-watered conditions. Breeding for enhanced drought resistance using conventional breeding strategies has been difficult due to the complexity of the trait, uncertainty of the environment, and the polygenic nature of drought resistance. Traditional genetic mapping techniques have identified putative QTLs for complex agronomic traits in rice that could benefit marker-assisted selection, candidate gene studies, and map-based cloning. However, other research groups have rarely repeated the majority of these studies for verification. Plant genetic mapping strategies routinely use marker genotype frequencies obtained from progeny of controlled crosses to declare presence of putative QTL on previously constructed linkage maps, even though a detected interval of 5-30 cM by this procedure may contain numerous genes. Based on the results of our recent investigation, we propose a robust, non-parametric multivariate model called discriminant analysis (DA) as a tool for detecting candidate markers associated with genes that confer drought resistance among inbred as well as pedigree lines of rice. DA allows the study of differences between two or more groups with respect to several variables and has potential for detecting markers associated with traits of interest. DA offers a complement to standard mapping techniques in controlled crosses as well as unrelated inbred lines. An example of DA analysis for six agronomic traits in an interspecific cross between *O. sativa* and *O. glaberrima* follow.

## Methods

Data on plant height (PHT), heading date (DTH), grain yield (GY) and 1000 grain weight (TGW) were collected from 312 doubled haploid (DH) lines. The data were transformed to achieve a normal distribution. Training samples were defined by taking materials at the tail of the distribution at 1, 2, or 3 standard deviations from the mean to represent low and high trait values (Figure 1). Molecular data were collected from the entire population. Molecular profiles of the training samples were tested for molecular variance using AMOVA (Excoffier et al., 1992). Population structure was checked using the model-based method of Pritchard (2002). Discriminant Analysis (proc STEPDISC, SAS Institute, ver. 9.0) was performed to identify marker(s) that best differentiate training samples within each subpopulation. Allocation of lines into predefined groups of low and high trait values was performed using the “K-nearest-neighbor” option within DISCRIM procedure (SAS Institute, ver. 9.0), and the percent correct classification was calculated with “validate” option within proc DISCRIM procedure (SAS Institute, ver. 9.0). Markers that best differentiate lines into groups of low and high trait values with high percent of correct classification were identified as candidate markers associated with the trait. If rice lines are scored for drought resistance using a scale of 1–7, the DA

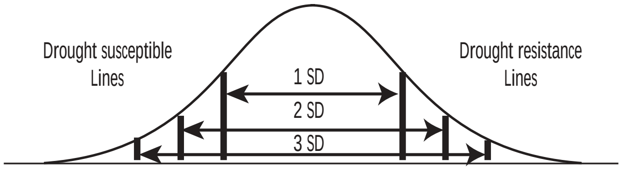

**Figure 1. Proposed scheme for selecting “training samples” or targeted groups for DA analysis. 1SD = 1 standard deviation, 2SD = 2 standard deviations, 3 SD = 3 standard deviations.**

procedure could be used to classify rice lines into groups of resistant (scores 1–3) and susceptible (scores of 4–7) based on their molecular profiles.

## Results

The DA procedure was found to properly assign DH lines between high and low groups using 5 to 10 SSR markers. Maximum differentiation between high and low groups (3SD) produced the highest percent correct classification using the fewest markers. Some markers selected by DA pointed to the same or nearby regions as those QTL intervals for GY, DTH, TGW, and PHT detected in the same cross (Figure 2). In addition, DA detected new markers associated with economically important traits. We propose this procedure for detecting markers associated with drought resistance in rice.

## Conclusions

Discriminant analysis (DA) can be used to successfully assign breeding lines in a segregating populations to pre-defined groups of interest. Markers selected by DA pointed to similar regions as traditional QTLs on the rice genetic map. New markers associated with agronomic traits, not previously identified by QTL analysis, were detected by DA method. Relatively few DA-selected alleles should allow for rapid gains in rice improvement via marker-assisted classification. DA should be used to complement traditional molecular and breeding approaches for detecting markers associated with quantitative traits in rice.

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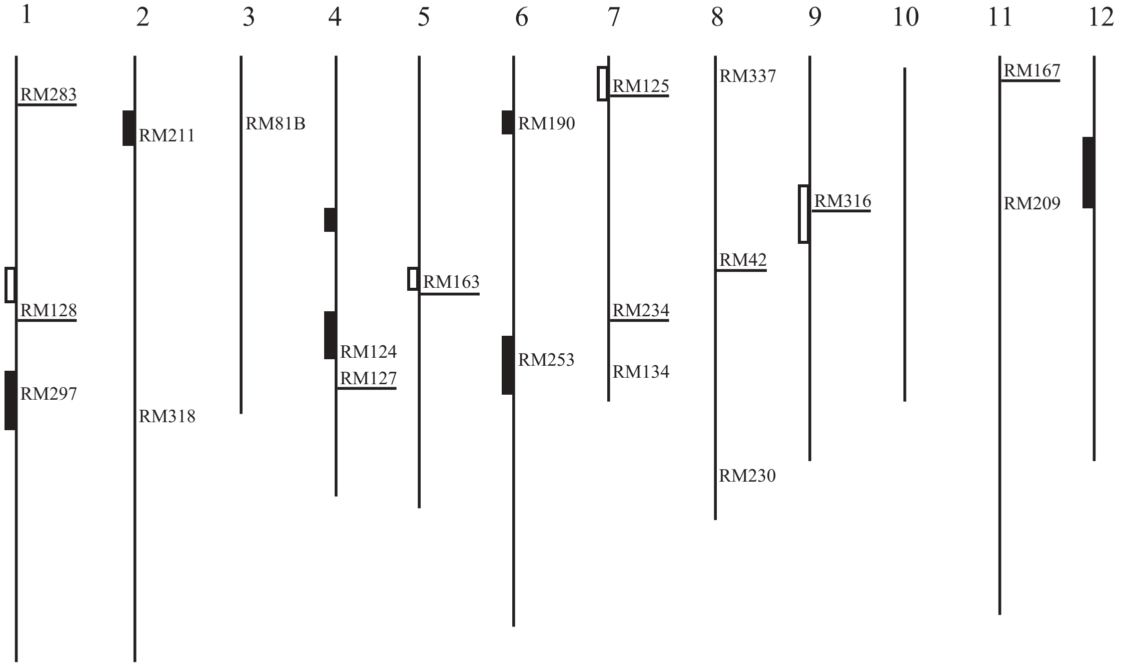

**Figure 2.** Chromosomal location of DA-selected markers compared with QTL mapping for heading date and grain yield. Filled boxes represent QTL regions for heading date; unfilled boxes represent QTL regions for grain yield. Markers detected by DA are indicated by their names. Underlined markers are DA-detected markers for grain yield.

# Heritability of rice yield under reproductive-stage drought stress, correlations across stress levels, and effects of selection: Implications for drought tolerance breeding

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To be useful in variety development, screening methods for tolerance to reproductive-stage drought stress need to be repeatable, applicable at a reasonable cost to large breeding populations, and predictive of grain yield under stress. Secondary anatomical and physiological parameters have generally not fulfilled these criteria in rice. As a result, there has been increasing interest in screening for yield under stress, either in easily-drained fields in the wet season or in managed-stress nurseries in the dry season. However, little guidance is available regarding how to incorporate such screens into cultivar development programs, and there are few reports on the effectiveness of direct selection for yield under stress. We present experimental evidence that direct selection for improved yield under severe reproductive stage stress is likely to be effective, based on information from the evaluation of populations of selected and unselected lines at IRRI and elsewhere. The results of a selection experiment conducted under severe upland stress are also presented, and the integration of drought tolerance screening in IRRI's aerobic rice breeding program is described.

## Heritability within and correlations across stress levels in unselected breeding populations

If selection for yield under drought stress is to be effective, yield in the stress treatment must be repeatably measurable. The stress treatment should also provide information about cultivar differences that is not available from screening under non-stress conditions. Information on this question has been assembled for 6 populations of unselected lines evaluated in a total of 10 experiments at locations in India, Thailand, and the Philippines under both well-watered conditions and severe stress. Broad-sense heritabilities (H) within water regimes and genetic correlations across them are presented in table 1. Yield under stress averaged 35% relative to the well-watered controls across these experiments. H estimates for grain yield in the stress environments were similar to those for yield in the non-stressed controls, indicating that selection for yield under stress is likely to be as effective as selection for yield under favorable conditions.

**Table 1. Repeatability (*H*) of grain yield estimates in well-watered and moisture-stressed treatments , and genetic correlations across stress levels ($r_G$), in trials evaluating unselected populations**

| Location | Year | Stress environ-ment | Non-stressenviron-ment | Population | Relative yield under stress | H | | $r_G$ | Data provided by: |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Stress | Non-stress | | |
| Bet Dagan, Israel | 1997 | Upland | Upland | CT9993/IR62266 | 0.26 | 0.81 | 0.63 | 0.35 | A. Blum |
| Coimbatore, Tamil Nadu | 1999 | Upland | Upland | CT9993/IR62266 | 0.31 | 0.60 | 0.56 | 0.86 | R. Chandra Babu |
| Paramakudi,Tamil Nadu | 2000 | Upland | Upland | CT9993/IR62266 | 0.41 | 0.76 | 0.23 | 0.91 | R. Chandra Babu |
| Ubon, Thailand | 2000 | Upland | Upland | CT9993/ IR62266 | 0.30 | 0.50 | 0.54 | 0.71 | G. Pantuwan |
| Raipur, India | 2000-02 | Lowland | Lowland | CT9993/IR62266 | 0.21 | 0.37 | 0.45 | 0.80 | R. Kumar |
| Los Banos,Philippines | 2003 | Upland | Lowland | Vandana/IR64 | 0.67 | 0.42 | 0.27 | 0.69 | R. Venuprasad |
| Los Banos,Philippines | 2003 | Upland | Lowland | Apo/IR64 | 0.13 | 0.24 | 0.45 | 0.35 | R. Venuprasad |
| Los Banos, Philippines | 2003 | Upland | Lowland | Apo/IR72 | 0.29 | 0.67 | 0.30 | 0.64 | R. Venuprasad |
| Los Banos, Philippines | 2003 | Upland | Lowland | Vandana/IR72 | 0.31 | 0.07 | 0.42 | 0.78 | R. Venuprasad |
| Los Banos, Philippines | 1998-9 | Upland | Upland | IR64/Azucena | 0.56 | 0.68 | 0.74 | 0.62 | B. Courtois |
| **Mean** | | | | | **0.35** | **0.51** | **0.46** | **0.67** | |

Genetic correlations across water regimes were consistently positive, averaging 0.67. This indicates that, within segregating populations of lines generated from a single cross, nearly half the genetic variation for yield under severe stress is accounted for by factors that also affect yield potential. The relatively high correlations observed across establishment methods and stress levels indicate that plant type and partitioning differences that are also expressed in non-stress environments are causing much of the yield variation observed under stress in unselected breeding materials. These results show that direct selection for yield under stress is likely to result in yield gains under stress, and that improvement in yield under stress can be combined with improvement in yield potential

## Heritability within and correlations across stress levels in advanced cultivar testing

The results presented above refer to unselected breeding lines from single crosses. In more diverse cultivar sets exhibiting a wide range of maturity, the correlation of genotype means across stress levels is affected by the magnitude of the relative yield in the stress and non-stress environments. This was observed in an experiment conducted at IRRI, wherein a diverse set of upland and lowland cultivars, breeding lines, and landraces was evaluated for two years under 4 water regimes: (1) full lowland irrigation, with transplanted management; (2) favorable upland conditions in the wet season; (3) moderate dry-season continuous stress; and (4) severe dry-season continuous stress. Means in the four environments were 2.7, 2,2, 1,1, and 0.5 t ha$^{-1}$, respectively. Genetic correlations stress levels are presented in table 2. Yields were positively and highly correlated among the non-stress and moderate stress treatments, but yields under the severest stress level were not associated with yields under non-stress conditions.

**Table 2. Genetic correlations across water regimes for 44 upland and lowland cultivars evaluated under 4 water regimes at IRRI, 2000-2003**

| Environment | Moderate upland stress | Upland non-stress | Lowland non-stress |
|---|---|---|---|
| Severe upland stress | 0.51 | -0.08 | -0.20 |
| Moderate upland stress | | 0.78 | 0.85 |
| Upland non-stress | | | 0.97 |

## Sources of variation in trials measuring yield under stress.

Although the heritability of yield is similar in stress and non-stress environments, it is rather low. To plan effective screening programs, an understanding of the sources contributing to the variance of cultivar means is needed. Major potential sources are within-trial field variability, which may be exacerbated by water stress treatments, and genotype x environment interaction. The latter source is likely to be especially important in drought screening trials conducted under natural stress in the wet season. This was illustrated in a set of 39 advanced Thai breeding lines and released varieties evaluated under stress and non-stress conditions in transplanted and direct-seeded trials at Ubon and Chumpae in 2003. Stress was applied by draining paddies 2 weeks before the onset of flowering and not re-watering. Very high genotype x environment interactions were observed, due to differences in stress timing and blast disease pressure among sites (Table 3). Residual within-trial variances were also very high resulting. This resulted in very low predicted heritabilities for both stress and non-stress trials.

By contrast, managed-stress screens for drought tolerance can have relatively high repeatabilities by controlling genotype x trial interaction. This is illustrated in the variance component and H estimates derived from the 44-entry cultivar set (described above) evaluated under upland and lowland management at IRRI. Moderate and severe stress treatments were applied in the relatively uniform dry season. In this experiment, genotype x environment interaction in the stress treatments was low relative to the genotypic variance, and relative to GxE interaction in the wet season. The main source of variation in the stress trials was within-trial residual field variation, indicating that useful levels of H can be achieved in a single managed-stress screening trial if it is well-replicated and designed (Table 4).

**Table 3. Variance component and broad-sense heritability (H) estimates for the combined analysis over Ubon and Chumpae in northeast Thailand for 4 types of screening trial (Wet season 2003)**

| | Variance components | | | H | |
|---|---|---|---|---|---|
| REGIME | Genetic | Genotype x environment | Residual | 2 sites, 3 replicates | 1 site, 3 replicates |
| Direct-seeded: non-stress | 0 | 983 | 3205 | 0.00 | 0.00 |
| Direct-seeded: stress | 32 | 393 | 966 | 0.08 | 0.04 |
| Transplanted: inon-stress | 161 | 0 | 1264 | 0.43 | 0.28 |
| Transplanted:Stress | 27 | 97 | 355 | 0.20 | 0.11 |

## Selection experiment

The results reported above indicate that direct selection for yield under stress is likely to be effective if conducted in a well-managed screening environment. To test this hypothesis, a direct selection experiment was conducted in two populations at IRRI in 2003-4. Populations were derived without selection from the crosses Apo / IR64 and Vandana / IR64. IR64 is an elite irrigated lowland variety. Apo (IR55423-01) is a high-yielding upland rice variety with moderate tolerance to continuous water stress in the field. Vandana is a highly drought-tolerant eastern Indian upland variety derived from a cross between an *aus* traditional variety and an improved Philippine tropical *japonica*. Two hundred and twenty-five (225) random $F_2$–derived $F_3$ lines from each cross were evaluated under severe intermittent upland stress as well as under non-stress irrigated lowland management in the dry season of 2003. Evaluation was conducted in 2 replicate alpha-lattice trials in which the experimental unit was a single plot, 2 m in length in the upland and 5.25 m in length in the lowland. The 25 highest-yielding lines were selected from each screening environment (giving a selection intensity of 12.5%) and compared with 25 random lines.

Results of the selection experiment are presented in table 5. Under severe upland stress, the tolerant upland parent, Vandana, yielded 104.6 g $m^{-2}$, whereas Apo and IR64 yielded only 14.8 and 3.6 g $m^{-2}$, respectively. The selected Vandana / IR64 population outyielded the random set of lines by nearly 20% under severe stress. The selected population derived from Apo / IR64, however, exhibited no improvement over the random set under severe stress. Neither upland-selected population exhibited reduced mean yield relative to the checks under lowland conditions.

It is important to note that, on average, random lines derived from Vandana / IR64 outyielded random lines from Apo / IR64 by 300%. The effect of using a highly tolerant donor on yield under severe upland stress was greater than the effect of selection.

Table 4. Variance components for 44 upland and lowland cultivars evaluated under 4 water regimes at IRRI, 2000-2003

| Environment | $^{2}_{G}$ | $^{2}_{GY}$ | $^{2}_{e}$ | H(one 4-rep trial) | H(two 4-rep trials) |
|---|---|---|---|---|---|
| Severe upland stress | 58 | 20 | 86 | 0.48 | 0.58 |
| Moderate upland stress | 86 | 36 | 138 | 0.46 | 0.55 |
| Upland non-stress | 337 | 168 | 184 | 0.76 | 0.61 |
| Lowland non-stress | 481 | 299 | 163 | 0.75 | 0.59 |

In general, this experiment demonstrates that direct selection for yield under severe stress in small plots can be highly effective in populations where adequate genetic variability for the trait exists. It also illustrates the importance of using highly tolerant donors when tolerance to severe stress is the breeding objective.

## Implications for drought tolerance breeding

Yield under drought stress is a moderately heritable trait with repeatability similar to that of yield in non-stress environments. Direct selection for yield under stress is effective, if stress tolerance screening is done in replicated field trials that effectively control within-trial field heterogeneity. This means that selection for yield under stress is best initiated after pedigree breeding lines have been fixed for plant type, disease resistance, quality, and other high-heritability traits, as is the case for selection for yield potential. This was demonstrated in a selection experiment. The moderate correlation usually observed between yields in stress and non-stress environments indicates that improvements in stress tolerance can be generated while maintaining high yield potential. Highly-tolerant donors are likely to be much more efficient in generating progeny tolerant to severe stress than donors with only moderate levels of tolerance. Such donors, however, are unlikely to be agronomically acceptable in high-productivity environments. This is a strong argument for pre-breeding highly drought-tolerant donor lines with acceptable agronomic traits under non-stress conditions. Such donors could be directly used as sources of drought tolerance in cultivar development programs that must generate cultivars combining high yield potential with moderate stress tolerance.

Table 5. Effects of direct selection for yield under upland stress in 2 populations at IRRI: 2003-4

| | Vandana / IR64 | | Apo / IR64 | |
|---|---|---|---|---|
| | Evaluation environment | | | |
| Entry | Upland | Lowland | Upland | Lowland |
| Upland-selected population | 68.9 | 182 | 16.7 | 191 |
| Lowland-selected population | 57.8 | 214 | 12.8 | 224 |
| Random population | 54.8 | 184 | 18.1 | 193 |
| IR64 | 3.6 | 286 | 5.8 | 293 |
| Apo | 14.8 | 240 | 17.8 | 242 |
| Vandana | 104.6 | 146 | 83.2 | 51 |
| $LSD_{.05}$ for checks | 59.0 | 49.4 | 21.4 | 91 |
| $LSD_{.05}$ for populations | 11.8 | 24.0 | 4.3 | 18 |

# Breeding approaches to develop drought tolerant maize hybrids

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The general process to develop maize hybrids starts with the creation of a source segregating breeding population that it is used to develop inbred lines through inbreeding and selection (Figure 1). Selected inbreds are then evaluated in hybrid combinations across locations to select superior hybrids and to estimate their combining abilities. Maize breeders use multiple-trait, multi-stage, and multi-environment screening methods, including evaluations under managed drought stress, to develop superior hybrids (Beck et al., 1997; Betrán et al., 2004).

Different types of segregating populations can be used as sources in line development: open pollinated cultivars, synthetic cultivars, single crosses, backcrosses, double crosses, related line crosses, and exotic germplasm. Populations originated from germplasm with a previous selection history for drought tolerance increase the probability of developing drought tolerant inbreds and hybrids (Edmeades et al., 1997). Major emphasis goes to the use of breeding populations created by hybridization of complementary inbreds and the selection of progenies possessing the desirable traits from the parents. In line recycling, crosses among elite lines from the same heterotic group with superior multiple-environment and multi-year general combining abilities and validated performance in hybrids under drought are preferred.

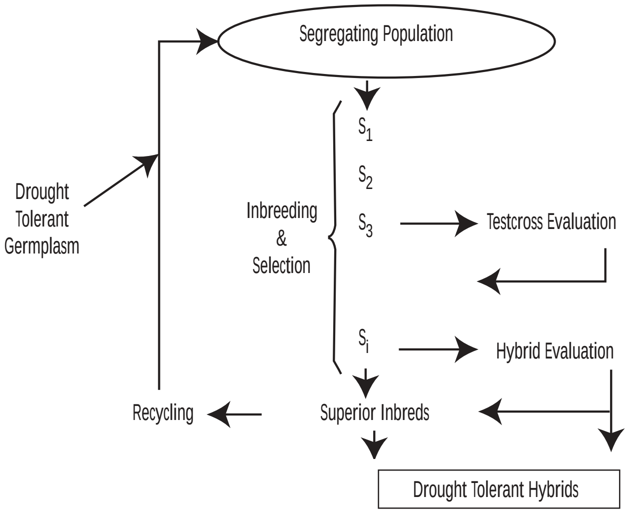

**Figure 1. General scheme for the development of drought tolerant maize inbreds and hybrids.**

The development of parental inbreds can follow different breeding methods such as pedigree breeding, backcross, bulk, single seed descent (SSD), double-haploids, etc. Typically, self-pollination is applied to the $F_1$ or segregating populations and subsequent generations to develop inbred lines that are superior to the parents (transgressive segregants) through genetic segregation and recombination. Selection is applied among progeny rows and among plants within families. It is common to have replicated nurseries for the selfed families exposed to drought stress as well as to prevalent biotic stresses. This process of selfing and selection is repeated in successive generations ($S_2$,$S_3$,$S_4$,$S_5$,….$S_n$) until homozygous elite inbreds are developed. Effective phenotypic selection and greater selection intensity can be applied in initial inbreeding stages for traits with high heritability such as maturity, anthesis-silking interval, morphological traits, pest resistance, grain type, etc. The consequences of inbreeding are homogeneous expression of traits and inbreeding depression (i.e., loss of vigor and productivity). Inbreeding depression for grain yield seems to be greater under drought stress than under optimal conditions. Vigor, plant size, grain yield components, and grain yield are reduced while time to flowering, anthesis-silking interval and barrenness normally increases with inbreeding.

Maize is a cross-pollinated species that shows high heterosis (i.e., superior performance of crosses relative to their parents) for grain yield. Heterosis is greater under drought stress than under optimal conditions (Betrán et al., 2003). Therefore, the identification and development of heterotic groups of elite inbreds can contribute to hybrid performance under drought. Testcross performance with representative testers is used to group large number of inbreds to known heterotic groups. Recently, DNA molecular markers have being effective for assigning inbreds to heterotic groups. The enhancement of heterotic response and hybrid performance is improved by subsequent cycles of inbred line development. The possibility to use inbred line information, as indicative of hybrid performance, is desirable to reduce the number of hybrid evaluations. In general, the inbred-hybrid correlation is relatively high for some additively inherited traits (e.g., plant morphology, ear traits, maturity, quality characters, etc.) but is relatively low for grain yield. The correlation for grain yield has been consistently positive and often significant, but not high enough to predict hybrid performance under drought.

In hybrid development, efforts are allocated in preliminary tests to evaluate as many hybrids as possible in few locations under managed drought stress where intensive selection is applied, leaving relative few hybrids to proceed to the more advance stages. Testcrossing with appropriate testers has been adopted extensively to evaluate the relative combining ability of experimental inbred lines. The level of inbreeding when testcross evaluation is conducted varies among breeders and depends on the traits under consideration and the effectiveness of visual selection of the lines per se. Evaluation of hybrids in late stages emphasizes wide-area evaluation with multiple environments that also include locations with managed drought stress. This extensive evaluation permits the selection of the hybrids for adaptation and stability. Besides managed drought, additional managed environments may include different densities, planting dates, fertilization levels, crop rotation, diseases, etc. More weight in decision-making during selection is applied to those environments more correlated with the target environment.

Characterization and selection of inbreds is a sequential process with some lines discarded either by early testing or by performance per se in early generations, and others discarded later by general and specific combining ability in hybrid combinations. Improvements of breeding technologies such as off-season nurseries, managed environments for screening against biotic and abiotic stresses, analysis of genotype x environment interaction and selection methods, adoption of experimental designs and equipment, and applications of molecular tools and biological research have increase the accuracy and efficiency of inbred and hybrid development (Bänziger and Cooper, 2001; Ribaut et al., 2002).

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# Improvement of maize populations for drought stress tolerance in Mozambique

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## Introduction

Maize (*Zea mays* L.) is one of the most important food crops in Mozambique, contributing more than 40% of the total calorie intake for human nutrition. More than 95% of the total maize area is occupied by open pollinated varieties grown mainly by small-scale farmers, which contribute 90% of the total maize production (1.2 million tons per year). Grain yield for smallholder farmers is very low (0.2–0.6 t / ha). Drought stress and low soil fertility are the two major abiotic factors affecting crop production in Mozambique. Therefore, one of the major activities of INIA Maize Program is the improvement of maize populations for drought and low-N stress tolerance.

## Material and methods

Four groups of maize populations with different backgrounds were evaluated in 2002 and 2003 under irrigated and randomized drought conditions, in two different levels of soil fertility, in three locations, Umbeluzi, Chokwe (low land), and Sussundega (midaltitude land). Collected data from the three locations were treated together and grouped according to trial type (irrigated or randomly stressed). Data for grain yield were analyzed on SAS software, while other agronomic characters where analyzed using the Alpha program.

## Results

Twenty-two (22) of 40 varieties from the four groups exhibited ratios above 60% between the grain yield under randomized drought stress and irrigated conditions (Figure 1). EV8430SR and ZM521 flint showed the highest ratio (almost 72%), but EV8430SR yielded less than 2 t / ha$^{-1}$ even under irrigation. For release, not only grain yield was considered, but other traits such as grain type, maturity, and disease resistance, were decisive. Following this approach, Matuba-1, ZM421 flint and ZM621 flint had cumulative advantages over the others. Many crosses were made involving these and other promising varieties. New selections for DT and LNT are underway. In 2003, Matuba-1, ZM421 flint, ZM521 flint, and ZM621 flint were released and named Changalane, Dzandza, Chinaca, and Tsangano, respectively.

**Table 1. Agronomic characteristic of DT&LNT varieties released in 2003 in Mozambique**

| | Grain yield across locations | | Ratio | Grain | Maturity | Diseases | | |
|---|---|---|---|---|---|---|---|---|
| Varieties | Irrigated | Drought | 100*(Dr / Ir) | Type | | MSV | DM | GLS |
| *Matuba-1* | 3.06 | 2.11 | 68.9 | Flint | Early | Toler. | Resist. | - |
| *ZM 421 F C3* | 3.20 | 2.07 | 64.9 | Semi-flint | Early | Toler. | Susc | Moder. |
| *ZM 521 C3* | 2.58 | 1.85 | 71.7 | Semi-flint | Interm. | Toler. | Susc | Resist. |
| *ZM 621 F C3* | 3.69 | 2.32 | 62.8 | Semi-flint | Interm. | Toler. | Susc | Moder. |

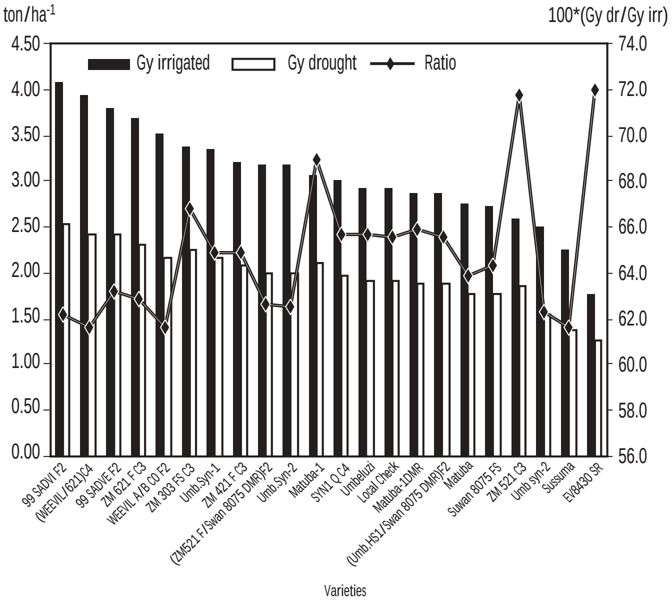

**Figure 1. Performance of selected Varieties under fully irrigated and under randomized drought stress conditions, across 3 locations x 2 years.**

# Screening for drought resistance in *Oryza glaberrima* varieties under floating and lowland conditions in Mali

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In Mali, rice is grown under four agroclimatic conditions (465,898 ha cultivated area): irrigated (14.34%), floating and deep water (25.97%) in the inland Niger River Delta, and rainfed lowland and upland (57.23%) in the southern part of the country. The production in the two latter ecosystems represents respectively 19.98 and 23.76% of the national rice production (717,116 t / yr). Apart from the irrigated ecosystem, rice production in the others ecosystems is affected by several biotic and abiotic stresses such as drought, weeds, diseases and insects, leading to low yields. Drought is the foremost constraint in these ecosystems. To overcome this constraint, it is necessary to use varieties that provide both high yield and drought tolerance. To reach this objective, *O. glaberrima*, known for its drought resistance can be used for *O. sativa* improvement. One-hundred and sixty-four (164) local varieties of African rice, *Oryza glaberrima,* an important crop in the inland Niger River Delta in Mali, were collected during the off-season 2002 with the financial support of The Rockefeller Foundation. These varieties were first characterized for some agronomic traits like plant height, days to 50% flowering, disease and insect incidence, and grain yield during the wet season 2002. During the off-season 2003, 121 varieties, of which 110 accessions were *Oryza glaberrima,* were screened for vegetative stage drought resistance to identify sources of resistance to drought under rainfed lowland condition in a testing collection design, with three replications. Each entry was represented by three rows, 5 m long. The varieties were grown under irrigation. Thirty days after emergence, irrigation was stopped for 30 days. Notable differences were observed among the varieties for leaf rolling, leaf tip drying, and regeneration capacity of tillers. Among tested varieties, 19 of *O. glaberrima* have shown good drought resistance. Interspecific hybridization between these varieties and elite *O. sativa* lines, which were used as male parents, were made during the wet season 2003 to develop populations segregating for drought resistance. F0 seeds of these crosses were collected from individual plants for their utilization in a backcrossing programme with recurrent parents, of the off-season 2004. Additionally, populations F3 and F5 selection was conducted to generate agronomically superior, drought resistant lines. The material comprised 35 interspecific segregated populations from multiple way crosses and backcrosses. One hundred to 500 plants by population were transplanted (1 plant / hill) in the field (rainfed lowland conditions), with a spacing of 20 cm x 20 cm. Two hundred $kg^{-1}$ NPK and 100 $kg^{-1}$ urea were applied. Based on the agronomic characteristics, 254 lines were selected for screening for drought resistance during the off-season 2004.

# The importance of experiment design and statistical analysis for genetic studies under water-limited conditions

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## Introduction

Variability is an omnipresent feature of field and laboratory experiments, consequently the experimenter needs to utilize procedures that control heterogeneity. Heterogeneity can arise from variation present in the experimental location and from effects occurring during the conduct of the experiment. Spatial variability in field, labs, and slides affects the detection of treatment differences by inflating the estimated experimental error variance. Researchers address this problem by using an appropriate design and layout for the experiment and by using suitable statistical methodology for statistical analyses. Starting with Sir Ronald A. Fisher's three design principles of blocking, randomization, and replication, many types of experimental designs (EDs) have evolved to meet the various situations encountered by researchers. Resolvable incomplete block designs such as alpha-lattice (Patterson and Williams, 1976) have become common in breeding trials. When the layout of the ED is in a row-column shape, EDs can be developed that control variability in two directions. When the entire experiment is laid out in a row-column arrangement, it may be desirable to assure that treatments do not occur more often than once in a row or a column of the experiment. The so-called "latinized designs" accomplish this. Also, it may be desirable to restrict randomization of treatments in such a way that certain groups of treatments do not occur together so that treatment interference can be avoided. Latinized alpha lattice and row-column designs. as well as neighbor-restricted designs, can be generated using different software packages. Optimal plot size and shape, border effects, competition between experimental units, and experimental techniques may be factors contributing to the variability in experimental results. The inclusion of points of reference, controls, or standards in an experiment is vital for the success of many experiments. Measuring related variates and using covariates technique helps to control variation.

## Variability in experiments

Typical variability existing in field experiments concerning soil micronutrients is shown figure 1, whereas a common spatial variability existing in a slide of a microarray experiment is depicted in figure 2. This variability due to experimental conditions can be controlled by using an appropriate experimental design and / or by performing a posteriori statistical analysis.

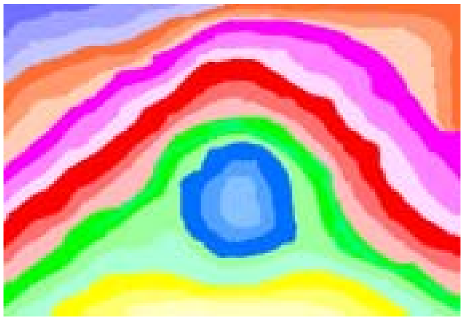

Figure 1. Variability in sodic soil.

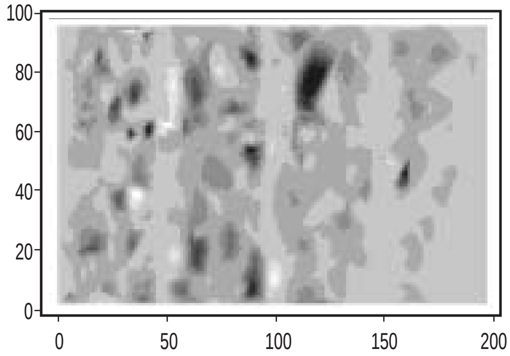

Figure 2. Variability in microarray.

## Statistical analysis

After the experiment is performed and data is collected, statistical analysis must consider the actual layout of the experiment (in the field, in the lab or in the slide) and account for the possible spatial variability. Variograms obtained after performing two different spatial analyses on grain yield of a field trial conducted under severe drought indicated that some control of soil heterogeneity has been achieved. However, variability in the direction of the rows and columns is still present (Figures 3 and 4).

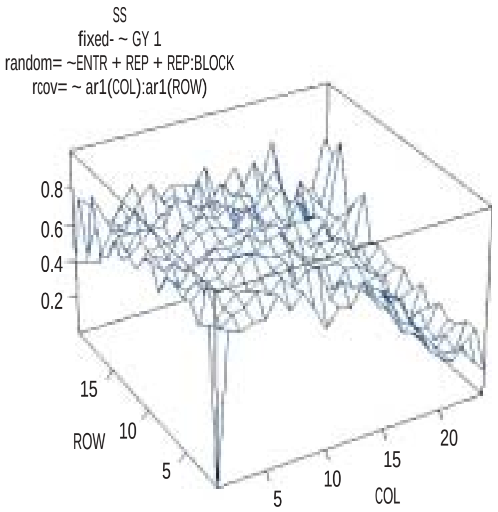

Figure 3. Variogram 1.

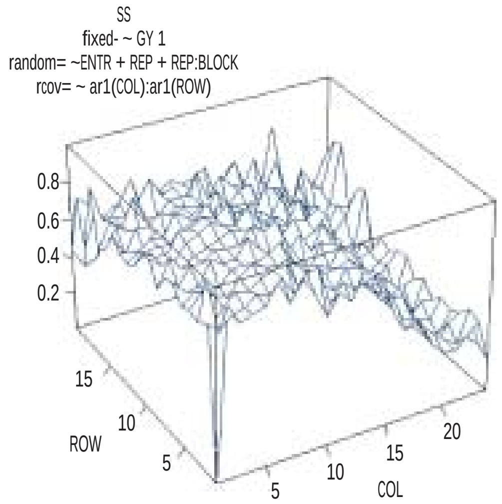

Figure 4. Variogram 2.

Once the experiment has been performed, the statistical analysis is an 'a posteriori' tool for controlling variability and adjusted means in the form of BLUE (Best Linear Unbiased Estimate) or BLUP (Best Linear Unbiased Predictor) are computed. These 'estimates' (BLUE) or 'predictor' (BLUP) are used to achieve further objectives such as QTL mapping, select candidate genes from microarray experiments, gene discovery from association mapping studies, and selection of the best genotypes (families) for breeding programs.

In this study we show the effect of using adjusted means based on incomplete block design and spatial analysis on an experiment conducted under severe drought stress with the objective of mapping QTL for yield and other traits in maize. Figure 5 shows that the QTL detected in chromosome 3 by using the unadjusted means (arithmetic mean) is not significant, whereas it becomes significant when using adjusted means based on the incomplete analysis and / or based on the spatial analysis.

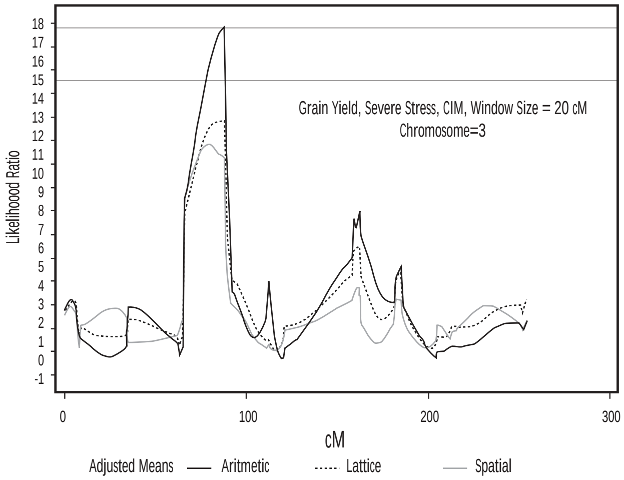

Figure 5. Likelihood ratio versus distance in chromosome. The levels of significances are marked in the two horizontal lines.

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# Development of drought tolerant lines for upland rice ecologies in the tropics of Africa: Preliminary results

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Rainfed upland is an important ecology for rice cultivation in West and Central Africa (WCA) accounting for 57% of the area sown and 40% of the regional production. Even though yield potential in the upland is estimated to be between 2.5 t / ha and 4.5 t / ha, farmers' yields often do not realize more than 1 t / ha, due to a range of production biotic and abiotic production constraints. Amongst these constraints, drought is one of the most important and can occur at any stage of crop development.

The African rice, *Oryza glaberrima* Steud. originated in West Africa around 3,500 years ago. Although it has been largely displaced by high yielding *O. sativa* lines from Asia, it is still cultivated in The Gambia, Senegal (Casamance), Guinea Bissau and Mali, because it is well adapted to local production systems and has resistance or tolerance to important biotic and abiotic production stresses. Funding from The Rockefeller Foundation to Institut d' Economie Rurale of Mali was used to collect a range of *O. glaberrima* accessions in 2002. Screening in the dry season of 2003 identified 21 accessions with good drought recovery ability.

The Rockefeller Foundation also provided research funds for a PhD study on the transfer of the drought tolerance of *O. glaberrima* into elite, high yielding, but drought sensitive material, and to elucidate the inheritance of drought tolerance and morph-physiological characters. Late 2003, four *O. glaberrima* accessions with the best drought recovery ability were used as donor parents in crosses with drought sensitive *O. sativa* and interspecific lines, the latter derived from earlier crosses between *O. glaberrima* and *O. sativa*. As sterility can be a problem in such crosses, and to introduce a wider range of genetic variation for drought tolerance, a further series of crosses were undertaken involving interspecific (drought tolerant) and *O. sativa* lines.

The $F_1$ plants were grown and 128 crosses with four tester lines were undertaken in 2004 in accordance to the North Carolina mating design II for genetic analyses of drought tolerance and morpho-physiological characters. Aside from the existence of genetic barriers between *O. y glaberrima* x *O. sativa* crosses, high day temperatures, ranging from $35^0$C to $43^0$C were observed to reduce seed set in $F_1$ crosses. Crossing success was low and seed set did not exceed 11% in any of the individual pollinations undertaken.

# Assessment of maize hybrids for drought tolerance in Mozambique

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## Introduction

Maize (*Zea mays* L.) is the most important food crop in Mozambique, occupying about 1.2 million hectares, which represent 40% of the total cultivated area. Maize is grown by commercial and small-scale farmers with average yields raging from 0.2 to 1.2 t / ha (DINA, 1995). Improved seed covers only about 9% of maize area, whereas hybrids represent 3% (Pingali, 2001). However, there is indication that in the future the demand for hybrid seed will increase. Drought is one of the major abiotic constraints affecting crop production in the country (Nunes et al., 1987). To minimize the impact of drought stress, the INIA maize program is collaborating with CIMMYT-Zimbabwe in the Southern Africa Drought Tolerance Project.

## Material and methods

During the 2002 / 03 hot season, three hybrid trials with drought tolerance background from CIMMIT-Zimbabwe were evaluated in two different environments: random drought stress (RS) in Sussundenga, and good rainfall (GR) in Nampula and Angonia. During the dry season, about 70 single cross hybrids from INIA's elite lines were tested under well-watered (WW) and severe water stressed (SS) conditions in Chokwe and Umbeluzi, respectively. Trial data on yield and other agronomic traits were analyzed using SAS software and the Alpha program, respectively.

## Results

Significant differences in grain yield between locations and within each trial were observed. Differences between locations were 30.1% for early to intermediate maturing hybrids (Table 1), 41.4% and 30.7% for intermediate to late maturing and QPM hybrids, respectively (data not shown). In the case of INIA single cross hybrids, the difference between locations was 74.6% (Table 2).

According to the relative yield for the three trials from CIMMYT, 11 entries performed above the trial mean for early to intermediate hybrids (Table 1), 16 for intermediate to late hybrids, and 12 for QPM hybrids (data not shown). Apart from grain yield, some secondary characteristics, such as anthesis-silking interval (ASI), ear per plant, relative stand, and shelling rate were considered. It seems that the differences between means of these characteristics are mainly influenced by plant stand. The same entries of INIA hybrids demonstrated big yield differences under WW and SS conditions (Table 2).

**Table 1.** **Early to intermediate maturing hybrids from CIMMYT-Zimbabwe, evaluated under random drought stress (RS) and good rainfall (GR), 2002 / 03 grown season**

| | | Relative grain yield | | ASI | | Ear per Plant | | Shelling Rate | |
|---|---|---|---|---|---|---|---|---|---|
| Entry | Pedigree | Sussu.(R S) | Nampula(GR) | Sussu.(R S) days | Nampula(GR) days | Sussu.(R S) | Nampula(GR) | Sussu.(R S) % | Nampula(GR) % |
| 14 | CZH01005 | 1.15 | 1.39 | 2.0 | 4.7 | 1.10 | 1.00 | 78.4 | 84.3 |
| 16 | CZH01008 | 1.15 | 1.28 | 1.7 | 4.7 | 1.14 | 1.00 | 84.1 | 83.5 |
| 15 | CZH01006 | 1.38 | 1.07 | 2.0 | 5.3 | 1.00 | 1.01 | 91.8 | 78.9 |
| 6 | PAN 31 | 1.30 | 1.11 | 2.0 | 4.3 | 1.15 | 1.03 | 86.1 | 75.7 |
| 11 | CZH00007 | 1.05 | 1.15 | 1.7 | 4.7 | 1.36 | 1.01 | 84.1 | 81.0 |
| 2 | 983WH78 | 1.18 | 1.06 | 2.0 | 5.0 | 1.12 | 1.01 | 86.1 | 84.0 |
| 9 | SC513 | 1.22 | 1.04 | 1.3 | 5.0 | 1.04 | 1.03 | 86.2 | 74.7 |
| 13 | CZH00013 | 1.11 | 1.09 | 1.7 | 4.7 | 1.04 | 1.03 | 85.2 | 77.9 |
| 28 | CZH02011 | 0.91 | 1.15 | 2.0 | 4.3 | 1.24 | 1.01 | 85.1 | 80.9 |
| 27 | CZH01002 | 1.09 | 1.06 | 1.7 | 5.0 | 1.15 | 1.00 | 83.2 | 79.4 |
| 12 | CZH00012 | 0.72 | 1.01 | 1.3 | | 1.05 | 1.02 | 87.8 | 87.6 |
| | n=36 | | | | | | | | |
| Mean * | | 1.00 (3.41) | 1.00 (4.89) | 1.63 | 4.62 | 1.12 | 1.02 | 84.1 | 78.8 |
| MIN | | 0.64 (2.17) | 0.76 (3.70) | 0.67 | 3.99 | 0.89 | 1.00 | 75.3 | 64.2 |
| MAX | | 1.38 (4.71) | 1.39 (6.78) | 2.33 | 5.33 | 1.46 | 1.06 | 91.8 | 87.8 |

* Data in brackets indicate grain yield in tones per hectare.

**Table 2.** **Single crosse hybrids evaluated under water stress (SS) and well watered (WW) conditions in Umbeluzi and Chokwe, respectively, during Mozambican 2003 dry season**

| | | Grain Yield | | Ear per plant | | Relative Stand | | Grain Type | |
|---|---|---|---|---|---|---|---|---|---|
| Entry | Pedigree | Chokwe(WW) t / ha | Umbeluzi(SS) t / ha | Chokwe(WW) | Umbeluzi(SS) | Chokwe(WW) % | Umbeluzi(SS) % | Chokwe(WW) (1-5) | Umbeluzi(SS) (1-5) |
| 5 | LP02 x LP52 | 6.57 | 0.84 | 0.95 | 0.6 | 93.04 | 69.18 | 1.0 | 2.2 |
| 23 | LP23 x LP22 | 10.51 | 1.13 | 1.13 | 0.5 | 89.62 | 77.53 | 3.0 | 3.2 |
| 50 | LP63 x LP31 | 6.60 | 1.76 | 1.02 | 0.7 | 87.63 | 78.38 | 4.5 | 3.5 |
| 70 | OPVs Check | 7.57 | 1.26 | 0.90 | 0.7 | 89.17 | 74.09 | 3.5 | 3.0 |
| | n=70 | | | | | | | | |
| Mean | | 7.81 | 1.25 | 1.00 | 0.64 | 89.87 | 74.80 | 3.0 | 3.0 |
| Min | | 6.57 | 0.84 | 0.9 | 0.53 | 87.63 | 69.18 | 1 | 2.2 |
| Max | | 10.51 | 1.76 | 1.13 | 0.72 | 93.04 | 78.38 | 4.5 | 3.5 |

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# A manual for breeding for drought tolerance in rice: Feedback and capture of experience by practitioners

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## Introduction.

At The Rockefeller Foundation conference held at IRRI Los Banos, 2002 we presented a working draft of a manual “Breeding for Drought Tolerance in Rice.” More than 30 practitioners attended the special workshop and provided useful feedback on the content and style leading to a published version ”Breeding Rice for Drought-Prone Environments” (Fischer et al., 2003), which has been distributed to many of you and is now available from IRRI. The manual has been adapted for use on the web or by CD. It is located on the IRRI Knowledge Bank web site (http://www.knowledgebank.irri.org/) under the Research Materials Methodology section. It can be downloaded as print or made into a CD (Mark Bell per.com.).

We now seek feedback on the interest of the user group for making the content of the manual available in an interactive CD and incorporating more experiences of practitioners.

## Materials and methods

There are two levels of information contained in the manual—practice and theory. The idea is that the information can be adapted to meet specific requirements for application and training. We are interested in meeting with user groups to learn how the manual has been used at the local level and what additional practices and illustrations are available that add clarity to the principles and practice outlined in the manual. Specifically, we wish to meet and explore the interest from the user group for capturing more experiences and using an interactive CD format to (i) keep the manual up to date and (ii) make it more appropriate for a learning and training environment.

## Conclusions

Based on the feedback from the users of the manual, we will explore the development of an interactive CD using the principles and practices outlined in the manual and incorporating additional illustrations and experiences.

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# Genetic improvement for drought tolerance in rice (*Oryza sativa* L.)

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Among production constraints, abiotic stresses like moisture stress in both upland and lowland rice ecosystems is very important. In India, about 58% of total rice area is under rainfed conditions (Siddiq, 1996). This situation is becoming precarious year after year due to inadequate precipitation, less water efficient irrigation methods in rice farms, lack of control on water deliveries and distribution, and poor establishment of water charge systems based on the actual amount of water used by the farmers.

With rice being a major consumer of water, shrinking water sources is bound to seriously affect its productivity. Global research priorities are pinned towards the genetic improvement of rice for drought tolerance combined with high yield (Widawsky and O' Toole, 1990).

Despite having proven sources of tolerance to moisture stress, no high yielding variety with the desired level of tolerance has yet evolved, owing to the complexity of drought tolerance. The major setback in drought tolerance breeding is the poor understanding of genetics and inheritance of drought tolerance traits and complete ignorance about the relationship between traits of physiological drought tolerance and plant productivity under stress (Blum, 1982).

Considering this aspect, line x tester analysis was carried out to study the combining ability, nature of gene action, heterosis, direction of association, and direct and indirect effects of yield and drought related traits in 28 hybrids and 11 parents under moisture stress conditions.

The study revealed that the importance of dominant gene action for most of yield, its components and drought tolerance traits viz., plant height, number of productive tillers per plant, number of grains per panicle, 100 grain weight, spikelet fertility, single plant yield, days to attain 70% RWC, leaf rolling, leaf drying, leaf area index, chlorophyll stability index, proline content, drought recovery rate, root dry weight, root shoot ratio, and harvest index.

Out of 11 parents evaluated, based on *per se* performance and *gca* effects, four genotypes viz., Norungan, Nootripathu, PM 9106 (PMK 3), and PMK 2 were judged as good combiners that could be utilized in a hybridization programme to infuse drought tolerance into elite lines.

Three hybrids viz., Nootripathu / PMK 2, Nootripathu / ASD 16.and Norungan / PMK 2 were identified as the best for recombination breeding to get desirable segregants for the improvement of yield and drought tolerance under stress. Two hybrids viz., Norungan / ASD 16 and PM 9106 (PMK 3) / PMK 2 were found to be amenable for heterosis breeding viz., *inter se* matings to break the undesirable linkages between yield and drought tolerant traits under stress and also exploit dominant gene action. Association analysis revealed that selection based on number of productive tillers per plant, number of grains per panicle, spikelet fertility, 100 grain weight, and proline content would be effective in improving the grain yield under moisture stress.

Success of any plant breeding programme depends on the extent of genetic variability present in a crop. Eighty-nine early duration recombinant inbred lines (RILs) of the cross IR 20 / Nootripathu in $F_7$ generation were evaluated for the extent of variability along with checks in an augmented design for 11 traits viz., canopy temperature, leaf rolling, leaf drying, days to attain 70% RWC, days to 50% flowering, chlorophyll stability index, drought recovery, plant height, number of productive tillers, panicle length, and grain yield under stress. The results revealed that most of the yield, its components, and drought tolerant traits were predominantly under the control of additive gene action since the traits showed high heritability with high genetic advance. An effective and early selection for the traits could be exercised due to fixable genetic effects except for two traits viz., leaf drying and number of productive tillers, wherein, the selection might be postponed to later generations. The association study also revealed that yield trait was significantly correlated with most of the traits under study. The intercorrelation analysis encouraged the breeders to exercise selection for drought tolerance, yield, and its components under stress.

We also reported the extent of variability and association analyses in three selected $F_2$ crosses involving drought tolerant parents under stress.

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# Genetic and physiological basis of breeding productive and drought tolerant genotypes in upland rice

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## Introduction

Local rice landraces are preferred in rainfed direct seeded situations because of their ability to withstand drought. These genotypes are low in productivity, but have good root systems and physiological parameters like RWC, epicuticular wax for drought tolerance, which are adaptive traits for drought prone environments (O' Toole, 1982). There is a need to use the sources of these attributes in breeding programmes to combine drought tolerance and productivity (Blum, 1979). Ultimately, the grain yield in rice under drought conditions is very important since survival alone is not sufficient based on economic reasons (Fukai and Cooper, 1995). Moisture stress during the reproductive stage leads to pronounced spikelet sterility due to impaired biochemical and physiological events (Cruz and O'Toole, 1984 and Ekanayake et al., 1989). Kobata et al. (1994) postulated that sterility in rice is because of dehydration of the root zone and hence suggested deep and thick roots and moderate tillering as varietal characteristics for drought tolerance. The results presented in this paper are based on an effort made to combine productivity and drought tolerance in upland rice.

## Methods

Breeding populations were generated using high productivity lines adapted to an irrigated ecology and landraces of upland rice with the objective of combining productivity traits and roots, as well as physiological traits conferring drought tolerance, to develop productive and drought tolerant rice genotypes for upland conditions of Karnataka, India. The breeding populations were handled by pedigree method in $F_2$ and $F_3$, focusing selections on productivity traits and drought tolerance behavior. The fairly stabilized 93 selections, representing 21 crosses from $F_4$ generation onwards, were evaluated in replicated trials during 2001–2003 in the target environment. The moisture stress was severe in vegetative (2001), reproductive (2002), and both stages (2003) in different growing seasons. This provided an opportunity to critically assess progress in combining productivity and drought tolerance.

## Results

The *per se* performance of top 10 productive lines during three years under drought conditions in the target environment indicated that they were more productive than high yielding variety (HYV) checks, MTU-1001 and Jaya,and were also drought tolerant (Table 1). These top yielding genotypes were superior for overall drought score and RWC at vegetative stress during 2001. Higher productivity in these genotypes during 2002 (drought at reproductive stage) was because of higher numbers of fertile grains and higher spikelet fertility, which could be due to deep and thick roots that avoid dehydration in the root zone (Kobata et al., 1994).

An examination of the genetic basis of these lines revealed that most of the top productive lines under drought were derivatives of crosses Rasi x Amruth or Dodiga x Sonamahsuri, which had high x high and high x low general combining ability (GCA) status, respectively, for important root and physiological traits related to drought tolerance as well as productivity traits (Figure 1). The least productive lines under stress during 2001 and 2002 belong to low / negative GCA status cross combinations, with respect to drought and productivity traits (Table 1 and Figure 1).

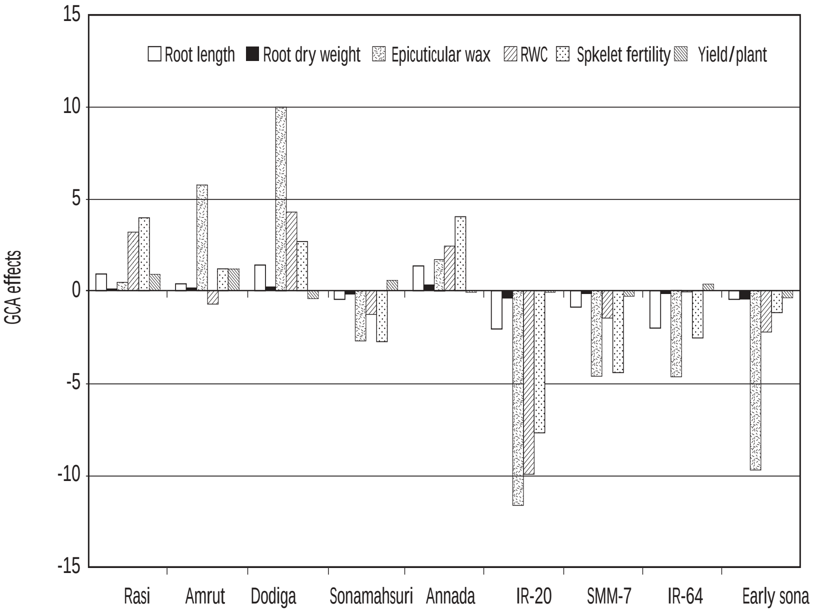

**Figure 1. GCA status of important root and physiological traits related to drought tolerance and yield in selected parental lines of rice.**

## Conclusion

The results indicated that the proper choice of parents, on the basis of their combining ability status for putative drought tolerant attributes, as well as productivity traits and selections in typical target environment, will help in combining complex traits such as productivity and drought tolerance.

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**Table 1. Performance of top high productive and low productive genotypes under moisture stress over years and their component traits for yield and drought tolerance**

| | | Grain yield (kg / ha) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Selection** | **Cross** | **2001#** | **2002** | **2003** | **Mean** | **Drought score (2001)** | **RWC (2001)** | **Fertile spike lets / panicle (2002)** | **% Spikelet fertility(2002)** |
| **High productive lines** | | | | | | | | | |
| MGD-33 | Rasi x Amrut | 6500 a | 3060 ab | 2950 a | 4170 a | 3 | 89.1 | 105 ab | 92.7 a |
| MGD-32 | Rasi x Amrut | 6000 ab | 3460 a | 2380 abc | 3940 a | 2 | 90.2 | 108 a | 93.1 a |
| MGD-13 | Dodiga x S. mahsuri | 5500 abc | 3600 a | 2740 ab | 3940 a | 2 | 90.5 | 106 ab | 95.6 a |
| MGD-31 | Rasi x Amrut | 5500 abc | 3430 a | 2260 abc | 3730 ab | 3 | 88.4 | 99 ab | 92.2 a |
| MGD-20 | Dodiga x S. mahsuri | 5500 abc | 2960 ab | 2260 abc | 3570 ab | 4 | 86.2 | 97 ab | 90.1 ab |
| MGD-29 | Rasi x Amrut | 5000 abc | 2900 ab | 2260 abc | 3380 abc | 2 | 89.8 | 94 ab | 90.7 ab |
| MGD-19 | Dodiga x S. mahsuri | 6000 ab | 1770 cde | 2330 abc | 3360 abc | 4 | 87.5 | 89 abc | 89.6 abc |
| MGD-30 | Rasi x Amrut | 5250 abc | 2900 ab | 1780 abc | 3310 abc | 4 | 86.1 | 90 abc | 88.6 abc |
| MGD-24 | Dodiga x S. mahsuri | 5000 abc | 2930 ab | 1900 abc | 3270 abc | 2 | 90.0 | 89 abc | 87.9 a-d |
| MGD-84 | Rasi x Annada | 6000 ab | 1870 cd | 1900 abc | 3260 abc | 5 | 85.3 | 85 bc | 87.1 a-e |
| **Low productive lines** | | | | | | | | | |
| MGD-9 | IR 20 x SMM-7 | 4500 bc | 900 ef | - | - | 6 | 71.2 | 65 d | 82.5 b-f |
| MGD-6 | IR 20 x SMM-7 | 4500 bc | 700 f | - | - | 6 | 70.5 | 70 cd | 81.2 c-f |
| MGD-3 | IR 20 x SMM-7 | 4500 bc | 625 f | - | - | 6 | 69.3 | 60 d | 79.8 def |
| MGD-93 | Amrut x Early sona | 4000 c | 1290 c-f | - | - | 6 | 70.1 | 72 cd | 74.9 f |
| MGD-88 | Amrut x IR 64 | 4000 c | 1140 def | - | - | 5 | 74.2 | 61 d | 78.6 ef |
| MGD-1 | IR 20 x SMM-7 | 3750 c | 1270 c-f | - | - | 5 | 80.2 | 64 d | 78.0 f |
| MGD-2 | IR 20 x SMM-7 | 4000 c | 1110 def | - | - | 5 | 71.0 | 59 d | 77.5 f |
| **Checks** | | | | | | | | | |
| MTU-1001 (HYV) | - | 4750 abc | 2160 bc | 1250 bcd | 2780 bc | 5 | 89.9 | 61 d | 82.2 b-f |
| Jaya (HYV) | - | 4000 c | 1690 cde | 1580 abc | 2420 cd | 5 | 85.6 | 60 d | 75.9 f |
| Dodiga (Land race) | - | 2180 d | - | 1040 cd | 1610 d | 3 | 88.4 | 56 d | 94.9 a |

# Observations pertaining to the years shown in each column
Means followed by same alphabets did not differ significantly
- data not developed

# Screening and selection of rice lines for drought tolerance in target production environment

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## Introduction

The districts of Ramanathapuram and Sivagangai, Tamil Nadu, India have a substantial area under rainfed rice, where drought is a serious yield-limiting factor. Drought stress can occur at any stage of crop growth, and is unpredictable. Drought stress is less damaging during the vegetative phase than during the reproductive phase because younger plants are better able to recover when the stress is relieved (O'Toole and Chang, 1979). Drought stress during the reproductive phase desiccates spikelets and anthers, reduces pollen shedding, inhibits panicle exsertion, and increases sterility rate (O'Toole and Namuco, 1983; Ekanayake et al., 1989). Sustained efforts are being made to develop drought tolerant rice cultivars by evaluating large numbers of germplasm under target production environment.

## Materials and methods

Doubled haploid (DH) lines of CT 9993–5-10–1–M x IR 62266–42–6–2, recombinant inbred (RI) lines of IR 58821–2–3–B–1–2–1 x IR 52561–UBN-1–2 (RILs 1) and IR 20 x Nootripathu (RILs II) and IR 64 x Azucena (NILs) and back cross progenies of Vandana x Moroberekan (BC lines) were evaluated in replicated trials for drought tolerance under rainfed conditions. The details of the experiments are furnished below:

| Population | No. of lines | Year of testing | Plot size |
|---|---|---|---|
| DHLs | 220 | 1999-2000 | 2 m x 0.4 m |
|  | 154 | 2000-2001 | 2 m x 0.4 m |
| RILs I | 155 | 2001-2002 | 2 m x 0.6 m |
|  | 155 | 2002-2003 | 2 m x 0.6 m |
| RILs II | 380 | 2003-2004 | 2 m x 0.4 m |
| NILs | 23 | 2003-2004 | 2 m x 0.4 m |
| BC lines | 30 | 2003-2004 | 2 m x 0.4 m |

Observations on days to heading, grain yield, biomass, and per cent spikelet sterility were recorded. In each population, out of the total lines evaluated, the lines that gave sustainable grain yield were considered for analysis.

## Results

The rainfall received during critical stage (panicle imitation to dough stage) during 1999–2000 was 128.7 mm and during 2000–2001, 294.4 mm; severe drought occurred during panicle initiation. The precipitation deviated very considerably from the ten year average. Among the lines, 45 lines exhibited sustainable yield during 1999-2000 and 38 lines during 2000-2001 in the DHLs. High harvest index, low spikelet sterility, and high biomass indicated that these lines are tolerant to drought (Table 1). The analysis of variance showed moderate genetic variance and high heritability for grain yield under drought (Table 2). In RILs I, with rainfall during the critical phase (2001–2002 and 2002–2003) the deviation from average is very low, which indicated that the drought was moderate. The genotypes' response to this moderate drought was moderate. During 2003–2004, the drought was severe. The deviation from mean rainfall was 54% (212 mm rainfall). All the genotypes of RILs II and NILs withered after panicle initiation, not withstanding the drought, but the response of genotypes in BC lines is very high due to their drought escape. The landraces, including Moroberekan, that are supposed to be adaptable and drought tolerant also withered, thus indicating that if there is insufficient rain at the panicle initiation, these races too could not withstand the drought. Appreciable yield level was obtained in the BC lines.

**Table 1. Range of different traits observed in the various rice populations**

| Population | Year | No. of lines with sustainable yield | Days to heading | Grain yield Grain yield(t/ha) | Biomass (t/ha) | Harvest index (%) | Spikelet sterility (%) |
|---|---|---|---|---|---|---|---|
| DHLs | 1999-2000 | 45 | 73-85 | 1.33-2.33 | 2.17-4.67 | 32-85 | 12.5-78 |
| DHLs | 2000-2001 | 38 | 84-94 | 0.3-1.0 | 1.5-5.9 | 10-48 | 12.5-78 |
| RILs I | 2001-2002 | 13 | 84-127 | 0.67-1.2 | 1.9-7.5 | 10-36 | 15-35 |
| RILs I | 2002-2003 | 8 | 84-79 | 1.07-1.48 | 6.4-10.8 | 12-43 | 18-44 |
| BC lines | 2003-2004 | 11 | 55-65 | 0.875-1.88 | 3.63-7.55 | 14-26 | 18-25 |

**Table 2. Variance and heritability in DHLs and BC lines for grain yield**

| Population | Year | Phenotypic variance | Genotypicvariance | Error variance | Heritability |
|---|---|---|---|---|---|
| **DHLs** | 1999-2000 | 0.80 | 0.70 | 0.1 | 0.90 |
| **DHLs** | 2000-2001 | 0.47 | 0.43 | 0.04 | 0.91 |
| **BC lines** | 2003-2004 | 0.75 | 0.55 | 0.2 | 0.73 |

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Ekanayake I.J., S.K. De Datta, P.L. Steponkus. 1989. *Ann. Bot.* 63: 257–64.

# Evaluation of early maturing maize hybrids for the low altitude areas of Malawi

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## Introduction

Maize (*Zea mays*) is a staple food in Malawi, where it is the most important food crop. It takes up 70% of the arable land with more than 1.2 million hectares planted to it (Zambezi, 1990). The major production constraints in Malawi are drought and floods, mostly in the lowland areas. With respect to drought, it is of erratic occurrence and favourable seasons are often interspersed with unfavorable seasons.

Two major categories of drought resistance can be distinguished: drought escape and drought tolerance (Blum, 1988). Under drought escape, the plant completes critical physiological processes before drought sets in, while drought tolerance implies the presence of physiological mechanisms that allow the plant a reasonable level of production despite the presence of drought (Blum, 1988). Short season early maturing cultivars frequently escape drought that occurs at either end of a rainy season, but normally have limited yield potential (Blum, 1988).

When drought stress occurs just before or during the flowering period in maize, a delay in silk emergence is observed, resulting in an increase in the length of anthesis-silking interval. Synchrony between male and female flowering dates is strongly associated with yield increase under drought (Bolanos and Edmeades, 1993).

## Methods

Inbred lines isolated from Matindiri OPV (an early maturing OPV) were crossed to ZEWA and ZEWB at Chitala Research site in in Salima District in 2002 winter. Thirty-one hybrids were evaluated in summer 2002/2003 along with three local checks in four locations characterized by a short season (early cessation of rains) and high temperatures etc.

**Table 1. Agronomic traits of the selected entries**

| Entry | DP | DS | PH<br>cm | EH<br>cm |
|---|---|---|---|---|
| MAT188-1XZEWA | 56.5 | 56.5 | 151.5 | 61.0 |
| MAT221-4XZEWA | 53.0 | 54.0 | 126.5 | 53.0 |
| MAT248-2XZEWA | 55.5 | 59.0 | 125.5 | 43.5 |
| MAT274-1-2XZEWA | 55.5 | 56.5 | 130.0 | 53.5 |
| MAT94-1-3XZEWA | 52.5 | 53.0 | 130.0 | 52.0 |
| MAT94-1-1XZEWA | 56.0 | 56.5 | 153.5 | 54.0 |
| MAT15-1-2XZEWB | 58.0 | 61.0 | 116.0 | 46.0 |
| SC403 (local check) | 60.0 | 62.0 | 138.0 | 54.0 |
| MH16 (local check) | 61.0 | 63.0 | 146.5 | 74.0 |
| MH18 (local check) | 59.5 | 60.0 | 150.0 | 64.5 |
| **Mean** | **57.0** | **57.6** | **140.0** | **58.78** |
| **CV%** | **3.75** | **5.3** | **7.6** | **12.8** |
| **LSD(0.05)** | **4.3** | **6.2** | **21.7** | **15.3** |
| **Significance** | ***** | ***** | ***** | ****** |

Key: DS=days to 50% silking; DP=days to 50% pollen shade; PH=plant ht. in cm; EH= ear ht. in cm

**Table 2: Grain yield of the selected entries**

| Entry | Chitala<br>t/ha | Bwanje<br>t/ha | Lupembe<br>t/ha | Ngabu<br>t/ha | Mean<br>t/ha |
|---|---|---|---|---|---|
| MAT188-1XZEWA | 5.0 | 3.0 | 2.5 | 6.3 | 4.2 |
| MAT221-4XZEWA | 5.0 | 3.3 | 1.8 | 4.8 | 4.2 |
| MAT248-2XZEWA | 5.0 | 3.9 | 2.2 | 5.2 | 4.1 |
| MAT274-1-2XZEWA | 5.3 | 3.6 | 2.2 | 4.7 | 3.9 |
| MAT94-1-3XZEWA | 4.8 | 2.8 | 2.1 | 3.0 | 3.2 |
| MAT94-1-1XZEWA | 4.5 | 3.7 | 2.6 | 3.9 | 3.6 |
| MAT15-1-2XZEWA | 3.3 | 3.8 | 1.9 | 4.8 | 3.5 |
| SC403 | 3.9 | 3.2 | 3.4 | 5.5 | 4.0 |
| MH16 | 4.5 | 4.3 | 3.1 | 4.1 | 4.0 |
| MH18 | 4.6 | 2.8 | 1.6 | 3.2 | 3.0 |
| Mean | 4.4 | 3.2 | 2.5 | 3.9 | 3.5 |
| CV% | 20.0 | 25.4 | 43.0 | 30.3 | |
| LSD(0.05) | 1.8 | 1.6 | 2.2 | 2.4 | |
| Significance | NS | NS | NS | NS | |

## Results

Significant differences ($p<0.05$) were observed in number of days to 50% silking and 50% pollen shed, plant height in cm, and ear height in cm. MAT 94-1–3 X ZEWA and MAT221-4 x ZEWA were the earliest in terms of maturity with a mean number of 53 and 54 days to 50 % silking, respectively. Apart from early maturing, MAT221-4 x ZEWA had a yield advantage over a local check MH18 with mean yields of 4.2t / ha and 3.03t / ha. MAT94-1-1 X ZEWA had the highest plant height and MAT15-1-2 X ZEWB had the shortest plant height of 153.5 cm and 116 cm, respectively. Their mean yields were 3.6t / ha and 3.5t / ha, respectively.

## Conclusions

MAT 94-1–3 X ZEWA and MAT221-4 x ZEWA have the potential to be introduced in short season agroecologies because they can escape moisture stress due to early cessation of rains.

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# Advanced evaluation yield trials of drought and low-N tolerant maize varieties for midaltitude areas of Tanzania

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## Introduction

Maize, *Zea mays* L., is one of the major cereal crops and thus plays a very important role in human and animal nutrition around the world. It is produced on about 100 million hectares in the developing world (Pandey, 2000). In Tanzania, over 80% of the population depends on it as food; average consumption is about 113 kg per year and it contributes about 60% of the total calories in the diet of Tanzanians. The crop is grown on an average area of 2.0 million hectares (Mduruma, 2002). The midaltitude zone of Tanzania has the largest area with high potential for maize production. The most important limiting factors to maize production are drought and low soil fertility. Average yield loss due to drought is estimated to be 17% but it may go up to 70% (Edmeades et al., 1994). Some parts of the country receive very little rain, even below the minimum requirement for maize plant growth. As such, losses caused by drought may reach 100%. Analysis carried out in the northern part of Tanzania on monthly rainfall totals from 1926 to 2003, revealed that all years ending with “3” have 75% probability of being dry (Lyamchai, 2003). Rain and other water resources for agriculture use has become more limiting; thus development of drought resistant and drought escape maize varieties has become more important. However, in developing countries, farmers in high yielding and high input conditions are a more attractive target for the private seed sector than resource-poor farmers. For this reason, commercial sector breeders ignore abiotic stress-tolerant maize development. Thus public breeders are also influenced, even if their responsibility and target environments usually include areas not served by private sectors (Bänzinger et al., 2000). The National Maize Research Program thus has the mandate to evaluate maize hybrids and open pollinated maize varieties (OPVs) from the private seed companies together with its own newly developed experimental varieties in comparison with existing commercial varieties to identify and recommend the best biotic and abiotic resistant varieties.

## Methodology

Newly developed maize lines, experimental OPVs and maize hybrids are initially screened under managed drought and low-N stress conditions. Based on results obtained from screening sites in Tanzania and across sites in the SADC and ECA regions, promising varieties are selected. These are finally evaluated in National Performance Trials (NTP) through the Tanzania Maize Variety Trials (TMVTs) and on farmers’ fields to assess farmer’s preference. The varieties that meet special criteria and farmers’ preferences are then submitted for release to the National Variety Release Committee of the Ministry of Agriculture ad Food Security. The NPTs in seasons 2001/02 and 2002/03, were tested in two separate experiments (TMVT1 and TMVT2). Twenty entries were evaluated in each of the two experiments (TMVT1 and TMVT2) in season 2001/02 while 12 and 15 were evaluated in the same way in season 2002/03. The design was Alpha lattice with two rows per plot, 5 m long. Plant spacing used was 0.75mX0.5m. The trials were managed under optimum conditions and without fertilizer. Recommended fertilizer used under optimum was 100 kg N/ha and 80 $P_2O_5$kg/ha.

## Results

Results obtained from TMVT1 season 2001/02, showed significant difference between yields across five sites under optimum conditions. Higher yielding entries were HMLO1 with 7.68t/ha, PAN 45 with 7.64t/ha, PAN 53 7.60t/ha, H21ML1 with 7.40t/ha PAN 57 with 7.33t/ha PAN 33 with 7.3t/ha H20M1 7.0 t/ha and X1379PW with 7.0t/ha (Figure 1). Yield from TMVT2 did not differ significantly. Results obtained from TMVT1 season 2002/03 were significantly different for yields across two sites. Higher yields were obtained from LONGE 2H with 5.5t/ha, X1391 LW with 5.1t/ha and ZA 7551 with 4.9t/ha (Figure 2). Yield from TMVT2 was not significantly different.

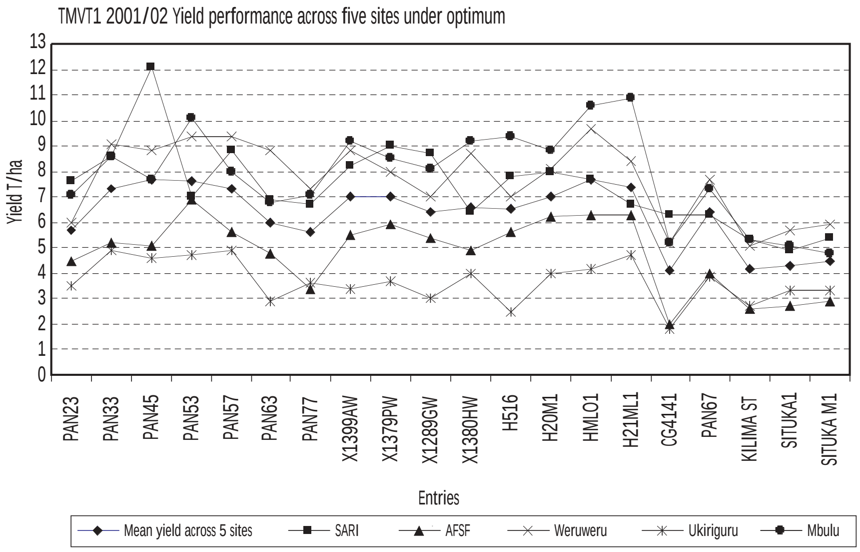

Figure 1. TMVT1 2001/02 Yield performance across five sites under optimum.

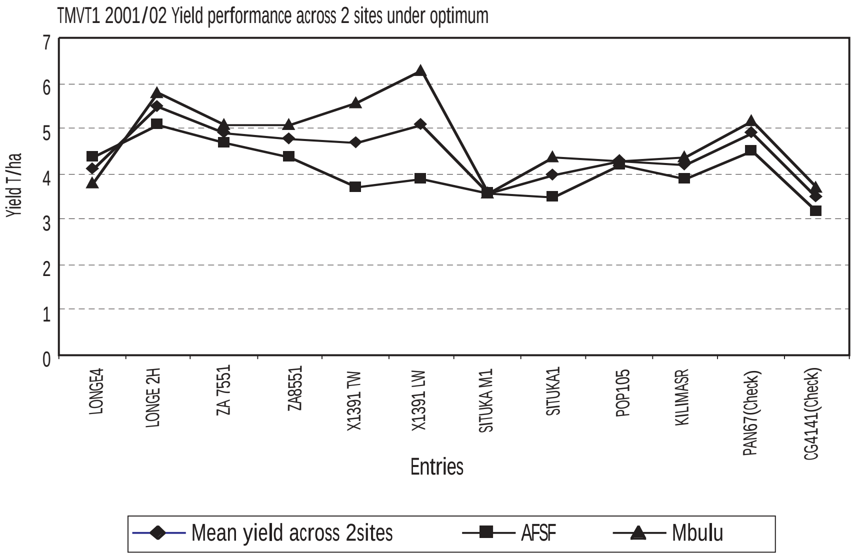

Figure 2. TMVT1 2002/03 Yield performance across 2 sites under optimum.

## Conclusion

Yield results from TMVTs2 were not significantly different; this could be due to late planting. However, based on combined analysis results across sites (green and yellow) and those from farmers' preferences, selected varieties are proposed for release to the National Variety Release Committee.

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# Evaluation of rice lines for drought tolerance in target production environment

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## Introduction

Cultivation of rainfed rice in the coastal district of Ramanathapuram, India is entirely dependent on the northeast monsoon (October-December). Successful crop yields have been obtained only in 21 years, despite 40 years of near normal seasonal rainfall, due to uneven distribution. The high yielding varieties suited to this region are of medium duration and have often suffered early drought and / or late drought at the critical stages of the crop growth. This resulted in poor yield and at times total crop failure. This has forced the farmers to cultivate drought and saline tolerant low yielding landraces. It is the need of the hour to develop short duration, drought and saline tolerant high yielding varieties. Thus local landraces, recombinant inbred lines (RILs), and doubled haploid (DH) and root QTL introgression lines were evaluated with the above perspective.

## Methods

During 2003-04, the drought tolerant but low yielding local landraces viz. Norungan, Nootripattu, Kallurundaikar, Chittraikar, Vellaichittraikar, and Poongar collected from different hamlets were evaluated for drought tolerance. RILs (156 lines) derived from the cross of IR 58821 x IR 52561 were evaluated under target environment. A subset of 23 IR64 / Azucena DH and introgression lines were evaluated for drought tolerance. A total of 20 saline and alkaline lines of Directorate of Rice Research (ICAR) were also evaluated. A plot size of three rows, each 2 m long was followed with three replications. Data for root length, root weight, days to 50% flowering, number of grains / panicle, grain yield, and panicle harvest index were recorded.

## Results

Among the landraces, Norungan excelled over others. Among the DH and root QTL introgression lines, five lines *viz.*, 17, 18, 15, 19, and 21 performed well under the target environment (Table 1). Of the total 156, 153 RI lines yielded only chaff as they were of medium duration group and were exposed to terminal stress. The grain set was recorded only in two RI lines. Three genotypes, SATVT-09, SATVT-03, and SATVT-19, recorded higher yield under drought environment.

## Conclusions

The exploitation of a narrow genetic base in the breeding programme has resulted in reduced gain in improvement. Plants have evolved several mechanisms to combat drought damage. Thus, it is important to explore the new donors exhibiting stable performance under water stress with good yield potentials. The six best DH and introgression lines identified will be included in the farmers' participatory plant breeding programme in the coming years. The best three lines identified in the saline and alkaline tolerant variety trial will be included as one of the parent for the development of rice varieties suitable for saline environments.

Table 1. Performance of IR64xAzucena DH and root introgression lines under rainfed conditions, Ramnad 2003-04

| Line | Root length (cm) / hill | Root weight (g) / hill | Days to 50% flowering | Grainspanicle$^{-1}$ | Panicle index | (%) | Grain yield (kg / ha) |
|---|---|---|---|---|---|---|---|
| 01 | 08.3 | 3.291 | 82 | 089 | 64.1 | | 3734 |
| 02 | 10.0 | 2.031 | 88 | 054 | 67.8 | | 1955 |
| 03 | 09.0 | 4.009 | 79 | 085 | 87.8 | | 3734 |
| 04 | 09.7 | 1.865 | 82 | 097 | 66.8 | | 3200 |
| 05 | 08.7 | 2.974 | 82 | 090 | 86.5 | | 2045 |
| 06 | 08.7 | 1.515 | 86 | 086 | 63.5 | | 3289 |
| 07 | 12.7 | 3.655 | 82 | 102 | 66.6 | | 3555 |
| 08 | 11.3 | 3.594 | 93 | 125 | 82.5 | | 1600 |
| 09 | 09.7 | 4.670 | 82 | 104 | 92.0 | | 1155 |
| 10 | 08.7 | 4.212 | 86 | 055 | 25.4 | | 3200 |
| 11 | 11.3 | 7.566 | 87 | 107 | 81.4 | | 2182 |
| 12 | 07.7 | 3.038 | 69 | 056 | 82.2 | | 2045 |
| 13 | 13.0 | 5.538 | 94 | 079 | 89.6 | | 1511 |
| 14 | 10.0 | 5.685 | 82 | 101 | 86.0 | | 3111 |
| 15 | 12.3 | 3.972 | 78 | 092 | 42.6 | | 4089 |
| 16 | 10.3 | 8.754 | 80 | 087 | 92.3 | | 3289 |
| 17 | 09.3 | 5.218 | 82 | 070 | 90.6 | | 4266 |
| 18 | 08.3 | 6.549 | 79 | 050 | 91.3 | | 4178 |
| 19 | 13.0 | 5.441 | 78 | 100 | 90.6 | | 4000 |
| 20 | 09.7 | 3.152 | 79 | 079 | 85.5 | | 3022 |
| 21 | 08.0 | 5.369 | 82 | 092 | 89.9 | | 4000 |
| 22 | 09.0 | 2.590 | 79 | 095 | 91.7 | | 3555 |
| 23 | 07.7 | 3.367 | 80 | 079 | 90.9 | | 4266 |

# Characterization of maize testing environments in the Southern Africa Development Community (SADC) region

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## Introduction

Maize is the most important food crop in the Southern Africa Development Community (SADC) region. Maize productivity increases by planting improved varieties developed by public and private breeding programs in the region. The goal of the maize breeding programs is the development and deployment of suitable varieties in the target environments characterized by specific climatic conditions, soil type, and crop management practices. Failure of maize genotypes to perform similarly across locations creates genotype-by-environment interactions and complicates the selection process especially in situations that result in re-ranking of genotypes across locations (Bradshaw, 1964). When allocating resources in SADC maize breeding programs, the selection and definition of target environments are important. Therefore, our goal is to use the historic database that is generated from years of multi-locational trials to conduct a retrospective analysis of the repeatability of certain types of genotype by environmental interaction and the selection of appropriate set of testing environments (Mirzawan et al., 1994; DeLacy et. al., 1990).

## Materials and methods

### *Material and environments*

This work is utilizes data from maize regional multi-locational trials from 1999 to 2003. The trials included 372 maize cultivars that were planted across 140 sites in the SADC region. The trials were classified into four categories: early to intermediate maturing open pollinated varieties (EPOP), intermediate to late maturing open pollinated varieties (ILPOP), early to intermediate maturing hybrids (EIHYB), and intermediate to late maturing hybrids (ILHYB). Five distinct biophysical environments were used, optimum (well fertilized rainfed), managed drought, low nitrogen, low pH and maize streak virus (MSV). Data collected included grain yield, anthesis date, plant height, lodging, grey leaf spot (GLS), MSV scores, number of plants, and grain moisture.

## Statistical analysis

Grain yield means per location adjusted for anthesis date through covariance analysis were used in Sequential Retrospective Pattern Analysis (Mirzawan, et al., 1994; DeLacy et al., 1996) to classify the test locations. The analysis was conducted with the SEQRET package Version 1.1 (DeLacy et al., 1998). The matrix used to stratify locations is using the incremental sum of squares (ISS) clustering algorithm (Ward, 1963). Geographical information systems (GIS) will be used to facilitate the classification of SADC testing locations based on long-term averages of temperature, precipitation, evapotranspiration and relative humidity, latitude, longitude, and soil characteristics. In addition, we will estimate which field management parameters for the different sites have a significant contribution to the differential response of maize genotypes.

## Preliminary results

Preliminary results for the classification of testing locations are shown in the dendogram in figure 1. The testing environments were grouped into six categories based on consistent GxE interactions across years and trials. Test locations within the same category were frequently from different countries. Drought and low nitrogen managed environments were each located in different categories. It seems that both long-term climatic conditions and crop management affected the categorization. Site similarity did not follow national boundaries, demonstrating a scientific rationale for regional development and deployment of maize varieties. Use of historic data that spans over four years ensured the reliability and durability of classification, making it a good basis for selecting maize testing locations in the region.

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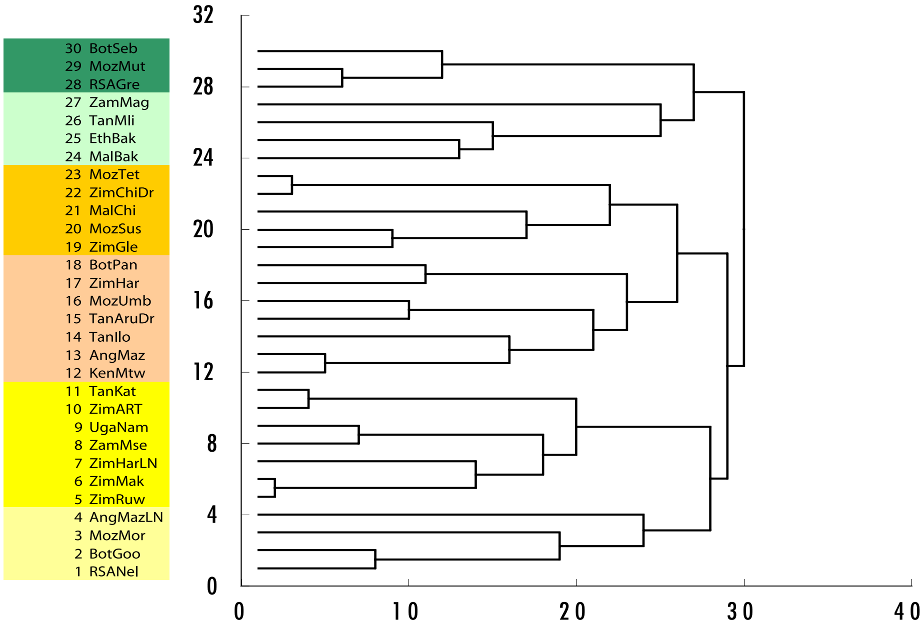

| **Leyend** | |
|---|---|
| BotSeb | Botswana Sebele |
| MozMut | Mozambique Mutarara |
| RSAGre | Republic of South Africa Greytown |
| ZamMag | Zambia Magoe |
| TanMli | Tanzania Mlingano |
| EthBak | Ethiopia Bake |
| MalBak | Malawi Baka |
| Moz Tet | Mozambique TeteAngonia |
| ZimChiDr | Zimbabwe Chiredzi (Drought) |
| MalChi | Malawi Chitedze |
| MozSus | Mozambique Sussundenga |
| ZimGle | Zimbabwe Glendale |
| BotPan | Botswana Pandamatenga |
| ZimHar | Zimbabwe Harare |
| MozUmb | Mozambique Umbeluzi |
| TanAruDr | Tanzania Aursha (Drought) |
| TanIlo | Tanzania Ilonga |
| AngMaz | Angola Mazozo |
| KenMtw | Kenya Mtwena |
| TanKat | Tanzania Katrin |
| ZimArt | Zimbabwe ART Farm |
| UgaNam | Uganda Namulonge |
| ZamMse | Zambia Msekera |
| ZimHarLN | Zimbabwe Harare (Low N) |
| ZimMak | Zimbabwe Makoholi |
| ZimRuw | Zimbabwe Ruwe |
| AngMazLN | Angola Mazozo (Low N) |
| MozMor | Mozambique Morrumbala |
| BotGoo | Botswana Goodhope |
| RSANel | Republic of South Africa Nelspruit |

**Figure 1. Dendogram for maize testing locations in SADC based on grain yield data from 1999 to 2002.**

# Diallel analysis of tropical maize inbreds under stress and optimal conditions

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Maize is an important staple crop in many tropical regions. Abiotic stress conditions, especially drought and low soil fertility (mainly low nitrogen stress), limit maize production in the tropics, where they frequently occur together. Maize inbred lines tolerant to drought and low nitrogen stresses have been developed by the International Maize and Wheat Improvement Center (CIMMYT). This study set out to investigate the genotype x environment interaction across stress conditions and testing locations, and combining ability of inbreds and hybrids.

Fifteen (15) inbred lines of tropical origins with a range of response to abiotic stresses were crossed in a diallel mating design. The resulting 105 hybrids were evaluated under the following environments: well-watered, water stressed, and low nitrogen conditions. There were four well-watered, three water-stressed, and two low nitrogen stress experiments. The experiments were planted at two locations in Mexico and Texas (USA), and two locations in Zimbabwe, in a replicated alpha-lattice design. Measurements for grain yield (GY), anthesis-silking interval (ASI), and ears per plant (EPP) were taken in each environment.

General and specific combining abilities effects were estimated following Griffing's (1956) diallel analysis. Both GCA and SCA effects across locations were significant for all the traits. GCA x environment and SCA x environment interaction effects were significant for GY and EPP. Inbred CML 343 had the highest GCA for grain yield across locations and

**Table 1. GCA effects across environments for male flowering (MF), female flowering (FF), plant height (PH), anthesis silking interval (ASI), ears per plant (EPP), and grain yield (GY) (t ha$^{-1}$)**

| | MF | FF | PH | ASI | EPP | GY |
|---|---|---|---|---|---|---|
| P502c1 | 0.254*** | -0.496** | -3.646*** | -0.637*** | 0.067*** | 0.059 |
| P501c1 | -0.836*** | -0.868*** | 3.061*** | -0.383* | -0.037*** | 0.112** |
| CML 78 | -2.995*** | -3.321*** | -1.280* | -0.717*** | -0.012 | 0.036 |
| CML 321 | -0.910*** | -1.421*** | 0.091 | -0.13 | -0.043*** | 0.059 |
| CML 311 | -2.172*** | -1.783*** | -2.918*** | 0.155 | -0.039*** | -0.032 |
| CML 202 | -0.639*** | -0.652*** | -1.519** | 0.460** | -0.005 | -0.116** |
| CML 206 | 1.613*** | 4.016*** | -6.559*** | 1.859*** | -0.081*** | -0.315*** |
| CML 216 | 0.008 | 0.515** | 7.974*** | 0.890*** | -0.079*** | 0.031 |
| CML 247 | 0.662*** | 2.411*** | -10.386*** | 1.312*** | -0.098*** | -0.446*** |
| CML 254 | 1.969*** | 1.915*** | 2.627*** | -0.034 | 0.071*** | 0.082* |
| CML 258 | 0.629*** | 0.831*** | 1.955** | 0.153 | -0.005 | 0.233*** |
| CML 339 | 1.032*** | 0.531** | 8.235*** | -0.717*** | 0.129*** | 0.182*** |
| CML 341 | 0.355*** | -0.796*** | 2.148*** | -0.974*** | 0.002 | 0.1657*** |
| SPLC7-F254 | -1.022*** | -2.528*** | -2.082*** | -1.066*** | 0.075*** | -0.287*** |
| CML 343 | 2.053*** | 1.646*** | 2.298*** | -0.169 | 0.054*** | 0.236*** |
| SE (gi) | 0.059 | 0.146 | 0.493 | 0.134 | 0.006 | 0.034 |

under low nitrogen conditions in Zimbabwe. Inbred CML 258 had the highest GCA for grain yield under water-stressed conditions, both in Mexico and Zimbabwe. CML 258 also had the second highest positive GCA for grain yield under well-watered conditions in Zimbabwe. Inbreds CML 206 and CML 339 had the highest GCA for ASI and EPP across locations, respectively. Inbred CML 206 had the highest GCA for ASI under water-stress conditions and well watered conditions in Zimbabwe. Inbred CML 339 had the highest GCA for EPP under well watered conditions. Hybrid CML 78 x CML 339 had the highest grain yield (3.9 t $ha^{-1}$) across locations. Of the ten best yielding hybrids, four involved inbred CML 343 and three involved inbred CML 339. These two lines, developed from a population improved for drought tolerance, La Posta Sequía, have a history of selection under managed drought environments.

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# Two years of selecting for drought and low-N tolerance in two populations of maize and two hybrid groups (early and intermediate in maturity)

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## Introduction

Maize is the most important food crop in Tanzania accounting for about 30% of the total food production during the last four years (Fews Net, 2003). The two priority stresses that impede maize production, food security, and economic growth in the southern Africa are drought and low soil fertility (Bänziger et al., 2003). Tanzanian maize is not an exception to the cited two stresses (Mduruma, 2003). Due to the importance of the two stresses, drought and low soil fertility breeding for maize tolerant to the stresses is inevitable. Selection for drought tolerance in tropical maize populations has shown progress (Edmeades et al., 1999; Chapman et al., 1999). Recently in Tanzania, Malawi, Zimbabwe, and South Africa two maize open pollinated varieties (OPVs) tolerant to low nitrogen (low-N) and drought have been released (Mduruma, 2003). In breeding for drought and low-N tolerant maize, the Tanzanian national research team is working in very close collaboration with the Southern African Drought and Low-N Soil Fertility project (SADLF) and with other NARS. This paper focuses mainly on two seasons of work done in Tanzania in selecting for drought and low-N tolerant maize from two populations and two hybrid groups of different maturity (early and intermediate).

## Methods

Four regional trials—namely early to intermediate maturing open pollinated varieties (EPOP), intermediate to late maturing open pollinated varieties (ILPOP), early to intermediate maturing hybrids (EIHYB) and intermediate to late maturing hybrids (ILHYB) —were planted under managed drought conditions and low-N conditions for two seasons (2001/02 and 2002/03). The materials tested were from CIMMYT-Zimbabwe and all testing was done at the Arusha Foundation Seed Farm in Tanzania, where the screening facilities are located. The drought materials were planted at the end of the rainy season (May/June), and stress was imposed to coincide with the flowering period. Planting for the low-N was done during normal season after soil N depletion using a maize crop and/or wheat crop. The number of varieties/hybrids tested were 40, 32, 20, and 16 for ILHYB, EIHYB, EPOP and ILPOP, respectively, in the 2001/02 season. In 2002/03, the number of varieties tested were 48, 36, 30, and 20 for ILHYB, EIHYB, EPOP, and ILPOP, respectively. In 2002/03, optimum yield trials were conducted on two to three locations. The yield data was included for selection. Selection was made for entries performing above mean for drought, low-N, and optimum conditions.

## Results

In the 2001/02 season, for EIHYB, the above average performing entries under drought and low-N were CZH00013, CZH99015, CZH01003, and the local check PAN 67 (Table 1). For ILHYB, the above average performing entries under drought and low-N were CZH99038, CZH99030, CZH01015, and CZH99044. For ILPOP, the above average performing entries were PAN 67, 99ADVLF2, and MASYNO1 MALAWI. For EPOP the above average entries for drought and low-N were S01SIWQF2 and 99ADVLF2 67 (Table 1).

Table 1. Best five performing entries for the drought trial (2001 / 02)

| **ILPOP02-D** | | | | **EPOP02A-D** | | | |
|---|---|---|---|---|---|---|---|
| | **Pedigree** | **t / ha** | **rank** | | **Pedigree** | **t / ha** | **rank** |
| | AC969A-SR(BestFS)F2 | 4 | 1 | | 99SADV1 F2 | 3.69 | 1 |
| | S01SIWQF2 | 3.96 | 2 | | 00SADV1 F2 | 3.39 | 2 |
| | ECAVL2-DLN | 3.79 | 3 | | LOCAL CHECK (TMV-1) | 3.2 | 3 |
| | ZM621 F2 | 3.65 | 4 | | 00SAVE F2 | 3.07 | 4 |
| | MASYN01 MALAWI# | 3.62 | 5 | | 99SAVE F2 | 3.07 | 4 |
| | LOCA CHECK (PAN 67) | 3.3 | 9 | | ZM521F2 | 2.9 | 6 |
| MEAN | | 3.27 | | MEAN | | 2.35 | |
| LSD | | ns | | LSD | | ns | |
| CV | | 23.6 | | CV | | 29.49 | |
| Min | | 3.3 | | Min | | 1.43 | |
| Max | | 3.27 | | Max | | 3.69 | |
| ILHY02A-D | | | | EIHYB 2A-d | | | |
| | Pedigree | t/ha | rank | | Pedigree | t/ha | rank |
| | CZH00026 | 5.17 | 1 | | CZH00007 | 4.39 | 1 |
| | CZH01014 | 4.85 | 2 | | CZH01003 | 4.06 | 2 |
| | CZH99021 | 4.54 | 3 | | CZH00014 | 4.04 | 3 |
| | CZH01020 | 4.47 | 4 | | GV470 IMPROVED | 4.01 | 4 |
| | CZH01012 | 4.26 | 5 | | LOCAL CHECK2 (PAN 67) | 3.86 | 5 |
| | LKL CHECK1(CG 4141) | 1.99 | 33 | Mean | | 3.14 | |
| | LKL CHEK2 (PAN 67) | 1.71 | 39 | Lsd | | ns | |
| | MEAN | 2.97 | | CV | | 32.17 | |
| | LSD | 1.85 | | F | | ns | |
| | CV | 34.8 | | min | | 2.26 | |
| | Min | 1.45 | | max | | 4.39 | |
| | Max | 5.17 | | | | | |

In the 2002 / 03 season for EIHYB, the above average performing entries under low-N were DK 8031, CG 4141 (local check), PAN 31, CZH01008, and SC513. Different entries performed better under drought conditions as indicated in the table. For ILHYB, the above average performing entries under drought and low-N were CZH02020 and CZH99021. For ILPOP, the above average performing entry was 99ADVLF2, which also performed above average for low-N and drought last year. For EPOP, the above average entries for drought and low-N were 01SADVLF2 and 99ADVLF2. 99ADVLF2 was one of the five top-ranking in the EPOP low-N and drought groups.

## Conclusions

The results of the experiment suggest that potential improvement for drought and low-N exists for the populations and hybrids. Inclusion of the promising materials to the advanced national variety trials and in the mother / baby trials should be considered. The trend was for the hybrids to perform better than the populations under both drought and low-N and optimum conditions, which suggests that in the future, use of hybrids should be promoted.

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# Early drought and low nitrogen tolerant double top cross maize hybrids for the dry midaltitude ecology of Kenya

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## Introduction

Low soil fertility and frequent drought are the most important constraints to maize (*Zea mays* L.) production in the dry midaltitude ecologies of Eastern Africa. In Kenya's dry midaltitude zone, the existing maize varieties (Katumani composite B and dryland composite) are not very productive under conditions of moisture stress and low soil fertility (KARI, 1992). With increasing settlement in the marginal areas, increased maize production will require better varieties—probably non-conventional hybrids, like double top cross hybrids—to meet the food demand of a growing population in an environment characterized by declining soil fertility and land holding size. Heterosis, as expressed in a double top cross hybrid, has been responsible for much of annual yield gains in maize (Duvick, 1997). Double top cross hybrid seed will be less expensive to produce. Furthermore, in instances when farmers recycle seed from the previous season, the yield loss will not be very drastic, as this will be, in effect, an open pollinated variety. Therefore, availability of early stress tolerant double top cross hybrids will enhance the ability and the scope of farmers to increase maize production in the dry midaltitude zone of Kenya .

## Methods

Seventy-two (72) early streak tolerant elite populations were crossed to a CIMMYT line (CML 78). The progenies were crossed to an adapted early open pollinated variety to form 72 non-conventional double top cross hybrids. In 2002, the 72 double top crosses were evaluated across ten sites in the dry midaltitude ecology of East Africa (Kenya, Tanzania, Uganda, and Ethiopia). The ten sites included optimum, controlled drought and low nitrogen, random drought and random low-N conditions. The trial was composed of two row plots with two replications and a density of 53,000 plants per hectare. The recommended agronomic practices were applied at the optimum sites to allow the genotypes to express their yield potential. For the low-N sites; established low-N blocks were used to screen the hybrids for their tolerance to low soil fertility. To screen for drought tolerance, the last irrigation was applied 40 days after planting. Grain yield data and important agronomic characteristics were recorded. Analysis of variance was carried out.

## Results

Highly significant ($p < 0.05$) differences were observed in three locations under optimal conditions. The best double top cross hybrid across ten sites yielded 5.7 t/ha while the best commercial check recorded 6.3 t/ha. The double top cross hybrids were earlier than the checks and also resistant to maize streak virus, a major disease in the region. A selection index was used to select four streak resistant, drought and low-N tolerant double top cross hybrids; they were earlier than the checks but had equal or better performance in yield and disease tolerance than the best commercial check hybrid (Table 1 and Figure 1). Two of these hybrids were nominated for Kenya's National Performance Trials in 2003 (28,62).

**Table 1. Yield(t/ha) of early streak and abiotic stress resistant double topcross hybrids tested across 10 sites including optimum, low n and drought in E. Africa, 2002a**

| Entry | Pedigree | Across Rel GY | Across Rank | | | OPT Across | LN | DR | RDR | Anth Date | ASI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % | Avg | Stdev | | t/ha | t/ha | t/ha | t/ha | d | d |
| 28 | Phil DMR61-2-#-#1-8-B-B/CML78/ZEWAC1F2 | 110 | 18 | 12 | | 5.6 | 4.4 | 1.1 | 2.7 | 73 | 3 |
| 62 | P300C5S1B-2-3-2#-#1-2-B-B/CML78/ZEWAC1F2 | 110 | 19 | 7 | | 5.6 | 4.8 | 1.0 | 2.8 | 72 | 3 |
| 37 | P100C6-26-1-2-#-#1-6-B-B/CML78/ZEWAC1F2 | 106 | 25 | 22 | | 5.5 | 4.5 | 0.7 | 2.7 | 72 | 4 |
| 29 | P300C5S1B-2-2-3-#-#1-1-B-B/CML78/ZEWAC1F2 | 109 | 21 | 19 | | 5.7 | 4.4 | 0.9 | 2.4 | 74 | 4 |
| 76 | PIONEER3253 | 116 | 24 | 40 | | 6.3 | 3.7 | 0.4 | 2.3 | 78 | 8 |
| 74 | Check2 | 103 | 33 | 10 | | 5.3 | 4.9 | 0.4 | 3.1 | 79 | 6 |
| **Mean** | | | **100** | **39** | **18** | **5.1** | | **0.8º** | **2.4** | **72.9** | **4.9** |
| **LSD (0.05)** | | | | | | | **0.7** | | | | **1.1** |
| **2.5** | | | | | | | | | | | |
| **MSe** | | | | | | **0.4** | **ns** | **ns** | **ns** | **2.9** | **4.5** |
| **CV** | | | | | | **12.0** | | | | **2.3** | **43.3** |

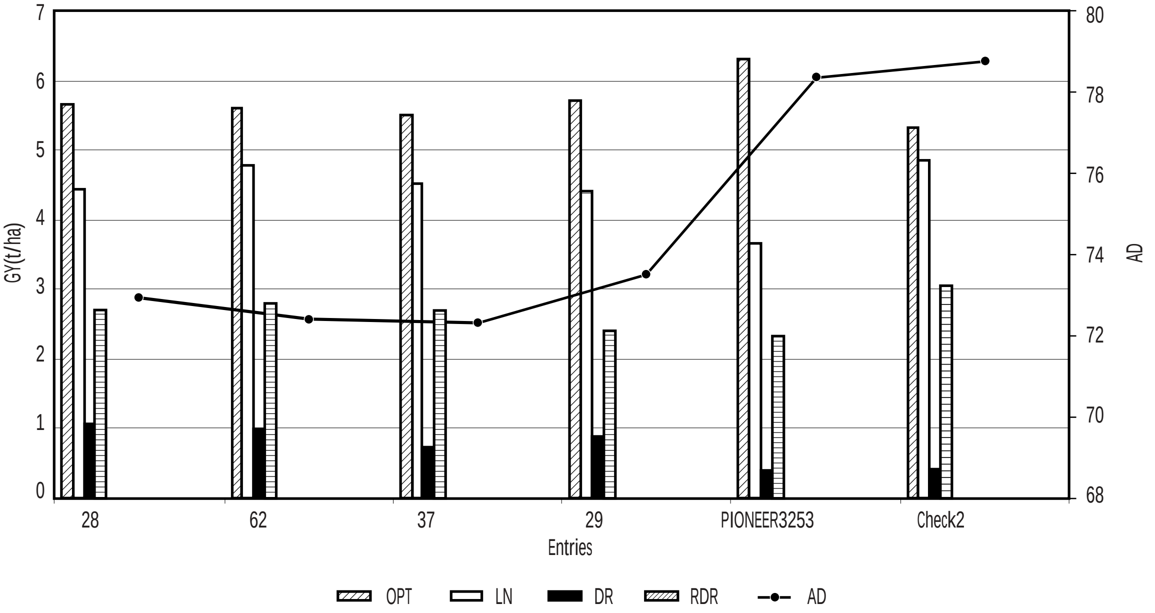

**Figure 1: Yield(t/ha) and days to anthesis of the best 4 early streak and abiotic stress resistant double topcross hybrids tested across 10 sites including optimum, low N and drought in E. Africa, 2002A.**

## Conclusion

Double top cross hybrids are more productive in dry midaltitude ecologies of Kenya than the currently grown open pollinated varieties. Double top cross hybrid seed will be less expensive to produce than the conventional hybrids. In instances when the farmers plant seed from the previous season's crop, their yields will not be reduced very dramatically, as the hybrid will function like an open pollinated variety. In conclusion, it is possible to increase maize yields by developing early adapted, drought and low-N tolerant double top cross hybrids for the dry midaltitude ecologies of Kenya.

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# Development of maize varieties under drought stress and non-stress conditions

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## Introduction

Zambia has continued to experience drought conditions since the worst drought in recent times, 1991 / 92 season. The National Maize Research Programme, in collaboration with the CIMMYT Regional Project, “Increasing the Tolerance of Maize in Southern Africa to Drought and Low Soil Fertility,” based in Zimbabwe, has been involved in the development of drought tolerant varieties since 1996. Methods developed at CIMMYT-Mexico of developing stress tolerant maize have been employed and are being replicated in southern Africa and other regions of Africa (Bänziger et al., 2002; Bolaños and Edmeades, 1996).

## Methods

One hundred (100) experimental maize varieties and checks were evaluated in four sets of 25 entry trials. Each set was evaluated in one drought stress and two non-stress environments during the 2002 / 03 season. The drought stress site was at Nanga (987 masl, 15°46”S latitude, 28°55”E longitude), while the two non-stress sites were at Golden Valley (1170 masl, 14°17”S latitude, 28°37”E longitude) and Msekera (1100 masl, 13°38”S latitude, 32°39”E longitude). The trials at Golden Valley and Msekera were planted during the rainy season of November 2002 to April 2003. The trials at Nanga were planted during the off-season (winter) of May to October, 2003. This was under controlled moisture stress under irrigation to mimic drought conditions. Water was withdrawn at flowering,

Entries were planted in two row plots of 5 metres under non-stress conditions and in one row plots of 5 metres under stress conditions. Trials were planted in three replications. A total of 155 kg N, 80 kg P, and 40 kg K was applied. Weed control was by chemical and mechanical control at Golden Valley and Nanga and by mechanical control at Msekera.

## Results

The analysis of non-stress sites was combined and compared to that of the stress site. On average, the drought treatment reduced yield by 33%. Four entries were found in the top 20% performance among the four sets under both stress and non-stress conditions (Government of the Republic of Zambia 2003). These varieties all had an ASI of 2 or less. Tables 1 and 2 show data from one set of 25 entries in both environments. Only the top five yielding entries are shown in each case. Three of the four entries (9, 17, and 24) can be seen in the set.

**Table 1. Combined results of T1 at Golden Valley and Msekera under non-stress conditions**

| | | ASREML | | | Standard analysis (e.g. ALPHA, MSTAT) | | | | | | LOCATIONS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Grain yield | | | Grain yield | | | | | | GOLDEN VALLEY | | MSEKERA | |
| Entry | Pedigree | t / ha | Avg Rank | Stdev | t / ha | Rank | Shelling % | Moisture % | Ears / plant | Plant no. | t / ha | rank | t / ha | rank |
| 2 | La Car x L12-280-3-3-2 X L913 | 7.67 | 4.50 | 4.95 | 7.59 | 3.50 | 0.84 | 26.10 | 0.99 | 39.33 | 9.58 | 1 | 5.75 | 8 |
| 9 | L2-8-3-1-1-6xL334-1-1 X L710 | 7.01 | 7.00 | 0.00 | 6.84 | 8.00 | 0.86 | 27.98 | 1.01 | 38.83 | 8.23 | 7 | 5.78 | 7 |
| 17 | L12'-2xL2-1-5-3 X L917 | 7.62 | 2.00 | 1.41 | 7.64 | 2.00 | 0.84 | 30.27 | 1.10 | 40.00 | 8.84 | 3 | 6.40 | 1 |
| 21 | (L914xL3233) X L917 | 7.35 | 6.00 | 5.66 | 7.19 | 6.00 | 0.85 | 28.65 | 1.03 | 39.17 | 8.98 | 2 | 5.73 | 10 |
| 24 | MRI 455 | 6.76 | 7.00 | 5.66 | 6.76 | 7.50 | 0.83 | 26.00 | 0.99 | 36.33 | 7.54 | 11 | 5.98 | 3 |
| Mean | | 6.29 | 13.00 | 5.15 | 6.32 | 13.00 | 0.84 | 27.50 | 1.00 | 36.98 | 7.40 | 13 | 5.19 | 13 |
| LSD | | | | | | | | | | | 1.31 | | 1.17 | |
| CV | | | | | | | | | | | 10.52 | | 13.37 | |

**Table 2. Results of T1 at Nanga under drought stress conditions**

| | | ASREML | | Standard analysis (e.g., ALPHA, MSTAT) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Grain yield | | Grain yield | | | | | | | | | |
| Entry | Pedigree | t / ha | rank | t / ha | rank | Shelling % | Moisture % | Ears / plant | Plant no. | Athesis days | ASI days | Plant ht CM | Ear ht CM |
| 9 | L2-8-3-1-1-6xL334-1-1 X L710 | 2.8 | 5 | 2.8 | 3 | 0.66 | 23 | 1.17 | 13.0 | 97.0 | 2.0 | 176.0 | 93.3 |
| 15 | L917 X L710 | 2.8 | 3 | 2.7 | 4 | 0.63 | 23 | 1.04 | 15.3 | 96.0 | 1.7 | 162.0 | 89.7 |
| 17 | L12'-2xL2-1-5-3 X L917 | 3.9 | 1 | 3.8 | 2 | 0.69 | 24 | 1.12 | 17.7 | 99.3 | 1.3 | 178.3 | 91.7 |
| 20 | L12 X L917 | 2.8 | 4 | 2.7 | 6 | 0.67 | 25 | 0.99 | 15.7 | 99.3 | 1.7 | 176.3 | 85.7 |
| 24 | MRI 455 | 3.8 | 2 | 4.0 | 1 | 0.67 | 24 | 1.22 | 16.3 | 98.0 | 1.7 | 162.0 | 92.3 |
| Mean | | 2.2 | 13.0 | 2.2 | 13.0 | 0.6 | 23.1 | 0.9 | 14.1 | 98.4 | 1.8 | 168.6 | 87.6 |
| LSD | | 0.9 | | | | | | | | | | | |
| **CV** | | **24.2** | | | | | | | | | | | |

## Conclusions

The results indicate that there is yield correlation between non-stress and stress environments for some entries. Entries that perform well under both stress and non-stress conditions can, therefore, be identified.

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# Meeting challenges of breeding for improved drought tolerance and other traits in maize in Kenya

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## Introduction

Maize (*Zea mays* L.) is the most important staple food crop in Kenya. It is grown over a wide range of agroecological zones ranging from sea level to over 2,100 masl. The average rainfall varies from 250 mm to more than 2,000 mm per year. The total land area under maize production is about 1.6 million hectares, of which 70–90% is from small-scale farms. Annual production is estimated at 3.3 million tons. According to the United Nations Food and Agriculture Organization (FAO), the area under maize (1972-1992) remained stagnant while production increased by 1.6% due to improved yield. In contrast, consumption increased at a rate of 2.1% per year.

## Germplasm development

Kenya's maize improvement programme has identified six maize agro-climatic zones; environmental determinants of these agro-climatic zones are elevation, rainfall, and temperature. The six zones are low tropics, dry midaltitude, moist midaltitude, dry transitional, moist transitional, and high tropics. Across these ecologies, farmers actual yield realization is about 2 t/ha, and in a bimodal rainfall situation, farmers may not harvest at all during the short rains season (September-December rainy season).

The Kenyan maize programme is principally concerned with the improvement of grain yield, biotic factors such as diseases, insect pests, weeds (*Striga*

**Table 1. Grain yield and other important agronomic characters of varietal hybrids, 2 OPVS and 2 commercial checks. Results of ECA-DTLN-VT03-12; Location: Embu, Kenya**

OPT

| Entry | Pedigree | FW | Rank | Anth Date | ASI | Lodging | | Ears / Plant | Husk Cover | Ear Rot | GLS | E.turc | Ear Aspect | Plant Aspect |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Root | Stem | | | | | | | |
| | | t/ha | Rank | d | d | % | % | # | % | % | 1-5 | 1-5 | 1-5 | 1-5 |
| | | GYF | Rk | AD | ASI | RL | SL | EPP | HC | ER | GLS | ET | EA | PA |
| 1 | ECAVL20/ECAVL1 | 7.2 | 29 | 75 | 0 | 2 | 0 | 0.9 | 16 | 18 | 1.6 | 1.4 | 2.6 | 2.4 |
| 2 | ECAVL20/ECAVL2 | 9.4 | 2 | 75 | 1 | 3 | 0 | 1.1 | 15 | 8 | 1.4 | 1.5 | 1.5 | 2.3 |
| 27 | ECAVL16/ECAVL18 | 8.2 | 16 | 73 | 1 | 2 | 0 | 1.0 | 14 | 8 | 2.0 | 1.8 | 2.1 | 2.2 |
| 28 | ECAVL17/ECAVL18 | 8.7 | 5 | 76 | 1 | 0 | 0 | 1.0 | 17 | 8 | 1.7 | 1.5 | 2.5 | 2.2 |
| 34 | NIP25/POP49SR-# | 7.2 | 30 | 73 | 0 | 2 | 0 | 1.0 | 9 | 14 | 1.7 | 1.3 | 2.7 | 1.5 |
| 35 | ECUPB(BC1)/SYNTHSR-# | 8.4 | 10 | 73 | 1 | 7 | 0 | 0.9 | 9 | 17 | 1.8 | 1.9 | 1.9 | 2.1 |
| 36 | KIPB/POP49SR-# | 7.6 | 25 | 76 | 0 | 4 | 0 | 1.0 | 14 | 11 | 1.6 | 1.5 | 2.8 | 2.2 |
| 37 | PIONEER3253 | 8.4 | 8 | 74 | 0 | 1 | 1 | 0.9 | 3 | 21 | 2.4 | 1.3 | 2.5 | 2.0 |
| 38 | H513 | 8.3 | 14 | 75 | 0 | 8 | 2 | 1.1 | 18 | 16 | 2.2 | 1.5 | 2.8 | 2.7 |
| 39 | LOCAL CHECK: EMCO | 6.8 | 37 | 73 | 1 | 5 | 1 | 0.9 | 15 | 10 | 1.7 | 2.0 | 2.6 | 2.5 |
| **Mean** | | **7.85** | | **75.1** | **0.6** | **3.1** | **0.3** | **1.02** | **14.3** | **13.4** | **1.7** | **1.5** | **2.1** | **2.1** |
| **LSD (0.05)** | | **1.33** | | **1.7** | **1.4** | **8.9** | **2.3** | **0.14** | **12.5** | **10.9** | **0.4** | **0.4** | **0.6** | **0.4** |
| **CV** | | **10.26** | | **1.3** | **134.4** | **173.7** | **415.5** | **8.20** | **52.8** | **49.6** | **15.4** | **16.7** | **15.8** | **10.7** |

spp), and abiotic stresses, which include drought and low nitrogen soils. Currently, a programme on Quality Protein Maize (QPM) has been initiated and directed toward some of the malnutrition problems in the country. Insect pests pose the greatest challenge amongst the biotic factors whilst drought is the foremost constraint in the drier parts of the country (KARI / CIMMYT database, 1994)

Two narrow base populations, a Tuxpeno'-derived Kitale station maize collected from farmers' fields (Kitale synthetic 11 ) and a high altitude flint collection, EC 573, form the basis of the maize breeding programme for Kenya, especially in the moist transitional, moist midaltitude and the high tropics zones. There is, therefore, a need for drought tolerant germplasm infusion in the maize programme to address drought issues.

## Status of breeding for drought

In the last seven years or so, development of germplasm for drought has been going on in the dry midaltitude ecological zone (800-1400 masl, 250-500 mm rainfall) in collaboration with CIMMYT. The varieties developed have been evaluated extensively in East and Central Africa. Currently, early / extra early open pollinated varieties (OPVs), varietal hybrids, and early three-way hybrids are available. What is needed is to get these materials through the National Variety Testing System in order to avail them to farmers in the midaltitude agro-ecozone.

## Way forward

Further testing of available varieties is needed in more areas of Kenya, especially during the short rains. Breeding for drought tolerance while incorporating streak resistance and nitrogen use efficiency in the early / extra early materials is paramount in order to reduce the yield gaps on-farm.

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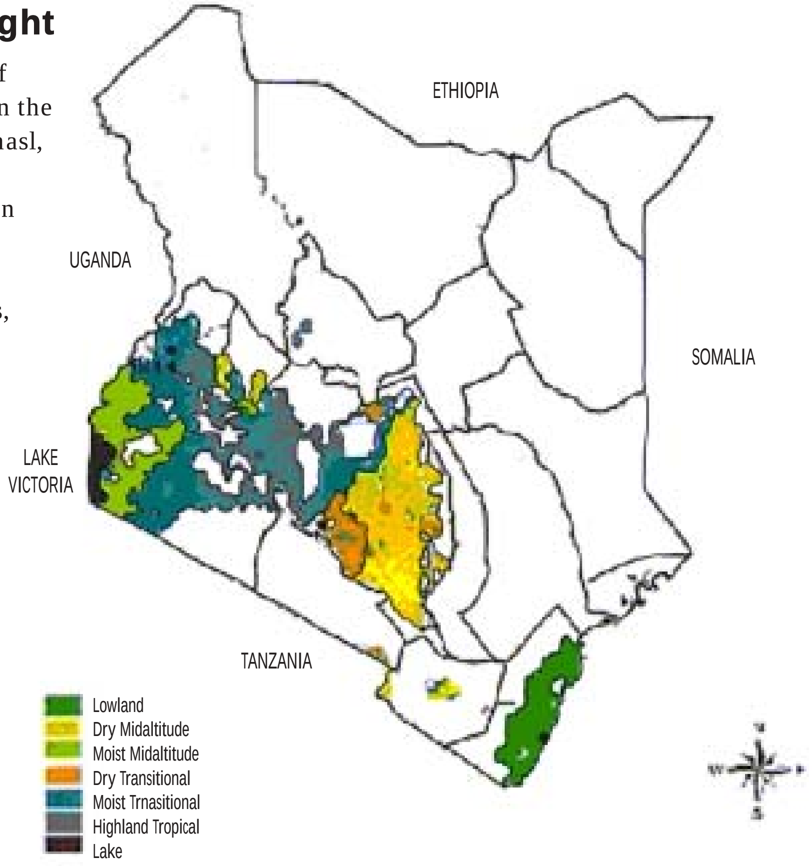

**Figure 1. Maize improvement zones in Kenya.**
Source: Kenya maize database project 1992-94.

# Breeding maize cultivars for drought tolerance in Malawi

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## Introduction

Maize is the main staple food in Malawi and is a major source of carbohydrates. The rising demand for food has also turned it into a cash crop. Maize is planted on 70% of arable land with an average yield of 1.3 million metric tons per year, with an average yield of 1.2 t / ha (Smale, 1999; CIMMYT, 1999). However, yield is low and drought is the principal factor influencing maize productivity in Malawi. Drought, mainly caused by insufficient rainfall and poor distribution during growth, is one of the most important abiotic stresses affecting maize production in Malawi and is the most important source of variation in yield over time. The development of varieties with high and stable grain yields under drought in Malawi, would therefore be a high priority as the use of drought-tolerant cultivars may be the only affordable option for small-scale farmers. Furthermore, it has been shown that germplasm developed for drought tolerance is likely to be as stable across environments as conventionally developed germplasm (Byrne et al., 1995). Two breeding approaches were used: (i) screening several germplasms from the regional trials under stressed artificial drought conditions, and (ii) $S_1$ recurrent selection for drought tolerance in four populations. Our objective was to identify economically desirable maize cultivars that will be able to establish, develop, and maintain themselves through drought periods by efficient and economic use of moisture.

## Methods

Recurrent selection for improved grain yield under drought was carried out in four populations in winter and the tolerant lines were selected based on an index in which anthesis-silking interval (ASI) and ears per plant were the major criteria. The best 30-40 $S_2$ lines from each population were recombined to form drought tolerant populations and these populations were screened under drought stress. Local and regional varieties were also screened under drought stress. The experimental design ranged from randomized complete block to alpha-lattices, with three replications. The plot sizes were 5.0 m x 0.9 m. The spacing between plants within a row was 30 cm, giving a plant population of 37,000 plants per hectare. The traits recorded were grain yield, number of ears per plant, senescence (1-10 scale), anthesis-silking interval (ASI) and other agronomic traits. Fertilizer was applied at the rate of 120 kg / ha N and 60 $P_2O_5$. Data was adjusted at 12.5% moisture content before analysis and data analyzed using alpha lattice.

## Results

The results of the drought screening trials are shown in table 1 for grain yield and other agronomic traits. There were no significant differences between varieties for grain yield, ears per plant, leaf blight, and rust. There was a negative genetic improvement for Chitibu and CCD whilst there was 16.8% and 5.37% genetic improvement for Matindiri and Sundwe, respectively. It can be construed from the data that only the Matindiri population performed well. For past seasons' screening, 23 varieties were identified with drought tolerance. As a result of this work, we have released five drought tolerant open pollinated varieties in Malawi: Giring'ande, Mpesi, Kalawe, Ngalonde, and Masuku.

## Conclusions

Five varieties have been released as a result of the drought project work. We focus our selection efforts at the flowering period when maize is vulnerable to stress. We use secondary traits such as anthesis-silking interval and ears per plant, which are highly heritable indicators (Bänziger and Lattife, 1997). We are using these traits along with yield to select for drought tolerance. In the future, our drought breeding program will receive increased attention,

**Table 1. Maize OPV trial at Chitala under drought stress, winter 2001**

Grain (t/ha) and Agronomic traits

| Var | Yield | rank | DP | DS | ASI | PH | EH | EPP | Senes | GI |
|---|---|---|---|---|---|---|---|---|---|---|
| Su c0 | 2533 | 9 | 74 | 76 | 2 | 176 | 88 | 0.81 | 5.6 | 2 |
| Su c1 | 2669 | 7 | 76 | 77 | 1 | 171 | 87 | 0.87 | 4.6 | 2.3 |
| ma c0 | 2563 | 8 | 76 | 77 | 1 | 165 | 78 | 0.83 | 5 | 2 |
| ma c1 | 2996 | 2 | 76 | 77 | 1 | 165 | 77 | 1.15 | 4.6 | 1.6 |
| ch c0 | 2963 | 3 | 79 | 80 | 1 | 179 | 98 | 0.85 | 4 | 2 |
| ch c1 | 2708 | 6 | 80 | 81 | 1 | 176 | 88 | 0.83 | 4.3 | 2 |
| ccd0 | 2871 | 4 | 70 | 71 | 1 | 136 | 63 | 0.96 | 5 | 3.6 |
| ccd1 | 2373 | 10 | 76 | 78 | 2 | 167 | 76 | 0.89 | 4.6 | 1.6 |
| Sylow | 2202 | 11 | 79 | 80 | 1 | 184 | 90 | 0.91 | 3.3 | 1.6 |
| Symid | 2142 | 13 | 80 | 81 | 1 | 176 | 84 | 1.06 | 3 | 2.6 |
| Opvla | 3064 | 1 | 76 | 77 | 1 | 188 | 87 | 1.0 | 5 | 3 |
| Opvm | 2184 | 12 | 76 | 79 | 3 | 176 | 83 | 0.97 | 4 | 2.3 |
| Kaful | 2788 | 5 | 79 | 81 | 2 | 189 | 96 | 0.75 | 4.3 | 2.6 |
| Mean | 2620 |  | 77 | 78 | 1.2 | 173 | 84 | 0.92 | 4.4 | 2.2 |
| CV% | 23.7 |  | 2.4 | 2.4 |  | 5.1 | 7.8 | 17.5 | 31.8 | 23.6 |
| Lsd | 1048 |  | 3.1 | 3.2 |  | 14.9 | 11.2 | 0.027 | 2.3 | 0.91 |
| P | Ns |  | *** | *** |  | *** | *** | ns | ns | ** |
| Min | 2142 |  | 70 | 71 |  | 136 | 63 | 0.82 | 3 | 1.6 |
| Max | 3064 |  | 80 | 81 |  | 189 | 98 | 1.15 | 5.6 | 3.6 |

EPP = number of ears per plant; Senes = senescence scores ( 1- 10 score); Mat = Matindiri
Su = Sundwe; Chi = Chitibu ; Ccd = Chitedze composite D

and will focus on the following goals: (i) to select locally adapted germplasm containing genetic variability for high yields, short ASI, and morphological and physiological traits associated with drought tolerance; (ii) to characterize lines developed in Malawi or introduced from abroad for drought tolerance, combining ability, and yield potential, with the aim of developing new hybrids and composites from these sources; and (iii) identify hybrids / open pollinated varieties with broad adaptation to highly variable growing conditions in Malawi.

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# Development of highland banana cell suspension system; A critical stage in genetic improvement of the banana

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## Introduction

Triploid *Musa* AAA-EA (East African highland banana) varieties are indigenous to and specially adapted to the highland conditions of the East African region. They are a major source of food known locally as Matooke. However, they lack resistance to pests and diseases. The development of resistant Matooke varieties through cross breeding and genetic engineering are the only possibilities for improving the crop. Cross breeding is hampered by female sterility, low genetic base, and the long cropping cycles of the crop. Biotechnological tools to supplement conventional breeding require a well performing in vitro cell suspension regeneration system through somatic embryogenesis from a regenerable target tissue. The cell suspensions are the material of choice for genetic transformation because regeneration through somatic embryogenesis tends to be less susceptible to somaclonal variation (Hanna et al., 1984), presumably because somatic embryos are not very tolerant of conditions that disrupt their ontogeny. This is of particular advantage for genetic transformation of individual genotypes, because changes other than the engineered trait are desirable (Parrot, 1992). Neumann (1999) emphasizes that the emerging application of gene technology to agriculture requires cell and tissue culture systems and is not feasible without them.

Three main procedures have been described for somatic embryogenesis in banana; all of them use vegetative tissues such as rhizome fragments and leaf bases (Novak et al., 1989; Ganapathi and Higgs, 1999), proliferating meristem cultures (Dhed'a et al., 1991; Schoofs, 1997) and immature male or female flowers (Escalant et al., 1994; Cote at al., 1996, Grapin et al., 1998). For most of the banana genome groups, embryogenic cell suspensions were obtained and plants regenerated from them (Dhed'a et al., 1991; Schoofs et al., 1997; Grapin et al., 1996, 1998). However, these methodologies have been reported for diploid bananas, plantains, Cavendish group and remain genotype specific (Vuylsteke et al., 1998). The objective of this research was to establish a system to initiate and regenerate embryogenic cell suspensions for East African (EA) highland bananas. Specifically, the task was to develop a method that can reduce the infertile banana plant to a single cell and reproduce a true-to-type banana from there.

## Method

Four representative varieties (Musa AAA-EA cvs 'Nakyetengu', 'Kisansa', 'Nakitembe', 'Namwezi') were selected from the Musa AAA-Ea clone sets (Karamura D.A., 1998) for screening to identify which of the infertile varieties can produce embryogenic cells. Immature male flowers were used as explants. The

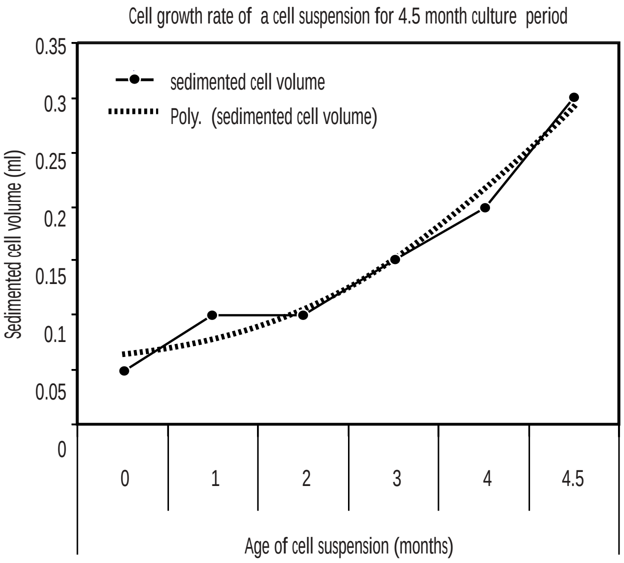

**Figure 1. Cell growth rate of banana cell suspension over 4.5 months.**

flowers were inoculated on callus induction medium modified for Musa AAA-EA bananas (Namanya, 2003), containing MS salts and vitamins (Murashige and Skoog, 1962) supplemented with growth hormones, antioxidant, and sucrose as a carbon source. Embryogenic callus was obtained and cell suspensions were initiated in liquid medium (Cote et al., 1996), maintained on a rotary shaker at 80 rpm in the dark at 28 ± $1^0$C and monitored for cell growth and proliferation. Plantlet regeneration was achieved on solid medium under the same growth conditions.

## Results

Of four Matooke cultivars, embryogenic callus was obtained from one, cv ‘Nakyetengu’. Subsequently, embryogenic cell suspensions were initiated and maintained in liquid medium.

The cell suspensions had a cell growth rate of 1.5-2.0 scv_per month. This is comparable to 2-5-fold pcu / month for Cavendish (Cote et al., 1996) and commonly observed in embryogenic cell suspensions of other monocotyledons. The cells maintained their embryogenic potential when plated on solid regeneration medium with a regeneration capacity of 60-100%. The plants derived from cell suspensions were planted in the field. In preliminary observations, their growth habits (vigor and stature) are comparable to the controls. All are morphologically normal and have completed the development stages of the cropping cycle (suckering, flowering, and fruit filling).

Improvement of infertile bananas can only be achieved through genetic engineering, therefore, introduction of genes of desired traits such as pest and disease resistance will be done using the in vitro cell regeneration system we have developed. With this success in development of cell suspensions, and recovering true-to-type plants, the future efforts to improve infertile *Matooke* varieties against the numerous constraints hold promise for the banana farmer and consumer.

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# Direct selection for grain yield under on-season natural moisture stress condition: Results from a large-scale screening nursery

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## Introduction

Indirect selection for grain yield through use of several morphological and physiological selection criteria is often advocated as being advantageous. However, each cultivar group is possessed with specific adaptive mechanisms for survival and reproduction in demand with the varying environments (Lafitte et al., 2002). A secondary trait should be strongly associated with grain yield under the target production environment if the trait has to be used as a selection criterion (Richards, 1996). Grain yield under a characteristic on-season natural stress is still considered as a reliable selection tool (Ceccarali et al., 1992). Collections of advanced breeding lines offer a range of adaptations to the moisture stress conditions and their interaction with the natural environment would result in differential yield potential of the genotypes. Exploitation of the wide genetic base to look for drought tolerant lines was attempted using a large-scale screening nursery.

## Methods

A collection of 432 advanced breeding lines mainly derived from national and provincial irrigated and rainfed rice breeding programmes was screened under on-season natural moisture stress environment at Paramakudi, a test site in the target production environment in the wet season 2002-03. The experiment was laid out in an augmented randomized complete block design (RCBD) in six blocks. Five varieties were tested across each block. The monsoon ended when most of the lines were in the flowering stage, thus creating a natural reproductive stage stress in the nursery. Data on heading days, plant height, productive tillers, panicle length, panicle harvest index, and grain yield were recorded. The association analysis was done between grain yield and other morphological traits. Based on the performance, a set of 30 lines with grain yield significantly higher than the median was selected. Trait association was also attempted within the subset.

The selected subset was tested in replicated yield trial under natural moisture stress environment at Ramanathapuaram, a second test site, in the same target production environment in the wet season 2003-04. The efficiency of selection in one season in a particular environment was studied in another environment in the subsequent season. The season received sufficient rains during the vegetative stage of the crop and the trial experienced a reproductive stage stress. The data were collected on heading days, plant height, productive tillers, panicle length and grain yield. The efficiency of selection was analysed through association between the two sites.

## Results

A positive relationship between grain yield and heading days within the whole population indicated the strong influence of duration on grain yield under stress (Figure 1). However, within the selected subset, the panicle harvest index is positively associated with grain yield and the lines were not significantly different in heading days. This explains the genetic variation for panicle harvest index, an indirect measurement of spikelet sterility within the same flowering duration group (data not shown). The best 30 lines were evaluated in 2003-04 at Ramanathapuram. A nonsignificant relationship was observed between the grain yield in 2002-03 at Paramakudi and that recorded in 2003-04 at Ramanthapuram (Figure 2). However, three cultivars viz., IET17495, IET17837, and IET17458 were found to be superior in yielding, at least 15% higher than the improved check, PMK2.

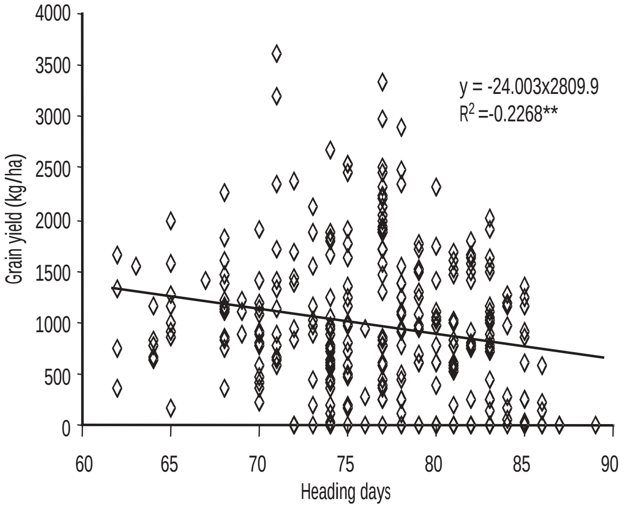

**Figure 1. Grain yiedl (kg / ha) vs heading days in 2002-03.**

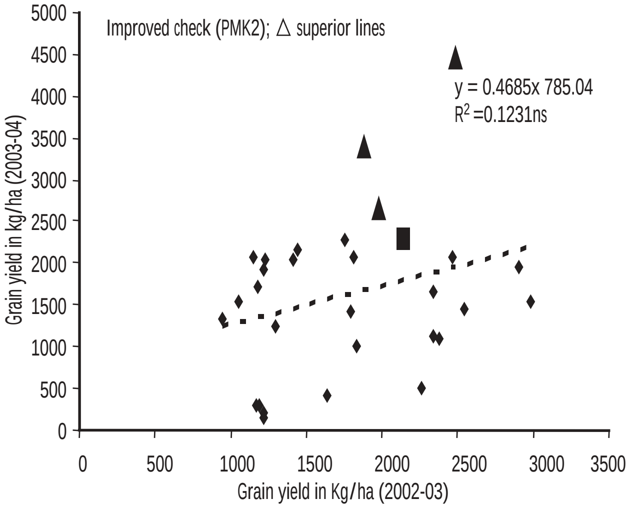

**Figure 2. Grain yiedl (2002-03) vs grain yield (2003-04).**

## Conclusions

Selection for drought tolerance based on grainyield under natural stress was effective in identification of superior genotypes. Large-scale germplasm screening can offer variations that can deliver adaptable genotypes after repeated field evaluations. The nonsignificant correlation between the seasons and locations was reflected by the spatial variations in rainfall and the heterogeneity within the target production environment. The study explained that broad selection intensity adopted in an unreplicated screening nursery followed by multienvironment testing might enable scientists to locate drought adaptable genotypes. The lines identified from this study are to be included in the multienvironment yield testing in the coming seasons.

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# Breeding for reproductive stage drought tolerance through development of near flowering lines in rice

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## Introduction

Breeding rice varieties with post flowering stage moisture stress tolerance is attempted. The definition of the target production environment with the analysis of annual rice production in relation to monthly rainfall deviation revealed that moisture stress occurring at the reproductive stage is the most devastating factor limiting rice yields in rainfed rice tracts of Tamil Nadu, India. The genetic analysis of reproductive stage moisture stress tolerance is always influenced by the heading days and the trait confers either advantage or disadvantage depending upon the specific requirement of the target production environment (TPE) in a particular year (Lafitte et al., 2002). Several putative traits were suggested as components enhancing performance under moisture stress prone environments (Fukai and Cooper, 1997). However, evidence for positive interaction between any specific trait(s) and grain yield under stress is still elusive, mainly due to the differential adaptations of genotypes under varied ecosystems (Lafitte, 2002). In this study, the populations are developed and characterized for phenologic similarity. The lines with closer phenology, hitherto referred to as near flowering lines (NFLs), were evaluated under natural stress. The field evaluation will be followed by molecular and physiological characterization of those lines that perform agronomically better than the parents. Attempts are made to develop an adaptable non-random population in order to locate the genomic regions of useful introgressions conferring drought tolerance during reproductive stage from the traditional variety into the high yielding varieties.

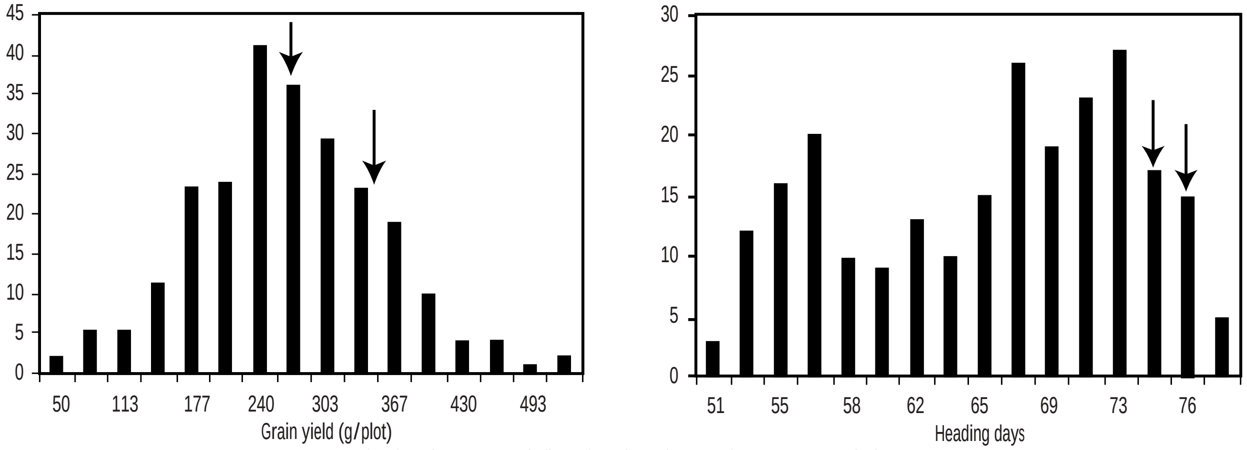
## Methods

The contemporary mapping populations derived out of diverse parents delivered DHLs or RILs with greatly differing phenology. The reproductive stage starting with panicle initiation to grain filling has varied response to moisture stress. Hence the populations with varying flowering duration will respond differently to moisture stress occurring under field conditions. To minimize the bias, mapping population with IR64/IR50, two elite lowland cultivars and Norungan, a traditional landrace, were made with the objective to develop recombinants of same phenology, since both parents are of nearly equal duration.

The population comprising of 241 $F_5$ families of IR64 x Norungan were evaluated at Ramanathapuram, the target production environment during wet season, 2003-2004 (Oct-Jan), along with the parents in two replications. The experiment was conducted under natural moisture stress condition so as to have realistic evaluation of the phenology from an on-season trial. The season experienced moisture stress during the reproductive stage. Grain yield apart from heading days, plant height, panicle length, number of productive tillers, and panicle length were measured and analyzed.

## Results

As the population significantly differed for all the traits studied, the lines also exhibited a normal distribution for grain yield, whereas lines skewed towards earliness in heading days (Figure 1). The heading days ranged from 51 to 78 days and the variation in phenology was stratified into three subsets of near flowering lines with the ranges of 59-64, 65-70, and 71-76.The mean and range of the traits studied were computed for individual subset and are furnished in table 1. Within the subset, the influence of heading days on grain set was insignificant.



Dotted and continuous arrows indicate the values of IR64 and Norungan respectively.

**Figure 1. Frequency distribution of IR64 x Norungan $F_5$ lines for grain yield and heading days under reproductive stage moisture stress.**

**Table 1. Range and mean of the subsets of NFLs of IR64 x Norungan for heading days, plant height, panicle length, number of productive tillers and grain yield (g/plot)**

| Parents/ subsets | | Heading days | Plant Height (cm) | Panicle length (cm) | Number of productive tillers | Grain Yield (g/m$^2$) |
|---|---|---|---|---|---|---|
| IR64 | | 77±1.41 | 78.85±2.18 | 21.83±1.18 | 9±2.83 | 285±63.64 |
| Norungan | | 71±2.12 | 120.10±0.47 | 24.50±1.65 | 8±1.18 | 345±35.46 |
| Subset I: | range | 59-64 | 63.16-128.33 | 17.83-25.33 | 5.0-16.0 | 75.0-455.0 |
| | mean | 61.63±0.29 | 98.68±2.90 | 22.27±0.29 | 8.61±0.38 | 254.54±13.15 |
| Subset II: | range | 65-70 | 63.16-137.50 | 17.83-25.33 | 5.0-17.0 | 50.0-440.0 |
| | Mean | 67.65±0.20 | 106.24±2.11 | 22.27±0.29 | 8.63±0.39 | 254.85±10.58 |
| Subset III: | range | 71-76 | 59.00-141.50 | 19.33-29.00 | 5.0-15.0 | 50.0-525.0 |
| | mean | 73.03±0.21 | 108.52±2.29 | 23.60±0.23 | 8.26±0.21 | 252.84±12.55 |

## Conclusions

The experiment did not experience severe drought and grain set was observed in all the entries. The entire population flowered within 80 days after sowing since the parents were of almost same duration. The strategy of reconstituting individual sets of NFLs is undertaken by inclusion of RILs from other population derived from the backcross programmes involving the same parent. Apart from these, germplasm screening has brought out breeding lines falling within the range of the NFLs in heading days. Attempts will be undertaken to carry out association mapping with the help of microsatellites for mapping the genomic regions associated with drought tolerance in rice.

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# Population structure of *O. glaberrima* Steud and its implications for breeding drought tolerance in cultivated rice

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## Introduction

African rice cultivars are adapted to widely different environments, including deep water, rainfed lowland and upland ecosystems (Portères, 1970). Today, both *O. glaberrima* and *O. sativa* are commonly grown in mixtures by farmers in upland and rainfed lowland environments, both of which are prone to drought. *O. glaberrima* has been cultivated in drought-prone environments in West Africa for more than 3,500 years and it is of interest to understand the population structure and evolution of this native African species. *O. sativa* was introduced from Asia to Africa by Arab traders and Portuguese explorers 300-500 years ago. We were interested in assessing the relative importance of introgression, admixture, geography and ecological specialization in shaping the pattern of genetic variation within *O. glaberrima*. In this study, we used 93 nuclear simple sequence repeat (SSR) markers to investigate population structure in *O. glaberrima.* We aimed to determine whether any genetically defined sub-populations were more likely to be associated with drought-prone environments. Results from this analysis can be used to establish a basis for association mapping in *O. glaberrima,* whereby collections of existing landraces and improved varieties could be evaluated for drought tolerance and used as the basis for gene and QTL discovery without the need to establish fixed bi-parental mapping populations.

## Methods

One hundred and ninety-eight (198) accessions of *O. glaberrima* where selected from a larger collection of 1,130 accessions maintained at WARDA. Selection was made to maximize geographical and morphological diversity. A set of 93 SSR markers was selected to provide good coverage of all 12 rice chromosomes. PCR conditions for each SSR primer pair were as described by Chen et al. (1997) and Temnykh et al. (2000, 2001). Amplification products of SSR were size separated using polyacrylamine gel electrophoresis and detected using silver staining as described by Panaud et al. (1996) or using an ABI fluorescent detection system as described by Coburn et al. (2002). Three different methods were used to assess population structure, including a Mantel test for “isolation by distance”, a distance-based and a model-based method (Pritchard et al., 2000; Rohlf 1997; Goldstein and Pollock, 1997).

## Results

Abundant genetic diversity was observed within the 198 accessions of *O. glaberrima*. An average of 9.4 alleles per SSR locus was detected, with a range of 2-27 alleles/locus. When this variation was analyzed, significant levels of linkage disequilibrium (LD) were detected among the SSR alleles. This was determined to be the result of population substructure. Using a model-based approach, five genetically distinct groups, or admixtures thereof, were identified within this collection of *O. glaberrima*. Three of the groups were characteristic of *O. glaberrima* while two groups (clusters 4 and 5 in Table 1) appeared to share a significant proportion of their ancestry with the *japonica* and *indica* subspecies of *O. sativa*. These can be classified as admixed sub-populations that contain introgressions from *O. sativa.* The remaining three populations of *O. glaberrima* were significantly differentiated from each other (Fst) and of approximately equal size and could be distinguished genetically, with two groups harboring relatively more genetic diversity than the other, as represented by number of alleles (A), polymorphism information content (PIC) and heterozygosity (*He*) values (Table 2). There was no evidence for isolation by distance (*p*-value = 0.40) among the sub-populations of *O. glaberrima*, suggesting that accessions collected in the same country are not necessarily more genetically similar than accessions found in geographically distant locations. This was evident from the fact that two of the sub-populations contained individuals collected from all 12 countries of origin, while one group contained individuals from only 4 (non-contiguous) countries. This emphasizes the importance of human activity in determining the distribution of rice accessions across West Africa. It will be of interest to evaluate the performance and phenotypic characteristics of the accessions in a range of different environments to determine whether these sub-populations show differential adaptation to different ecological environments, such as the upland, rainfed lowland and deep water environments described by Porteres (1970). Knowing something about the population structure within *O. glaberrima* makes it possible to undertake association mapping aimed at identifying genes and QTLs related to drought tolerance using existing germplasm resources. It also facilitates selection of genetically diverse *O. glaberrima* accessions to be used as parents in interspecific breeding programs.

**Table 2. Genetic diversity and pair-wise differentiation among the three groups identified within *O. glaberrima* using a Bayesian analysis (composed of individuals with ancestry equal or greater than 75%).**

| | | Diversity | | | Differentiation (Fst values) | | |
|---|---|---|---|---|---|---|---|
| **Population** | **Size** | **A** | ***He*** | **PIC** | **Group1** | **Group3** | **Overall** |
| Group1 | 3 | 3 | 0.29 | 0.27 | - | | |
| Group2 | 27 | 4 | 0.27 | 0.25 | 0.16 | 0.18 | |
| Group3 | 34 | 3 | 0.22 | 0.20 | 0.09 | - | |
| Mean | | | | | - | - | 0.14 |

A= allele number; *He* = heterozygosity; Fst value are all significant (p<0.0000)

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**Table1. Bayesian partitioning of ancestry within a population of SSR alleles from a screening of 207 cultivated rice accessions (including 198 accessions of *O. glaberrima* and 9 cultivars of *O. sativa* ) using "Structure".**

| | | | Ancestry grouping | | | | |
|---|---|---|---|---|---|---|---|
| | | | **1** | **2** | **3** | **4$^a$** | **5$^b$** |
| **Country of origin** | **Species** | **# of individuals** | Proportion of ancestry | | | | |
| Burkina Faso | *O.glaberrima* | 2 | 0.37 | 0.5 | 0.02 | 0.1 | 0.01 |
| Cameroon | *O.glaberrima* | 2 | 0.41 | 0.5 | 0.03 | 0.07 | 0 |
| Ivory Coast | *O.glaberrima* | 6 | 0.73 | 0.16 | 0.02 | 0.05 | 0.04 |
| Ghana | *O.glaberrima* | 10 | 0.47 | 0.36 | 0.09 | 0.07 | 0.01 |
| Guinea Bissau | *O.glaberrima* | 11 | 0.7 | 0.15 | 0.1 | 0.05 | 0 |
| Guinea Conakry | *O.glaberrima* | 6 | 0.31 | 0.02 | 0.01 | 0.07 | 0.59 |
| Liberia | *O.glaberrima* | 67 | 0.28 | 0.08 | 0.6 | 0.04 | 0.01 |
| Mali | *O.glaberrima* | 10 | 0.42 | 0.22 | 0.25 | 0.1 | 0.01 |
| Nigeria | *O.glaberrima* | 67 | 0.3 | 0.56 | 0.09 | 0.04 | 0.01 |
| Senegal | *O.glaberrima* | 9 | 0.63 | 0.25 | 0.08 | 0.03 | 0.01 |
| Sierra Leone | *O.glaberrima* | 6 | 0.4 | 0.12 | 0.06 | 0.03 | 0.39 |
| Zimbabwe | *O.glaberrima* | 2 | 0.88 | 0.09 | 0.03 | 0 | 0 |
| Africa-Asia | *O. sativa* (check) | 9 | 0 | 0 | 0 | 0.08 | 0.91 |

1,2,3 represents *O. glaberrima* ancestry identified. 4,5 represents *O. sativa* ancestries ($^a$ *O.sativa* var. indica ancestry; $^b$ *O.sativa* var japonica ancestry)

# Role of stress tolerant germplasm in increasing maize productivity in drought-prone areas of Angola

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## Introduction

Maize, together with cassava, are the most important staple foods grown in dry-prone areas of Angola. About 30,000 to 50,000 hectares of arable land constitute the area planted to maize, mostly for human consumption and animal feeding. These areas are mainly located in the coastal and northern agroecological zones and are characterized by sandy soils with low soil pH and erratic rainfall (less then 600 mm). Maize production is limited mainly by the use of local varieties, lack of water at emergence and during flowering and grain filling stage. The latter is due to a frequent pronounced mid-season gap in rainfall. As a result, yields remain very low, 350-750 kg/ha at the small-scale farmers’ level and 1500-3500 kg/ha for commercial farmers. As rainfalls are variable, both between years and in their timing within a season, it is desirable to employ additional breeding strategies that improve yield stability under drought. In collaboration with CIMMYT and other SADC NARS, the Angola Maize Program has developed, evaluated, and selected several open pollinated varieties (OPVs) and hybrids (Syn01E2 F2, 01SADVE F2, ZM421, ZM521, ZM621, ), which have been recommended for seed multiplication and release.

## Methods

Drought screening was conducted at Mazozo Research Station (60 masl, 9$^{0}$10’ S, 13073’E). Drought stress was managed by irrigation during the dry season of 2001 to 2003. Irrigation was stopped two weeks before flowering. Its intensity varied from intermediate to severe at flowering and grain filling stages, respectively. Screening under drought was done according to Bänziger et al. (1999) and used high yield as the primary criteria. The following secondary traits were also taken into consideration: decreased ASI, delayed and less leaf senescence (stay-green), less leaf

**Table 1. Average performance of early maturing populations under drought conditions. Mazozo, 2001-2003**

| Entry | Pedigree | Origin | GYW, t/há | ASI; days | SLODG, % | LROL | LSEN | EROT |
|---|---|---|---|---|---|---|---|---|
| | 5 Syn01E2 F2 | CIMMYT | 3.17 | 1.2 | 14 | 1.32 | 1.38 | 2.17 |
| | 8 ZM521 F2 | CIMMYT | 2.90 | 2.1 | 7 | 1.77 | 1.35 | 1.67 |
| | 9 ZM421-FLINT# | CIMMYT | 2.67 | 2.5 | 20 | 1.46 | 1.15 | 1.67 |
| | 13 01SADVE F2 | CIMMYT | 2.53 | 3.3 | 7 | 1.53 | 1.63 | 1.83 |
| | 27 KEP | BOTSWANA | 2.51 | 3.3 | 26 | 1.55 | 1.33 | 2.67 |
| | 14 01 SADVI F2 | CIMMYT | 2.36 | 2.6 | 22 | 1.68 | 1.39 | 2.67 |
| | 19 AMEDIN-1# | ANGOLA | 2.26 | 5.4 | 22 | 1.9 | 1.83 | 2.83 |
| | 18 ZM303 | CIMMYT | 2.23 | 4.5 | 2 | 2.05 | 2.41 | 1.67 |
| | 3 VV023 | CIMMYT | 2.21 | 5.2 | 25 | 1.67 | 1.75 | 2.67 |
| | 1 VV021 | CIMMYT | 2.17 | 5.6 | 2 | 1.09 | 1.38 | 2.33 |
| | 22 KAFULA (MALAWI)# | MALAWI | 2.15 | 4.5 | 16 | 1.35 | 1.69 | 1.83 |
| | **Mean** | | **1.93** | **3.6** | **16** | **1.7** | **1.55** | **2.1** |
| | **LSD** | | **2.43** | **5.0** | **15** | **12** | **3.11** | **4.21** |
| | **CV** | | **48.33** | **134.2** | **52** | **37.45** | **33.87** | **34.76** |
| | **FSIG** | | **1.11** | **1.0** | **5** | **0.67** | **2.65** | **0.99** |
| | **REFF** | | **1.47** | **1.1** | **1** | **1.43** | **1.07** | **0.96** |

rolling, less barreness, increased prolificacy, and decreased shoot and root lodging. Other data collected were plant and ear height, husk cover, ear aspect and disease scoring. For sake of comparison, testing was conducted as well under well-fertilized / rainfed conditions during the main cropping season at several locations in the country.

## Results

Early-maturing populations were, on average, higher yielding than intermediate- to late-maturing OPVs under drought stress. EPOP produced grain yields of 2.15 to 3.17 t / ha under drought while ILPOP entries produced 1.5 to 1.7 t / ha. Earlier flowering of EPOP entries probably allowed them to escape the negative effects of stress at critical stages of plant development. Best EPOP entries (Syn01E2 F2, 01SADVE F2, ZM421, ZM521, ZM621) had a short ASI of 1 to 5 days. The best ILPOP entries (ZM621) had an ASI of 2 and 9 days, respectively. Increased ASI in ILPOP resulted in increased barreness and lower yields. We were interested in selecting entries that were suitable to a range of conditions and therefore calculated average rank per entry in each of the trials. Low average rank is a better indicator for yield stability than high average yield because entries typically differ much more under favorable conditions than stress conditions. Yield differences under well-fertilized / rainfed conditions thus would over-rule yield differences under drought and N stress if simply the average yield across trials were calculated.

## Conclusions

Among the regional and national trial entries that were evaluated under drought stress and well-fertilized / rainfed conditions, the Angolan National Program selected the following germplasm for future breeding work, seed multiplication efforts, and release purposes: Syn01E2 F2, 01SADVE F2, ZM421, ZM521, ZM621. Some breeding work was started in the 2000 / 2001 season with two local and seven CIMMYT and NARS populations. They are undergoing recurrent full-sib selection (Hallauer and Miranda, 1988) under managed drought stress.

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# Guinea sorghum hybrids: Bringing the benefits of hybrid technology to a staple crop of sub-Saharan Africa

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While the guinea sorghums appear to be well adapted to the harsh climate and poor soils of West Africa, considerable efforts have been made to breed higher yielding sorghums using exotic non-guinea germplasm. The varieties and hybrids developed, however, due to several factors, are not a viable option for producers of guinea sorghums, except in special niche conditions. Many problems limited the exploitation of the first hybrids: lodging, severe leaf diseases, head bugs damage, grain mold, *Striga* and grain quality. Our strategy has been to develop the guinea hybrid parent. Guinea genetic materials from diverse sources were characterized for fertility reaction by crossing into an A1 source of cytoplasmic male-sterility. The backcrossing procedure for developing guinea male-sterile lines from varieties and breeding lines with the maintainer reaction were conducted. The establishment of a second off-season generation in this project accelerated the breeding process by artificial control of the day length. More than 400 testcross F1s with A1 cytoplasmic tester lines with guinea-race landraces and landrace derivatives and breeding lines with guinea-race parentage were evaluated for their fertility reaction. These testcross F1s were planted at Sotuba and Samanko; each hybrid F1 was planted in one row plot with 5 hills with 2 plants each. Five heads in each plot were bagged to observe seed production. Five non-bagged heads were evaluated for anther form and anther dehiscence and flowering date in each plot. The results showed high frequency of maintainer reactions among varieties. Significant progress was made to develop male-sterile lines from the maintainer lines identified; guinea-race A / B pairs have been developed. Five generations of backcrossing were conducted to develop male-sterile lines for seven lines during the rainy season 2003. The sixth backcross generation was completed for CSM 335, CSM 207, CSM 219, and Fambé. A total of 55 BC5F1 are made from 97-SB-F5DT-150, 97-SB-F5DT-154, 97-SB-F5DT-160, 98-BE-F5P-82, 99CLo634, and N'Tenimissa. A total of 33 R lines (guinea, and guinea derivatives) have been crossed to six developed A lines A97-SB-F5DT-150, A97-SB-F5DT-154, A 98-BE F5P-82, A N'TENIMISSA, ACSM 219E and 99CLo634 to produce F1 hybrids. These hybrids will be evaluated for heterosis and combining ability in the 2004 growing season. Future research will be focused on broaden genetic base of hybrid parents using agronomic trait characterization data and molecular genetic characterization of structure of genetic diversity from diverse collections evaluations. We will determine ideotype for guinea-race hybrid parents to better target hybrids by farmer participatory in identifying traits for future hybrids.

# Managed drought stress environments in Mexico and their association with global wheat growing environments

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## Introduction

Wheat is grown in many diverse environments around the world, spanning an area from the equator to more than 60∫ of latitude. The growing conditions across these diverse environments range from no drought stress to severe drought stress depending on annual precipitation and water availability for irrigation. Drought stress is a variable phenomenon and can occur at any time during the growth cycle. However, the probability of certain types of drought stress occurring based on long-term weather records and / or availability of irrigation, indicate that many regions have dominant drought stress patterns (Rajaram et al., 1996). In an effort to better target the development of spring wheat germplasm for the frequently drought stressed environments, which comprise more than half of the wheat growing areas in the developing world, managed stress environments were generated at The International Maize and Wheat Improvement Center's (CIMMYT) research station in the Sonoran desert in northwestern Mexico (latitude 27∫ N, 60 masl). A common set of spring bread wheat genotypes was grown under both managed drought stress in Mexico and globally in drought affected areas, and the association among environments was explored.

## Materials and methods

Twenty spring bread wheat genotypes, selected for their global performance in dry environments and their relatively narrow variation in flowering time, were tested under eight different primary stress environments generated in the field in Sonora using gravity and drip irrigation over a four-year period (Table 1). A total of 32 managed stress environments were generated in this way. Yield trials were arranged as alpha-lattices of 2 reps each. Yield data on the performance of 19 of the 20 genotypes, sown head-to-head in the same trial in 29 different drought prone environments around the world, was collected by CIMMYT's regional collaborators. These data from the 29 global trials and the 32 managed stress trials in Mexcio were analyzed using SAS (1988). The shifted multiplicative model (SHMM) was then used to cluster genotypes without rank change into groups with reduced crossover interaction (Crossa et al., 1993). A dendrogram of the associations among environments was then constructed using the adjusted yield means from each trial.

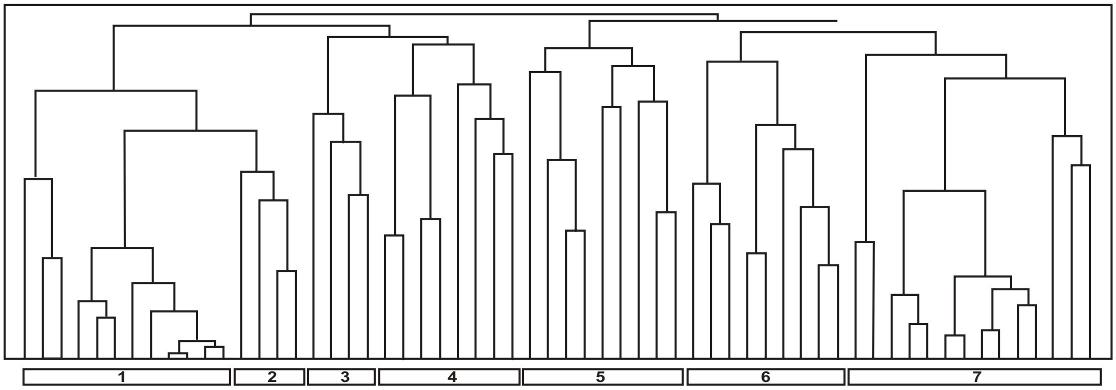
**Table 1. The different irrigation regimes generated in Sonora, Mexico, using limited irrigation**

| Form of irrigation | Type of stress generated | Total water applied (approximate in mm) |
|---|---|---|
| Gravity fed | Non-drought stressed | 600 |
| Gravity fed | Post-anthesis drought stress | 240 |
| Gravity fed | Continuous drought stress | 120 |
| Gravity fed | Late heat stress | 600 |
| Drip | Non-drought stressed | 360 |
| Drip | Post-anthesis drought stress | 160 |
| Drip | Continuous drought stress | 80 |
| Drip | Pre-anthesis drought stress | 160 |

Note: on average there was between 90–120mm of water available in the soil to 1.2m at planting

## Results

The dendrogram of site associations produced seven distinct groups or clusters (Figure 1). The dominant Mexican managed stress environment within each cluster was identified (see stress generated in Mexico in Figure 1), as were the global locations that clustered within each group (see international sites in Figure 1).



| Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Stress generated in Mexico | Gravity continuous stress | Drip Terminal stress | Gravity No Stress | Drip Continuous stress | Heat and terminal moisture stress | Drip Moisture stress pre-flowering | Gravity no stress and terminal stress |
| International Sites | Brazil Spain<br>Algeria Bolivia<br>Pakistan | No sites | No sites | Saudi Arabia<br>Argentina<br>South Africa<br>Egypt<br>Canada | Zimbabwe<br>Iran<br>Pakistan | Nepal Brazil<br>Pakistan<br>Iran Canada | Iran<br>Bangladesh<br>Saudi Arabia<br>Spain<br>Afghanistan |

**Figure 1. The associations among managed drought stress environments in Mexico and global wheat growing areas.**

## Conclusions

The managed drought stress environments generated in Mexico clearly associated with different global wheat growing areas on the basis of the SHMM analysis. Two of the seven primary clusters or groups of sites generated in the dendrogram—terminal stress generated using drip irrigation and gravity irrigation without drought stress—did not associate with any of the global environments. These two stress environments are unlikely to offer plant breeders the same gains in selection as the remaining five managed stresses. Sites in Spain, Pakistan, Brazil, Saudi Arabia, Canada, and Iran appeared in more than one group, indicating the presence of distinctly different wheat growing conditions within these countries at the sites where trials were grown. Clearly, these results indicate that managed stress environments generated in Mexico can be used to better target spring wheat germplasm to the many and diverse wheat growing environments of the world.

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# Comparative genetic variability in rice accessions for water limited environments

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## Introduction

Approximately 60% of rice area in India is rainfed, which entails many natural stresses, but also an endowment of rice varietal diversity. Great genetic variability exists in nature regarding stress tolerance in rice genotypes, which is helpful for increasing tolerance as well as productivity. Extensive research in relation to breeding for drought tolerance of rice has been widely addressed by many scientists (O'Toole, 1982; Cabuslay et al., 2002; Joyce et al., 2003). Genomic changes, either by conventional breeding or by biotechnological innovations, may alter the phenotypic variations of adaptive nature to withstand drought stress conditions. It is imperative to understand the true genetic relationship and diversity of segregants (DHLs and ILs) and cultivated rice. A better understanding of the genetic diversity pattern and relationship of the close relatives of rice genotypes will facilitate more effective conservation and utilization of rice germplasm for the improvement of drought tolerant rice varieties. An attempt was made to identify the suitable genotypes to augment better drought tolerant rice donors for improving tolerance as well as yield under stress conditions.

## Methods

The experiment, with 59 doubled-haploid (DHLs, including IR64) and 45 introgressed lines (ILs) of IR64 collected from IRRI, Manila, Philippines) was conducted in augmented design with two subsets (one in rainout shelter-stress and another in natural field condition-irrigated) during the wet season 2003. Water scarcity was created by withholding water at 40 days of plant growth up to 20 days drought at the vegetative stage. The morpho-physiological traits viz., germination (%), yellowish tip of leaves, leaf tip drying, leaf rolling, recovery, and root traits, supported by physiologist (only introgressed lines), initiation of heading and infection of major diseases (brown spot-BS, blast- BL and narrow brown leaf spot-NBLS), were observed as per standard evaluation system (SES of IRRI) in DHLs and ILs. None of these segregants could flower due to reductions in soil moisture and temperature (Figure 1). Soil moisture was recorded by tensiometer. In addition, 38 selected genotypes / cultivars (including two checks in two different blocks) of rice collected from different eco-geographical regions were assessed (in augmented design) for genetic variability during dry season 2003 under natural field conditions. The yield and its related morphological traits viz., plant height, panicle length, ear bearing tillers (EBT), number of fertile grains / panicle, number of sterile grains / panicle, L / B ratio along with major disease infection, were

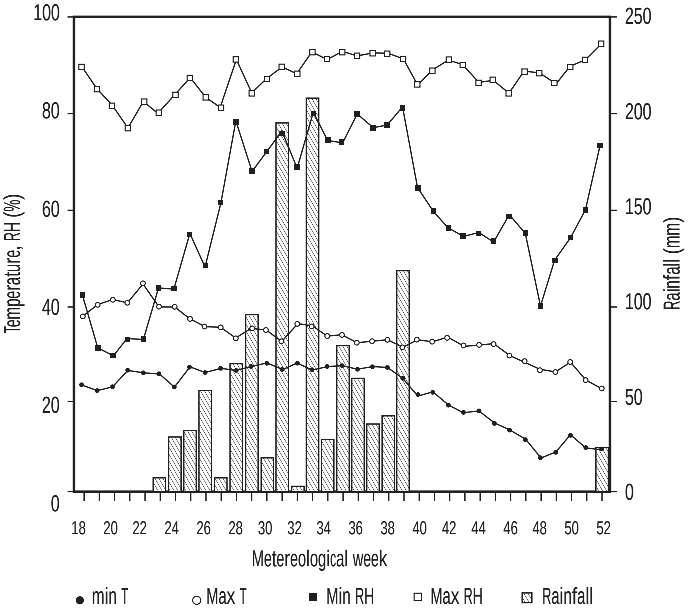

Figure 1. Changes in weather parameters during experimentation.

recorded. A dendrogram showing genetic relationship (similarity index) of all rice accessions (59 DHLs, 45 ILs and 38 diverse eco-cultural types) was generated separately by using the Unweighted Pair-Group Method with an arithmetic mean (UPGMA), following Lu et al. (2002).

## Results

Genetic diversity was considerably high among all accessions. Similarity coefficient ranged from 26.78 to 97.89 (DHLs); 27.68 to 98.50,(ILs); and 55.92 to 96.04 (germplasm under stress conditions) (Figures 2,3,4). The dendrogram clearly shows the genetic variation

**Figure 2. Dendogram generated from cluster analysis of morpho-physiological traits in DHLS.**

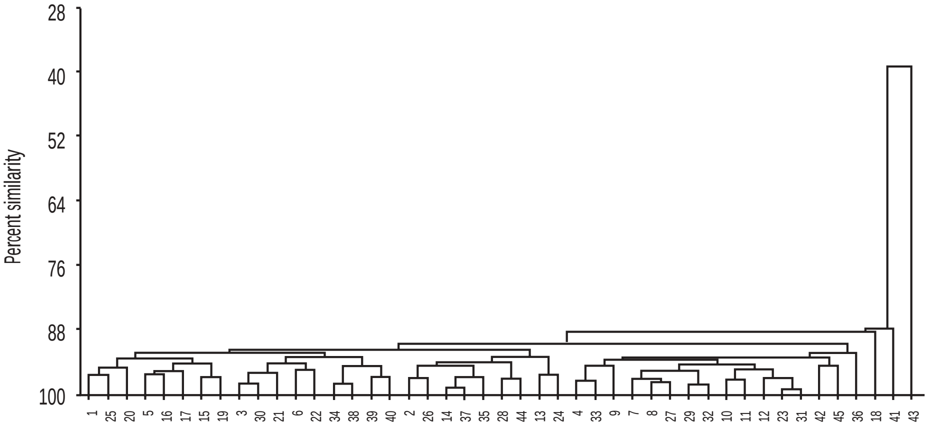

**Figure 3. Dendogram generated from cluster analysis of morpho-physiological traits in ILS.**

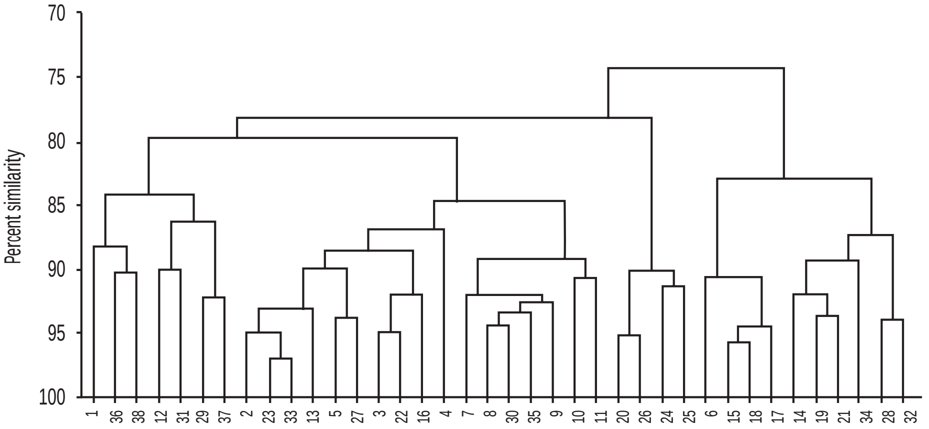

**Figure 4. Dendogram generated from cluster analysis of morpho-physiological traits germplasm.**

pattern of the *Oryza* accessions. Certain accessions of DHLs with small differentiation indicated unique genetic status, possessing homogeneity or less complexity for drought tolerant traits. Significant variation was found among DHLs and ILs for the drought traits, phenology (plant stature), initiation of heading, and root traits, under both control and stress conditions. Panicle of shoot initiation outside leaf was delayed in rainout shelter compared with irrigated plots. Twenty days drought exposure in the rainout shelter was found to be detrimental to DHLs and ILs at the vegetative phase. Certain accessions were found in an independent cluster, including Azucena and OS 6 (Japonica group). The accessions collected from the same eco-geographical region or nearby eco-geographical regions tended to stay together in the same group. Six distinct clusters were grouped for germplasm and in cluster V, Annada, Lalnakanda, Laloo 14, IET 16706, and Gaurav, and found promising under severe drought conditions. These lines are being used in our crossing programme for drought tolerant traits. Twenty-eight (28) DHLs and 16 ILs out of the tested entries maintained high recovery potential. Mean, range, standard deviation, and correlation coefficient with grain yield values of various attributes further reflected a wide range of diversity in the descriptors (Table 1). Grain yield was significantly reduced under water scarcity conditions in germplasm. This did not conform with those of Cabuslay et al. (2002).

## Conclusions

Large genetic variation existed in drought tolerance and disease infestation within the segregating population and germplasm. Lines that showed superiority over others have to be incorporated in multidisciplinary research programmes to generate new rice genotypes for drought tolerance. Maximum homogenic similarity index for the majority of the genotypes was recorded in DHLs, followed by ILs and germplasm. It reflects that more emphasis should be given to DHLs for drought screening, following ILs and germplasm. Breeding populations involving parents of different eco-geographical regions/ genetic background for drought tolerant and yield potential with grain quality traits and disease resistant types are being developed in order to pyramid the desired quantitative traits in widely adopted genotype/ cultivar and to obtain maximum number of molecular markers due to high levels of polymorphism at the DNA level.

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**Table 1. Mean, range, standard deviation and correlation coefficient of different traits in local germplasm evaluated under stress (SS) and well watered (ww) conditions**

| Traits | Mean | | Minimum | | Maximum | | SD | | r* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SS | WW | SS | WW | SS | WW | SS | WW | SS | WW |
| Plant height(cm) | 95.61 | 105.74 | 57.25 | 60.50 | 146.00 | 157.00 | 3.34 | 3.48 | 0.10 | 0.31* |
| Panicle length (cm) | 23.20 | 25.92 | 17.50 | 18.50 | 30.00 | 31.40 | 0.57 | 0.57 | 0.35* | 0.51** |
| Ear bearing tillers | 13.85 | 19.04 | 5.50 | 7.00 | 21.50 | 27.00 | 0.61 | 0.71 | 0.25 | 0.28 |
| No. of fertile grains/ panicle | 82.70 | 99.43 | 27.00 | 36.00 | 188.00 | 262.00 | 7.29 | 8.59 | 0.54** | 0.67** |
| No. of sterile grains/ panicle | 14.96 | 9.40 | 3.00 | 2.0 | 40.00 | 23.00 | 1.50 | 0.92 | 0.07 | 0.30 |
| L: B ratio | 2.87 | 3.01 | 2.13 | 2.39 | 5.22 | 5.54 | 0.09 | 0.79 | 0.01 | 0.17 |
| Brown spot | 2.54 | 2.00 | 1.45 | 1.00 | 5.00 | 5.00 | 0.24 | 0.20 | -0.13 | -0.11 |
| Blast | 2.70 | 2.50 | 0.00 | 0.00 | 5.00 | 5.00 | 0.29 | 0.27 | -0.16 | -0.14 |
| NBLS | 2.04 | 1.58 | 0.00 | 0.00 | 5.00 | 5.00 | 0.26 | 0.22 | -0.12 | -0.10 |
| Grain yield/ plant (g) | 13.88 | 18.00 | 6.25 | 8.66 | 25.50 | 35.54 | 0.79 | 1.03 | | |

r*= Correlation coefficient with grain yield, *significant at *p*=0.05; **significant at *p*=0.05

# Evaluation of advanced backcross populations for non-flooded, irrigated conditions and other associated stresses

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## Introduction

Water is increasingly becoming scarce in most Asian nations including India (Gleick, 1993), warranting its efficient use through integrated approaches. The cultivation of aerobic rice aims to minimize the water requirement of rice while maintaining yield levels close to the irrigated lowlands. Traditionally, aerobic rice is grown in fragile rainfed uplands with low or no inputs and uncertain water availability. As fresh water is becoming scarce, aerobic rice cultivation is expected to expand to the regions of more intense cropping with high external inputs and productivity (Lafitte et al., 2002). The available evidence suggests that higher yielding aerobic cultivars have resulted from crosses between improved upland and elite irrigated varieties. High yielding varieties suited to irrigated conditions are known to contribute high yield potential, input responsiveness, lodging resistance, and high harvest index, while improved upland cultivars contribute adaptation to aerobic conditions. (Atlin and Lafitte, 2002). Generally, the aerobic situation in itself implies a low level of stress for rice, particularly if the relative humidity is low (Dingkuhn, 1989). Depending on the soil where aerobic rice is expected to be grown, the problems can differ. For example, in a typical saline sodic soil, the plant could experience osmotic stress. In flooded soils, roots develop superficially as a mat facilitating absorption of nutrients from the flood water, while in aerobic soils, root growth is more dispersed (Yoshida, 1981). In addition to the soil water availability, other major factor that differs in aerobic soils is nitrate replacing ammonia, making it important for management of nitrogen. The problems of pests / diseases, including blast and nematodes, are expected to be significant in aerobic soils. With this in mind, 555 $BC_2F_6$ drought tolerant introgression lines of IR64 and Teqing were screened for various biotic and abiotic stresses including limited water stress. The results of this evaluation are presented briefly.

## Methods

The material for the study consisted of 555 $BC_2F_6$ drought tolerant introgression lines of IR64 and Teqing, developed in collaboration with International Rice Research Institute (IRRI), Philippines. The lines were screened under non-flooded irrigated conditions during the wet season 2003. The details of field screening are described in the related article elsewhere in this publication. Apart from limited water stress, the material was also screened for 'P' deficiency tolerance and resistance to blast, brown planthopper (BPH), and root knot nematodes. For tolerance to 'P' deficiency, the material was screened by growing in a field that was maintained as a 'P' zero plot for more than 12 years. Blast and BPH screening was done under screenhouse conditions by following standard methods. For screening against root knot nematodes, seeds were germinated in trays and each plant was inoculated with 200 infective juveniles and observed for development of galls on the roots 45 days after inoculation. In another experiment, some of the selected IR64 introgression lines were evaluated under flooded and limited water regimes at two 'N' levels (75 and 150 kg 'N / ha) and two spacings (20x15 cm *vs* 10x15 cm). Tolerance to blast and BPH was scored using 0-9 scale, and for nematodes the actual number of galls formed were counted. The restorers and maintainers were identified by evaluating the testcrosses made between the CMS lines (WA based) and the introgression lines.

## Results

The summary of screening results of drought tolerant introgression lines for various stresses is presented in table 1. Of the 385 IR64 introgression lines screened, about 100 showed better performance under non-flooded conditions. Among these, seven were identified as restorers and two as maintainers of WA

**Table 1. Evaluation of drought tolerant in trogression lines under limited water and other related stresses**

| Stress | $BC_2F_6$ Introgression Lines | |
|---|---|---|
| | IR 64 (385) | Teqing(170) |
| 1. Limited water | | |
| (a)Restorers | | |
| (b) Maintainers | ~ 100 | ~ 75 |
| | 7 | 9 |
| | 2 | 1 |
| 2. 'P' deficiency | 35 | 11 |
| 3. Blast~ 350 | ~ 150 | |
| 4. Brown plant hopper | 75 | 35 |

cyto-sterility. More than 90% of them were found to be resistant to blast and about 75 showed resistance to BPH. Nearly 35 entries performed better than the check variety Rasi under 'P' deficient conditions. With regard to Teqing introgression lines, of 170 screened, 75 were found to be good under limited water conditions, of which nine were identified as restorers and one as a maintainer. Nearly 150 entries showed resistance to blast and 35 to BPH. Under 'P' deficient conditions, 11 entries showed better performance over the tolerant check variety Rasi.

The multiple stress tolerance features of selected introgression lines of IR64 and Teqing are presented in table 2 (a, b). A selected combination of these lines as parents should enable the plant breeders to cut short the breeding cycle in pyramiding the genes for different stresses. For example, a cross between line 175 and line 310 within the Binam donor will allow plant breeders to develop new restorers combining limited water tolerance with all other stresses in two cycles of selection. Since all these lines are expected to share 87% of the recurrent parent genome, their progenies will segregate at only a few loci and attain homozygosity rapidly. Similarly, the level of tolerance to water stress of Teqing introgression line 9, which is a maintainer, can be improved with ease by crossing it with another maintainer line, 20.

On the other hand, the screening against root knot nematodes of nearly 200 introgression lines of IR64 and Teqing indicated that Teqing introgression lines were relatively more susceptible compared to IR64 introgression lines. Of the 100 Teqing lines screened, only four recorded less than 3 galls / plant and, interestingly, all of them were derivatives of Teqing / Binam cross.

**Table 2 (a). Performance of selected introgression lines of IR64 against multiple stresses**

| Donor | Line No. | Limited Water | Screening tests | | | Restorer / Maintainer |
|---|---|---|---|---|---|---|
| | | | 'P' Deficiency | Blast | BPH | |
| Binam | 175 | √ | √ | √ | √ | R |
| | 205 | √ | √ | √ | √ | - |
| | 310 | - | √ | √ | - | R |
| BR 24 | 22 | √ | - | √ | - | R |
| | 43 | √ | - | √ | √ | R |
| | 51 | √ | √ | √ | √ | - |
| | 321 | √ | √ | √ | - | - |
| OM 1723 | 37 | √ | √ | - | √ | - |
| | 159 | √ | √ | - | - | - |
| | 232 | √ | √ | √ | - | R |
| Khazar | 166 | √ | √ | √ | - | - |
| | 203 | √ | √ | √ | - | - |
| | 210 | √ | √ | √ | - | R |
| | 301 | √ | √ | √ | √ | - |
| Type 3 | 59 | √ | √ | √ | - | - |
| | 68 | √ | √ | √ | - | M |
| | 148 | √ | - | √ | - | - |
| | 388 | √ | √ | √ | - | - |

R = Restorer; M = Maintainer ; √ = tolerant / resistant

**Table 2(b). Performance of selected introgression lines of Teqing against multiple stresses**

| Donor | Line | Limited Water | Screening tests | | | Restorer / Maintainer |
|---|---|---|---|---|---|---|
| | | | 'P'Deficiency | Blast | BPH | |
| Binam | 9 | - | - | √ | √ | M |
| | 19 | - | √ | √ | √ | R |
| | 20 | √ | √ | √ | √ | - |
| | 39 | √ | - | √ | √ | - |
| | 52 | - | √ | √ | - | R |
| | 58 | √ | - | √ | - | M |
| | 92 | √ | - | √ | - | R |
| | 108 | √ | - | √ | √ | - |
| | 156 | √ | √ | √ | - | - |
| BR 24 | 43 | - | √ | √ | - | R |
| | 106 | √ | √ | √ | - | - |
| FR 13A | 68 | √ | √ | √ | - | R |

R = Restorer; M = Maintainer ; √ = tolerant / resistant

Selected IR64 introgression lines along with some varieties and hybrids were evaluated in the wet season 2003 to generate information on crop management under different field water situations. Limited water conditions, in general, decreased the mean yield by 13%. The test lines showed differential response to applied 'N' under both water regimes. Among introgression lines studied, the line 123 (derived from IR64 and BR24 cross) was consistent in yield level and ‘N response. With regard to spacing, except for a few entries, closer spacing produced higher yields, irrespective of water regimes. However, the response to closer spacing under limited water conditions was substantial.

## Conclusions

The screening study identified a number of drought tolerant introgression lines possessing tolerance to multiple biotic and abiotic stresses, which are of significant value in further breeding programs and development of varieties and hybrids for limited water situations. Teqing introgression lines were relatively more susceptible to root knot nematodes than IR64 introgression lines. Some of the introgression lines appear to be efficient in nitrogen use under water stress and closer spacing is found to be good under limited water conditions.

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# Challenges and opportunities for maize improvement for drought stressed areas of Ethiopia

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## Introduction

Owing to its high yield per unit area and adaptability to diverse agroecologies, maize (*Zea mays* L.) is among the leading cereals selected to achieve food self-sufficiency in Ethiopia (Benti et al., 1993). It is widely produced in most parts of the country and covers 21% of the nearly 7 million hectares of land under cereal cultivation. Maize production and productivity in Ethiopia has reached the highest level in high potential areas (midaltitude, subhumid agroecological zones) of the country through the use of improved packages generated by the National Maize Research Project. The high potential area covers about 60% in hectarage and contributes more than 80% to the total maize production (Mandefro et al., 1995). On the other hand, the drought stressed maize growing areas occupy about 40% of the maize growing areas, but contribute less than 20% to total maize production.

## Maize production constraints in stressed areas

Most of the improvement work done in the past emphasized the high potential area, and as a result, many improved varieties / hybrids with improved management practices have been made available to farmers in these areas. In contrast, in most of the drought stressed areas, there are few improved varieties released and those few being grown are usually under low levels of management. The reason is that maize research for moisture stressed areas began late and proceeded slowly with very limited facilities and resources; the breeding activities were handicapped by a narrow genetic base of available germplasm, and the selection criteria for drought tolerant maize varieties were not well established (Mandefro et al., 1995). Additionally, the maize productivity gaps between stressed and high potential areas are not only an issue of technology per se, but also differences in climatic factors. The stressed areas are characterized by erratic rainfall conditions that significantly affect the production and productivity of maize with grain yield normally not exceeding 1.3 t / ha. Unavailability of suitable varieties is responsible for this significant yield gap. The amount of grain being lost due to drought in moisture stressed areas every year could have supported millions of people for daily sustenance.

## Maize breeding for drought stress

Germplasm development for drought stressed areas of Ethiopia was started by Awasa College of Agriculture in 1976 and the Nazareth Maize Program in 1993, to reduce farmers' risk of crop failure. To date, one extra-early variety (Melkasa-1) was released for moisture stress areas, and Pool 17 EEV was identified as the most promising for an extra-early variety. Four early

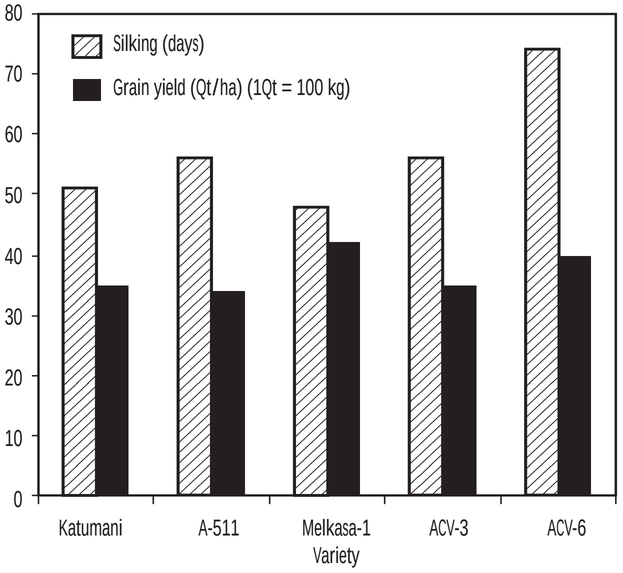

Figure 1. Grain yield and days to silking for popular maize varieties grown by farmers in drought stressed areas of Ethiopia.

varieties (Fetene, Tesfa, Degaga (SEW-1), and Katumani) were released. Two medium maturing open pollinated varieties (ZM-521 and SADVE $F_2$), noted for their high yield under stress were released very recently . Two drought tolerant populations (A-511 and Melkasa-3) were released as a source for potential varieties for moisture stressed areas. In addition to these, BH140 is recommended for irrigated areas because of its early maturity. A Quality Protein Maize (QPM) variety (CML144 x CML159 x CML176), already released for high potential maize growing area, can also be used for growing under supplemental irrigation. DTP-1 x Dendane was identified as a potential nonconventional hybrid for its higher heterosis and grain yield (66 q/ha) (Mandefro et al., 2001). From recent trials, promising genotypes were identified based on yield potential, desirable agronomic traits, and tolerance to major biotic and abiotic stresses (Table 1).

**Table 1. Mean grain yield, days to anthesis and silking of promising genotypes identified through evaluation at different drought stressed locations over years (1Qt = 100 kg)**

| | | Days to | |
|---|---|---|---|
| **Pedigree** | **Grain yield Qt/ha** | **Anthesis** | **Silking** |
| MS-Comp. 1 | 25.2 | 57 | 59 |
| MS-Comp. 2 | 25.8 | 60 | 62 |
| MS-Comp. 3 | 26.2 | 60 | 62 |
| MS-Comp. 5 | 26.6 | 60 | 61 |
| Nubaria 9146 | 25.1 | 55 | 58 |
| Across 9146 | 29.0 | 57 | 57 |
| Across 9346 | 28.2 | 55 | 56 |
| Pool 16 BN C5 | 27.8 | 59 | 59 |
| Pool 16 BN Seq. C0 225 (FAM) | 27.5 | 59 | 61 |
| Melkasa-I (check) | 25.1 | 51 | 51 |
| [92 SEW-2]#-# | 34.4 | 53 | 56 |
| TEWD SR- DR. TOL/SYN POOL27 (W-FLINT) F3-# | 36.8 | 52 | 55 |
| POOL 15 C7 QPM | 31.8 | 56 | 59 |
| Acr. 95 DMSRE-W | 34.2 | 64 | 69 |
| TEWD-SR Dr tol Synth/NAW5867/P30-SR($S_2$)#-# | 36.4 | 65 | 71 |
| Mean | 29.2 | 57.4 | 59.5 |

## Future directions for drought stress breeding

Maize improvement for drought stress in Ethiopia should be considered as the major challenge for future maize breeders. To maximize the contribution of the maize improvement program to dry land agriculture, the following points should be emphasized:

- The materials already identified should be intensively used in breeding program for stress tolerance.
- Augment the breeding work with agronomic practices (soil and water conservation techniques). There will be a need to develop packages of agronomic practices associated with the development of varieties.
- Refine selection strategies for drought tolerance.
- Strengthen farmers participation in developing drought stress tolerant varieties.
- Strengthen collaborative work with CIMMYT, IITA, and national programs of the neighboring countries.
- Convert promising genotypes to drought tolerant versions.

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# Using near-isogenic introgression lines to map rice genes conferring drought tolerance

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## Introduction

Rice is the main staple food crop worldwide. Drought stress is the major constraint to rice production and yield stability in water-limited environments. Improving drought tolerance of high-yielding rice is a serious challenge to breeders. In order to better understand the mechanism underlying drought tolerance and to improve water-use efficiency of elite rice cultivars, large-scale backcross breeding activities have been initiated in China and other Asia countries (Yu et al., 2003). The objective of the program is to construct near-isogenic introgression lines (NIILs), and use the NIILs to map genes associated with drought tolerance, with the goal to develop elite hybrids that are well adapted to drought-prone environments. In our study, more than 120 varieties of diverse origins were backcrossed as donors to two elite hybrid parents: Zhenshan 97B, a maintainer of the most widely cultivated elite three-line hybrid (Shanyou 63), and 93-11, the restorer of a currently used two-line hybrid in China. A series of NIILs containing only small DNA segments derived from different donors in the same genetic backgrounds were obtained by massive backcrossing.

## Methods

Initial evaluation of the NIILs under terminal stress was performed during the dry season at Hainan and normal growing season at Wuhan in 2002-2003. At the two screening sites, recurrent parent as check was grown together with every 20 NIILs in an experimental plot, and a tolerant check variety (IRAT 109) was planted in the same experimental field. Each line was planted in two rows with 12 individuals per row. A stress management scheme as described previously was applied on the NIILs during reproductive stages (Saxena and O'Toole, 2002). In brief, removing water or withholding irrigation was started one week before panicle emergence, and maintained for two weeks. Severe stress was thus developed during the flowering time. After all heading completed, the stress was relieved by surface irrigation. The percent of spikelet fertility and grain weight per plant were visually scored at maturity. Those introgression lines with more filling grains than neighboring checks under the terminal drought stress were selected. Another screening of NIILs was conducted at seedlings stage by plotted experiment. The seedlings were stressed one week at the beginning of three-leaf stage by adding 0.3% NaCl solution into the pots. A total of 180 SSR markers, evenly distributed on the rice chromosomes according to the published map (Temnykh et al., 2000), were used for genotyping the selected NIILs.

## Results

More than 4,200 introgression lines with the same genetic background were evaluated at the stress environments. The NIILs with more grains per plant and less reduction in grain filling than the recurrent parent and the check variety (thus indicating tolerance to the terminal drought stress) were chosen. One hundred and five (105) promising NIILs that derived from various donors (Table 1) in the genetic background of Zhenshan 97 showed tolerance to drought stress, both in the reproductive stage and the seedling stage; they were obtained for further trials and also for molecular genotyping. The preliminary results of the genotyping suggest that at least 20 different genomic regions are associated with drought tolerance, with many of the loci having multiple alleles reflecting allelic polymorphisms on the same chromosomal segments from various donors. Meanwhile, through searching homologous sequences of two known functional genes of salt / drought tolerance in gramene database, we found three tagged markers, respectively, on chromosomes 4 and 12, which detected polymorphisms among the NIILs and the recurrent parent. These markers may be useful for germplasm screening and marker-assisted pyramiding of different favorable alleles for drought tolerance.

**Table 1. Some putative tolerant NIILs containing only small segments derived from various donors in the genetic background of Zhenshan 97.**

| Donors | Origins | Subspecies | Selected NIIL numbers |
|---|---|---|---|
| SADAJIRA 19-303 | Bangladesh | *Indica* | 5 |
| PUSA | India | *Indica* | 5 |
| KANALAMK | India | *Indica* | 6 |
| IR65482-4 | IRRI | *Indica* | 6 |
| TUN SART | Vietnam | *Indica* | 6 |
| SLG-1 | China | *Japonica* | 10 |
| CHAOJI-HANDAO | China | *Japonica* | 4 |
| BASMATI 370 | India | *Japonica* | 8 |
| IRAT26 | Indonesia | *Japonica* | 10 |
| YUE-GUANG | Japan | *Japonica* | 5 |
| KHOLE MARSHI | Nepal | *Japonica* | 6 |

## Conclusions

The development of NIILs with a uniform genetic background provides valuable genetic resources for the discovery of genes associated with drought tolerance. One of the advantages using the population of NIILs is that it overcomes the limitations of asynchronic flowering time that frequently occurs in segregating populations that often confound straightforward evaluation of tolerant performances under terminal stress. Another merit of the NIILs is as a useful start point to produce near isogenic lines for fine mapping and cloning the genes of interest. Moreover, those NIILs carrying favorable alleles in the elite genetic backgrounds might be good candidate varieties with enhanced tolerance to drought.

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# Root growth responses to water: Interaction between hydrotropism and gravitropism in *Arabidopsis* wild-type and no-hydrotropic response mutant

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Roots are capable of constructing a three-dimensional perspective of their local space by sensing and responding to a variety of stimuli. By doing this, roots actively forage resources from their environment. Roots have positive hydrotropic response and modify their growth direction in search of water overcoming their positive gravitropic response. Using a screening system with a water potential gradient, we isolated a non-hydrotropic response (*nhr*) semi-dominant mutant of *Arabidopsis* that continued to grow downwardly into the medium with the lowest water potential, contrary to the positive hydrotropic and negative gravitropic response seen in wild-type roots. The lack of hydrotropic response of *nhr1* roots was confirmed in a system with a gradient in air moisture. The root gravitropic response of *nhr1* seedlings was significantly faster in comparison with those of the wild-type. The enhanced gravitropic response of *nhr1* roots might suggest that the reduction in the strength of hydrotropism increases the net effectiveness of gravitropism.

# Carbohydrate accumulation and remobilization of upland and lowland rice in response to water deficit at various development stages

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## Introduction

Rice yields are most susceptible to reduction by stress at flowering (Chaturvedi et al., 1996). Drought at flowering causes irreversible loss of leaf area and reduced photosynthesis, which in turn increases the proportion of unfilled spikelets. When rice suffers flowering stage drought, grain growth is largely supported by reserve materials (carbohydrate) accumulated in culm and leaf sheath before stress and remobilized during stress (Yang et. al., 2001, Garg et. al., 2002, Cabuslay et. al., 2002, and Chaturvedi and Ingram, 1989).

## Methods

Pre-germinated seeds of upland and lowland rices were sown in plastic buckets (20 kg capacity) filled with typical upland and lowland soil. The lowland soil was puddled before seed was sown. Fertilizer was applied at 30-20-20 mg NPK/kg dry soil in both upland and lowland soil. The five upland cultivars were, UPLRi 5, IRAT140, Kinandang Patong, N22, and Sathi 28229. The five lowland cultivars were IR64 Salumpkit, IR46, IR54, and Mahsuri. Four water treatments were (i) control, (ii) water deficit at tillering, (iii) water deficit at booting, and (iv) water deficit at flowing. Water deficit was imposed by withholding water for 7-8 days until soil moisture tension at 15 cm soil depth reached 70 Kpa in the lowland and 80 Kpa in the upland trial. Apparent Translocation Rate (ATR), the post anthesis decrease in culm dry weight relative to increase in panicle dry weight was calculated by the formula of Reyniers et al. (1982).

## Results

In general, cultivars that had higher levels of carbohydrate before flowering also had higher yield under both normal and water deficit conditions. A strong positive correlation was observed between flowering stage carbohydrate in all plant parts and yield (Tables 1-3) No significant relationship was found between tissue carbohydrate at tillering and yield. Among different treatments of single cultivars, yield was inversely correlated with apparent translocation rate in both upland and lowland conditions. Among cultivars, yield was directly correlated with maximum ATR. Among lowland cultivars, IR46 maintained better translocation under

**Table 1. Correlation coefficient between filled grain/ hill and carbohydrate content of different parts. (Stress and control data combined)**

| | Time of water deficit | | | | | |
|---|---|---|---|---|---|---|
| | Tillering | Booting | Flowering | Tillering | Booting | Flowering |
| Plant part | | Upland | | | Lowland | |
| Leaf blade | 0.60 | 0.42** | 0.76** | 0.23 | 0.42** | 0.78** |
| Leaf sheath | 0.20 | 0.48** | 0.73** | 0.20 | 0.49** | 0.51** |
| Culm | 0.13 | 0.49** | 0.73** | 0.14 | 0.19 | 0.46** |

**Significant at P<0.01

**Table 2. Correlation coefficient between yield and carbohydrate content of various plant parts. (stress and control data combined)**

| | Time of water deficit | | | | | |
|---|---|---|---|---|---|---|
| | Tillering | Booting | Flowering | Tillering | Booting | Flowering |
| Plant part | | Upland | | | Lowland | |
| Leaf blade | 0.02 | 0.50** | 0.80** | 0.21 | 0.47** | 0.85** |
| Leaf sheath | 0.19 | 0.51** | 0.82** | 0.20 | 0.41** | 0.66** |
| Culm | 0.12 | 0.45** | 0.72** | 0.20 | 0.26* | 0.52** |

*, **Significant at P<0.05 and 0.01, respectively.

**Table 3. Yield and yield attributes of three rice genotypes under well watered (c) and water–deficit (WD) conditions (80 kPa) at flowering**

| Cultivar | Grain yield g/pot | | Spikelet/ panicle | | Individual grain weight (mg) | | Unfilled grain (%) | |
|---|---|---|---|---|---|---|---|---|
| | C | WD | C | WD | C | WD | C | WD |
| IR-46 | 20.2 | 14.0 | 105 | 83 | 23 | 20 | 16.5 | 30.5 |
| N-22 | 16.4 | 12.4 | 87 | 71 | 19 | 18 | 20.0 | 30.5 |
| IR 52 | 17.9 | 9.0 | 107 | 87 | 24 | 19 | 19.7 | 63.8 |
| LSD 0.05 | 1.1 | 3.0 | 1.1 | 3.1 | | | | |

stress than did other cultivars. Cultivar IRAT140 had higher translocation under stresses among upland cultivars. Higher yields of these cultivars can be attributed to greater increases of ATR under stress. One goal of plant breeders is to produce germplasm with stable yields. One measures of yield stability is the slope of ATR *vs* yield regression, the smaller the slope, the more stable the yields (Figures. 1-3). In this experiment, N22 had the lowest slope and a low crop susceptibility factor, but relatively low yield. On the other hand, IR64 had the greatest slope and a higher susceptibility factor, but greater yield than N22. Yield stability, while related to soluble carbohydrate accumulation and remobilization, does not assure higher yield.

## Conclusion

A preferable cultivar would be one with high yield potential and moderate yield stability, for example, IR64 for lowland and IRAT 140 for upland conditions. These cultivars also had the greatest increase in ATR under stress conditions.

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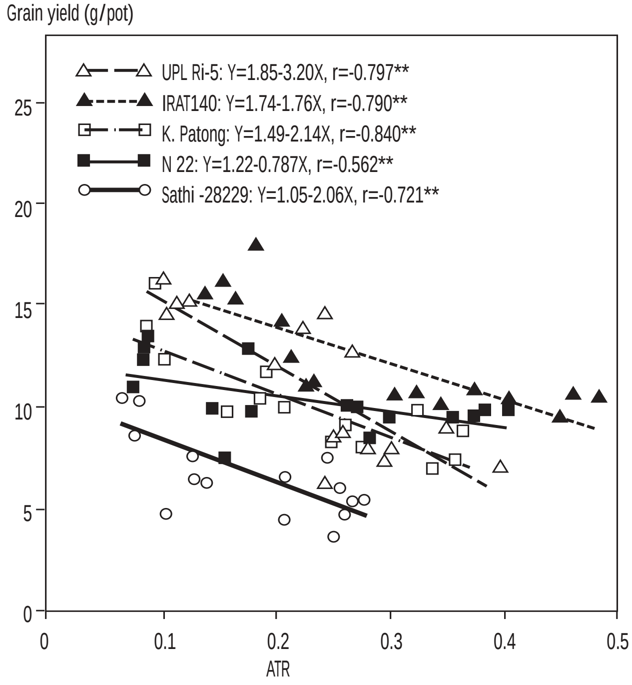

**Figure 1. Relation ship between yield and apparent translocation rate in five upland rices subjected to water deficit at different developmental stages.**

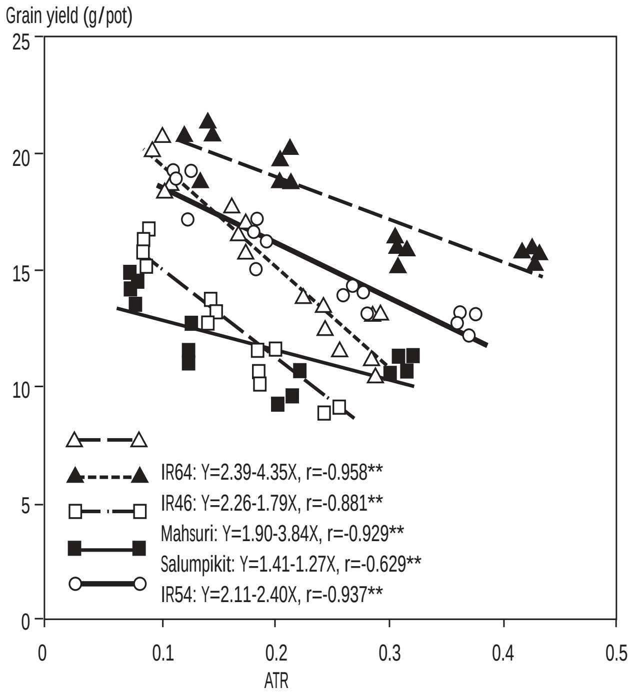

**Figure 2. Relationship between yield and apparent translocation rate in five lowland rices subjected to water deficit at different developmental stages.**

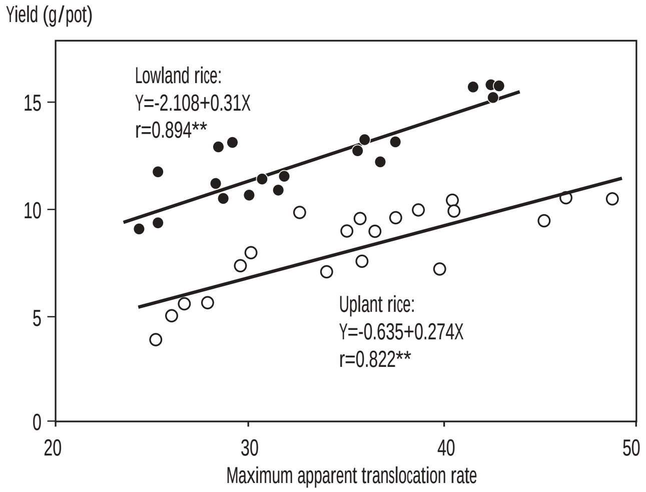

**Figure 3. Relationship between maximum apparent translocation rate and yield in upland and lowland rice genotypes. Data points are for individual pots from treatments greatest apparent translocation rate for each cultivar and regressions are across cultivars from the same ecosystem.**

# Relation between carbohydrate metabolism and drought resistance in 'Pinto Villa,' a drought resistant common bean variety

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## Introduction

In Mexico, most of the common bean (*Phaseolus vulgaris* L) production is concentrated in arid and semiarid regions. For this reason, it has been important to develop varieties with an improved tolerance to water-limited environments, as well as to elucidate the mechanisms involved in the drought tolerance process in this crop. Drought escape is one of the drought adaptive mechanisms observed in common bean by which plants shorten their life cycle to avoid water stress. This response to water deficit involves a faster remobilization of assimilates from leaves to seeds (Samper and Adams, 1985; Acosta-Gallegos and White, 1995). Although there is a wealth of information about the translocation of assimilates from source to sink in plants and the different players in the process, there are still many key points that remain to be investigated. Among those are the components that trigger the activation of this process under water stress conditions as well as its regulation.

One of the products of the common bean improvement program at INIFAP was the drought resistant cultivar 'Pinto Villa.' Its characterization has shown that this variety leads to higher productivity and crop index when compared to drought susceptible cultivars, such as 'Canario 60,' when they are grown in semiarid regions in Mexico (Rosales-Serna et al., 2000). It has been observed that in response to terminal drought a common pattern in this cultivar is an acceleration of its life cycle concomitant with the loss of mature leaves. These results have led us to propose that drought resistance in this variety is related to an increased assimilates mobilization from leaves (source tissues) to pods (sink tissues) in response to a moderate terminal drought.

## Methods

To get insight into this phenomenon, we designed greenhouse experiments with eight repetitions, in which plants of two common bean cultivars, 'Pinto Villa' (drought resistant) and 'Canario 60' (drought sensitive), were grown under optimal irrigation or water deficit conditions. Bean plants were grown under well-irrigated conditions (field capacity) until flowering; at this point, watering was restricted up to 60% of the field capacity, to produce a moderate drought. Plants were incubated during 2 h in the presence of $^{14}CO_2$. After 6 days, the radioactive $CO_2$ assimilates (starch and soluble carbohydrates) were quantified in source and sink tissues. In parallel, similar determinations were obtained from well-irrigated plants, which were maintained as controls of the experiment.

## Results

The results from these experiments indicated that the imposed water limitation treatment induced starch accumulation in pods from both varieties and accelerated carbon assimilates distribution in 'Pinto Villa' (drought resistant) when compared to 'Canario 60' (drought sensitive). Figure 1 shows that $C^{14}$ incorporated in pods from Pinto Villa increased in response to mild drought treatments. Consistent with this observation, these stress conditions simultaneously induced a reduction in 'Pinto Villa' leaves starch levels, in contrast to those found in the susceptible cultivar.

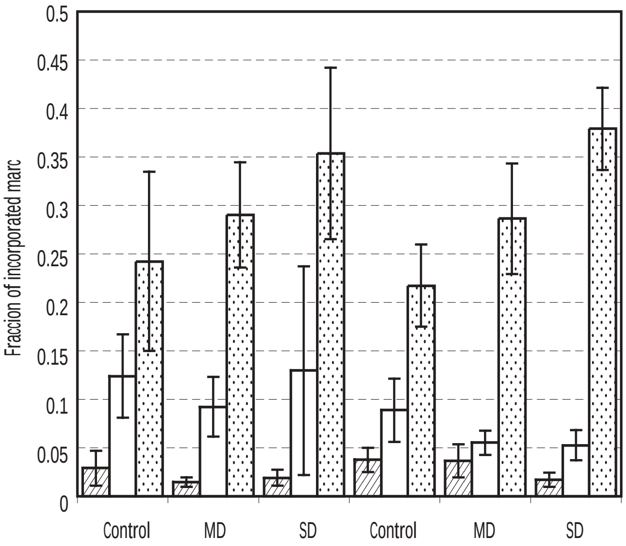

**Figure 1. C$^{14}$ partition in plants of ‘Canario 60’ and ‘Pinto Villa’, common bean varieties drought susceptible and drought tolerant respectively. Plants (4 weeks) of ‘Pinto Villa’ and ‘Canario 60’ were exposed to drought treatments: 60% of the water need for normal field capacity for Moderated Drought (MD); 30 % of the water need for normal field capacity for Severe Drought (SD). The controls were grown in well-irrigated conditions. The error bars show standard deviations of 6 plants.**

## Conclusions

The data in this work support the hypothesis that drought resistance in ‘Pinto Villa’ is related to an increased assimilates mobilization from leaves (source tissues) to pods (sink tissues) in response to a moderate terminal drought, and suggest that the modulation of carbon mobilization has been a successful adaptive strategy during exposure to drought conditions usually present in the field.

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# A potentially new screening method for tolerance of plants to limiting growing conditions

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This paper aims to stimulate interest in, and increase knowledge of, the concept of screening of plant species for limiting growing conditions.

A simple new screening method was designed somewhat in accordance with the approach followed by Marie Curie, for the discovery of the element radium around 1900. Breeding material of South African land race collections of pearl millet was tested (71 entries, 4 to 8 plants per entry) and sorghum (61 entries, 6 to 10 plants per entry). Plants were left to survive in polystyrene young-plant-trays, on a floor mat. The tray had 200 cells, each with a small volume (40-45 cc), limiting the root volume and the water quantity. The composted pine bark (seedling mix), had a low water holding capacity and excess water almost immediately passed through the plug. The overhead irrigation was applied at 10 mm / h, for short periods (10 to 15 minutes per application), and at low daily quantities (< 6 mm per day). The relatively small cell area to receive water was partly covered by growing plants, further reducing the availability of water to the roots. Tunnel temperatures rose up to a maximum of 45∫C, resulting in high daily evaporation. Thus water and nutrients available to the plants were probably severely limited.

At 120 days after sowing, 53% of the pearl millet plants survived, versus 5% of the sorghum plants. Root quantity tended to be larger and the quality (% white root area with less necrotic tissue) tended to higher in sorghum (95% white) than in pearl millet (65% white). The development stage (Decimal Code Zadoks) of the plants differed between 32 and 54 in sorghum and between 32 and 63 in pearl millet. Plants frequently remained vegetative (development stage < 35): 80% in sorghum and only 68% in pearl millet. Several volunteer weed species also survived, both mono- and dicotyledons. Many of the surviving ‘pearl millets’ after transplantation in the field appeared to be offspring from crosses between pearl millet and wild grass species, and often exhibited seed shattering.

Large quantities of plants could be tested in a small-contained environment. Pearl millet appeared to survive more frequently than sorghum. The relationship between root quality and crop survival could be studied easily. The healthy sorghum roots enabled the uptake of water and nutrients. With the relatively healthy roots after limiting conditions, surviving sorghum plants remained able to recover. However, a portion of the plants died or became generative. With the healthy roots, sorghum can also produce new tillers and heads. At 120 days after sowing, already 32% of pearl millet plants were in the generative stage, and some were even flowering. Pearl millet roots were partly degenerated and this likely forced the plants into seed production for survival. These findings are in correspondence with field tests and literature.

Control and measurement of effective water and nutrients were missing. Polystyrene easily degenerates after extended use. However, this artificial method created differentiating conditions for screening. It may possibly be used to test GMOs and conventional plants of different plant species for tolerance against drought or nutrient deficiency or surplus. Probable improvements for the screening method are young-plant-trays made of durable hard plastic, using air pruning (tray resting on wire support, or tray on legs). We should continue the use of very small plugs (200 cells per tray, 40-45 cc), but define the water holding capacity of the medium. Better control of the quantity and quality of the water and the additional nutrients could be obtained by using drip irrigation.

# Improving drought resistance in rainfed rice for the Mekong region: Defining target population of environments (TPE), characterizing the available water and breeding for better adaptation to the variable water supply including an overview of the project "Improving drought resistance in rainfed lowland rice for the Mekong region"

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## Introduction

Rainfed lowland rice is grown in highly heterogeneous drought-prone environments throughout Asia (Fukai et al., 1997; Wade et al., 1999). The large genotype-by-environment (GxE) interaction for yield has complicated the definition of the appropriate breeding objectives. Genetic improvement of adaptation to the abiotic stresses associated with drought, submergence, and nutrient toxicities and deficiencies has been identified as a breeding target to achieve higher and more stable yields (Fischer, 1996; Mackill et al., 1999). This paper focuses on the variation in water supply as it determines the target population of environments (TPE) where a given genotype will be grown and as it defines the strategy and traits for breeding and selection. Fukai and Cooper (2001) have reviewed the complexity of breeding for the variable supply of water in the rainfed ecosystem and have proposed an "adaptation model"(Figure 1) that shows the interaction of three selection objectives—increased yield potential, matching phenology to water supply, and drought tolerance—under different intensities of drought.

The paper reports the results of large number of multi-environment trials (METs) conducted in three countries in the Mekong region to identify TPE and to evaluate the robustness of the adaptation model shown in figure1. The paper also reports on a regional project "Improving drought tolernace in rainfed lowland rice for the Mekong region: use of new conventional approaches and molecular tools."

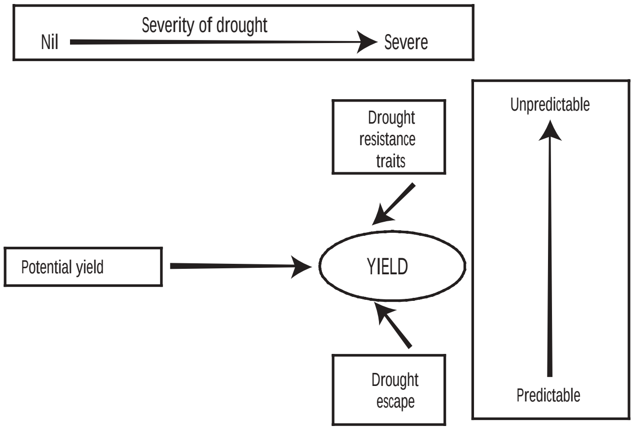

**Figure 1.** Schematic diagram of 3 components, potential yield, phenology and drought resistant traits and yield relationship in different types of drought in rainfed lowlands.

## Materials and methods

a) ***Defining TPE and breeding approach for rainfed lowland environments.***
Over 100 multi-environment trials (METs) have been conducted and analyzed to define the TPE for the rainfed lowland rice systems in Thailand, Cambodia, and Laos. In each site, the water supply was also measured as the depth of water above and below the soil surface. In a number of sites, additional experiments of imposed drought and irrigated treatments were also conducted to assess the response of materials to drought and to use a measure of the water environment to “predict” the intensity of the drought.

b) ***Selection for drought tolerant traits, drought tolerant donor lines, and new populations for drought tolerance in the Mekong region.***
One thousand, one hundred and forty-nine (1,149) mainly landrace rice materials were evaluated under imposed drought and irrigated water treatments at five locations in the Mekong region in 2002 and six in 2003. The lines were evaluated for the drought tolerance traits of drought response index (DRI), leaf water potential (LWP), flower delay, and spikelet sterility.

## Results and discussion

The components for variation in yield for each of the countries are shown in table 1. In each country, the GxE component was large. However, the G component was also significant, demonstrating an opportunity to develop varieties with broad adaptation to the environments. In Thailand and Laos, the GxY component of the GxE was large, necessitating testing across years. In Cambodia, the GxL component was large, allowing for the definition of TPE by locations.

**Table 1. Estimated components of variance for grain yield (t ha$^{-1}$) of genotype, genotype-by-year, genotype-by-location, genotype-by-year-by-location interactions, residual for the multi-location trials conducted with random rice populations in three countries in SE Asia**

| Source of variance | Thailand (MET) | Laos (toposequence) | Cambodia (MET) |
|---|---|---|---|
| Genotype (G) | 0.060 | 0.634 | 0.053 |
| G´Year (Y) | 0.049 | 1.161 | 0.010 |
| G´Location (L) | 0.003 | 1.963 | 0.051 |
| G ´ Position (P) | - | 0.231 | - |
| G´Y´L | 0.259 | 2.151 | 0.189 |
| Residuals | 0.440 | 22.341 | 0.047 |
| Proportion G: G´E | 6.18 | 9.6 | 5.7 |

In Laos, the GxL was also large, with some G x toposequnce position variance.

The relationship between grain yield reduction and water levels (below ground) during flowering (3 weeks early and 3 weeks after flowering) in nine drought screening experiments with 15 genotypes in Cambodia is shown in figure 2. The stress intensity ranged from moderate (30%) to severe (75 %) reduction in yield. This simple measure of the water environment can account for 43% of the variation in grain yield. The method can describe the water environment for each genotype and thus reduce the effect of phenology on the evaluation of the G x drought response analysis.

Physiological based screening studies have identified the integrative drought tolerant traits of DRI, LWP, flower delay, and spikelet sterility as useful for differentiating genotypes responses to drought in the different TPEs in the region.

## Conclusions

1. These studies have identified TPEs for each country and groups of genotypes that perform well within the TPE across the years. The study has also validated the robustness of the adaptation model (data not shown); genotypes with high yield potential contribute to broad adaptation and to higher yields under less severe drought, and

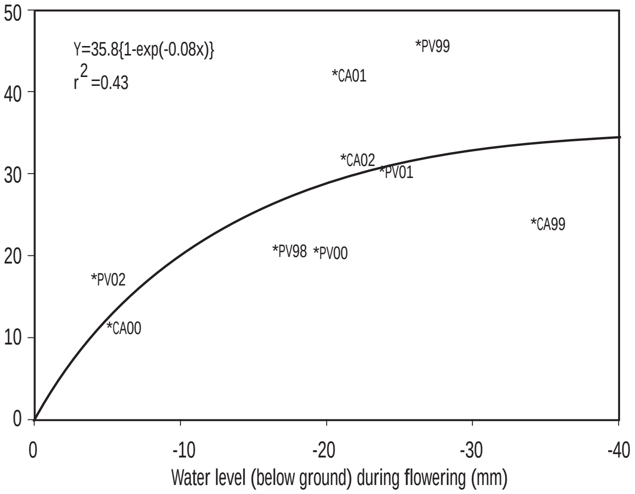

**Figure 2. Relationship between grain yield reductions and water levels (below ground) during flowering (3 weeks early and 3 weeks after flowering) in nine drought-screening experiments with 15 genotypes in Cambodia.**

genotypes of appropriate duration and with drought tolerant traits perform well under severe and terminal drought.

2. A method to characterize the water environment independent of yield has been developed for use in adaptation analysis. Now the water environments of each genotype can be described allowing for a better comparison of the performance under the same drought intensity
3. Approximately 15 new populations have been developed based on donor lines that have been selected and validated for the drought tolerant traits. The plans for the selection and improvement of these populations, and for their molecular analysis of the drought traits, will be discussed.

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# Improving drought resistance in rainfed rice for the Mekong region; the experience from Laos in the selection of drought tolerant donor lines for the target population of environments (TPE) based on yield and on leaf water potential (LWP), flowering delay, and drought response index (DRI)

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## Introduction

In Laos, rainfed rice area occupies more than 67% of the total rice grown area and drought is a major problem. Earlier work in rainfed lowland rice in Thailand (Cooper et al., 1999) has recommended modifications to the plant breeding process based on, in part, (i) better assessment of the target population of environments (TPE), (ii) early yield selection in the TPE, and (iii) the judicious combination of yield potential, appropriate flowering and drought tolerance adaptive mechanism, depending on the severity of the drought in the TPE (Fukai and Cooper, 2001). The work of Fukai et al. (1999) and Pantuwan et al. (2002) has identified the putative traits of maintenance of leaf water potential (LWP), reduced flower delay, reduced spikelet sterility and high drought response index (DRI) as the most useful for selection for improvement of yield under moderate to severe drought stress.

This paper reports on the identification of appropriate TPEs for the development and delivery of improved rice cultivars for the rainfed lowlands in Laos. It examines the relationship among putative drought traits and grain yield under imposed drought in the TPE and the genetic variation for the putative drought traits in the local land race materials. The paper also reports on the development of populations using putative drought tolerant lines as the donor parent and on the plans for further phenotyping and selection and molecular analysis of these populations.

## Materials and methods

### *A. Identification of the TPE*

A series of multi environment trials (METs) comprising 30 genotypes (G) (mainly from the Laos breeding program and including materials from the ACIAR-Thai project) were grown at two locations in 2000, at four locations in 2001, and at two locations in 2002. At each location (L) a separate trial was established at the upper, middle, and lower position (P) of the natural toposequence. The plants were established and fertilized using conventional methods. The water table and rainfall were monitored at each site. Data of grain yield and flowering were collected for analysis.

Because of the imbalance of locations and years (Y) the data were initially analyzed with locations, years, and position as environments (E). The G´E variance for grain yield was used to cluster the environments using pattern analysis. A second analysis was conducted in one location (Phongthong), which was representative for rainfed cultivation. Here the analysis examined the G, G´Y and G´P effects.

### *B. Phenotyping for putative drought tolerant traits*

Eighty lines, including 22 from local (landrace) materials, were evaluated under imposed drought at Vientiane and Pakse in Laos in the 2002 wet season. The trials at both locations consisted of two water treatments (an irrigated control and an imposed drought). The experiments were established somewhat later than the normal sowing date to increase the likelihood of drought occurring during grain filling. The water was drained from the field 25 days after transplanting to impose the drought treatment. There was poor germination of some entries in the Pakse experiment and data were collected on only 68 entries.

Measurements were taken on flowering date, grain yield under irrigated and drained conditions, and LWP under drought. The drought response index (DRI) was estimated as described by Bidinger et al. (1982). The lines were grouped by the duration of exposure to drought (days from the commencement of drought to flowering) and selection for drought tolerant liens was made within groups (i.e., groups exposed to similar drought conditions)

The screening trial was again repeated with some additional landrace materials in the 2003 wet season at three sites. The data are not yet available for analysis but will be presented at the workshop.

## Results

### *A. Identifying the TPE*

There was a significant G and G´E effect with the variance for the G´E being 25% of that of the G. The grouping of the environments was truncated at the three levels and this accounted for 56% of the total variance. The different environmental groups appeared to be due to location and to the use of supplementary irrigation, particularly in the upper toposequence, which is often near the household. Thus the locations Nkaou and Kanthbouli and the locations Pakse, Sonkhong, and Phongthong (only when flooded) formed two TPEs, largely irrigated or with supplemental irrigation. The Phongthong location formed the third environmental group and appears to be a TPE subjected to some drought.

The analysis of the G, G´Y and G´P effect in the drought prone TPE showed that there were significant G, G´P and G´Y effects. The G´P and G´Y appeared to be associated with difference in maturity—the later flowering material performing poorly under the upper toposequence positions and low rainfall years (Figure 1).

### *B. Phenotyping for drought tolerant traits, 2002 wet season*

The levels of water above and below the ground for the two water treatments for Pakse and NARC are shown in figure 2. The draining was effective in developing drought stress from flowering onward only at the Pakse site, where yield was reduced by 17%, providing an opportunity to select lines for the drought tolerance traits of flower delay, LWP, and drought response index (DRI).

There was no significant genotype by water interaction under the mild drought stress, and grain yield under stress was significantly correlated with yield potential ($r^2$ =0.83). There was a large range in

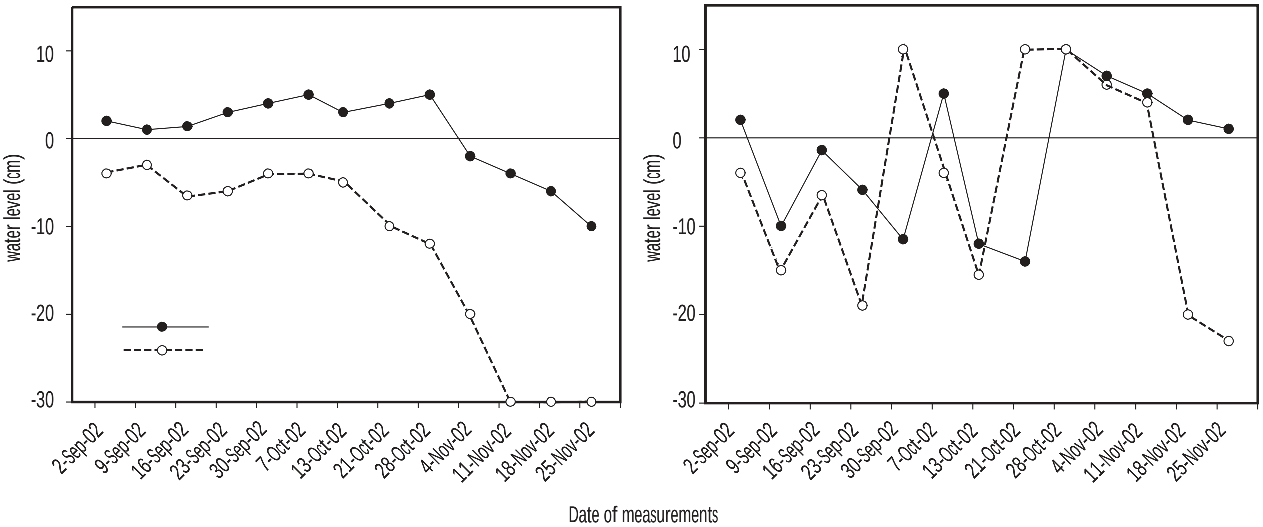

**Figure 1. The relationship between flowering date and grain yield of late flowering genotypes (long exposure) grown under a mild drought stress and irrigation around flowering at the Pakse 2002 wet season.**

flowering among the materials, which were grouped by the duration of exposure to drought from its commencement to flowering. There was no significant effect of flowering date on yield of the genotypes in the short and medium exposure groups. There was a significant difference among entries in DRI, which ranged from –2.41 to +3.06 (Table 1). There was also a significant difference among entries in LWP. Six (6) putative drought donor lines were selected based these drought tolerant traits (Table 1) and used to develop new populations.

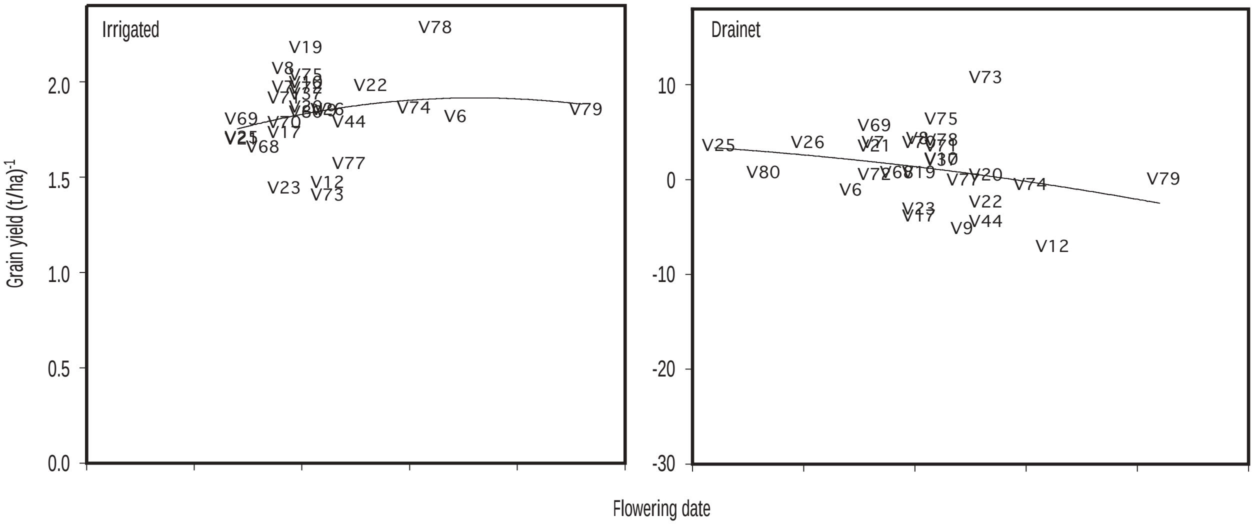

**Figure 2. Water levels in the irrigated (filled symbols) and drained (open symbols) experiments at Pakse and NARC in Laos in the 2002 wet season.**

**Table 1. Flowering date under irrigated conditions, delay in flowering under drought, LWP, grain yield under irrigation and drought, and drought response index (DRI) for rice genotypes at Pakse in the 2002 wet season, The lines were grouped by the duration of exposure to drought (days from the commencement of drought to flowering). Data for the 4-7 most drought tolerant in each exposure group along with checks are presented.**

| | | | | | Grain yield (t ha$^{-1}$) | | |
|---|---|---|---|---|---|---|---|
| *Cultivar* | Drought exposure | Flowering date | Delay flower (days) | LWP (MPa) | Irrigated | Drained | DRI |
| MACK KHAM KON | Short | 14 Oct | -6 | -1.9 | 1.52 | 1.82 | 0.84 |
| SANPATONG | Short | 12 Oct | 4 | -2.3 | 1.15 | 1.56 | 0.34 |
| ANG DO | Short | 15 Oct | -7 | -2.2 | 1.71 | 1.63 | 0.17 |
| I CHANG | Short | 16 Oct | 0 | -1.9 | 1.72 | 1.60 | 0.15 |
| HANG V1 | Short | 15 Oct | 0 | -2.3 | 1.66 | 1.53 | -0.03 |
| **CHAO AMERICA** | Medium | 21 Oct | 9 | -2.2 | 2.03 | 2.02 | 1.43 |
| IR71505-16-1-1-TDK-1-2-B | Medium | 23 Oct | -1 | -2.6 | 1.87 | 1.90 | 1.07 |
| CHAO DENG | Medium | 20 Oct | -1 | -1.9 | 1.85 | 1.87 | 0.94 |
| IR72666-5-TDK-1-1 | Medium | 22 Oct | 2 | -2.2 | 2.69 | 1.98 | 0.88 |
| **IR68101-TDK-31-1** | Long | 30 Oct | 0 | -2.5 | 1.80 | 1.78 | 0.84 |
| **TDK21-B-6-2-1-B** | Long | 2 Nov | 2 | -2.2 | 1.40 | 2.04 | 1.88 |
| TDK21-B-20-16-1-B | Long | 31 Oct | 2 | -2.7 | 2.03 | 1.82 | 0.85 |
| **IR 68796-27-3-B-2-1-TDK-1-B** | Long | 1 Nov | 0 | -2.2 | 1.79 | 1.69 | 0.59 |
| **IR68105-TDK-B-B-6-1** | Long | 1 Nov | 1 | -2.4 | 2.07 | 1.71 | 0.51 |
| IR72738-3-TDK-1-2-B | Long | 2 Nov | -5 | -2.2 | 1.85 | 1.69 | 0.50 |
| **IR57514-TDK-9-1-2** | Long | 1 Nov | -1 | -1.9 | 1.97 | 1.69 | 0.47 |
| RD6 (check) | Medium | 22 Oct | 2 | -2.1 | 1.70 | 1.67 | 0.56 |
| RD10 (check) | Medium | 23 Oct | -5 | -2.8 | 1.91 | 1.21 | -1.04 |
| TDK4 (check) | Long | 6 Nov | -1 | -2.5 | 1.98 | 1.38 | -0.43 |
| TDK1 (check) | Long | 31 Oct | 5 | -2.4 | 1.79 | 1.28 | -0.64 |
| Mean[b] | | | 2 | -2.2 | 1.85 | 1.53 | 0.49 |
| Lsd (p=0.05)[b] | | | 10.7* | 0.45* | 0.57* | 0.46* | 1.45* |

[a] Duration of drought exposure from draining to flowering. [b] Calculated from all 68 cultivars. Lines in **bold** letters were used as donor parents.

## Conclusions

The limited data from the METs suggests that genotypes can be selected for broad adaptation to all environments. The primary target for yield improvement (in addition to quality, pest resistance, and acceptance by the users) is to improve the yield potential while matching the appropriate phenology with the water supply. There may, however, be a need to consider the upper / middle toposequences as a TPE where yields may be reduced by up to 50% (data not shown) by drought and where some selection for drought tolerance (along with matching phenology and improved yield potential) would enhance performance. There were significant differences among lines for DRI and LWP even when differences in the duration of exposure to drought from its commencement to flowering were considered. Populations based on the crosses of the putative donor lines and recipient parents with high yield potential and good quality are being developed and will be available for further phenotyping and selection and molecular analysis.

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# Effects of drought stress during reproductive stages on grain yield and quality of different genotypes in rice (*Oryza sativa*)

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## Introduction

With fast economic development and urbanization, China is facing a serious crisis of water shortages. The estimated annual loss to the national economy from water shortage reaches more than $25 billion in China (Deng, 1999). Rice production alone consumes about 50% of the water resources in China. There is an urgent need for reducing water consumption in rice production. Traditionally, the Yangtze River basin is the major rice production area (70% of the total) in China. Although the wet monsoon brings ample rainfall to Yangtze basin in most years, its arrival date and duration are unstable. The rainfall is erratic and frequently causes drought and / or flooding in this area. Drought has been the single most devastating factor that affects rice production (Lin, 1996). Drought in summer is prevailing and drought stress often occurs at the reproductive stages of the rice crop. Drought tolerance is a complex trait that involves numerous aspects of developmental, physiological, biochemical, and molecular adjustments (Blum, 2002). Therefore, it is essential to understand the effects of the prevailing drought stress in the target environment on both the yield and quality traits in order to undertake the genetic improvement of drought tolerance of the rice cultivars for this region.

## Methods

Seven different rice cultivars (5 upland rice varieties: Caiapo, Carajas, Rio Paranaiba, Canastra; and 2 paddy rice: *shanyou* 64, an *indica* hybrid rice; Ewan 11, a *japonica* variety) were sowen on 16 June and transplanted on 12 July to two locations, respectively. One was in well irrigated paddy field (keeping shallow water layer) and another in a drought screening site under movable rainout shelter. Starting at one month after transplanting, the later location was subjected to moderate to severe drought stress during panicle development and moderate drought stress after flowering. Soil moisture during drought stress treatment was monitored and recorded. Normal yield trial design was used for both locations, with 3 replications of randomized blocks consisting of 3x4 m plots. Variance analysis was made on data of both yield components and grain quality traits of the tested varieties.

## Results

The difference of grain yields between drought stress treatment and well irrigated control was significant at 0.01 probability level. Yield loss was caused by drought stress mainly due to the reduction of panicle size (Table 1). Moderate drought stress during grain filling, on the other hand, may improve grain quality through the increase of head rice and the reduction of grain chalkiness (Table 2). Genotypic differences in drought stress response were also observed. Spikelets filling percentage of upland rice seemed less affected compared with that of the paddy rice; and among paddy rice, the *Indica* rice seemed more susceptible than *Japonica* rice.

## Conclusions

Althought it is difficult to draw conclusions from an experiment conducted in only one year, it showed a few evident impacts: (1) severe drought stress in panicle development stage caused great yield loss by reducing spikelets per panicle, not only in paddy rice but also in upland rice, though yield loss of paddy rice decrease by a bigger margin; (2) upland rice is better adapted to mild drought stress during grain-filling stage. There were no apparent differences in spikelets filling percentage and grain weight between drought stress treatment and normal irrigated control. While both spikelets filling percentage and grain weight of paddy rice decreased under the stress; and (3) Moderate drought stress during grain filling may have positive effects on grain quality.

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**Table 1. Grain yield and yield components of rice cultivars grown under draught stress and well irrigated paddy fields (Wuhan, China,2002)**

| | Grain yield (t ha$^{-1}$) | | Panicles per m$^{2}$ | | Spikelets per panicle | | Spikelets per m$^{2}$ (x10$^{3}$) | | Spikelets filling (%) | | Grain weight (mg) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cultivar** | **DS$^{1)}$** | **CK** | **DS** | **CK** | **DS** | **CK** | **DS** | **CK** | **DS** | **CK** | **DS** | **CK** |
| Shanyou 64 | 2.64 | 7.18 | 355 | 385 | 75 | 114 | 26.6 | 43.9 | 56.1 | 87.5 | 21.9 | 26.0 |
| Ewan 11 | 2.64 | 6.77 | 313 | 352 | 59 | 108 | 18.5 | 38.0 | 85.3 | 87.8 | 24.3 | 27.4 |
| Caiapo | 2.48 | 5.37 | 188 | 213 | 81 | 147 | 15.2 | 31.3 | 79.1 | 78.5 | 28.0 | 28.9 |
| Carajas | 1.88 | 4.81 | 175 | 198 | 60 | 106 | 10.5 | 21.0 | 83.0 | 76.2 | 31.7 | 30.9 |
| Gvrani | 2.58 | 4.79 | 174 | 216 | 71 | 113 | 12.4 | 24.4 | 75.6 | 70.0 | 35.2 | 34.2 |
| Rio Paranaiba | 1.62 | 4.63 | 156 | 206 | 86 | 145 | 13.4 | 29.9 | 78.9 | 73.1 | 34.2 | 32.7 |
| Canastra | 2.26 | 4.40 | 176 | 230 | 60 | 123 | 10.6 | 28.3 | 83.9 | 71.1 | 29.3 | 30.5 |
| Mean | 2.30 | 5.42 | 220 | 257 | 70 | 122 | 15.3 | 31.0 | 77.5 | 77.7 | 29.2 | 30.1 |
| *Analysis of variance* | | | | | | | | | | | | |
| Treatment | 138.16** | | 17.20** | | 258.97** | | | | 0.40$^{NS}$ | | 8.08** | |
| Cultivar | 3.96** | | 34.18** | | 8.93** | | | | 24.37** | | 97.2** | |
| Treatment_cultivar | 1.67$^{NS}$ | | 0.90$^{NS}$ | | 2.12$^{NS}$ | | | | 3.16* | | 7.36** | |

$^{1)}$: DS=drought stress; *, **: *F* values significant at the 0.05 and 0.01 probability levels respectively;
$^{NS}$ = non-significant at the 0.05 probability level

**Table 2. Grain quality and quality components of rice cultivars grown under draught stress and well irrigated paddy fields (Wuhan, China, 2002)**

| **Cultivar** | Brown rice (%) | | Milled rice (%) | | Head rice (%) | | Chalkiness (%) | | Amylose content (%) | | Kernel L/W | | ASV-GT (grade) | | Gel consistency (mm) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **DS$^{1)}$** | **CK** | **DS** | **CK** | **DS** | **CK** | **DS** | **CK** | **DS** | **CK** | **DS** | **CK** | **DS** | **CK** | **DS** | **CK** |
| Shanyou 64 | 77.6 | 79.7 | 65.5 | 55.7 | 53.2 | 31.3 | 2.8 | 19.2 | 28.4 | 29.1 | 2.5 | 2.5 | 3 | 3 | 28 | 28 |
| Ewan 11 | 75.4 | 80.5 | 69.7 | 70.1 | 68.3 | 68.7 | 1.0 | 3.6 | 19.1 | 19.2 | 1.8 | 1.8 | 7 | 7 | 55 | 67 |
| Caiapo | 80.7 | 79.9 | 68.5 | 67.6 | 66.5 | 65.2 | 0.1 | 1.4 | 25.5 | 23.7 | 2.8 | 2.9 | 3 | 3 | 33 | 38 |
| Carajas | 82.1 | 79.9 | 66.5 | 66.5 | 61.2 | 61.0 | 7.3 | 8.1 | 26.3 | 25.0 | 2.6 | 2.8 | 4 | 4 | 34 | 37 |
| Gvrani | 79.6 | 78.7 | 67.0 | 67.6 | 60.4 | 61.0 | 3.9 | 4.6 | 28.4 | 25.9 | 2.9 | 3.1 | 4 | 3 | 38 | 37 |
| Rio Paranaiba | 80.0 | 79.2 | 68.6 | 69.4 | 65.0 | 64.7 | 1.8 | 4.9 | 20.0 | 18.3 | 2.7 | 2.7 | 3 | 3 | 50 | 56 |
| Canastra | 78.1 | 79.3 | 66.5 | 66.4 | 63.4 | 62.3 | 0.6 | 1.0 | 18.5 | 18.0 | 3.0 | 3.1 | 2 | 2 | 63 | 67 |
| Mean | 78.3 | 79.3 | 67.5 | 66.2 | 66.6 | 59.2 | 2.5 | 6.1 | 23.7 | 22.7 | 2.6 | 2.7 | 3.7 | 3.6 | 43 | 47 |
| *Analysis of variance* | | | | | | | | | | | | | | | | |
| Treatment | 0.40NS | | 1.31NS | | 42.49** | | 22.82** | | 6.52* | | 4.08NS | | 3.27NS | | 5.68* | |
| Cultivar | 24.37** | | 4.84** | | 155.84** | | 14.78** | | 70.17** | | 112.84** | | 60.24** | | 47.77** | |
| Treatmentxcultivar | 3.16* | | 1.78NS | | 34.68** | | 8.46** | | 1.29NS | | 0.55NS | | 1.58NS | | 1.05NS | |

# Improving drought resistance in rainfed rice for the Mekong region: the experience from Thailand with a focus on the use of leaf water potential (LWP) and spikelet sterility as indirect drought tolerant traits

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## Introduction

Earlier work on the adaptation of rice to rainfed lowland conditions in northeast Thailand highlighted the importance of panicle water potential (PWP) as a useful selection trait for drought tolerance in addition to yield under drought (Pantuwan et al., 2002). It was shown that genotypes that can maintain high PWP produce higher yield (r=0.55**) under relatively severe late season drought common in the region. Jongdee (1998) found that genotypes with high leaf water potential (LWP) had low spikelet sterility and produced higher yield. The studies of Fukai et al. (1999) have indicated that among many putative drought traits, LWP is the one most consistently related to sterility and indirectly to yield under drought and, as such, is a useful trait for selection. Until recently it has been difficult to measure LWP in sufficient numbers to be of use in a practical breeding program. The Thailand Rice Improvement Program now has the capacity to screen replicated experiments with up to 2000 entries for LWP.

This paper reports on the phenotyping of advanced and landrace materials for drought tolerant traits with a particular focus on the use of LWP and spikelet sterility as useful indirect selection traits for target population of environments (TPE) of severe reproductive drought stress.

## Materials and methods

### *A. Screening of advanced lines*

A total of 191 advanced lines grouped into early maturity glutinous, early non-glutinous, medium glutinous and medium non-glutinous were evaluated under a water drainage system to induce drought stress at the reproductive stage of plant growth at Chum Phae (Northeast) and Phrae (North) in Thailand in the 2002 wet season. The materials were evaluated for LWP, spikelet sterility, flowering date, grain yield, and drought response index (DRI), and other agronomic traits. The drought stress that was imposed is shown in table 1.

### *B. Screening of mainly landrace materials*

In the 2002 / 2003 dry season, 803 local lines (mainly landrace materials) were grown at Ubon and evaluated for LWP when subjected to a vegetative stage drought. There were three replications and the plants were evaluated for LWP when there was visible leaf drying in most entries.

In the 2003 wet season, 56 genotypes (10 lines from screening of advanced lines (“A”, above) and 46 from screening mainly landrace materials (“B”, above) were evaluated under a range of drought conditions using a line source sprinkler irrigation system at Ubon and Chum Phae (Northeast) and Phrae (North). There were two replications and data for LWP, flowering delay, spikelet sterility, grain yield, and DRI were again recorded.

**Table 1. The drought intensity measured as a reduction in grain yield for screening different sets of advanced lines at Chum Phae and Phrae under line source sprinkler in Thailand, wet season 2002**

| Location | Drought intensity (%yield reduction) in: | | | |
|---|---|---|---|---|
| | Early maturity non glutinous | Early maturity glutinous | Medium maturity non-glutinous | Medium maturity glutinous |
| Chum Phae | 38 | 9 | 24 | 39 |
| Phrae | 2 | 3 | 6 | 2 |

## Results

### *Wet season 2002*

At Chum Phae, the drought reduced yield by 38, 9, 24, and 39% in the four subsets of materials (Table 1). There was no drought at Phrae. Only data from Chum Phrae are considered in the analysis. The drought stress is considered a moderate reproductive drought for the subsets early maturity non-glutinous, medium glutinous, and medium non-glutinous. In each subset, there was a considerable range in flowering date. Thus, entries that flowered early were exposed to fewer days from the imposition of drought to flowering than entries that flowered late. In order to partly address this issue in comparing performance among all lines, the entries were grouped using cluster analysis, for the interval from the beginning of drought to flowering, and comparisons were made within groups (i.e., within groups that experienced a similar duration of exposure to drought).

There were significant differences in the drought tolerant traits of LWP, spikelet sterility, and DRI in each of the four subsets. The relationships among drought tolerance traits and yield were examined using the lines from the medium exposure to drought group. Under the moderate drought of three of the subsets, the yield was correlated with DRI ($r^2$=>0.75). However, in a set with little drought, there was significant correlation ($r^2$=>0.30) of yield under drought with the yield potential. In the early maturing sets (1 and 2), spikelet sterility influenced grain yield under moderate drought conditions ($r^2$=>0.34) and there was a significant relationship between LWP and sterility ($r^2$=>0.30 and 0.49, respectively) for glutinous and non-glutinous materials (Figure 1). However, there was no

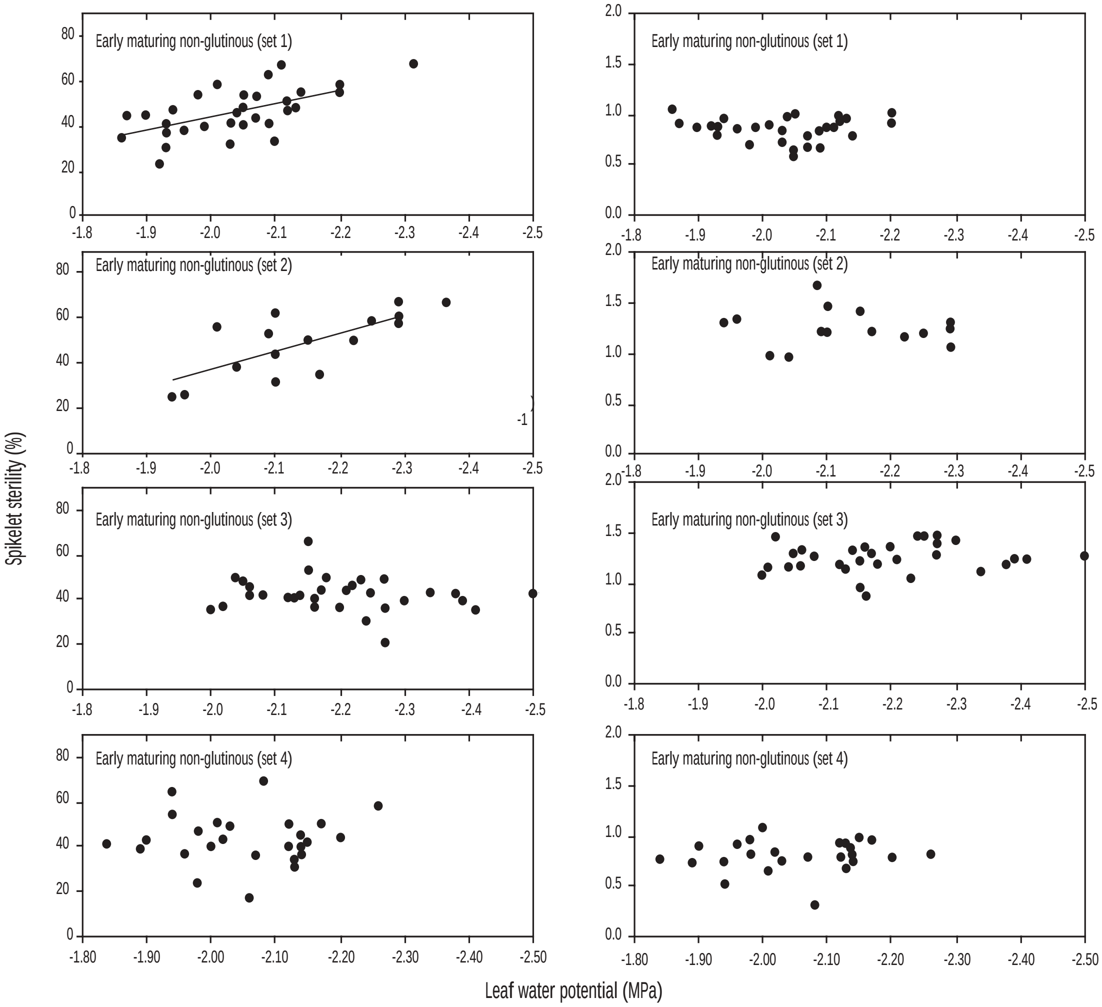

**Figure 1. Relationship between LWP and spikelet sterility and grain yield among advanced lines tested from four different groups (based on maturity and glutinous content) under medium drought conditions in Chum Phae, Thailand, and wet season 2003.**

relationship between LWP and yield under stress. Lines with high LWP, low fertility, and high DRI were chosen as putative drought tolerant donor lines for use as parents to develop new populations for TPEs with moderate to severe drought stress.

## Dry season 2002/2003

There were significant differences in the LWP among the 803 mainly landrace materials with values ranging from -2.0 MPa to -3.6 MPa. (Table 2). The LWP values are the mean of two samples on different days and of three replicates. Fifty lines with LWP values higher than the check (RD6) chosen for known drought tolerance were selected and used as putative donor lines to develop new populations. These lines are listed in table 2. These lines were also reevaluated under drought in the 2003 wet season (mentioned above, data not yet available).

**Table 2. The leaf water potential (LWP) of 50 mainly landrace materials selected for crossing and further testing based on performance in the 2002/2003 dry season screening for vegetative stage drought tolerance. Data for the check entries are also included.**

| Lines | LWP (MPa) | Lines | LWP (MPa) |
|---|---|---|---|
| Puang Tong | -1.79 | Saeng Daow | -2.20 |
| Puang Kradone | -1.82 | Pra Ruang | -2.21 |
| Boonma Luang | -1.84 | NIAW GARIANG | -2.21 |
| Hom Toong | -1.93 | NGLAY | -2.21 |
| Luang Pratan | -1.95 | Pin Kaew | -2.21 |
| Nang Nee | -1.96 | C 4 | -2.22 |
| Daw Pong Aew | -1.99 | Daw Noi | -2.22 |
| PHALABAN | -2.02 | Jao Daeng | -2.22 |
| Chang Rak | -2.04 | Lao | -2.24 |
| Hom Bang | -2.07 | PAN TAWNG | -2.25 |
| Luang Boonma | -2.07 | Boon Ma | -2.25 |
| Kiew | -2.07 | Mae Puang Tong | -2.26 |
| TAWNG SAI | -2.08 | Tak Haeng | -2.27 |
| Papo-Nampu | -2.11 | Khao Kaew | -2.27 |
| Luang Ema | -2.11 | MAHOS SOTH | -2.27 |
| Pang Hang Ma | -2.12 | E- KAAO | -2.28 |
| IR57514-PMI-5-B-1-2 | -2.13 | Hom Bai | -2.28 |
| RD4 | -2.15 | Hawm Jan | -2.28 |
| E- NAWN | -2.16 | Daw16 | -2.29 |
| NIAR TEW | -2.16 | Luang Klong | -2.30 |
| Nak Khaw Loy | -2.18 | Aew On | -2.31 |
| Niew Pama | -2.19 | Jek Chouy | -2.31 |
| LEUANG GON JUD | -2.19 | Hang Yi 71 | -2.31 |
| Khao Rai 2 | -2.20 | Pong Aew | -2.31 |
| RD17 | -2.20 | RD7 | -2.32 |
|  |  | RD6 | -2.30 |

Lsd 5% =0.31

## Conclusions

Screening of large numbers of advanced lines and landrace materials for the putative drought tolerance traits of LWP, spikelet sterility, flower delay, grain yield, and DRI is feasible in a plant breeding program. The draining of water, the use of a line source sprinkler, and the planting at different sites in the wet season, allowed for the exposure of materials to a moderate reproductive drought, at least at one site.

There were significant differences among lines for all putative drought tolerant traits, even when differences in the duration of exposure to drought from commencement to flowering were considered. LWP and spikelet sterility are useful indirect traits for the TPE of moderate reproductive drought. These traits can be measured on large numbers of materials particularly aimed at identifying putative drought tolerant donor lines as parents.

Populations based on the crosses of the putative donor lines and recipient parents with good yield and quality are being developed and will be available for further phenotyping and molecular analysis.

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# Mapping root traits using a RI population of rice under well-watered conditions

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## Introduction

Water stress is a major constraint for many rice production areas. Several mechanisms for water-limited stress are drought escape, drought avoidance, drought tolerance, and drought recovery (Fukai and Cooper, 1995; Price et al., 2002). The root system is regarded as significant in the response of rice to drought. Several rice root characteristics, such as root length, branching of root system, depth of rooting, root thickness, root to shoot ratio, root density, and the dry weight of roots below 30 cm are commonly considered to play an important role in water deficits (O'Toole, 1982; Fukai and Cooper, 1995; Blum, 1996; Nguyen et al., 1997). Most studies on roots under drought conditions reveal the possibility of enhancing water availability through adaptation of the root system. Several studies have mapped chromosomal regions for various root traits in several genetic populations, as reviewed by Price et al. (2002). Our objectives are to gain insight into the genetic basis of root characteristics, to identify and map the QTLs associated with the root system, and to screen lines with extreme performance in their root system for further research.

## Methods

A population of 190 recombinant inbred lines derived from Zhenshan 97 (*indica*)x IRAT109 (*japonica*) was employed. A cylindrical pot made of polyvinyl chloride (PVC), of 20 cm internal diameter and 100 cm depth, with a plastic bag insert was filled with sieved silty soil mixed thoroughly with fine sand (20%). A total of 2.3 g nitrogen per pot was supplied and mixed thoroughly into the soil. After filling and before planting, pots were watered and then refilled with soil until the saturated soil level came within 7 cm of the top. The experiments followed a randomized complete block design with four replicates in 2003.

Three germinated seeds were sown separately per pot, one of which was placed in the middle of the pot. Only one healthy seedling per pot remained and was located in the middle of pot approximately 10 days after sowing. Standing water above the soil surface was maintained by watering daily.

Tiller number was counted in vivo one day before sampling. In 2003, sampling date was at 2 to 4 days after heading. Plants were cut at the soil surface for above-ground dry weight one day before sampling roots. The soil mass with roots inside the plastic bag was slowly pulled out of the PVC pots. The soil was cut into three layers of 0-30 cm, 30-60 cm, and 60-approximately 90 cm from the soil surface.

First, the third layer from the surface was searched in order to identify the maximum rooting depth. If the deepest root tip was not found, the search extended to the second layer, or the first layer. Second, roots were carefully separated from the soil on a 2 mm sieve screen by rinsing, and collected. Roots attached to a stem base were cut. After measuring root volumes in each layer by water replacement, roots were oven-dried for root dry weight. Phenotypic values of other derived traits were calculated. A linkage map covering 1733.1 cM with a total of 240 SSR markers was constructed. The chromosomal locations of putative QTLs were determined based on a mixed model method, using QTLMapper developed by Wang et al. (1999). Sampling was carried out simultaneously at mid-tillering stage (45-47 days after transplanting for first replication, 48-50 for the second one, 51-53 for the third one) in 2002.

## Results

Data for 2003 are presented. The two parents, Zhenshan 97 and IRAT109, showed significance in all traits between two parents in 2003. IRAT109 is a parent with high phenotypic scores except for root: shoot ratio. Performance of the same trait in 2002 and 2003 are generally similar.

One QTL was found to control maximum rooting depth (MRD), accounting for 17.12% of phenotypic variations, at which allele from IRAT109 increased MRD.

Four, four, and four QTLs were detected for volume of shallow root (0-30cm, RVs), volume of deep root (>30cm, RVd), and total volume of all roots (TRV), respectively. A chromosomal region on chromosome 1(rm428-rm495) has effects on the three traits related root volume. A QTL for RVs and one QTL for TRV were located on the similar region on chromosome 5 (rm421-rm305). Alleles for increasing root volume at 12 QTLs detected were dispersed between the two parents. Four, three, and four QTLs were mapped for dry weight of shallow root (RDWs), dry weight of deep root (RDWd), and total root dry weight (TRDW), respectively.

Three, three, and five QTLs were identified for shallow root volume per tiller (RVsTN), deep root volume per tiller (RVdTN), and total root volume per tiller (TRVTN), respectively. The region flanked by rm317 and rm451 on chromosome 4 was observed to affect the three traits, an allele at which came from the high-value parent IRAT109, increasing the phenotype. Two, four, and three QTLs were detected for shallow root dry weight per tiller (RDWsTN), deep root dry weight per tiller (RDWdTN), and total root dry weight per tiller (TRDWTN), with explanation of 46.32%, 34.73%, and 37.94% of total variations, respectively. The region, rm317-rm451, erected its effects on the three traits.

Two and eight QTLs were identified for ratio of deep root volume to shallow root volume (RVds) and ratio of deep root dry weight to shallow root dry weight (RDWds), and explained 25.98% and 66.76% of total variation, respectively. Additionally, three QTLs for ratio of root to shoot on the basis of dry weight were located on chromosome 1, 4, and 9, respectively.

Generally, eight regions were identified to control related traits. Four regions, rm104-rm302 on chromosome 1, rm131-rm451 and rm470-rm273 on chromosome 4, rm2-rm125 on chromosome 7, were detected to simultaneously harbor QTLs in 2002 and 2003. QTLs located in the two regions, rm428-rm495 on chromosome 1, rm331-rm544 on chromosome 8 were only detected in 2003. However, QTLs clustered on the regions, rm240-rm324 on chromosome 2 and rm489-rm523 on chromosome 3 were only identified in 2002. Considering the different stages sampled for measuring root traits, regions only detected to harbor QTLs in a year may be related stage-specific expression of genes controlled root traits, whilst regions detected to carry QTLs in both years may be associated with constitutive expressions of genes for root traits.

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# Evaluation of rapid drought stress protocol to predict field performance of rice under drought stress conditions

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## Introduction

Accurate field phenotyping for drought tolerance is a limiting factor in our ability to detect underlying genetic factors affecting the trait (Bidinger, 2002). The yield reduction due to drought stress depends very strongly on the timing of the stress (Garrity and O' Toole, 1994). Drought stress around flowering time causes dramatic yield reduction primarily due to spikelet sterility (Lafitte, 2002). Screening of breeding lines for flowering stage drought tolerance in the field is complicated because of differences in phenology and rains during stress periods. The application of uniform drought stress to all the lines in screening is also critical. Unless all the genotypes in the screen are exposed to a similar stress, the measured differences among them are more likely to reflect differences in stress experienced than differences in stress tolerance/susceptibility (Blum, 2002). Well-developed root systems can extract water too rapidly and create severe water deficit at the flowering stage (Pantuwan et al., 2002). Shoot-related drought tolerance could be studied by eliminating the effect of deeper roots. Good recovery potential after severe drought stress can also improve grain yield. There is a need for a managed drought stress screen in which stress of similar intensity can be applied to each genotype at a particular phenological stage. Such a screen should be safe from rains, repeatable, and predictive of grain yield under field drought stress.

## Methods

A rapid stress protocol was evaluated at IRRI with 44 lines grown under glass house conditions in plastic trays (52.5 x33.0 x 24.0 cm, filled with 33 kg of farm soil). Six plants were maintained in each tray. Drought stress was created when inter-auricle distance between flag and penultimate leaf was 0 to 1 cm in three plants of a tray. Plastic trays were allowed to dry to achieve a weight loss of 9 kg. This weight reduction was achieved in about one week. A rapid and severe drought stress developed over this period that was reflected in severe leaf rolling and drying. After achieving the 9 kg weight reduction, trays were re-irrigated. Relative water content, membrane stability, leaf rolling, leaf desiccation, and soil moisture were estimated immediately prior to re-irrigation. Recovery was estimated after one week of relieving drought stress. Pollen was collected from the three main tillers, which were tagged prior to the onset of stress. Plant dry weights and yield components were recorded at maturity. The 44 lines had been previously screened in replicated small-plot trials planted in the field at IRRI under aerobic conditions during the dry season of 2002. In these trials, severe stress was intermittently imposed from canopy closure until more than 80% of plots in the trials had flowered.

## Results

Significant variation was observed among lines for injury due to drought and recovery ability. Membrane stability at the end of stress cycle varied from 15 to 62% among lines. Membrane stability was strongly associated with RWC at the end of drought. Recovery scores ranged from 1.1 to 5.0 after one week of re-irrigation. Recovery from drought was associated with RWC, membrane stability and leaf desiccation at the end of the drought period (r = 0.54**, 0.60**, 0.70** respectively). Grain yield among lines ranged from 0 to 11 g/hill and was associated with membrane stability and RWC at the end of the stress period (r = 0.51** and 0.48**, respectively) and ability to recover from drought stress. Pollen fertility in rapid stress studies was associated with heading delay due to severe drought stress observed in the field (r = - 0.69 **). Pollen fertility and spikelet sterility in the glasshouse assay using rapid stress was significantly correlated with yield in the field under severe drought stress (Figures 1, 2).

## Conclusions

Maintenance of plant water status by shoot-related mechanisms during drought helps in achieving membrane stability, better recovery after re-irrigation and stable grain yield under rapid stress conditions. Similar intensity of drought stress can be imposed at the same phenological stage in all lines using this protocol. The rapid stress protocol may be useful in predicting field performance of breeding lines under drought stress.

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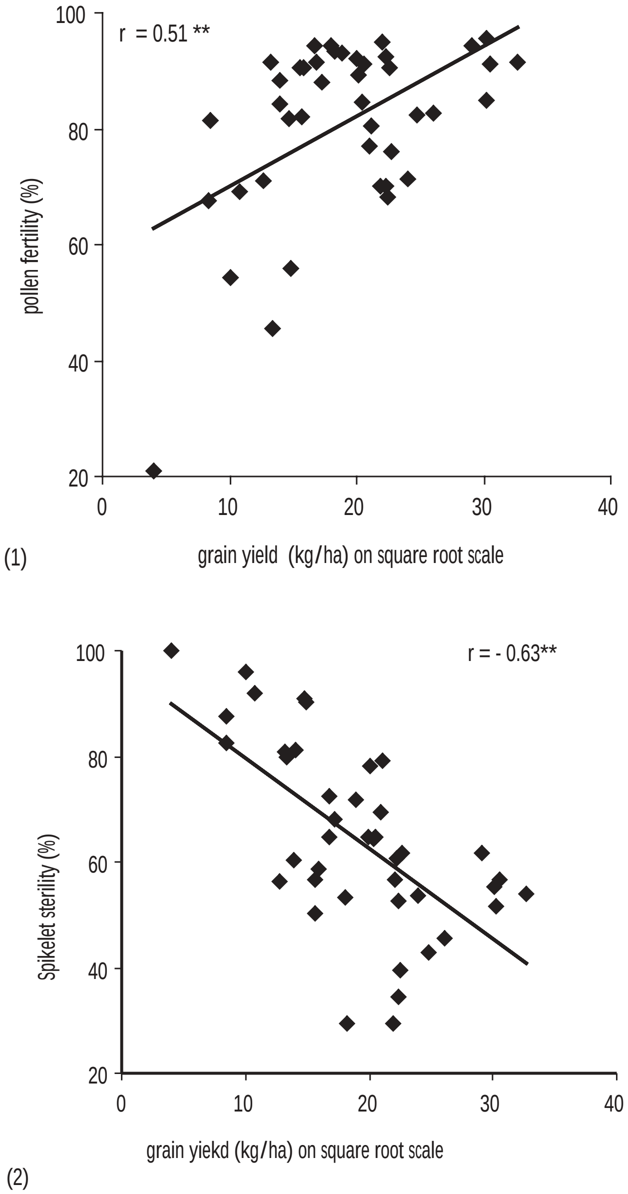

**Figure 1. Relationship of grain yield in field under severe drought stress with pollen fertility in glass house (1) and spikelet sterility in glass house (2) using rapid stress protocol.**

# Response to direct selection for yield under reproductive stage stress in rice backcross populations

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## Introduction

Drought regularly results in significant yield losses across some 23 million hectares of rice that is grown in unfavorable environments. In addition, water shortages are developing in traditionally irrigated areas due to competition between urban and rural users of fresh water. Varieties that are better able to tolerate drought are known, but it has been difficult to combine drought tolerance with good yield potential. A backcross breeding program based on highly productive cultivars that includes direct selection for improved yield under drought provides an opportunity to combine these traits. In addition, molecular markers can be used to identify those introgressions that result in improved performance in selected lines (Li et al., this volume). This information can be used to identify superior alleles in genomic regions from unimproved germplasm.

## Methods

Backcrossing was used to develop $BC_2$ populations from three elite lowland rice cultivars: IR64, Teqing, and a first-generation New Plant Type (NPT) line. The donors for this program comprised over 100 rice cultivars from diverse origins, most of which are modern or currently grown cultivars. The segregating $BC_2F_2$ generation was exposed to severe reproductive-stage stress in managed lowland nurseries or chronic water deficit in upland nurseries. Individual plants that set grain better than the recurrent parent were harvested and increased (Lafitte et al., 2004). Selected lines were evaluated as $BC_2F_4$ families in single row x 3 m plots under managed stress in the dry season, and better lines were re-tested under rainfed conditions in the wet season (Table 1). The lowland stress experiment was established by transplanting, and the field was maintained under flooded conditions until about 60 days after sowing. At that time the field was drained and no further irrigation was applied. In the upland stress experiments, plots were established by transplanting into wet soil in the dry season, followed by sprinkler irrigation. Around mid-tillering, basin irrigation was initiated, applied at a frequency of every 10 to 12 d. In the wet season, plots were dry seeded and established by sprinkler irrigation. After establishment, the crop received only rainfall. In replicated nurseries, lines were sown in three replications with an alpha-(0,1) lattice arrangement, with the recurrent parent present in multiple copies as a check. Superior lines were identified as those yielding equal to or more than the recurrent parent. In unreplicated nurseries, lines were sown with the recurrent parent interspersed every 20 rows. Superior lines were those with yields that exceeded the recurrent parent. Flowering dates and plant heights were also recorded.

## Results

Grain yields differed significantly across irrigation regimes. Within the 2002 dry season experiments, average yield ranged from 375 g $m^{-2}$ in the irrigated control to 135 g $m^{-2}$ in the lowland stress and 120 g $m^{-2}$ in the upland stress treatment. In the unreplicated observations, yields were 270 g $m^{-2}$ in the lowland stress experiment compared to 170 g $m^{-2}$ in the upland experiment. Stress was more severe in the 2003 dry season observations, and the average yield was 54 g $m^{-2}$ in the lowland and 28 g $m^{-2}$ in the upland.

The number of lines selected for testing varied among years and genetic backgrounds. Nonetheless some trends were apparent. For example, among the lines selected on the basis of grain quality, most yielded less than the recurrent parent whether the evaluation was carried out under fully irrigated, lowland stress, upland stress, or rainfed conditions (Figure 1). In contrast, more than half of the selected IR64 lines yielded more than IR64 in seven of the eight experiments. The proportion of Teqing lines with yields better than the recurrent parent was generally lower than for IR64 lines. In several cases, selection under upland conditions produced a greater proportion of superior lines than selection under terminal lowland stress. The majority of the lines tested flowered earlier than the recurrent parents. The number of superior lines recovered from a given donor was not associated with the per se performance of the donor under drought.

We conclude that selection for drought tolerance, by exposing segregating populations to stress near flowering, was effective in identifying backcross lines with superior grain yield under stress. Superior lines could be recovered from crosses with apparently susceptible donor cultivars.

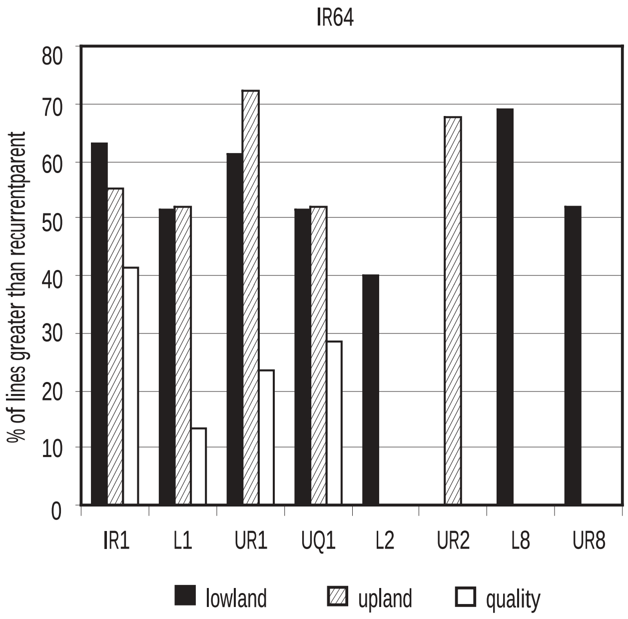

**Figure 1. Proportion of superior lines identified in IR64 background in 8 managed stress experiments. Lines were selected for either performance with lowland stress, performance with upland stress, or for grain quality. Trials L1, L2, and L8 were conducted with lowland stress; UR1, UR2, and UR8 were with upland stress; IR1 was fully flooded; UQ1 was aerobic and rainfed.**

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# Studies on phenotype of Zhenshan 97B/IRTA109 RILs population in field screen facility

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## Introduction

The performance of a plant's drought tolerance is extremely complex because of interaction between the genotype and environment. Blum (1999) argued that accurate assays of drought should be conducted on whole plants in the field with effective water control, especially in the reproductive stage because it is the most sensitive period for rice (Cruz and O'Toole, 1984). To better understand the drought tolerance of rice, a rainproof screen facility was established in Shanghai Agrobiological Gene Center. A mapping population and several rice accessions with different drought tolerance (DT) ability was evaluated on the morphological and physiological traits as well as the yield and its components in this facility.

## Methods

The field screen area (1600m$^2$) was separated into two islands by 1.8 m-depth channel in a rainproof greenhouse. The sprinkler irrigation and drainage system was installed in the roof and channel, respectively. The TDR system was also installed in the screen area to measure the soil water content. Each island is 76 m in length and 6 m wide and a drip irrigation tube was built in the center of islands. Before the stress, the roof was opened and the sprinkler irrigation kept the materials well watered. When the stress was initiated, the sprinkler irrigation was stopped while the drip irrigation was still working and underground water was drained out through the channel.

A set of 195 F10 recombinant inbred lines were developed from Zhanshan 97B (drought sensitive paddy rice) and IRAT 109 (drought tolerance upland rice) and its parents were arranged in the island in a random block design with three replications. Each line was seeded directly in two-rows plots with 15 plants per row. To synchronize the heading date, the RI population was separated into three subsets and seeded at three different dates with seven days interval. Normal irrigation was supplied until most of lines were at microspore stage. After supplying stress for four weeks, the plants in both ends of each row (one was near the channel, implying the drought region and one was near the drip tube implying the well-watered) was investigated on following traits: plant height (PH), panicle length (PL), leave rolling score (LR), relative water content (RWC), leaf water potential (LWP), canopy temperature (CT), panicle neck diameter (PND), photosynthetic rate (PR), chlorophyll content (CC), stomatal resistance (SR), transpiration rate (TR), water use efficiency (WUE), biomass, grain yield (GY) and its components, panicle number (PN), spikelets per panicle (SN), spikelet fertility (SF), and 1000-grain weight (GW).

All the collected data was primarily managed using Microsoft Excel. Standard analyses of variance were performed to check the genetic variance among the RI lines for the investigated traits. The phenotypic correlation was calculated using S-PLUS statistics software.

## Results

1. The distinct gradient of soil water content was formed after the stress. In the center of the island, the soil water content was about 45%, maintaining the plant in this area at a normal condition. In the margin of the island, the soil water content was below 20%, keeping the plant in a water stressed condition. In this way, all DT related traits measured from the plants at both ends of plots were well represented.
2. Yield and its components: the mean values of biomass, GY, PN, SN, SF, and GW under normal conditions were higher than that under stress conditions. However, no large difference was

observed for some lines under both conditions, indicating that these lines have better drought tolerance. The path analysis on yield and its components indicated that the spikelet fertility makes a larger contribution to yield under stress conditions while the spikelets per panicle was the largest contributor to yield under normal conditions.

3. **Drought related physiological traits:** there were clear differences in CT, LWP, and RWC between two parents in stress. For the RI population, the mean values of CT and RWC were higher but the LWP was lower under stress than in normal conditions. The correlation between CT and LWP was negative significant (-0.255**). No significant correlation was found between CT and RWC. The correlation between LWP and RWC was positive significant (0.200*).
4. **Photosynthesis related traits:** the mean values of PR of population under normal condition were much higher than under stress. The CC were relatively stable under different water conditions. There was significant correlation between SR and WUE in both conditions.
5. **Panicle neck diameter and leaf rolling score:** there is range of variation on PND and LR in the population under stress. The relationship between leaf rolling score and yield related traits are all negative with two (LR / SN, LR / GY) reaching significant levels. There was a significant positive correlation between PND and GY, SN, GW in both conditions. In the stress, the PND was negatively correlated with the CT and leaf rolling score.

## Conclusion

The facility with effective water control can form a gradient of soil water content and worked well for evaluating drought tolerance of rice. The phenotype on a RI population indicated most of morphological and physiological traits as well as the yield and yield components had different degrees of changes under different water conditions. As the CT and PND has significant correlation with GY and it is easier to measure in the field, they can be used as an easy indictor in DT related research and DT rice breeding programs.

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**Table 1. The correlation among all the traits in Zhenshan 97B / IRAT109 RIL population**

| 0 | 0 | PN | SN | SF | GW | PND | CT | LWP | RWC | PR | CC | TR | SR | WUE | LR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GY | s | 0.5679$^{**}$ | 0.6460$^{**}$ | 0.6930$^{**}$ | 0.3605$^{**}$ | 0.3049$^{**}$ | -0.3387$^{**}$ | 0.0788 | -0.0971 | -0.0334 | -0.2238$^{**}$ | 0.0038 | 0.0739 | 0.0811 | -0.2667$^{**}$ |
|  | n | 0.2723$^{**}$ | 0.7468$^{**}$ | 0.5662$^{**}$ | 0.3493$^{**}$ | 0.3980$^{**}$ | -0.2573$^{**}$ | -0.1913$^{*}$ | -0.0979 | -0.1067 | -0.3440$^{**}$ | 0.0278 | -0.0568 | 0.0223 |  |
| PN | s |  | 0.6101 | 0.2759$^{**}$ | 0.0588 | -0.0771 | -0.1904$^{*}$ | 0.0014 | -0.0598 | 0.1170 | -0.1549 | -0.0038 | 0.0648 | 0.0472 | -0.0830 |
|  | n |  | 0.4926 | -0.1043 | -0.0929 | -0.1496 | 0.0346 | 0.0666 | 0.0390 | -0.1616$^{*}$ | -0.0035 | 0.0200 | -0.0126 | -0.0988 |  |
| SN | s |  |  | 0.0733 | 0.0443 | 0.3348$^{**}$ | -0.1521 | -0.1141 | -0.1669$^{*}$ | 0.0784 | -0.1898$^{*}$ | 0.0931 | 0.0055 | 0.0480 | -0.2834$^{**}$ |
|  | n |  |  | -0.0036 | 0.0630 | 0.4012$^{**}$ | -0.0448 | -0.2463$^{**}$ | -0.1778$^{*}$ | -0.0892 | -0.1623$^{*}$ | 0.1198 | -0.0225 | 0.0001 |  |
| SF | s |  |  |  | 0.0945 | 0.0098 | -0.2323$^{**}$ | 0.2258$^{**}$ | 0.0488 | -0.0706 | -0.109 | -0.0554 | 0.0754 | 0.0235 | -0.0564 |
|  | n |  |  |  | 0.1400 | 0.0649 | -0.2445$^{**}$ | -0.0193 | 0.0768 | -0.0817 | -0.3872$^{**}$ | -0.0042 | -0.0656 | -0.0308 |  |
| GW | s |  |  |  |  | 0.3144$^{**}$ | -0.2053$^{**}$ | 0.0331 | -0.0001 | -0.0889 | -0.0061 | 0.0060 | 0.0692 | 0.0721 | -0.0938 |
|  | n |  |  |  |  | 0.2672$^{**}$ | -0.3181$^{**}$ | -0.0688 | -0.0756 | 0.0175 | -0.0078 | -0.0753 | 0.0539 | 0.1491 |  |
| PND | s |  |  |  |  |  | -0.1657$^{*}$ | -0.0853 | -0.0832 | 0.1350 | -0.0147 | -0.1050 | -0.0223 | 0.0965 | -0.1977$^{*}$ |
|  | n |  |  |  |  |  | -0.1495 | -0.1858$^{*}$ | -0.1227 | 0.2190$^{**}$ | -0.0075 | 0.0612 | -0.0523 | -0.0102 |  |
| CT | s |  |  |  |  |  |  | -0.2549$^{**}$ | -0.0246 | 0.0195 | 0.0066 | -0.0853 | 0.0008 | 0.0867 | 0.1115 |
|  | n |  |  |  |  |  |  | 0.1065 | -0.0261 | -0.0343 | 0.0657 | -0.0330 | 0.0799 | -0.0199 |  |
| LWP | s |  |  |  |  |  |  |  | 0.1995$^{*}$ | -0.0266 | -0.0818 | -0.0460 | 0.0120 | -0.0043 | -0.0470 |
|  | n |  |  |  |  |  |  |  | -0.1164 | 0.0378 | 0.114 | -0.0106 | -0.0110 | -0.1134 |  |
| RWC | s |  |  |  |  |  |  |  |  | -0.0034 | -0.0751 | -0.0094 | -0.1480 | -0.0247 | -0.0445 |
|  | n |  |  |  |  |  |  |  |  | 0.0297 | 0.0131 | -0.0660 | -0.0885 | -0.1923$^{*}$ |  |
| PR | s |  |  |  |  |  |  |  |  |  | 0.0938 | 0.1062 | -0.0105 | 0.1018 | 0.0230 |
|  | n |  |  |  |  |  |  |  |  |  | 0.1730$^{*}$ | 0.1395 | 0.0193 | 0.0419 |  |
| CC | s |  |  |  |  |  |  |  |  |  |  | 0.0637 | 0.0807 | 0.1060 | 0.1750$^{*}$ |
|  | n |  |  |  |  |  |  |  |  |  |  | 0.0341 | -0.0835 | 0.1150 |  |
| TR | s |  |  |  |  |  |  |  |  |  |  |  | -0.0957 | -0.1777$^{*}$ | 0.0503 |
|  | n |  |  |  |  |  |  |  |  |  |  |  | -0.0529 | -0.1860$^{*}$ |  |
| SR | s |  |  |  |  |  |  |  |  |  |  |  |  | 0.4475 | -0.0016 |
|  | n |  |  |  |  |  |  |  |  |  |  |  |  | 0.2932 |  |
| WUE | s |  |  |  |  |  |  |  |  |  |  |  |  |  | 0.0163 |
|  | n | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |  | 0 |

# Phenotypic diversity of southern Africa maize landraces

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## Introduction

Maize dominates the food economy of southern Africa, where it is by far the dominant staple crop grown by the vast majority of rural households. Per capita annual consumption of maize averages more than 100 kg in Zimbabwe, Zambia, and Malawi (Aquino, 2001). Local maize populations have been cultivated under natural and human selection in different environments and cultural methods in the three countries since the introduction of the crop about 100 years ago (Miracle, 1966). The main sources of introduced maize into the three countries were open pollinated varieties (OPVs) from the USA directly or through South Africa (Weinmann, 1972). Over time, new landraces have been created from the original introduced populations, through adaptation to local conditions as well as hybridization brought about by continuous exchange and trade. Farmers in the three countries have continued to cultivate and maintain “local” OPVs until hybrids and improved OPVs were developed and disseminated (Weinmann, 1972). The environments and farming systems in Malawi, Zambia, and Zimbabwe where maize is produced are extremely diverse with production varying considerably between years, and showing a close dependence on rainfall and soil fertility (Aquino, 2001). This study characterizes the phenotypic diversity among landraces, assesses the impact of farmers’ selections on adapting landraces to specific agroecologies in Zimbabwe, Zambia, and Malawi, and compares the impact of farmers’ selection with progress from formal selection under abiotic stress conditions.

## Methods

A total of 278 landraces collected from farmers in diverse agroecological zones in Zambia, Zimbabwe, and Malawi, 31 improved varieties from the southern African region, and six landrace varieties introduced from the USA were evaluated for phenotypic diversity in a field trial conducted at Harare during the summer of 2004. The varieties from the USA are presumed to be among the original varieties introduced into the three countries about 100 years ago and formed the basis of the current local landraces. The trial was planted on 26 November 2003 as a randomized complete block design with two replications. Plot size was 3.375 $m^2$ and each plot consisted of 17 planting stations in one row 75 cm apart and 4 m long, resulting in a density of 5.3 plants per $m^2$. Fertilizer equivalent to 170 – 56 - 24 kg $ha^{-1}$ of $N\text{-}P_2O_5\text{-}K_2O$ was applied as per standard local practices. Weeds were controlled by application of Atrazine (4.5 l / ha) and Dual (1.8 l / ha, 96% Metalachlor) pre-emergence herbicides. Escaped weeds were controlled by hand hoeing and application of Basagran (3 l / ha, 48% Bentazon). For protection against maize streak virus vectors, Furadan (100kg / ha, carbofuran) was applied at planting, while fungal diseases, (*Cercospora zeamaydis*, *Excerohilum turcicum*, and *Puccinia sorghi*) were controlled using Tilt 250EC (0.5 l / ha). Maize stalkborers (*Busseola fusca* and *Chilo partellus*) were controlled using Thiodan 1G (4 kg / ha) and Thionex (230 g / ha, endosulfan). Cutworms were controlled with Karate (5 g / ha, Lambda-cyhalothrin) applied at emergence.

During the growing season, data was collected as follows; (i) on a plot basis: number of days from planting to 50% of the plants shedding pollen; number

**Table 1. Number of maize landraces, improved OPVs and hybrids grown at Harare during the summer of 2003 / 04 for phenotypic evaluation**

| Country | Landraces | Improved | Total |
|---|---|---|---|
| Zimbabwe | 109 | 24 | 133 |
| Zambia | 114 | 7 | 121 |
| Malawi | 55 | 0 | 55 |
| USA | 6 | 0 | 6 |
| Total | 284 | 31 | 315 |

of days from planting to 50% of the plants having silks at least 1 cm long; silk coloration recorded as red or white (%); number of root lodged plants (%); number of stalk lodged plants (%); number of ear shoots per plant; number of plants with no ears (%); (ii) on five plants taken at random within each row: tassel length (cm); number tassel branches; ear leaf length (cm); ear leaf width (cm); leaf erectness (1 = lax to 9= erect); (iii) at milk stage: plant height (cm). ear height (cm), stalk diameter (cm). Further characterization of the varieties was also aided through the use of eight ecological variables associated with the collection sites for the landraces: latitude; longitude; altitude; mean January temperature; mean June temperature; soil type; mean annual rainfall; mean evapo-transpiration.

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# Breeding for aerobic rice adapted to non-flooded irrigated conditions

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## Introduction

The abundant water environment in which rice grows best differentiates it from all other important crops. But, water is becoming increasingly scarce. Per capita availability of water resources declined by 40-60% in many Asian countries between 1955 and 1990 (Gleick, 1993). The poor people continue to be the worst affected as more than 50% of the population in developing countries is exposed to polluted water resources. For various reasons, the availability of water for agriculture will diminish both in quantity and quality in the years to come (Tuong and Bhuiyan, 1994). Yet more rice needs to be produced with less and less water to feed the ever-increasing population. Of all the crops grown under irrigation, more than 50-60% of the irrigation water is used for rice, which is the staple food for nearly half the world's population. Since more than 90% of the world's rice is produced and consumed in Asia, water scarcity would particularly threaten food security in this region. Therefore, the future of rice production will depend heavily on developing and adopting strategies that will use water more efficiently in irrigation schemes.

Significant advances have been made in irrigation management, but the genetic research has not altered the basic water requirement of rice. In Brazil and Latin America, the traditional uplands were converted into intensive, market integrated cultivation systems with sprinkler irrigation facilities suitable for high input management. This system of rice cultivation with aeration became popularly known as aerobic rice. Traditionally, aerobic rice is grown in rainfed uplands with 'no' or low inputs. In India, of the 697 high yielding varieties released so far, 336 were developed specifically for the fragile ecosystem (Figure 1). A major proportion of these were released for rainfed uplands (96) and rainfed lowlands (136). Although, there is a slow but steady progress, the average productivity of rice in these systems is low, particularly in rainfed uplands (7.3 per hectare) (Mishra et al., 2003). With declining water availability for agricultural use, aerobic rice cultivation is expected to expand into the regions with intensive cropping and high productivity (Lafitte et al., 2002). The genetic improvement of rice for aerobic (non-flooded) environments has not been understood well and there have been no major efforts on this front. However, there are varied opinions on the use of the term 'aerobic rice' under puddled soil conditions, which is invariably followed to minimize irrigation. Keeping this in view, we conducted preliminary evaluation studies involving more than 2,600 improved germplasm lines. The results are briefly presented here.

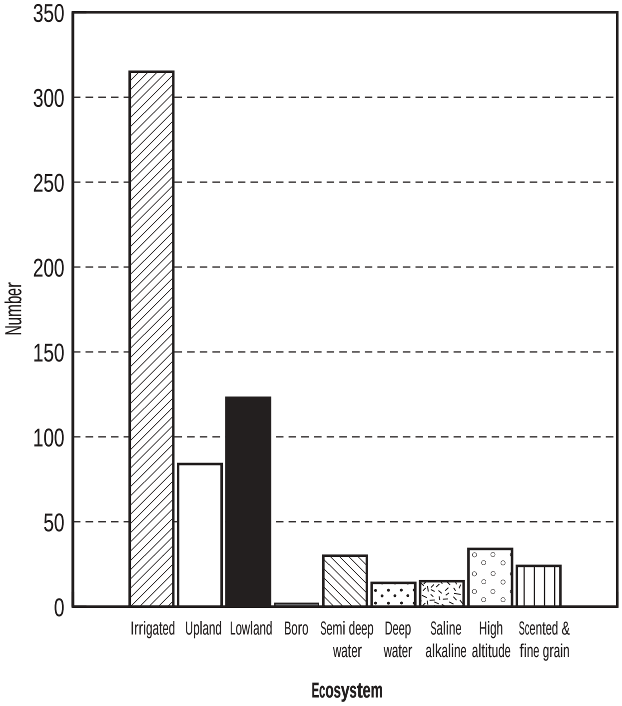


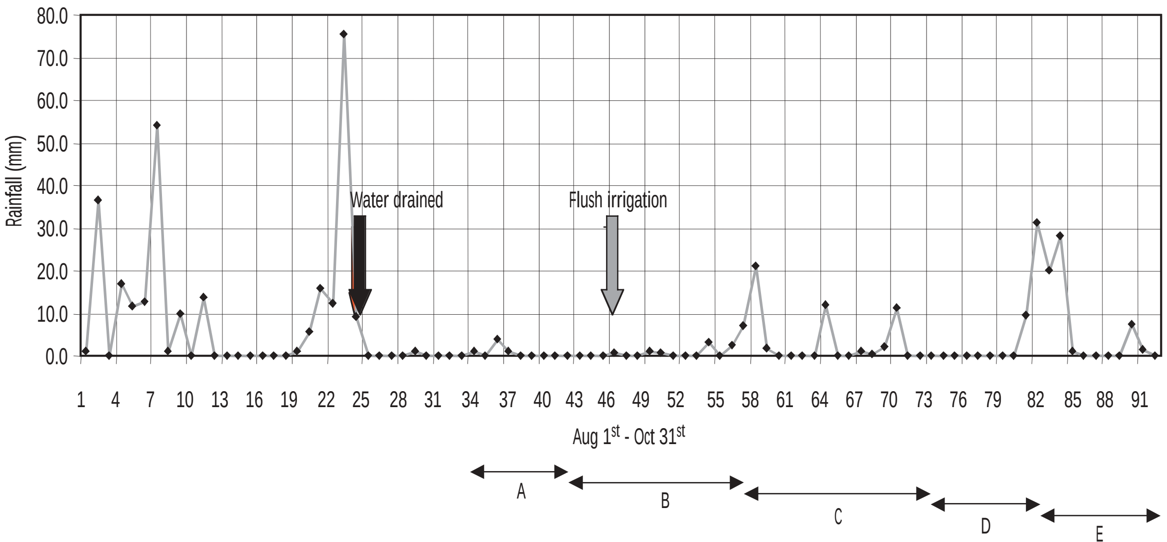
**Figure 1. Rice varieties released for various ecosystems in India till 2003.**

## Methods

During wet season 2003, more than 2,600 improved germplasm lines, including 600 drought tolerant introgression lines, 800 *indica / japonica* derivatives, and 800 hybrid rice breeding materials (detailed in Table 1) were evaluated in field under limited water conditions. The seeds were sown on 30 June, 2003 and seedlings transplanted into the main field on 4 August, 2003 under puddled conditions. Each entry was represented by a single row of 3.5 m length. After every ten entries, standard checks Rasi and Vandana, known for their tolerance to water stress, were planted. After planting, the experimental plot was not given any irrigation and was maintained as rainfed. Water accumulated due to a few rains was completely drained on 23 August, 2003 and the field was allowed to dry for about 20 days. On 12 September, a flush irrigation was given followed by top dressing with urea. Subsequently, the crop was not given any irrigation until its maturity. Each entry was evaluated for plant height, tillering ability, heading, and yield traits. The details on rainfall distribution during the crop growth and flowering of entries is depicted in figure 2.

**Table 1. Category of genetic material screened under aerobic(non-flooded, irrigated) conditions**

| **Drought tolerant introgression lines** | **600** |
|---|---|
| Indica/tropical japonica derivatives | 800 |
| Hybrid rice breeding lines | 800 |
| Released varieties | 225 |
| Released hybrids and their parents | 50 |
| DH lines and some donor germplasm | 175 |
| **Total** | **2650** |



| Group | # Entries | Varietal | Stage of Stress occurrence |
|---|---|---|---|
| | | | |
| A | 6 | Vandana | Mild-Post flowering |
| B | 77 | Rasi | Panicle Development |
| c | 316 | IR 64 | Tillering and Panicle Development |
| D | 172 | P. Basmati | Vegetative |
| E | 25 | Ahalya | Vegetative |

**Figure 2. Preliminary evaluation of improved germplasm lines under limited water stress: Distribution of selected entries for flowering and probable stage of stress period.**

Leaf rolling is one of the important observations used for evaluating desiccation tolerance in rice. As it is influenced by several environmental factors, its use as a reliable criterion is limited. Therefore, an attempt was made to refine the technique so that a large number of germplasm lines could be evaluated. Initially, leaf rolling was studied for three consecutive days at an hourly interval. The flag leaf was detached from the mother shoot and scored. The score of the same entries varied considerably with time of sampling. However, at noon (2-3 p.m.) the results were fairly consistent, which could be due to lower water uptake coupled with lower transpiration losses as a result of stomatal closure. It was also observed that when the flag leaf was cut along with ligules, it reduced the response rate, while its detachment initiated leaf rolling. Based on these observations, the technique and time for recording leaf rolling was standardized. The flag leaf was cut near the base without ligules. The time taken for cut leaf to roll was noted with the help of a stopwatch. Thus, nearly 1,200 lines were scored during the season.

## Results

On the basis of visual performance of entries for their tillering, spikelet fertility, and yielding ability, 596 entries were selected for confirmation during the dry season 2003-04. There was wide variation among the entries for heading. Vandana was the earliest, flowering just 67 days after sowing, while one of the ‘Teqing’ introgression lines took 122 days to flower. There was general delay in heading by about 10 days. Based on the heading date of selected lines and the distribution of rainfall during the season, five groups were made (Figure 1). The group-A included six entries, where Vandana, a rainfed upland variety flowered earliest. The entries in this group are expected to have experienced mild stress during post-flowering period. The group-B consisted of 77 entries with Rasi, another early variety known to adapt to water stress, as a representative. Group ‘C’ was the largest, having 316 entries with varieties Annada and IR64 as the representatives, which started flowering 55 days after planting. It is expected that these varieties would have undergone some stress during tillering and panicle developmental stage. *Indica* / tropical *japonica* derivatives and drought tolerant introgression lines predominated this group. Group ‘D’ was the second largest with 172 entries, predominantly the *indica* / tropical *japonica* derivatives. These belonged to maturity group of Pusa Basmati-1. Group ‘E’ was the late-maturing group with 25 entries. The earliest entry in this group took 80 days to flower after planting (i.e., 115 days after sowing). Of the 225 released varieties tested, about 30 could be considered as relatively better adapted to limited water conditions. Most of the hybrids tested (released + experimental) were found in middle three groups viz., B, C and D. The basmati group of varieties including Pusa Basmati-1, CSR30 (Yamini), Taraori Basmati, IR 58025B, and Binam appeared to have some tolerance for limited water conditions.

Using the standardized technique and time for leaf rolling studies, about 1,200 lines were scored during the crop season. The two check varieties, Vandana and Rasi, were studied in greater detail as they were planted after every ten entries in each block. These two varieties showed contrasting features of leaf rolling in relation to time and temperature as depicted in figure 3. The leaf samples of Vandana consistently took over 200 seconds to roll; the time taken for leaf rolling showed an increasing trend with rising mid-day temperatures. On the contrary, the variety Rasi took about 100 seconds, but the time required for leaf rolling exhibited a decreasing trend with rising mid-day temperatures. Although the relationship between leaf area and time for rolling was not significant, it was observed that the varieties with greater leaf area took less time for leaf rolling , while those with lesser leaf area needed more time (Figure 4).

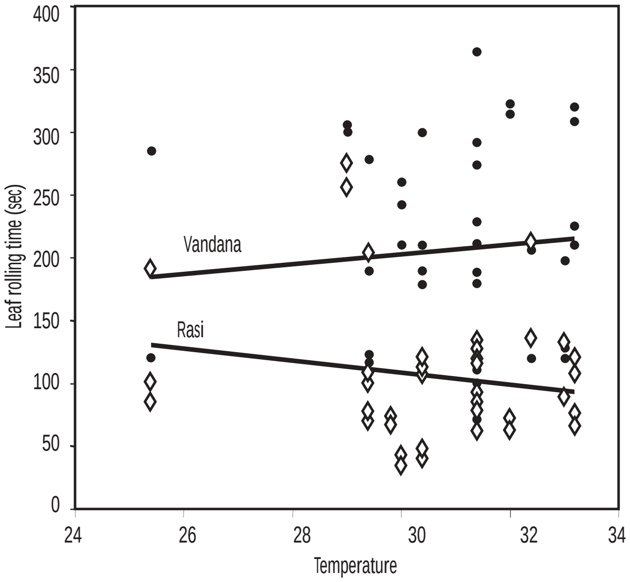


**Figure 3. Differential behaviour of two varieties. Vandana and Rasi for leaf rolling against temperature.**

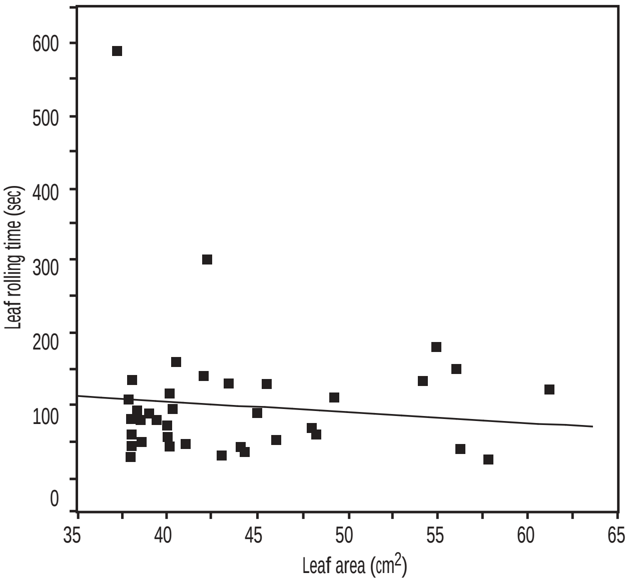


**Figure 4. Differential behaviour of two varieties. Vandana and Rasi for leaf rolling against temperature.**

## Conclusions

Based on the preliminary evaluation of a large number of improved germplasm lines, it is suggested that a majority of the varieties that were bred for irrigated lowland conditions were not suitable for limited water conditions. Most of the *indica* / tropical *japonica* derivatives and drought tolerant introgression lines showed better adaptability. The Basmati group of varieties appeared to have some level of tolerance to limited water situations.

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# Improving drought resistance in rainfed lowland rice for the Mekong region: The experience from Cambodia and on the use of drought resistance index (DRI) as an integrative drought tolerance trait

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## Introduction

Recent work on adaptation of rice to the rainfed lowlands of Cambodia has suggested a need to focus on three-target population (TPE) of environment, which account for 76% of the total variance due to environments (Basnayake et al., 2004). One of these TPEs is characterised by predictable terminal drought where yields can be reduced by around 50%. Fuaki and Cooper (2001) suggest that under these conditions, better crop performance can be achieved by selecting for appropriate phenology and for drought tolerant traits.

While many putative and specific traits for drought tolerance such as osmotic regulation, root length, and root penetration have been suggested for selection for drought tolerance, there is yet no clear evidence of their contribution to improved yield in the TPE. A more practical approach is to use a measure or index of the relative performance of genotypes under stress to that under well-watered conditions as a measure of the complex traits. For this purpose, Bidinger et al. (1982) developed a drought response index (DRI) for use in pearl millet. Pantuwan et al. (2002) successfully adapted this method for rainfed lowland rice in Thailand. They found genotypes differed in DRI, but the estimate of DRI was inconsistent across different environments. Ouk (2003) measured significant differences in DRI among 25 rice genotypes representing the breeding materials being used in Cambodia. The materials were evaluated under drought conditions that reduced yield by up to 46%. The DRI values ranged from –0.79 to +0.58. In addition to DRI, Panthuwan (2000) has shown that leaf water potential, spikelet fertility, and flower delay are also useful, integrative measures of drought tolerance.

This paper measures the variation in drought tolerance traits of LWP, DRI, flower delay, and spikelet sterility in improved and landrace materials in Cambodia. The paper also examines the robustness of DRI as a selection trait appropriate for the TPE in Cambodia.

## Materials and methods

### *A. Assessing the robustness of DRI as a measure of drought tolerance*

Fifteen genotypes (six Cambodian varieties, one Cambodian breeding line, seven lines from the Thai ACIAR project, and one popular variety; three genotypes are photoperiod sensitive, four mildly sensitive, and the others insensitive) were grown under two water treatments including well watered (WW) and a controlled drought (WS)[1] at two locations over five years. The depth of standing water and the distance to the water table in both water treatments was monitored along with rainfall. Data of flowering and grain yield were recorded and the value of DRI calculated as described elsewhere (Ouk, 2003).

### *B. Phenotyping for drought tolerant traits among mainly landrace materials*

Seventy five (75) rice genotypes, including 68 local landrace materials, were grown under a WW and WS treatment in the 2002 wet season and evaluated for DRI, flower delay, spikelet fertility, and LWP, as well as for yield under the different water treatments. The WS treatment effected a yield reduction of 75%, thus providing a severe drought in the grain filling stage. Most entries were again subjected to a vegetative drought in the 2002 / 2003 dry season and evaluated for LWP. Selections of these materials, along with additional landrace materials, were again screened under WW and WS at three locations in the 2003 wet season. The data from these trials is not yet available.

## Results

### *A. Robustness of DRI for use in selecting for drought tolerance*

The WS treatment reduced yield from 12 to 46 % and thus provided a range in drought intensity. There was significant effect of genotype on DRI in seven of the nine trials (Table 1). There was a significant difference in mean DRI, which ranged from –0.54 for IR46332-PMI-32-2-1-1 to 0.47 for CAR3, and the mean heritability was 0.51. A two-way cluster analysis of the genotype DRI values was truncated at the three levels of genotypes and the three levels of environments (data not shown). One genotype group of five genotypes had high (0.29) and consistent values of DRI (i.e., drought tolerant) across the three environmental groups, which differed in drought intensity.

### *B. Variation in drought tolerant traits among mainly landrace materials*

There was a large range in flowering date among the genotypes, suggesting that they were exposed to different durations of drought at the time of flowering. In order to minimize this effect on the selection of drought tolerant lines, the entries were clustered into groups (using cluster analysis) based on the duration of drought from draining to flowering under WW. Three groups were characterised: short, medium, and long duration of exposure were chosen. There was no significant effect of days to flower on yield in the short and medium duration groups and thus selections for drought tolerant lines were made in these two groups only. There were significant differences among the entries within each of the groups for DRI, spikelet fertility, and flower delay under drought. There was no correlation between grain yield under drought and yield potential. However, yield was correlated with DRI ($r^2$= 0.32). The delay in flowering among the short and medium duration groups explained about 60% of the variation in grain yield under drought conditions. Yield was also related to spikelet sterility ($r^2$ =0.36).

**Table 1. The drought response index (DRI) of 15 rice genotypes, the LSD5% values, the genotype mean heritability ($h^2$) and the genotype-by-environment / genotype ratio of the estimated components of variance ($^{2}_{GEI}$ / $^{2}_{g}$) when grown at two locations and for a number of years in rainfed lowlands in Cambodia. ns = not significant.  Bold and shading indicates higher than 1 and lower than –1, respectively.**

| Genotype | PV98 | PV99 | PV00 | PV01 | PV02 | CA00 | CA99 | CA01 | CA02 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| CAR3 | 0.32 | 0.14 | 0.43 | -0.14 | 1.00 | -0.48 | 0.14 | 1.71 | 1.09 | 0.47 |
| IR57514-PMI-5-B-1-2 | 0.76 | -0.33 | 1.35 | -0.20 | -0.11 | 0.94 | 0.39 | 0.39 | 0.70 | 0.43 |
| CAR6 | -0.68 | -0.04 | 0.89 | 0.14 | 0.21 | 0.46 | 0.79 | 0.32 | 0.21 | 0.26 |
| IR66327-KKN-25-P1-3R-0 | 0.30 | 1.24 | 0.98 | -0.16 | 1.04 | -0.39 | -0.53 | 0.41 | -0.60 | 0.26 |
| IR66327-KKN-10-P1-3R-0 | 0.01 | -0.58 | 0.41 | 0.63 | 0.49 | 1.34 | 0.04 | 0.05 | -1.28 | 0.12 |
| IR66327-KKN-8-P1-3R-0 | 0.66 | -0.36 | -0.77 | 1.09 | 0.57 | 0.42 | -0.03 | -0.09 | -0.41 | 0.12 |
| Somaly | -0.06 | -0.20 | -0.21 | 0.57 | 0.45 | -0.43 | 0.38 | 0.00 | -0.29 | 0.02 |
| Check | -0.35 | 0.51 | -0.86 | 0.47 | 0.54 | -0.49 | 0.10 | 0.15 | -0.35 | -0.03 |
| IR66368-CPA-84-P1-3R-0 | 0.02 | -0.42 | -0.81 | 0.45 | -0.69 | -0.32 | 0.41 | -0.31 | 1.31 | -0.04 |
| IR66327-KKN-54-P1-3R-0 | -0.55 | 0.52 | 0.02 | -0.41 | -0.28 | 0.59 | -0.17 | -0.45 | -0.36 | -0.12 |
| Khpor Daung | 0.25 | 1.25 | -0.93 | -0.62 | -0.36 | 0.14 | 0.21 | -0.17 | -0.98 | -0.14 |
| Bang Kuy (acc. 2865) | -0.21 | 0.15 | -0.13 | 0.26 | -0.42 | -0.87 | 0.76 | -1.27 | -0.11 | -0.20 |
| CIR158-B-B-SB-8-3-2 | -0.39 | -0.07 | 0.16 | 0.41 | -0.72 | 0.34 | 0.12 | -0.70 | -1.08 | -0.21 |
| CAR4 | 0.21 | -1.07 | -0.74 | -0.37 | -0.08 | -1.21 | -1.54 | -0.01 | 0.32 | -0.50 |
| IR46331-PMI-32-2-1-1(P1) | -0.29 | -0.70 | 0.19 | -2.12 | -1.65 | -0.03 | -1.05 | -0.03 | 0.83 | -0.54 |
| LSD5% | ns | 0.94 | 0.95 | 0.95 | 1.29 | 0.98 | 0.90 | ns | 1.19 | 0.47 |
| Heritability ($h^2$) | 0.23 | 0.58 | 0.72 | 0.74 | 0.67 | 0.60 | 0.57 | 0.50 | 0.75 | 0.51 |
| $^{2}_{GxE}$ $^{2}_{g}$ | | | | | | | | | | 4.73 |

The performance of the 3-5 lines with the highest DRI in each duration group is shown in table 2. The lines were evaluated for LWP under vegetative drought in the 2002 / 2003 dry season. However, rain data were taken only for one replication. The LWP ranged from –2.0 to –3.4 MPa (data not shown) and was used along with the data on all drought traits from the 2002 wet season to identify putative drought tolerant donor lines.

## Conclusions

There were significant differences in DRI among improved and landrace materials. DRI was significantly correlated with yield under the severe stress, suggesting that it is a useful measure of drought tolerance for some TPE in Cambodia. Some genotypes had high and stable DRI values over a range of drought conditions, showing that DRI is a robust trait but requires evaluation at a number of sites.

In addition to DRI, the traits of spikelet fertility and delay in flowering were associated with yield under drought and these and LWP were used to select putative drought tolerant donor lines. New populations for further phenoyping and selection and molecular analysis have been developed.

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**Table 2. Flowering dates under irrigated conditions, drought response index (DRI), delay in flowering and grain yield and spikelet sterility under drought for rice when grown under irrigated and imposed drought conditions at CARDI in the 2002 wet season, and leaf rolling score (LRS) and LWP under vegetative drought stress at CARDI in the 2002 / 03 dry season. The lines were grouped by the duration of exposure to drought (days from the commencement of drought to flowering) in the 2002 wet season. Data for the 3-5 most drought tolerant genotypes (based on DRI) in each group along with checks are presented.**

| Cultivars | Drought exposure duration from draining to flowering | Flowering date | DRI | ºDelay in flowering (days) | Grain yield (t ha$^{-1}$) Irrigated | Grain yield (t ha$^{-1}$) Drought | Spikelet sterility (%) | LWP (MPa) | LRS |
|---|---|---|---|---|---|---|---|---|---|
| **Buon Kuon (acc 1211)** c. | Short | 8 Dec | 1.96 | 6 | 2.59 | 1.15 | 24.4 | -3.02 | 3 |
| **Neang Nou (acc 636)** | Short | 29 Nov | 1.39 | 4 | 1.92 | 1.16 | 35.1 | -2.47 | 5 |
| **Neang Gnenh (acc 1535 B)** | Short | 4 Dec | 1.26 | 2 | 2.88 | 1.06 | 31.6 | -2.44 | 5 |
| **Gneng (acc 387**) | Short | 4 Dec | 1.24 | 3 | 1.76 | 0.94 | 29.1 | -2.08 | 5 |
| **Kong Neam (Acc No 26)** | Short | 7 Dec | 1.11 | 3 | 1.82 | 1.10 | 23.0 | -2.71 | 5 |
| Neang Kaun (acc 1540) | Medium | 9 Dec | 1.11 | 3 | 1.87 | 0.75 | 36.7 | -2.08 | 5 |
| Chamreak Phdau (acc 1106 A) | Medium | 9 Dec | 0.75 | 8 | 2.34 | 0.74 | 21.0 | -2.51 | 5 |
| **Neang Sar Thom (acc 152)** | Medium | 15 Dec | 0.68 | 5 | 2.06 | 0.44 | 34.1 | -2.38 | 3 |
| Damnoeub Krachak Sess (acc 1416 B) | Long | 26 Dec | 0.43 | 18 | 2.36 | 0.17 | 32.4 | -2.88 | 5 |
| Neang Chem (acc 1013) | Long | 24 Dec | 0.31 | 13 | 2.22 | 0.28 | 28.2 | -2.92 | 5 |
| Phcar Sla (acc 1014) | Long | 28 Dec | 0.27 | 20 | 1.92 | 0.16 | 32.8 | -2.44 | 3 |
| IR57514-PMI-5-B-1-2 (regional check) | Medium | 9 dec | -0.77 | 8 | 3.24 | 0.44 | 24.0 | -2.20 | 5 |
| NSG19 (local check) | Short | 4 Dec | -0.31 | 3 | 2.22 | 1.48 | 29.1 | -2.21 | 3 |
| Mean[a] | | | -0.07 | 8 | 2.13 | 0.55 | 28.5 | -2.54 | 4.47 |
| Lsd (p=0.05) [a] | | | 1.21* | 4.7* | 0.58** | 0.27* | 4.9* | ns | SE=1.2 |

**[a] Calculated from all 75 cultivars. Lines with bold letters were selected as putative drought tolerant donor**

# Screening method and phenotypic evaluation of rice genotypes resistance to drought in rainfed drought prone environments in north and northeast Thailand

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## Introduction

Drought stress, varying in severity during the reproductive stages, frequently reduces grain yield of rainfed lowland rice in north and northeast Thailand. Breeding for adaptation to drought in target population environments (TPE) has been attempted by the national breeding program. Selection for drought resistance for rainfed lowland drought stress conditions using morpho-physiological traits has been suggested (O'Toole, 1982; Fukai and Cooper, 1995). Before further progress can be made in utilizing morpho-physiological adaptations to drought, however, suitable screening methods must be developed (O'Toole and Chang, 1979). Furthermore, for any plant trait to be used in a breeding program, it must be demonstrated that it contributes to yield under drought conditions (Bidinger and Witcombe, 1989). Earlier studies indicated that cultivars with greater leaf water potential (LWP), high drought response index (DRI, Bidinger et al., 1987), and small delay in flowering time produced greater spikelet fertility and grain yield under rainfed lowland drought conditions in northeast Thailand (Pantuwan et al., 2001)

## Materials and methods

An experiment was conducted at Ubon Ratchathani Rice Research Center, northeast Thailand in 2000 wet season using 219 double haploid lines (DHL), derived from a cross between CT9993-5-10-1-M and IR62266-42-6-2. All DHL are targeted to be at the same stage of phenological development, i.e., flowering time, and experienced different degrees of drought stress during the reproductive stage. To synchronize flowering time, all DHL were grouped into four maturity groups and four seeding times were then applied. The first seeding date was about four weeks later than normal planting time, in order to avoid rain during the stress period. The experiment was under rainfed lowland conditions, with supplemental surface irrigation after seeding until DHL reached the panicle initiation stage (PI). Surface water was drained out of the field and line source sprinkler irrigation (LSS) was applied until maturity. LSS was used to generate a gradient of moisture regimes, i.e., from well-watered control to different levels of water deficit. Using cited procedures, DHL were evaluated across managed stress conditions for grain yield and yield components and some secondary traits.

## Results and discussion

LSS distributed water uniformly, however, seasonal winds that blew in from the northeast to the southwest moved water droplets away to replication 2, on the right side of the sprinkler line (Figure. 1a). Therefore, determination of areas for different water deficit treatments was adjusted using the amount of water received in the catch cans. Using water distribution generating from LSS, five water treatments, i.e., well-watered control in W0 and four levels of water deficit treatments, from mild to severe water deficit in W1, W2, W3, and W4, were assigned (Figure 1a). the amount of water decreased linearly from W0 to W4 areas. Water received in each area was 9.4 mm, 5.4 mm, 2.9 mm, 0.9 mm, and 0 mm per day in W0, W1, W2, W3, and W4, respectively, while average pan evaporation was about 6 mm per day. Staggered planting dates brought about 80% of DHL flowering at the same time, hence they experienced the same severity of water deficit. Biological yield, grain yield, and harvest index of DHL declined sharply with decreases in water applied, i.e., increase in severity of water deficit (Figure. 1b).

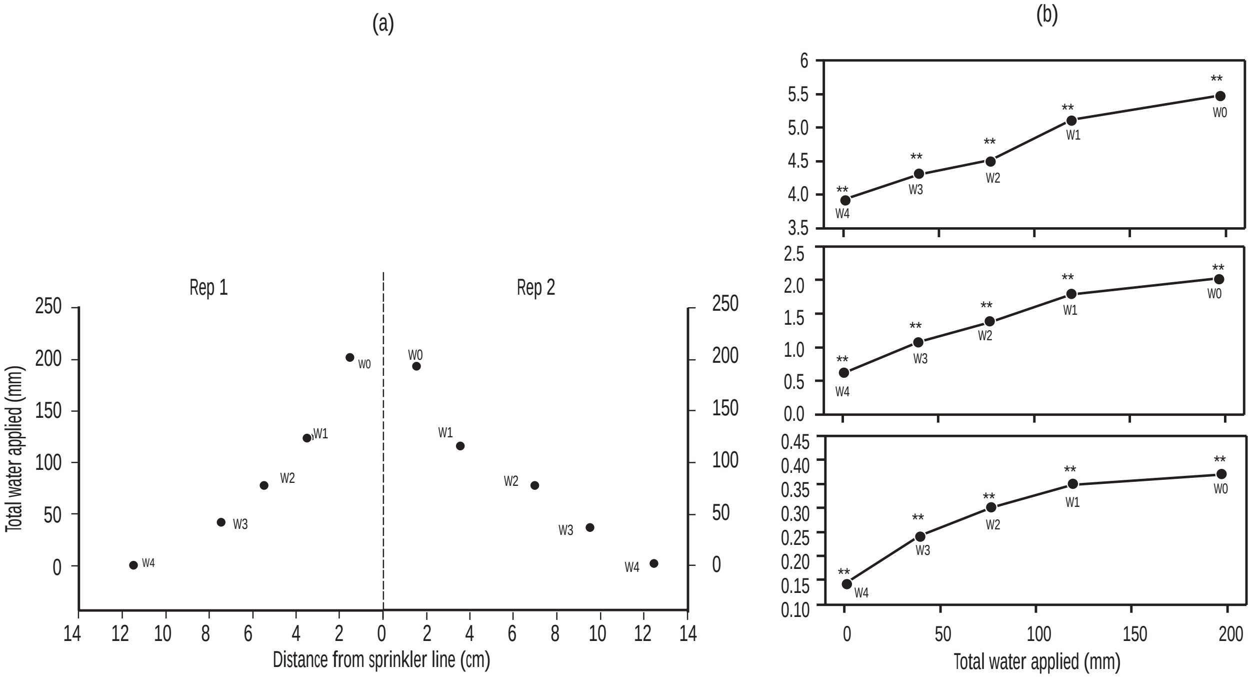

**Figure. 1. (a) Determination of treatments for W0 (well-watered control) and 4 drought stress treatments (W1, W2, W3 and W4) based on total amount of water applied using line source sprinkler. (b) Response of DHL for biological yield, grain yield and harvest index to 5 levels of water applied using line source sprinkler.**

Phenotypic characters evaluated are shown in table 1. Significant genetic variation existed (P £ 0.00) for grain yield, biological yield, harvest index, and spikelet sterility under well-watered control and the four stress treatments. Using DRI, 16, 22, 22, and 19 drought resistant genotypes were selected among 219 DHL in W1 (DRI 1.72±0.09), W2 (DRI 1.96±0.11), W3 (DRI 1.74±0.12), and W4 (DRI 2.03±0.14), respectively. LWP and drought score determined during stress period in W3 and W4 varied significantly among DHL (P £ 0.000). LWP highly correlated with drought score (r = -0.50**). Drought stress caused DHL to delay flowering time and the delay varied greatly, i.e., 0-5 days, 0-15 days, 0-27 days, and 0-40 days in W1, W2, W3, and W4, respectively.

In conjunction with other research for the breeding program, a marker assisted selection (MAS) scheme is in progress. Using data from this work, quantitative trait loci (QTL) for grain yield and its components and other agronomic traits were identified (Lanceras et al., 2004). Combining data from other studies, QTL detection and dissection of primary and secondary traits responsive to late drought stress has been exercised and six donors; IR68586-F2-CA-31, IR68586-F2-CA-43, IR68586-F2-CA-54, IR68586-F2-CA-109, IR68586-F2-CA-137 and IR68586-F2-CA-143 have been selected (Theerayut Toojinda, unpublished data). Introgressions of drought resistant QTLs into some rainfed lowland varieties via MAS is ongoing.

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## Acknowledgements

Financial support for this work by The Rockefeller Foundation is acknowledged.

**Table 1. Minimum, maximum, mean and standard error of the mean of phenotypic characters of DHL and parents evaluated under well watered control (W0) and 4 levels of stress conditions (W1, W2, W3 and W4) using the line source sprinkler (LSS) at Ubon Ratchathani Rice Research Center in 2000 wet season**

| | Min | Max | CT9993(parent) | IR62266 (parent) | Mean | Std.Err |
|---|---|---|---|---|---|---|
| **Grain yield (t / ha)** | | | | | | |
| W0 | 0.68 | 4.17 | 1.57 | 3.43 | 2.01 | 0.046 |
| W1 | 0.15 | 3.62 | 1.42 | 2.76 | 1.79 | 0.043 |
| W2 | 0.27 | 2.81 | 1.30 | 1.79 | 1.38 | 0.040 |
| W3 | 0.01 | 2.96 | 1.18 | 0.73 | 1.07 | 0.039 |
| W4 | 0.00 | 2.17 | 0.52 | 0.20 | 0.61 | 0.033 |
| **Harvest index** | | | | | | |
| W0 | 0.15 | 1.29 | 0.36 | 0.42 | 0.37 | 0.007 |
| W1 | 0.12 | 0.53 | 0.34 | 0.40 | 0.35 | 0.006 |
| W2 | 0.08 | 0.56 | 0.29 | 0.30 | 0.30 | 0.007 |
| W3 | 0.02 | 0.45 | 0.33 | 0.12 | 0.24 | 0.007 |
| W4 | 0.00 | 0.47 | 0.15 | 0.03 | 0.14 | 0.007 |
| **Biological yield (t / ha)** | | | | | | |
| W0 | 2.62 | 9.62 | 4.28 | 8.16 | 5.47 | 0.089 |
| W1 | 2.05 | 9.51 | 4.06 | 6.95 | 5.10 | 0.085 |
| W2 | 2.36 | 7.62 | 4.34 | 5.92 | 4.50 | 0.076 |
| W3 | 2.51 | 7.72 | 3.49 | 5.40 | 4.30 | 0.068 |
| W4 | 1.62 | 8.47 | 3.03 | 4.74 | 3.92 | 0.073 |
| **Drought response index (DRI)** | | | | | | |
| W1 | -5.70 | 2.32 | 0.34 | -0.07 | 0.00 | 0.071 |
| W2 | -2.23 | 3.42 | 1.55 | -0.52 | -0.01 | 0.070 |
| W3 | -2.24 | 3.64 | 1.58 | -1.33 | -0.02 | 0.070 |
| W4 | -2.30 | 3.86 | 2.27 | -1.45 | -0.02 | 0.071 |
| **Spikelet sterility (%)** | | | | | | |
| W0 | 14.10 | 96.50 | 33.73 | 28.26 | 36.68 | 0.919 |
| W1 | 10.80 | 89.70 | 32.57 | 33.14 | 38.18 | 0.947 |
| W2 | 10.20 | 84.70 | 34.53 | 41.89 | 42.83 | 0.989 |
| W3 | 16.90 | 96.90 | 49.10 | 56.21 | 51.62 | 1.092 |
| W4 | 14.60 | 95.00 | 49.55 | 62.43 | 59.22 | 1.082 |
| **Midday leaf water potential (MPa) determined in W4** | | | | | | |
| 09-Nov-00 | -2.19 | -1.26 | -1.68 | -1.92 | -1.83 | 0.012 |
| 15-Nov-00 | -2.30 | -1.48 | -1.86 | -2.07 | -1.96 | 0.013 |
| 22-Nov-00 | -2.25 | -1.10 | -1.52 | -1.82 | -1.70 | 0.014 |
| 22-Nov-00 | -2.65 | -1.50 | -2.04 | -2.40 | -2.16 | 0.016 |
| **Drought score determined in W4** | | | | | | |
| 9-Nov-00 | 0.00 | 5.00 | 1.46 | 3.25 | 2.23 | 0.072 |
| 16-Nov-00 | 0.00 | 6.50 | 2.52 | 4.13 | 3.06 | 0.097 |
| 24-Nov-00 | 0.00 | 8.00 | 4.55 | 6.38 | 5.31 | 0.095 |
| 30-Nov-00 | 0.00 | 8.50 | 5.25 | 6.50 | 5.65 | 0.091 |
| **Root mass density (mg / cm$^3$) at flowering stage determined in W4** | | | | | | |
| 0-15 cm depth | 0.23 | 1.44 | 0.75 | 0.58 | 0.75 | 0.014 |
| 15-30 cm depth | 0.00 | 0.52 | 0.09 | 0.05 | 0.07 | 0.004 |
| 30-45 cm depth | 0.00 | 0.05 | 0.01 | 0.01 | 0.01 | 0.001 |
| **Canopy temperature (Tc,˚C) and canopy minus air temperature (Tc-Ta, ˚C) determined in W4** | | | | | | |
| 20 Nov 00 Tc | 31.10 | 35.80 | 32.80 | 34.03 | 33.36 | 0.063 |
| 20 Nov 00 Tc-Ta | -1.90 | 3.30 | -0.20 | 1.03 | 0.63 | 0.070 |
| 24 Nov 00 Tc | 30.70 | 35.80 | 33.05 | 32.80 | 32.59 | 0.062 |
| 24 Nov 00 Tc-Ta | -0.10 | 4.80 | 2.30 | 1.86 | 1.46 | 0.057 |

# Using “smart” physiological-trait based crossing strategies to accumulate drought-adaptive genes

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## Drought environments

While drought is a problem worldwide, its consequences are most serious in terms of human welfare in the developing world, where wheat is cultivated under moisture stress on approximately 60 million hectares with national average yields ranging from 0.8 to 1.5 t/ha, a fraction of their irrigated yield potential (Morris et al., 1991). In terms of water distribution profiles within the drought prone environments, the three most distinct patterns are: (i) post-anthesis stress, (ii) pre-anthesis stress, and (iii) continuous stress. The post-anthesis drought stress is prevalent in the Mediterranean region, southern Africa and southern Australia, whereas the pre-anthesis stress scenario occurs in the Southern Cone of South America. A third scenario is that experienced by wheat grown on residual moisture following monsoonal rains, such as in regions of South Asia where irrigation water in unavailable. The realization of genetic gains in response to trait or gene selection to improve drought adaptation will be dependent on whether the choice of experimental environment adequately matches that of the target crop environment.

## “Smart crossing” to accumulate drought adaptive genes

Most of the traits currently mentioned in the literature associated with drought adaptation of wheat are shown in figure 1. However, the potential value of each trait needs to be considered with respect to the type of drought environment in which a cultivar must be adapted. For this purpose, traits can be divided into groups whose genes and/or physiological effects are likely to be independent such that when parents with contrasting traits are crossed, drought-adaptive genes will be pyramided.

## Group-1: Traits relating pre-anthesis growth

### *Crop establishment*

Vigorous crop establishment is agronomically desirable as it helps to shade the soil and suppress weeds that compete for water. Rapid groundcover is one important component and can be achieved by breeding for thinner, wider leaves and a more prostrate growth habit (Richards et al., 2002). Large seed and embryo size may help to achieve early vigor. These traits would be especially important in the Mediterranean type of drought environment where rainfall during the early part of the cycle would be protected from evaporation by a vigorous stand. Long coleoptiles are desirable for the environments where the crop grows exclusively on stored soil moisture, such as in S. Asia, to avoid extremely hot soil surface temperatures and rapid soil drying.

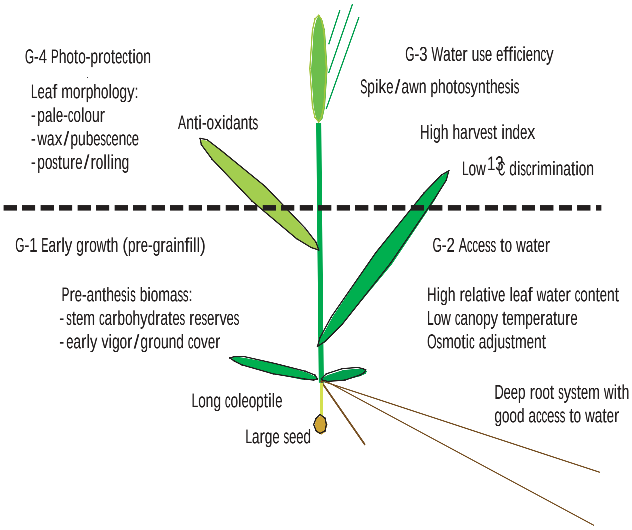

**Figure 1. Drought-adaptive traits groups associated with different physiological & genetic mechanisms**

### *Pre-anthesis biomass and partitioning of assimilates to stem reserves.*

In a predominantly post -anthesis drought scenario, vigorous growth prior to heading can theoretically give two advantages: (i) assimilates may be partitioned to a better root system that could help relieve subsequent moisture stress, (ii) assimilates may be partitioned to stems from jointing onwards for remobilization during grainfilling to supplement assimilates generated in the drier post-anthesis period. Where conditions are reasonably favorable prior to heading, stored fructans can contribute substantially to grainfilling, especially when canopy photosynthesis is inhibited by drought post-anthesis (Blum, 1998).

## Group-2: Traits relating to access to water

### *Roots*

A root system that can extract whatever water is available in the soil profile is clearly drought adaptive (Hurd, 1968), but difficult to measure directly. Traits that are indicative of the water status of a plant, especially when measured during periods of peak stress, are useful indicators of a plants capacity to match evaporative demand through the ability to explore and extract soil water.

### *Stomatal aperture related traits*

Instantaneous measurement of traits affected by the water relations of the plant, such as relative leaf water content (RLWC), stomatal conductance (Gs), and canopy temperature (CT) can give indications of water extraction patterns (Figure 2). Of these, by far the easiest to measure in the field is CT, which shows good association with other water relations parameters (Blum et al., 1982) as well as with performance under drought of random sister lines (Reynolds et al., 2000). Measurement of these traits in conjunction with soil moisture sampling may be useful in selecting deep-rooted germplasm in the appropriate environments.

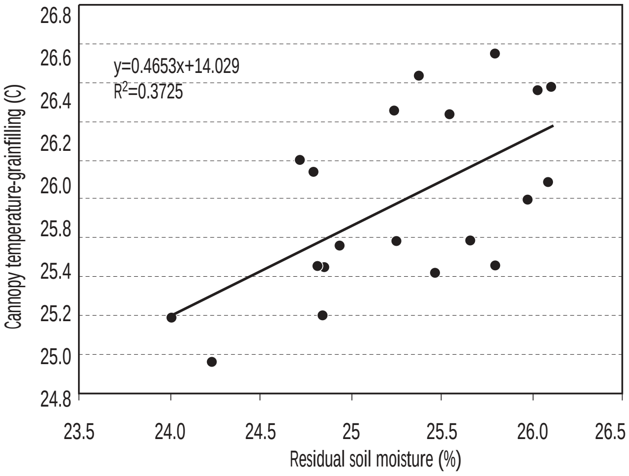

**Figure 2. Relationship between canopy temperature during grainfill and residual moisture post-harvest at 90-120cm for 20 wheat genotypes.**

### *Osmotic adjustment*

Solute concentration in the cell is intimately tied to plant water status as can be seen from the following definition of water potential: $\psi_{water} = \psi_{solute} + \psi_{turgor}$ That is, leaf water potential ($\psi_{water}$) is the sum of solute potential ($\psi_{solute}$) and turgor potential ($\psi_{turgor}$). Therefore, processes that depend on a favorable gradient of water potential, such as cell expansion, may in theory continue despite water deficit if osmotic adjustment creates a more negative water potential in the growing tissue than outside it. Theoretically, metabolic reactions that require fluxes of water or solutes between cells and organelles could also be maintained by osmotic adjustment when thermodynamically favorable. Therefore, under drought, osmotic adjustment may facilitate critical growth functions such as root growth, meiosis, and pollen development, thus mitigating some of the most detrimental effects of plant water deficit. Genetic variation in osmotic adjustment is well established in wheat. A number of experiments have shown that wheat lines selected for high osmotic adjustment in response to lowering of leaf water potential have higher grain yields in field experiments and in the study by Morgan and Condon (1986), high osmotic adjustment was strongly associated with greater soil water extraction. Osmotic adjustment is believed to be controlled by a single gene in wheat and rice (Morgan, 2000).

## Group-3: Traits relating to water use efficiency

### *Carbon isotope discrimination*

Measurement of $^{13}$C isotope discrimination (CID) or ash content of grain or other tissues can be used to estimate WUE of the crop since their signal is based on the integration of plant water status over a period of time (Condon et al., 1993). However, these data must be interpreted with care since although most field studies have shown that better performance of wheat cultivars under drought is associated with lower water use efficiency (Condon et al., 1993). A recent study in Australia (Rebetzke et al., 2002) indicates an advantage of low CID, high WUE genotypes under conditions where crops survive exclusively on stored soil moisture.

### *Harvest-index*

Genes that affect a greater relative partitioning of assimilates to the sink, resulting in a higher harvest index, would be expected to improve yield under drought since this would not be associated with the water cost of generating additional biomass.

### *Spike photosynthetic capacity*

Under extreme drought stress where the leaf-canopy may have mostly senesced, spike photosynthesis can play a major role in grain-filling and has been shown to contribute up to 40% of total carbon fixation under moisture stress in controlled environments (Evans et al., 1972). This is because spikes have higher WUE than leaves due to the fact that they can re-fix respiratory carbon (Bort et al., 1996). This stay-green-spike trait is currently being introgressed into elite drought tolerant backgrounds to see if it can be combined with yield responsiveness, such that the trait is facultative responding only in drier years.

## Group-4: Traits relating to photo-protection

### *Anti-oxidants*

Decreased stomatal conductance in response to drought leads to warmer leaf temperatures and insufficient $CO_2$ to dissipate incident radiation both of which increase accumulation of harmful oxygen radicals and photoinhibitory damage. Photoinhibition can be ameliorated by antioxidants such as superoxide dismutase and ascorbate peroxidase, and these have been shown to increase in response to drought stress in pea for example (Mittler and Zilinskas, 1994). Thermal dissipation through the xanthophylls cycle is another protective mechanism that can dissipate as much as 75% of absorbed light energy (Niyogi, 1999). In a study comparing a drought adapted barley landrace with a modern cultivar, the former displayed two mechanisms of photo-protection, rapid xanthophyll cycling, and up to 50% less leaf chlorophyll resulting in a passive reduction of light absorbance (Havaux and Tardy, 1999).

### *Leaf anatomy*

In addition to pale leaf color, a number of leaf traits such as glaucousness, pubescence, rolling, and erect posture can potentially decrease radiation load to the leaf surface, thereby lowering evapo-transpiration rate and reducing the risk of irreversible photo-inhibition.

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# Morphological traits for vegetative stage drought tolerance in rice (*Oryza sativa*)

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## Introduction

In South and Southeast Asia, about 21% of total rice area is drought prone. Yield losses due to drought ranged from 30 to 70%. In eastern Uttar Pradesh, yield loss in drought prone areas is due to mainly early stage drought coupled with later stage encounters with low temperatures, which leads to poor crop establishment and grain sterility. Results of several studies showed that field screening for tolerance to water deficit at vegetative stage has been difficult to demonstrate a consistent relationship between seedling / vegetative stage drought score and yield performance in target environments (Mackill et. al., 1996; Mitchell et al., 1998). Yield improvement in water-limited environments could be achieved through selection for secondary traits, which are more reliable and constitutive in contributing to drought resistance in breeding programmes. Therefore, preliminary field testing trials were conducted in a rainout shelter for phenotypic selection and to determine their contribution viz. drought resistance and stability performance of genotypes.

## Materials and methods

A field trial was carried out in a rainout shelter in two sets of environments: (i) water stress and (ii) irrigated conditions at the Main Experiment Station, Kumarganj in anaugmented block design. IR64 was kept as check and repeated in each block. Fifty-eight (58) DH lines and 23 IR64 introgressed lines, including Azucena (check) varieties were collected from TNAU, Tamil Nadu, India were used. Six rows of each line were direct sown in the field on 3 September 2003. Irrigation was withheld in 13 October 2003 for 30 days (water stress duration was extend due to low temperatures). Various growth parameters like plant height, tiller number, leaf area, relative water content (RWC), and specific leaf weight (SLW) were measured before and after stress and at recovery (20 days after re-watering). Leaf rolling and disease infestation in the field were scored on the basis of the IRRI scale.

## Results

### *Evaluation of 59 (including IR64) DH lines in rainout shelter*

The analysis of variance for environments were found significant for all morphological traits, whereas variation for genotypes were found non-significant. Relative water content showed significant a positive relationship between shoot biomass ($R^2$=0.50), leaf rolling ($R^2$=0.61) and weak relationships were obtained with plant height ($R^2$= 0.006) and tiller per hill ($R^2$=0.035); however, traits like specific leaf weight did not show significant relationship with other traits under water limited environment and irrigated conditions (Figure 1). Recovery growth might be another important component for screening of vegetative stage drought tolerance in rice. Crops currently being studied (wet season, 2003) were subjected to low temperatures (15-21°C) during recovery growth; severe infestation of BLB, BS, ShR, and zinc deficiency was observed in the field as well as in the rainout shelter experiments.

### *Evaluation of introgressed lines for drought tolerance*

Among 23 introgressed line, eight lines performing well under water-stress and showed faster recovery. Attempts were made to explore relationships between relative water content and variables such as biomass, specific leaf weight, leaf rolling, and plant height. Regression analysis showed linear relationship among the variables. $R^2$ values for specific leaf weight (0.13) and plant height (0.03) were very low. It indicates that relative water content over these plant growth traits were non-significant. Meanwhile, significant $R^2$ value (0.40) between relative water content and specific leaf weight were found during recovery growth (20 days after re-watering). However, biomass per plant and leaf rolling showed strong relationship with relative water content of the leaf during water stress period. $R^2$ values were 0.38 & 0.58, respectively (Figure2).

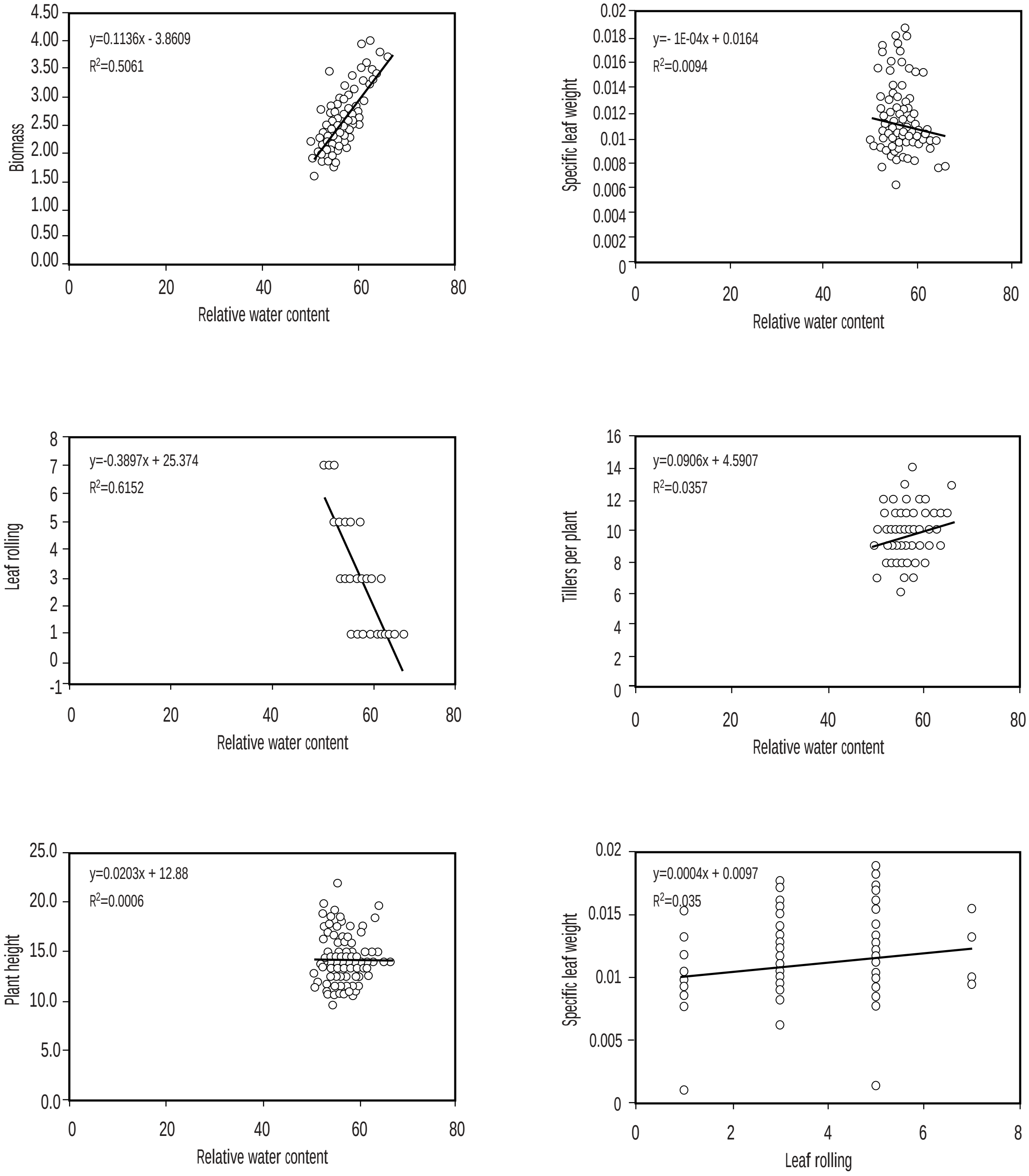

**Figure 1. Relationship among relative water content and other morphological traits of DH lines grown under water-stress environment.**

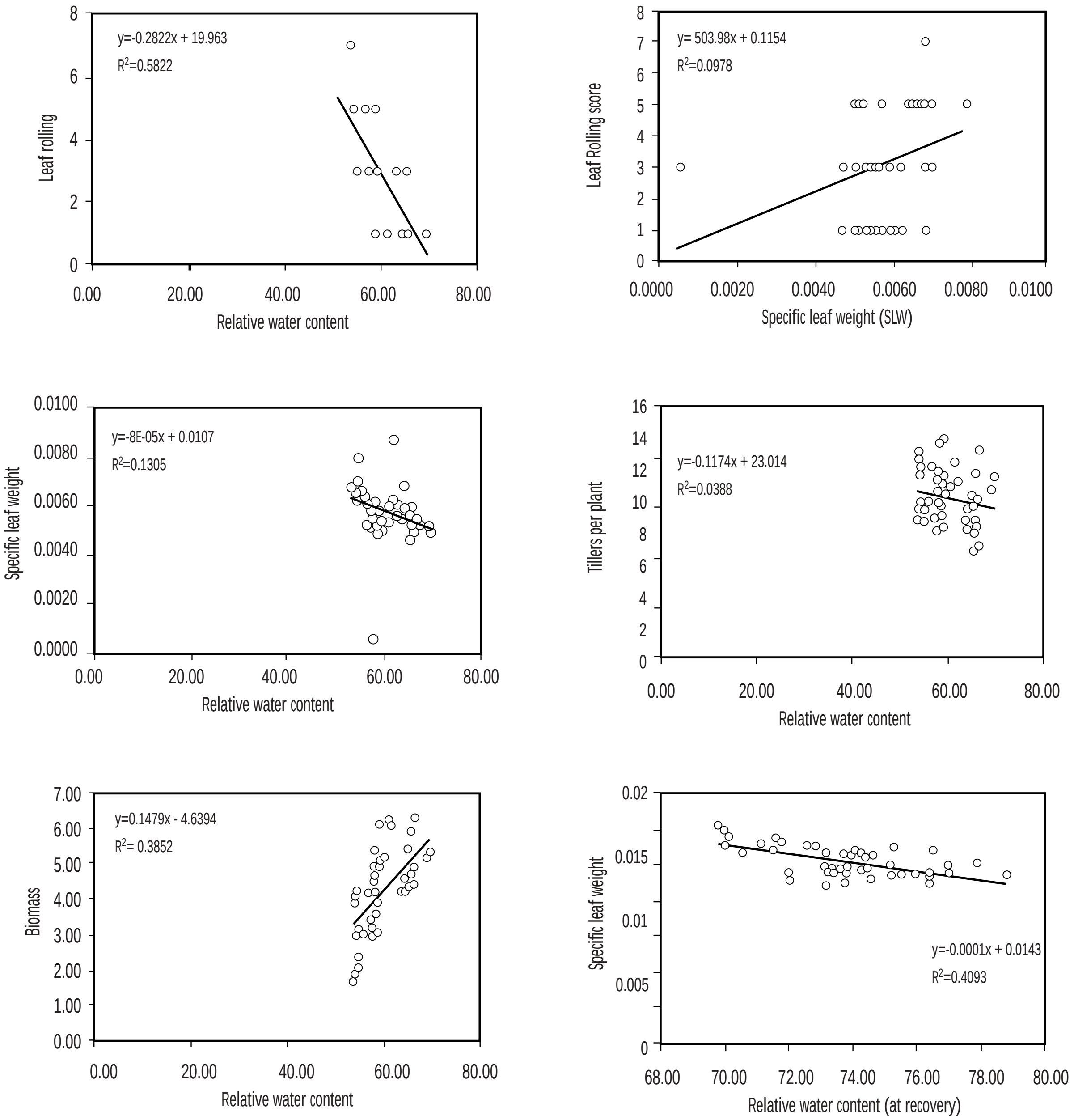

**Figure 2. Relationship among various morphological traits of 23 introgressed lines during water-stress and recovery (20 days after re-watering).**

## Conclusions

Data from this nvestigation indicate that relative water content (RWC) can be used a reliable and simple screening techniques for evaluating larger DH lines and introgressed lines for vegetative stage drought. Recovery growth is important for yield under water limited environments when rice crops cope with vegetative growth stage water-stress. Our preliminary study with IR 64 introgressed lines showed marked differences regarding new root formation after re-watering of the stressed plants. This research is ongoing.

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# Root study of traditional rice cultivars and IR64 introgressed lines for vegetative stage drought

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## Introduction

Drought is major problem for rice grown under rainfed upland conditions. Progress in breeding for drought resistance has been slow; mainly because of the wide range of water stress environments found in rice growing areas; drought conditions differ in time, intensity, and hydrology (Fukai and Cooper et al., 1994). From the perspective of a plant physiologist agronomist, plant water status has a major impact on plant function and adoptive processes in the plant under water limited environments. Several studies in the past reported that the major control of plant water status in crop plants subject to drought is exerted mainly by constitutive traits like root length, plant phenology, and leaf surface properties (Bloom, 2000). These traits may help to maintain high relative water content, and tend to retain higher leaf area and low leaf rolling. Traditional cultivars or landraces have unique tolerance strategies appropriate to specific growing condition. There are several putative traits that confer drought resistance in rice, therefore, this investigation was designed to identify morphophysiological traits and the inter -ssociation with drought tolerance in target environment.

## Materials and methods

***Experiment 1.***

Fifteen traditional rice cultivars and genotypes were collected from the rainfed area of eastern India. The cultivars have natural adoption through a long period of cultivation in specific environments. Field experiments were conducted at two sites: the Crop Research Station Masodha (27.47°N and 82.12°E) and the Main Experiment Station, Kumarganj, Faizabad, with three replicates during the hot season 2003. Temperature ranged between 36 and 44°C in both sites at early growth stage. The crop received deficit rain (April-June) whereas at a later stage, surplus rain was recorded in the field. Two seeds of each cultivars / genotypes were direct sown in a polythene bag (75x35 cm) with three replicates. Irrigation was withheld 30 days after sowing for 20 days in the field conditions. At the end of the drought period, root characters, shoot biomass, plant height, and shoot soluble-CHO were measured. Yield and yield attributing characters were measured at physiological maturity.

***Experiment 2.***

Validation of the above traits and identification of other traits for drought tolerance was undertaken. Forty-five IR64 introgressed lines (from IRRI) were direct sown in PVC pipe (1.25 m length and 6” dia) under rainout shelter at the Kumarganj site during the wet season 2003. Drought were created in field condition and controlled conditions (PVC pipe), irrigation was withheld 14 days after sowing for 20 days. Root traits were measured at the end of drought. Plant height, relative water content (RWC), specific leaf weight (SLW), leaf rolling, shoot biomass, and shoot soluble-CHO was measured before and after drought. Recovery was measured 20 days after re-watering.

## Results

***Grain Yield***

Mean grain yield of these rice cultivars were significantly reduced under non-irrigated conditions. A significant positive relationship among root length and grain yield of irrigated (R=0.47) and water limited ($R^2$=0.55) environments were obtained among traditional cultivars (Figure 1). Similar strong positive correlations (r=0.45 & 0.64) were obtained between shoot soluble carbohydrate (end drought) and grain yield of water limited in irrigated condition, respectively. Other root characters like root dry weight (r=0.20), shoot dry weight (r=0.21), and root:shoot ratio (r=0.01) showed a weak relationship with grain yield. Moreover, cultivars,

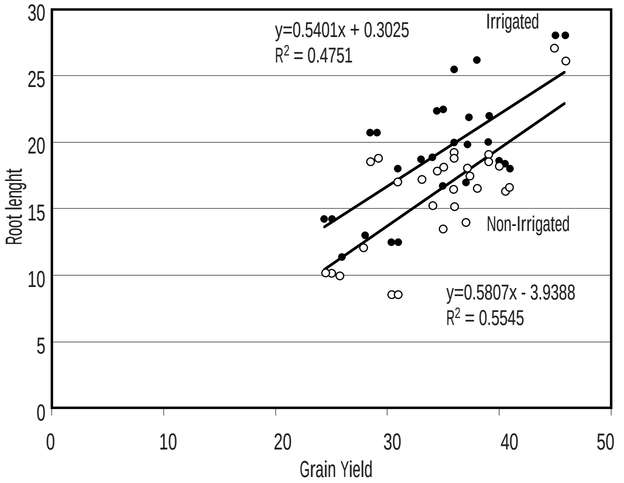

Figure 1. Relationship between root length and grain yield (non irrigated and irrigated conditions).

Nagina 22, Lalanakanda-41, Sathi 34-36, Laloo-14, and genotype RR 47-4001, RR 347-167, and RR 161-101 showed stable yield (reduction < 7 %) compared to other cultivars. The cultivars differed greatly for intertrait associations. Large variability in root traits was observed among traditional cultivars (Table 1).

### *Identification of morphophysiological traits*

Large variability was observed among IR64 introgressed lines for various root traits during water limited conditions. Analysis of variance for environments were found significant, whereas genotypes were found non-significant. Strong positive relationships were obtained between root length and shoot biomass ($R^2$=0.63), relative water content ($R^2$=0.59), and a significant negative relationship with leaf rolling ($R^2$=-0.54). Similarly, strong positive relationships were also obtained between RWC and biomass ($R^2$=0.50) and leaf rolling ($R^2$=-0.35). Shoot soluble CHO showed a very weak relationship with leaf rolling ($R^2$ 0.04) under water limited environments (Figure 2).

Table 1. Morpological traits and yield of traditional rice cultivars grown under water limited environment

| S.No. | Variety / Genotypes | Plant height | Total Tiller / plant | Root length | Root dry wt. | Shoot dry wt. | Root / Shoot ratio | Total Solublesugar | Irrigated | Non irrigated yield |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | RAV1401-1-1-2-3-4-5 | 66.25 | 33.1 | 49.7 | 41.4 | 45.5 | 49.7 | 47.6 | 20.66 | 18.66 |
| **2** | Azucena | 75.75 | 37.9 | 56.8 | 47.3 | 52.1 | 35.9 | 44.0 | 19.89 | 18.25 |
| **3** | Morobrakan | 54.75 | 27.4 | 41.1 | 34.2 | 37.6 | 37.6 | 37.6 | 18.85 | 15.16 |
| **4** | RR-161-101 | 57.25 | 28.6 | 42.9 | 35.8 | 39.4 | 51.1 | 45.2 | 18.33 | 17.12 |
| **5** | Ratna | 77.80 | 38.9 | 58.4 | 48.6 | 53.5 | 35.2 | 44.3 | 22.33 | 17.93 |
| **6** | IET-16706 | 53.65 | 26.8 | 40.2 | 33.5 | 36.9 | 41.3 | 39.1 | 14.14 | 10.20 |
| **7** | RR-348-6 | 63.00 | 31.5 | 47.3 | 39.4 | 43.3 | 47.9 | 45.6 | 18.88 | 18.60 |
| **8** | RR47-4001 | 73.00 | 36.5 | 54.8 | 45.6 | 50.2 | 59.7 | 55.0 | 28.00 | 26.50 |
| **9** | Lalkmanda-14 | 91.00 | 45.5 | 68.3 | 56.9 | 62.6 | 38.4 | 50.5 | 25.85 | 16.51 |
| **10** | Annada | 58.50 | 29.3 | 43.9 | 36.6 | 40.2 | 46.1 | 43.2 | 16.83 | 13.76 |
| **11** | Laloo-14 | 70.25 | 35.1 | 52.7 | 43.9 | 48.3 | 53.0 | 50.7 | 18.17 | 16.42 |
| **12** | Sathi 34-36 | 80.80 | 40.4 | 60.6 | 50.5 | 55.6 | 51.1 | 53.3 | 21.92 | 18.27 |
| **13** | Solumpikit | 77.85 | 38.9 | 58.4 | 48.7 | 53.5 | 62.3 | 57.9 | 12.49 | 18.50 |
| **14** | RR165-1160 | 95.00 | 47.5 | 71.3 | 59.4 | 65.3 | 49.5 | 57.4 | 19.59 | 18.95 |
| **15** | RR347-167 | 75.50 | 37.8 | 56.6 | 47.2 | 51.9 | 0.0 | 26.0 | 12.13 | 11.00 |

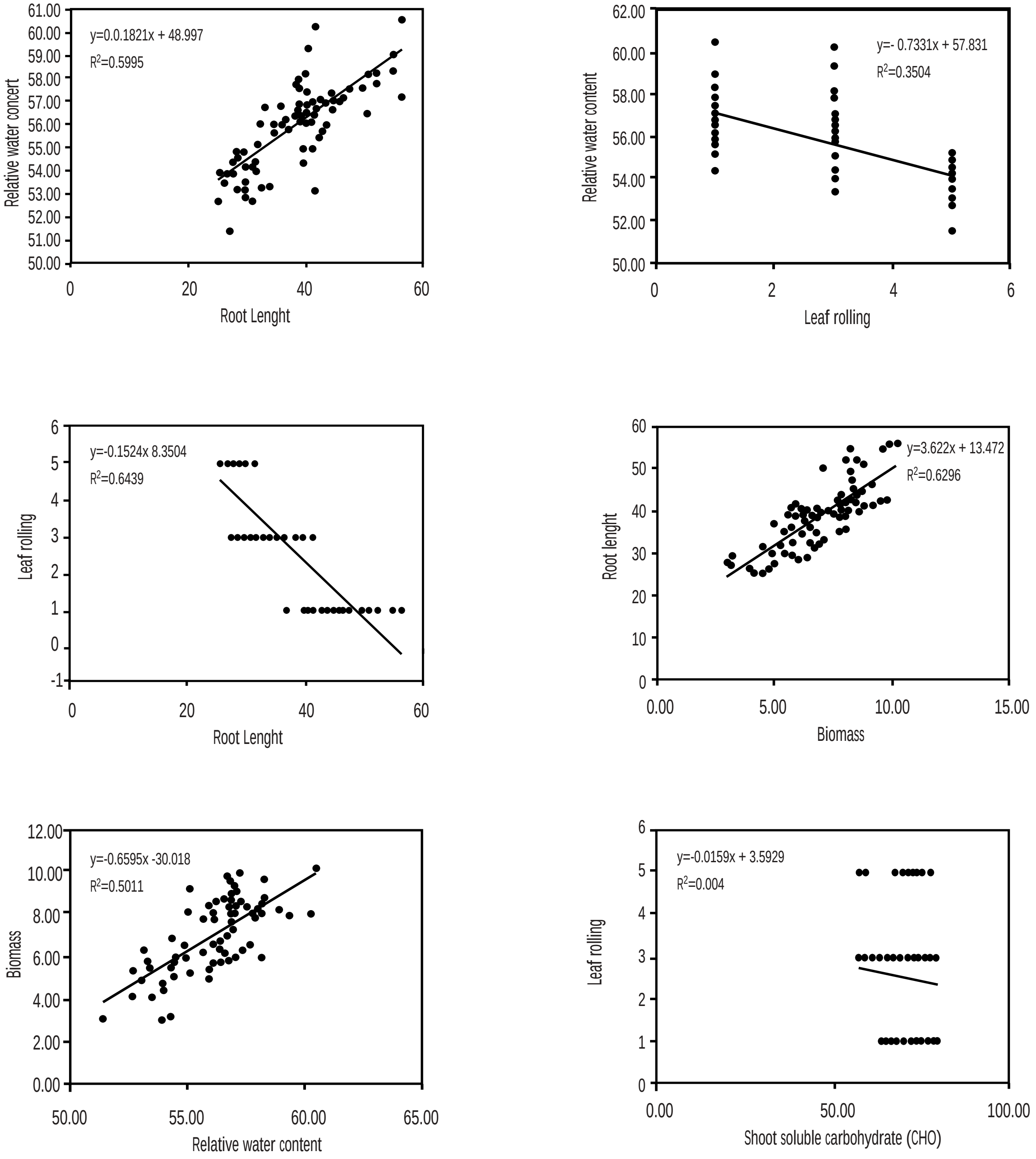

**Figure 2. Regression lines among some contitutive and indicators of rice crop under limited environment (wet seasons - 2003).**

## Conclusion

Preliminary evaluation indicates that constitutive traits like root length showed a strong relationship with grain yield as well as stress indicators (RWC and leaf rolling) and phenology (root: shoot ratio and biomass) under a water limited environment among cultivars and IR64 introgressed lines of rice. Further validation is needed. A hot season study is in progress.

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# Genetic analysis of physio-morphological traits associated with drought tolerance in rice across the environments

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## Introduction

Drought is the major constraint limiting rainfed rice production in the southern parts of India. Although rice is highly susceptible to drought, still there is a scope to improve this crop for drought tolerance due to the inherent capacity and inbuilt genetic variability for wider adaptations in varied ecosystems. Genotypic variation for different traits contributing to drought tolerance in rice has been reported by Babu et al. (2001). Hence it is necessary to collect and evaluate the available landraces and improved cultivars for their genetic variability for drought tolerance traits and assess their stable performance over varied environments. In the present study, attempts were made to collect landraces and cultivars developed at different research stations in India and elsewhere and to assess the extent of genetic variability (Genetic Coefficient of Variation, GCV; Phenotypic Coefficient of Variation, PCV and Environmental Coefficient of Variation, ECV), heritability ($h^2$) for physio-morphological traits exhibited by them and their stability over diverse environments.

## Methods

Rice accessions collected from various research stations and farmers' fields in India and IRRI, Philippines were field tested in two environments viz., Coimbatore (managed stress) during summer 2003 (Feb-May) and Paramakudi (target environment) during monsoon 2003-04 (Sep-Jan) in Tamil Nadu, India. Forty-six genotypes in location one (Coimbatore) and 36 genotypes in location 2 (Paramakudi) were evaluated for several traits related to drought tolerance. Observations were recorded among 32 varieties on plant height, number of tillers per plant, leaf rolling score, leaf drying score, stress recovery index, basal root thickness, canopy temperature, relative water content, chlorophyll stability index, proline content, SPAD value, and biomass yield. Observations on canopy temperature, leaf rolling score, leaf drying score, biomass yield were recorded in both the environments. Variability parameters (GCV, PCV, ECV, $h^2$) and genetic advance and stability parameters (mean, $s^2d$ and bi) were computed using standard statistical package (INDOSTAT).

## Results

Moderate to high PCV and GCV were observed for most of the traits. High heritability coupled with medium to high genetic advance was observed for all the traits except leaf rolling, canopy temperature, and SPAD value (Table 1). The variances due to genotypes and environments were significant for biomass and canopy temperature and G x E interaction was significant only in the case of biomass yield.

## Conclusion

Knowledge on the extent of genetic variability, heritability estimates, and stability over environments will facilitate the development of drought tolerant varieties. The mean sum of squares due to genotypes was significant for most drought resistant traits, indicating the presence of a considerable amount of variability among the genotypes for traits related to drought tolerance. In general, the ECV was lower than

**Table 1. Genetic variability estimates revealed for different traits in rice over different environments**

| | Variability parameters | | | |
|---|---|---|---|---|
| Traits | PCV (%) | GCV (%) | $h^2$ | GA (% of mean) |
| Plant height (cm) | 21.42 | 20.43 | 0.90 | 51.45 |
| Tillers per plant | 19.64 | 13.69 | 0.48 | 25.22 |
| Leaf rolling score | 14.11 | 11.23 | 0.61 | 24.80 |
| Leaf drying score | 24.60 | 20.20 | 0.67 | 44.62 |
| Stress recovery index | 52.21 | 50.44 | 0.93 | 128.64 |
| Basal root thickness (mm) | 25.66 | 25.03 | 0.95 | 64.43 |
| Canopy temperature (˚C) | 04.46 | 04.27 | 0.89 | 10.84 |
| Relative water content (%) | 26.29 | 22.64 | 0.74 | 51.50 |
| Chlorophyll Stability Index (%) | 28.79 | 28.67 | 0.99 | 75.38 |
| Proline content stress (mg/g) | 17.97 | 17.91 | 0.99 | 47.14 |
| Proline content control (mg/g) | 16.61 | 16.08 | 0.93 | 41.1 |
| SPAD value | 7.76 | 4.38 | 0.31 | 6.50 |
| Biomass Yield (g/row) | 66.00 | 62.50 | 0.89 | 156.50 |

PCV: Phenotypic coefficient of variation; GCV: Genotypic coefficient of variation; GA: genetic advance

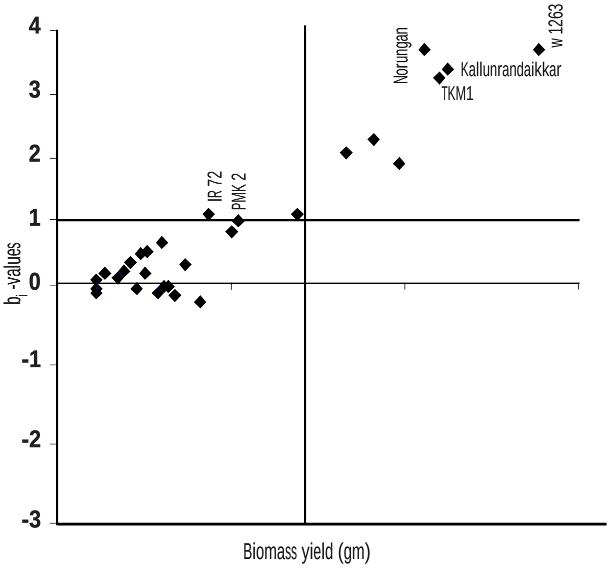

**Figure 1. Stability parameters of rice accessions for biomass yield.**

the PCV and GCV for all the traits except number of tillers per plant and SPAD value, indicating the lesser influence of environment over these traits, thus, improvement of these traits could be possible by simple selection. In both trials, grain yield could not be recorded due to severe drought. The biomass yield was taken as an indicator of plant production. The stability analysis for biomass over two environments revealed that the genotypes IR72, PMK 2, and TKM 2 proved to be more stable, based on their higher mean over the experimental mean, unit regression coefficient, and low $s^2d$ values (Figure 1). Reddy et al. (1998) also stressed the importance of these three parameters while selecting the stable genotypes in rice. In contrast, four genotypes viz., Kallurundaikar, Norungan, W1263, and TKM1 were found to be suitable for favourable environment (managed stress is considered as the favourable environment in the present case), recording high biomass yield with higher linear regression coefficients ($b_i > 1$) and high $s^2d$ values indicating their adaptation to the drought condition. Hence, these seven genotypes could be successfully utilized in the breeding programme for the improvement drought tolerance in rice.

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# Proteomic analysis of drought responsiveness in rice

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## Introduction

Drought is a major limitation to rice productivity especially when it occurs during reproductive stage. The drought responses are often mediated by abscissic acid (ABA), which accumulates during drought, which has an antagonistic effect on GA mediated gene expression particularly on panicle emergence. The phytohormone ABA regulates many important physiological and developmental processes in plants (Leung and Giraudat, 1998). It also mediates stress responses such as adaptation to salinity, low temperature and water deficiency (Ingram and Bartels, 1996). The ABA level in plant is simultaneously regulated by opposing forces of catabolism and / or biosynthesis (Zeevaart, 1999). There are many reports on ABA biosynthesis but the catabolic process is not yet studied in detail. The role of ABA in stress responses is also poorly understood. Various genetic and molecular technologies are available for the identification of ABA regulated genes, which will be useful in understanding the role of ABA in stress response. The recent molecular biological techniques such as microarrays and proteomics can be used as powerful tools to dissect out the molecular responses of plant to drought and hormone mediated drought responses. Limited reports are available regarding the effect of ABA on gene expression / proteins. Here we report the proteomic analysis of drought / ABA responsiveness in rice.

## Materials and methods

### *1. Plant material and ABA application*

Rice plants (Variety IR64) were grown in pots in the green house. After 25 DAS, ABA was applied daily (continuously for four days at 100 micromolar concentration) as foliar spray to one set of plants by maintaining a set of control plants (Jiang and Huang, 2002) Photosynthesis and transpiration rate were measured at about 11 AM (3 hrs after spraying).

### *2. Pattern of ABA accumulation*

The pattern of ABA accumulation and degradation upon exogenous application was studied by means of quantifying the ABA content by HPLC (Krochko et al., 1998). Leaf samples were collected from both control and ABA sprayed plants. In the ABA sprayed plants, samples were collected at the following time points; Before 4th spray, 2, 4, 6, 24, 36 and 48 hrs after fourth day spray.

### *3. Identifying proteins responsive to ABA application and drought*

Leaf samples were collected from both control and ABA treated IR64 rice plants (six hours after 4th day spraying). The total leaf protein extract (Hosseini et al., 2002) and the crude micromal extracts (Gitte Frandsen et al., 1996) were prepared. Proteins extracts were prepared from the peduncle tissues of IR64 plants grown under well-watered, drought subjected and rewatered conditions. The protein extracts were fractionated by 2D-PAGE separation. The first dimension electrophoresis was done by IEF (using IPG strips of pH 4-7). About 100-microgram protein was loaded for the separation. The second dimension (12% SDS-PAGE) gel was stained by silver staining method. The silver stained gel was read by densitometer for spot detection and quantification. Protein gel images were captured and analysed using the Melanie 3 proteomic analysis software.

## Results

The ABA content increased further after the fourth spray but eventually declined, showing that spraying leads to only a transient increase in ABA content. The decline is due in part to increased activity of ABA 8'-hydroxylase, a cytochrome P450-dependent enzyme for ABA degradation. ABA application had significant effects on transpiration, conductivity and spikelet fertility. In the two dimensional gel electrophoretic analysis of total leaf proteins, about 128 protein spots were found to be differentially expressed upon ABA application. From the already published data (Hosseini et al., 2002), the possible matches for 16 spots were identified tentatively based on the spot position in the gel, molecular weight and pI. The proteins namely, Transcription elongation factor, Ribisco activase, fructose 1,6-biphosphate aldolase and GSH dependent dehydroascorbate reductase were found to be up-regulated and proteins such as Putative isoflavone reductase homolog, S-like RNase homologs, Triphosphate isomerase, Rieske Fe-S precursor proteins, actin depolymerising factor, superoxide dismutase (chloroplast) and nucleoside disphosphate kinase were found to be down regulated. Apart from this 9 protein spots showed significant upregulation (Abundance ratio of >1.5) and 4 spots showed significant down regulation (Abundance ratio of <0.5) across biological replications. Characterization of remaining spots by mass spectrometry is on progress. Proteomic analysis rice microsomal proteins showed that about 60 protein spots were responsive to ABA (Down-regulation: 36 spots; Up-regulation: 24 spots). Further fractionation of crude microsomal proteins will give information on the microsomal membrane proteins associated with ABA catabolism. Proteomic experiments to identify proteins controlling the responsiveness of the peduncle to drought, re-watering and treatment with hormones such as ABA and GA are on progress. In IR64, 21 protein spots were found to be responsive to drought and rewatering.

## Conclusions

Further characterization of ABA responsive proteins will be useful in revealing the ABA mediated stress responses and ABA regulated gene expression. Proteomic experiments towards identification and characterization of genes associated with ABA catabolism and panicle emergence (peduncle elongation) are on progress. Identifying the proteins/ genes involved in ABA catabolism and peduncle elongation will be a crucial element in breeding programs for reproductive stage drought tolerance because the evidence is that although ABA is essential for drought tolerance in leaves, it interferes with panicle emergence.

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# Aquaporins during osmotic stress: Regulation and evidence for endomembrane trafficking

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## Introduction

It seems obvious that plant water channels, aquaporins (AQP), ought to play an important role in maintaining cellular water homeostasis under conditions that necessitate modifications in water flux. Changes in water flux and allocation would be required to balance alterations in the cellular osmotic potential and therefore, AQP activity and / or expression should be tightly regulated. Environmental stimuli, including drought, dehydration, desiccation, and salinity, as well as a rise in abscisic acid (ABA), which accompanies the perception of osmotic stress, have been shown to regulate the expression of both tonoplast (vacuolar membrane; TP) and plasma membrane (PM) AQP at the transcript level (Maurel et al., 2002).

Few studies have addressed the direct regulation of AQP expression by osmotic stress at the protein level, and fewer still have focused on AQP dynamic behaviour. In the ice plant, regulation of AQP protein amount by salinity stress was observed using peptide-specific antibodies. McMIPF amounts in leaf TP decreased during salt stress and levels of McMIPC in root PM fractions were induced, while McMIPA showed no change (Kirch et al., 2000). For McMIPF, the decrease in protein amount in the presence of NaCl was contrasted by an increase in the presence of mannitol (Vera-Estrella et al., 2000), suggesting precise deciphering and discrimination of osmotic and ionic signalling pathways. Here, we further characterize the regulation of the ice plant AQP McMIPF under osmotic stress, and begin to dissect the mechanisms responsible for the stress-induced changes in McMIPF membrane distribution.

## Materials and methods

*Mesembryanthemum crystallinum* L. plants and cell suspensions were grown as previously described (Barkla et al., 1999; Vera-Estrella et al., 1999). Membranes were isolated from *M. crystallinum* plants and cell suspension cultures as previously described (Barkla et al., 1999; Vera Estrella et al., 1999). Microsomes were layered onto either continuous or discontinuous sucrose gradients and membranes were isolated by centrifugation (Vera-Estrella et al., 1999). Protein content in microsomal and purified PM or TP fractions was measured by a modification of the dye binding method of Bradford (1976). Water permeability of aquaporins was assayed by their heterologous expression in Xenopus oocytes injected with 50 ng of cRNA, 2 to 5 d post injection. Swelling was measured by video imaging on a Nikon Eclipse TE 300 microscope, equipped with a Hitachi KP-D50 colour video camera. Membrane proteins were separated by SDS PAGE and protein immunoblotting was performed as previously described (Vera-Estrella et al., 1999). Immunofluorescence of aquaporins and membrane markers were prepared according to Brown and Lemmon (1995) with some modifications. The cells were imaged with a BioRad Confocal laser scanning microscope with a 40x magnification objective, with a 488 nm argon ion laser and a 488 bypass filter. Alexa fluor 458 and 568 were excited with a 488 and a 568 nm filters, respectively.

## Results

Aquaporin (AQP) protein regulation and redistribution in response to salt and osmotic stress were investigated. *Mesembryanthemum crystallinum* (Ice plant) McMIPA (PIP-subfamily) and McMIPF

(TIP-subfamily), mediated water flux when expressed in *Xenopus* oocytes. Salt- and mannitol-induced stress affected the expression of McMIPA and McMIPF proteins differently. Specifically for McMIPF, osmotic stress resulted in increased protein amounts and a shift in protein distribution in sucrose density gradients, suggesting aquaporin trafficking. Indirect immuno-fluorescence labelling also supports a change in membrane distribution of McMIPF, indicating that McMIPF may reside, like BP80, in a prevacuolar compartment and in a compartment with unique characteristics. Mannitol-induced redistribution of McMIPF was arrested by pretreatment with brefeldin A, wortmannin, and cytochalasin D, inhibitors of vesicle trafficking. Evidence suggests a role for glycosylation and involvement of a cAMP signalling pathway in membrane redistribution of McMIPF. McMIPF redistribution to endosomal compartments may be part of a homeostatic process to restore and maintain cellular osmolarity under osmotic stress conditions.

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# Controls of leaf growth and stomatal conductance under water deficit: Combining genetic and ecophysiological analyses

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## Introduction

Phenotype analysis is often seen as an accumulation of trait measurements, whose quality depends on the genetic variability of studied traits and on their correlation with yield. In the case of tolerance to water deficit, the choice of relevant traits has been the object of a long controversy. This approach has several drawbacks:

- A trait which is favourable in a given pedoclimatic scenario is not necessarily so in another scenario. For example, plants subjected to water deficit reduce transpiration by both stomatal closure and reduction in leaf expansion rate. This trait is favourable in very dry scenarios because it maintains leaf water status and saves water for late periods of the crop cycle, but it also reduces growth and photosynthesis. It is therefore counter productive in moderately dry climatic scenarios.
- A phenotypic trait usually changes with environmental conditions. Transient traits, such as stomatal conductance, concentration of hormones, or of proteins, are therefore complex to analyse. The analysis of permanent traits such as final organ size or yield components is not straightforward either, because of the high genotype x environment interaction that lowers the overall heritability of these traits.

It is proposed that a modelling approach is an appropriate way to deal with these problems. Ecophysiological models are based on physical laws and on experimental relationships accounting for plant controls. Algorithms of plant control describe quantitatively the plant responses to environmental conditions, e.g., soil water deficit, air humidity, and temperature. They predict when, where in the plant, and to what extent these controls operate. They can therefore help to identify when and where molecular processes involved in controls should be looked for. Furthermore, because the parameters of these models are (or at least should be) unique for a given genotype, they can be used to analyse the genetic variability of the plant controls that result in the genotype x environment interaction.

## The "one gene–one parameter" paradigm

Stomatal aperture is controlled by several mechanisms, which all vary very rapidly with time of the day. They involve (i) hydraulic mechanisms which control the water status of leaves and the turgor of guard cells, (ii) chemical messages which participate to the control and at least partially originate from roots, and (iii) the carbon status of the leaf, which affects stomata when carbohydrates accumulate in the leaf. Although all the variables that characterise these processes rapidly change with time in a given genotype (such as root water potential, leaf water potential, ABA concentration or flux etc), some of these variables follow stable relationships that describe controls at the plant level. These relationships are common to several experimental conditions within a genotype, including experiments in controlled and field conditions and experiments with special manipulations of the plants. It has been proposed to call them "metamechanisms" at plant level (Tardieu, 2003), and to use them as a base for analysing the genetic variability of plant responses to environmental conditions.

A test of this approach was carried out by applying a model of stomatal control to a family of transgenic plants affected in their ability to synthesise ABA. Manipulation of the zeaxantin epoxidase gene, which is involved in ABA synthesis, causes a large variety of phenotypic responses, including wilting, changes in stomatal conductance, changes in leaf water potential, etc. The response of a series of transgenic lines of *Nicotiana plumbaginifolia* to water deficit has been analysed using a model of stomatal control (Borel et al., 2001). The overall conclusion is that the transformation has affected one parameter of the model, the one that describes the ability of plants to synthesise ABA. It did not affect the other processes of the model. In this case, one parameter in the model played the same role as one gene in a plant. This allowed us to predict the behaviour of transgenic plants under any climatic scenario.

## Genetic analysis of parameters of response curves for modelling the genotype x environment interaction

If a correspondence can be drawn between genes and parameters of models, it should be possible to carry out a genetic analysis of these parameters in segregating populations. In maize, leaf elongation rate is decreased by both evaporative demand and soil drying. For a given genotype, response curves to vapour pressure deficit and to soil water potential are common to several experiments carried out in the field, in the greenhouse and in the growth chamber, provided that rates are expressed per unit thermal time. These response curves are, therefore, a characteristic of the studied genotype (Tardieu et al., 2000; Reymond et al., 2003). Response curves of leaf elongation rate to meristem temperature, water vapor pressure difference, and soil water status were established in 100 recombinant inbred lines (RILs) of maize in six experiments carried out in the field or in the greenhouse. All responses were linear and common to different experiments, consistent with the model. A QTL analysis was carried out on the slopes of these responses. Most QTLs were specific to one response only. QTLs of raw phenotypic traits (leaf length and leaf width) differed between experiments with contrasting environmental conditions, but colocalised with parameters of the model.

Each parameter of the ecophysiological model was then computed as the sum of QTL effects, allowing calculation of parameters corresponding to RILs that had not been taken into account in the genetic analysis. The ecophysiological model could therefore be tested on genotypes only known by their allelic values at QTL positions. In a growth chamber experiment, the combined ecophysiological + genetic model accounted for 74% of the variability of leaf elongation rate of these genotypes. We therefore believe that simulating the leaf growth of virtual genotypes in a large series of climatic scenarios is feasible. Combined with other functions of the plant, this could be an avenue for an *in silico* design of plants adapted to a given climatic scenario (Tardieu, 2003).

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# The capacity and genetic base of canopy temperature (CT) as indicator on drought tolerance (DT) in rice reproductive stage

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## Introduction

Although there have been an abundance of studies on drought tolerance of plants, progress on drought tolerance (DT) related to rice gene discovery and cultivar development has been very limited and slow. This is mainly because of the extreme complexity of this phenomenon as well as the lack of the simple and effective methodologies (Blum, 1999). Several morphological and physiological traits related the water status of plants have been suggested to indicate the DT performance of plants in many published papers (Fukai et al., 1995; Babu et al., 1999; Turner et al., 1997). However, for the large diversity of germplasm, especially for mapping populations, it is very difficult to accomplish the measurement on the physiological traits such as leaf water potential in a short time to meet the demand on homogeneity. Dennes and O'Toole (1995) found that there was significant correlation between visual drought tolerance and mean canopy temperature (CT) in rice under moderate water stress. In this study, by using a recombinant inbred population and molecular map technology, we investigate the capacity of CT as a drought tolerance measurement indicator, as well as try to understand the genetic basis of CT in plants at the reproductive stage under both normal and water stress conditions

## Methods

A set of 195 $F_{10}$ recombinant inbred lines was developed from Zhanshan 97B (drought sensitive paddy rice) and IRAT109 (drought tolerance upland rice) and its parents, and was studied for morpho-physiological traits under drought and controlled conditions in the drought screen facility at Shanghai Agrobiological Gene Center. Each genotype was seeded directly in two-row plots in random block design with three replications. Each row in a plot consisting of 15 plants with a spacing of 18 cm, which formed a gradient of soil water content after stress. As the stress increased, the plants at both ends of the row were in normal (well-watered) and stress (drought) conditions, separately. After stress, 2-4 representative plants at both ends of plots were selected as normal and stress treatment to measure DT related traits such as CT, leaf water potential (LWP), leaf rolling (LRol) and so on.

Standard analyses of variance were performed to check the genetic variance among the RI Lines for the investigated traits. The phenotypic correlation was calculated using S-PLUS statistics software. The genotyping was conducted according the published procedures (Luo et al., 2001). An integrated genetic linkage map with 186 SSR markers was constructed using Mapmaker version 3.0 (Lincoln and Lander, 1992). The putative quantitative trait loci linked to the traits were identified using Windows QTL Cartographer V2.0 (Basten et al., 2001) with a threshold LOD score of 2.0.

## Results

1. The CT of DT parent IRAT109 under stress and normal conditions was 33.7 and 31.8, respectively. The CT of Zhenshan 97B was 35.1 and 32.0, respectively. The significant difference on CT between two parents was observed only under the stress conditions. All lines in the RIL population had higher CT under the stress and were normally distributed with transgressive segregation in both normal and stress conditions (Figure 1).

2. There was significant correlation between CT and spikelet fertility (r =-0.2330**), leaf water potential (-0.2550**), grain weight per plant (r = -0.152*), and leaf rolling score (r =0.153**), respectively, in stress. There was a significant negative correlation only between the CT and spikelet fertility (r =-0.220**), grain weight per panicle (r =-0.279**) under normal conditions.
3. A total of six putative QTLs related to canopy temperature were located on 4 of the 12 rice chromosomes (Table 1). Two QTLs on chromosome 3 (*QCt3a, QCt3b*) and one on chromosome 11(*QCt11*) were detected under normal conditions. In total, these QTLs explained about 29.45% of total variation. The other two QTLs on chromosome 4 (*QCt4a and QCt4b*) and one on chromosome 5(*QCt5*) were detected under water stress conditions.

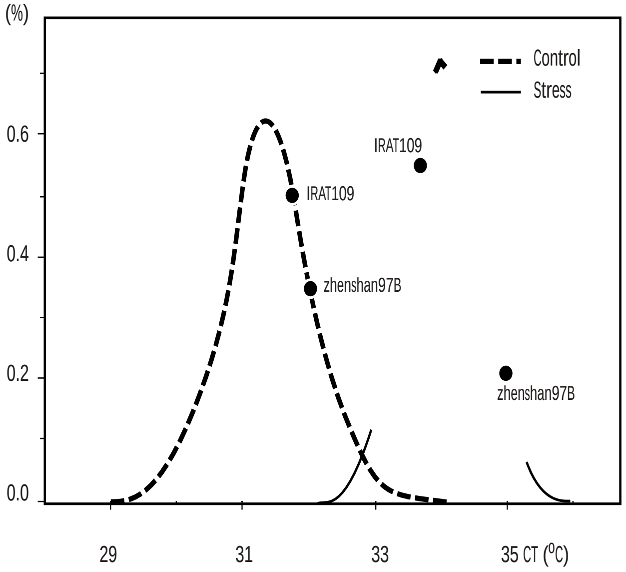

**Figure 1. The frequency distribution of canopy temperature in Zhenshan 97B / IRAT109 RIL population in both normal and water stress conditions.**

**Table 1. Putative QTLs for canopy temperature in Zhenshan 97B / IRAT109 RIL population**

| Trait | QTL | Chr | Water status | Marker interval | LOD | a | $R^2$% |
|---|---|---|---|---|---|---|---|
| Canopy temperature (CT, ! ) | *QCt3a* | 3 | Normal | RM426-RM203 | 4.13 | 0.2704 | 11.34 |
| | *QCt3b* | 3 | | RM130-RM514 | 2.39 | -0.2036 | 6.28 |
| | *QCt4a* | 4 | Stress | RM273-RM252 | 3.54 | 0.2252 | 9.55 |
| | *QCt4b* | 4 | Stress | RM317-RM255 | 2.32 | -0.1665 | 5.47 |
| | *QCt5* | 5 | Stress | RM459-RM161 | 2.07 | 0.1421 | 4.34 |
| | *QCt11* | 11 | Normal | RM20B-RM167 | 3.69 | 0.2518 | 11.83 |

## Conclusions

It is easier and simpler to measure canopy temperature in the field than other DT related physiological traits. Correlation analysis in this study indicated the CT was significantly associated with spikelet fertility and grain yield, ultimately implying the drought effect at reproductive stage in the stress. A total of six putative QTLs were found related to CT in the Zhenshan97B / IRAT 109 RIL population.

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# Mapping quantitative trait loci for drought tolerance in rice: Comparison across environments, genetic backgrounds and validation

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## Background

Drought stress is the major constraint to rice production and yield stability in the rainfed regions. Identifying genomic regions contributing to drought resistance will help develop rice cultivars suitable for rainfed regions through molecular marker assisted breeding. In a previous study, quantitative trait loci (QTLs) linked to plant water stress indicators, phenology and production traits under irrigated and drought stress conditions were mapped using a doubled-haploid (DH) population of 154 rice lines from the cross CT9993-5-10-1-M / IR62266-42-6-2 (Babu et al., 2003). The DH lines were subjected to water stress before anthesis in three field experiments in managed stress environment (MSE) and target production environment (TPE). The region RG939-RG476-RG214 on chromosome 4 identified for root-related drought resistance component QTLs also had pleiotropic effects on yield under stress in MSE. Root traits had significant positive correlations with yield and yield components under drought stress at this site. However, a key challenge faced by scientists involved in marker assisted breeding for drought resistance in rice is determination of QTLs that enhance drought tolerance in TPEs (Toenniessen et al., 2003).

## QTLs linked to drought tolerance in target production environment

Thus the above DH lines were further evaluated in three separate field experiments in MSE and TPE. Moderate to severe water stress occurred starting from pre-anthesis in these trials. The DH lines showed significant variation for plant water stress indicators, phenology, plant biomass, yield and yield components under irrigated control and water stress. A total of 38 QTLs were identified for various plant water stress indicators, phenology and production traits under control and water stress conditions in the field, which individually explained 8-49% of the phenotype variation . The details of selected QTLs are given in table 1. The region RG939-RG476-RG214 on chromosome 4 identified for root-related drought resistance component QTLs and those of yield under stress in MSE was also linked to grain yield under drought stress in TPE. Further, this region was consistently associated with plant production under stress in MSE. Common QTLs for yield under stress were detected across MSE and TPE. This study also indicated that QTLs for root traits had pleiotropic effects on yield under stress in TPE as well. Co-location of QTLs for root traits and those of yield under drought stress in TPE are also identified and are useful in marker-assisted selection for rainfed rice improvement. Consistency of these QTLs across experiments is also test verified by evaluating a subset of these DH lines under MSE. A significant positive correlation between root thickness and biomass under stress (r = 0.57**) indicated a causal relation between root traits and rice performance under drought stress in the field. Near isogenic lines developed for this QTLs region would validate and further characterize its effect on yield under drought stress in TPE.

**Table 1. List of selected putative QTLs detected with for plant production traits under water stress in a doubled-haploid rice population from CT9993-5-10-1-M and IR62266-42-6-2**

| Traits | QTL | Chr# † | Interval | LOD | Effect†† | $R^2$††† |
|---|---|---|---|---|---|---|
| **TPE, Paramakudi** | | | | | | |
| Biomass under stress | *pbms1.1* | 1 | C813-ME4_18 | 3.46 | 25.58(I) | 9.5 |
| | *pbms4.1* | 4 | EM18_18-RG163 | 4.60 | 34.37(C) | 17.1 |
| Days to flowering-stress | *pdhs2.1* | 2 | EM18_13-TGMSP2 | 3.84 | 2.29(C) | 24.8 |
| **Grain yield under stress** | ***pgys4.1*** | **4** | **RG476-RG214** | **4.30** | **5.94(C)** | **14.0** |
| Spikelet fertility -stress | *psfs10.1* | 10 | G333-EM16_9 | 5.50 | 5.06(C) | 18.9 |
| **MSE, Coimbatore** | | | | | | |
| Grain yield-stress (g/m2) | *gys6.1* | 6 | RZ682 – EM14_9 | 2.05 | 14.74(C) | 20.3 |
| | *gys7.1* | 7 | RG417 – EM17_3 | 3.04 | 18.48(C) | 31.8 |
| **Biomass-stress (g/m2)** | ***bms4.1*** | **4** | **RG939 – RG476** | **5.20** | **78.38(C)** | **36.2** |
| | *bms9.1* | 9 | ME5_9 – ME5_8 | 3.23 | 62.09(C) | 21.7 |
| | *bms12.1* | 12 | ME6_12 – RG9 | 3.08 | 59.55(C) | 20.8 |

†: Chromosome number; ††: Letters I and C in parentheses indicate that positive or favorable alleles for the effects are from IR62266 and CT9993, respectively; †††: Relative contributions of the detected putative QTLs to the phenotypic variation.

## Comparative QTL mapping for drought resistance

It is important to emphasize the relevance of a comparative QTL analysis for drought resistance in different genetic backgrounds and across a number of environmental conditions to identify the presence of ‘major’ QTLs. These QTLs should receive priority for the application of MAS and/or positional cloning (Tuberosa et al., 2002). Thus, QTLs linked to plant water stress indicators and production traits under irrigated and drought stress conditions were mapped using a recombinant inbred (RI) line population of 166 rice lines from the cross IR58821-23-B-1-2-1/IR52561-UBN-1-1-2. These two indica lines differ for root traits (Babu et al., 2001). The RI lines were subjected to water stress during anthesis in three field experiments in MSE and target production environment TPE. The RI lines showed significant variation for plant water stress indicators, plant biomass, yield and yield components under irrigated control and water stress. A total of 75 QTLs were identified for various traits under control and water stress conditions in the field, which individually explained 6-59% of the phenotype variation. The region C499 – PC11M1 on chromosome 2 identified for root-related drought resistance component QTLs (Ali et al., 2000) also had pleiotropic effects on yield traits under stress in TPE. Similarly, the region RZ448 – CDO122 on chromosome 3 identified for root trait was also linked to spikelet fertility under stress in TPE. In all these traits, the positive alleles came from IR58821-23-B-1-2-1, a line with deep and thicker roots. Thus the co-location of QTLs for root traits and those of yield traits under drought stress is verified across genetic backgrounds.

## Validation of putative QTLs in target production environment

An important follow-up of any QTL study is the validation of the putative QTLs. Ideally such QTLs should be associated with a phenotypic value, especially in TPE in such a way that breeding gains can be predicted through the introduction of the QTLs into elite varieties (Tuberosa et al., 2002). The potential impact of root trait QTLs on drought tolerance was studied by evaluating IR64 root introgression lines (Shen et al., 2000) under water stress in TPE. The introgression lines had higher drought tolerance index than IR64.

## Prospects

The potential implications for introgression of root QTLs for genetic improvement of rice for water-limited environments and the progress made in this direction are presented.

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# Highly efficient fine mapping of QTLs for drought tolerance using overlapping introgression lines of rice

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## Introduction

Drought has been the single largest factor limiting rice yield and stability in many parts of Asia. Past breeding efforts to develop drought tolerance (DT) rice cultivars have not been successful largely because of the complexity of the genetic control of DT in rice. Recent efforts using molecular markers have identified many quantitative trait loci (QTL) associated with DT in rice, but most conventional QTL mapping studies do not produce sufficient genetic information about identified DT QTLs to allow an effective application of marker-assisted selection (MAS) to the genetic improvement of DT. In particular, defining a target DT QTL allele in a small genomic region by closely linked DNA markers has been one of the most challenging problems to overcome. In this study, we have developed a new strategy for highly efficient fine-mapping of important DT QTLs using overlap DT introgression lines (ILs).

## Methods

In a large-scale backcross (BC) breeding effort, we have developed large numbers of DT ILs from $BC_2F_2$ populations derive from the crosses between three elite recurrent parents (IR64, Teqing and NPT) and 120 donors from worldwide. Of these DT ILs, 442 DT ILs from crosses between the three recurrent parents and nine donors were used to identify DT QTLs / genes. Previous results have revealed more than 50 chromosome regions (QTLs) for DT, of which 12 QTLs appeared to be more important based on their high frequencies in the DT ILs. To further fine map these QTLs, the 442 ILs and subsets of more than 100 SSR markers around the 12 target regions were used to genotype the ILs.

## Results

Our preliminary results that some of these QTLs could be clearly defined to small genomic regions less than 4cM based on the genotypic data of the overlap ILs and the test statistics ($X^2$) at the overlapping regions of target QTLs (Figure. 1). Bioinformatic analyses of the DNA sequences of these QTL regions from the public domain allowed identification of a relative small number of positional candidate genes associated with some of the DT QTLs in rice.

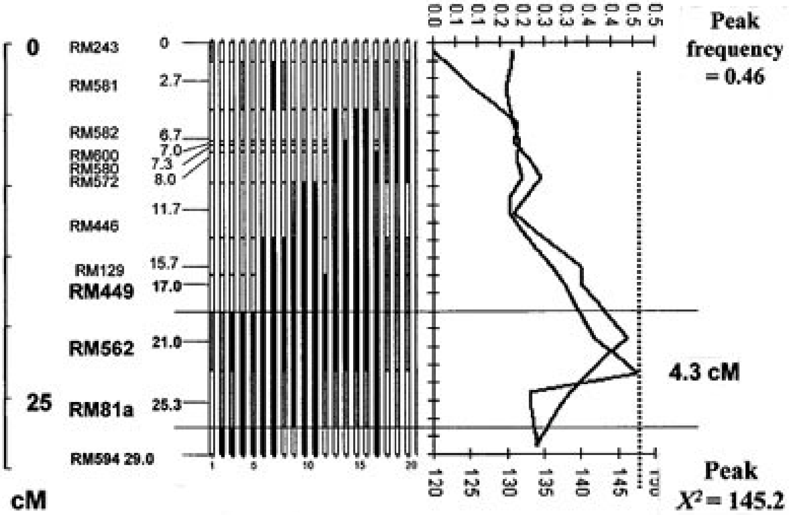

Figure 1. Fine mapping of a DT QTL on chromosome 1.

## Conclusion

Our results indicated that high-resolution QTL mapping can be achieved more efficiently using the unique genetic stocks and bioinformatic tools without large-scale phenotyping. Together with the possible presence of multiple functionally differentiated alleles at some of the DT QTLs, this study provided useful information on these DT QTLs required for MAS and map-based cloning.

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# Isogenization and characterization of *root-ABA1,* a major QTL affecting root traits and leaf ABA concentration in maize

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## Introduction

In the mapping population derived from Os420 x IABO78, two lines differing widely in the concentration of leaf ABA concentration (L-ABA) under a broad range of water regimes (Tuberosa et al., 1994), 16 QTLs were identified for L-ABA (Tuberosa et al., 1998). A major QTL near *csu133* on bin 2.04 accounted for 32% of the total variability (mean of four environments) for L-ABA. At this QTL, Os420 and IABO78 carry the allele increasing (+) and decreasing (-) L-ABA, respectively. The cause-effect relationships between quantitatively inherited traits can be more properly and accurately investigated through the evaluation of congenic strains (e.g., NILs, BDLs) sharing the same genetic background and differing only for the parental alleles at the target QTL affecting the traits in question. The isogenization of a QTL in more than one genetic background provides the opportunity for evaluating the QTL in a hybrid background, an important prerequisite for appropriately testing QTL effects for traits, such as yield, in species affected by inbreeding depression. The objectives of this study in maize were (i) to investigate the effects of the QTL near *csu133* on grain yield and other agronomic traits at two water regimes and (ii) to elaborate a model accounting for the effects of the QTL on a number of morpho-physiological traits.

## Materials

Four different pairs of Os420 and IABO78 BDLs for the QTL on bin 2.04 were obtained by reciprocal backcrossing (Landi et al., 2004). The four Os420 BDLs were factorially crossed to the four IABO78 BDLs, thus producing 16 near-isogenic hybrids (NIHs), which were tested (four reps) in 2002 and 2003, adopting two irrigation volumes corresponding to ca. 40 or to 120% of the estimated evapontranspiration after accounting for rainfall. The following traits were considered: L-ABA at mid-pollen shedding, leaf relative water content (RWC), stomatal conductance (SC), leaf water potential, pollen shedding and silking dates, anthesis-silking interval, plant height, root lodging (consequent to windstorms occurred in 2002 at ca. 10 days after silking and in 2003 at mid-stem elongation), grain yield, and its components. For the *F*-test of the ANOVA, the effects of years were considered as random and the effects due to irrigation volumes and hybrids as fixed.

## Results

The ANOVA showed no significant “genotype x year” interaction and a significant “genotype x water regime” interaction only for L-ABA, mainly due to changes in magnitude of the differences rather than in ranking of the genotypes. Therefore, except for L-ABA, table 1 reports the mean values of the three groups of NIHs [(+/+), (-/-) and (+/-)] at the target QTL across years and irrigation volumes. The intensity of the drought treatment was adequate for our objectives, as shown by the reduction in yield observed in the water-stressed treatment (-39%; data not shown). Irrigation volumes significantly affected most traits (data not shown), the most noticeable exception being RWC, probably as a consequence of the significantly higher SC of the well-watered treatment. The QTL significantly affected L-ABA, SC, root lodging, and kernel weight. The value of the eight hybrids heterozygous at the QTL (+/-) did not differ significantly from the mean of the two groups of hybrids homozygous at the QTL [(+/+) and (-/-)], indicating a prevalently additive effect of this QTL. Notably, the additive effects of the QTL on L-ABA and SC were similar to those reported in previous studies (Tuberosa et al., 1998; Sanguineti et al., 1999), indicating the consistency of the effects of this QTL.

The QTL showed a sizeable effect on root lodging, with the Os420 allele providing higher resistance to root lodging. Interestingly, a significant concurrent effect of the *csu133* region on root pulling force and L-ABA was reported by Lebreton et al. (1995), with additive QTL effects of similar sign for both traits. If the associated effects on root characteristics of the QTL for L-ABA were at least partly due to pleiotropy, a plausible explanation could be that these traits are causally related and regulated by the QTL acting directly on one trait only (according to the general model described by Lebreton et al., 1995, and Tuberosa et al., 2002b). As an example, it has been shown that ABA influences positively root elongation under conditions of water deficit (Sharp et al., 1996). To better elucidate the effects of the QTL on roots, a detailed analysis was carried out on root traits of plants of the parental BDLs, which were grown in soil columns. The QTL showed a significant effect on root mass and the angle of insertion of brace roots (Giuliani, 2004; Giuliani et al., unpublished) with the Os420 allele providing a higher root mass and a wider (i.e., more horizontal) insertion angle. Based on these findings, we hypothesize that the QTL primarily affects root density in the soil layers, with the Os420 allele enhancing root proliferation in superficial soil layers. Consequently, the Os420 allele, as compared to the IABO78 allele, would also enhance root lodging and would account for the higher L-ABA values as a result of the enhanced ABA flux from the roots undergoing dehydration in the superficial soil layers. A concurrent effect on root lodging resistance was also observed when the (Os420 ´ IABO78) $F_2$ population was divergently selected for L-ABA, with the high L-ABA population being more resistant to root lodging than the low L-ABA population (Landi et al., 2001). Bolaños et al. (1993) reported that direct selection in maize for yield under drought conditions was followed by a reduction in soil anchorage (as measured by root pulling resistance) and by a decrease in the root density in the superficial soil layers. In our study, the absence of a significant effect of the QTL on grain yield could partly be attributed to the counterbalancing effects of the concurrent effects of the QTL on ABA concentration and root lodging resistance. It remains to be ascertained to what extent the QTL affects ABA concentration in the reproductive organs and in the developing kernel. This notwithstanding, it should be noted that following the divergent selection for L-ABA of the (Os420 ´ IABO78) $F_2$ population, the low L-ABA population, as compared to the high L-ABA population, showed a significant increase in grain yield due to a higher kernel number / ear (Landi et al., 2001). The agronomic relevance of the chromosome region near *csu133* has also been documented in Lo964 ´ Lo1016 (Tuberosa et al., 2002a) and other genetic backgrounds (for a review, see Tuberosa et al., 2003). Recurrent selection for grain yield under drought conditions significantly altered allele frequencies at *csu133* in two maize populations

**Table 1. Mean values of the three groups of NIHs [(+/+), (-/-) and (+/-)] at the target QTL across two years and two irrigation volumes (corresponding to 40 and 120% of evapotranspiration, respectively)**

| Trait | (+/+) | (-/-) | a | Mean | (+/-) | b |
|---|---|---|---|---|---|---|
| L-ABA (ng $g^{-1}$ DW) Water-stressed (40%) | 554 | 405 | ** | 480 | 482 | ns |
| L-ABA (ng $g^{-1}$ DW) Well-watered (120%) | 373 | 290 | ** | 331 | 323 | ns |
| L-ABA (ng $g^{-1}$ DW) Mean | 464 | 348 | ** | 406 | 402 | ns |
| Relative water content (RWC) (%)[c] | 92.3 | 92.2 | ns | 92.2 | 92.7 | ns |
| Stomatal conductance (cm $s^{-1}$)[c] | 0.41 | 0.47 | ** | 0.44 | - | - |
| Leaf water potential (MPa) [c] | -1.40 | -1.39 | ns | -1.39 | - | - |
| Pollen shedding date[d] | 31.4 | 31.0 | ns | 31.2 | 31.1 | ns |
| Anthesis-silking interval (d) | 6.9 | 6.7 | ns | 6.8 | 6.9 | ns |
| Plant height (cm) | 215 | 213 | ns | 214 | 214 | ns |
| Root lodging (%) | 18.0 | 43.7 | ** | 30.9 | 26.9 | ns |
| Grain yield (Mg $ha^{-1}$) | 6.27 | 6.13 | ns | 6.20 | 6.41 | ns |
| Kernels per plant (no.) | 402 | 379 | ns | 391 | 397 | ns |
| Kernel weight (mg) | 308 | 316 | * | 312 | 313 | ns |

* and **, significant at *P* 0.05 and at *P* 0.01, respectively; ns, not significant.

[a] Comparison between the (+/+) and (-/-) NIH groups.

[b] Comparison between the mean of the (+/+) and (-/-) NIH groups and the (+/-) NIH group.

[c] Measured on only two (+/+) hybrids and on the two corresponding (-/-) NIHs. [d] 1 = June 1st.

developed at CIMMYT (Quarrie et al., 1999). Exploiting syntenic information, we have compared the QTL results for root traits of four maize populations (reviewed in Tuberosa et al., 2003) with the root QTLs data of seven rice populations. Interestingly, the highest frequency of QTLs for root characteristics reported in rice occurred in the region syntenic to maize bin 2.04, an encouraging finding as to the possibility of using rice as a model species for facilitating the positional cloning of the gene/s responsible for this QTL.

## Conclusions

Our results validate the effects of *root-ABA1*, a major QTL on bin 2.04 near *csu133,* influencing root characteristics and leaf ABA concentration. Such associated effects could in part be due to a primary effect of the QTL on root architecture. To further elucidate the genetic basis (linkage or pleiotropy) of this association, we have undertaken the fine mapping of the QTL. The fine mapping coupled with the synteny information available from rice may eventually allow us to identify the candidate gene/s underlying the QTL.

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# Validation of QTLs for drought resistance in near isogenic introgression lines of rice

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## Introduction

Rice (*Oryza sativar* L.) is most susceptible to drought especially during reproductive growth stage. One way of developing drought resistant cultivars is to accumulate the appropriate combination of genes responsible for drought resistance (Fukai et al., 1999). Three drought resistance mechanisms; drought escape, dehydration avoidance and dehydration tolerance, were proposed by Turner (1986). Among dehydration avoidance traits, a deep, well-developed root system appears beneficial for upland rice because deep soil allows the plants to express the full potential of a deep root system. O'Toole (1982) suggested that under conditions providing a large soil water reservoir and without root growth barriers, rooting should be dense and deep, while with a small soil water reservoir, increased root penetration of physical and chemical impedance may be required. Although root traits are beneficial under drought stress, they are however, difficult to incorporate into conventional breeding programs because they are difficult and time-consuming to measure. Molecular marker technology can be used to overcome this barrier mentioned above. Genetic markers are being developed, with a view to use marker-aided selection (MAS) in routine breeding programs. Several studies have been undertaken to map quantitative trait loci (QTLs) for root traits which presumably related with drought resistance in rice (Champoux et al., 1995; Ray et al., 1996; Price et al., 1997; Yadav et al., 1997). Recently, these QTLs were introgressed through MAS into elite rice variety, IR64 to develop near-isogenic lines for deeper and thicker roots (Shen et al., 2001). Our objectives are to evaluate the IR64-NILs for agro-physiological performance under late season drought in the lowland field of the northeastern Thailand and to validate the effect of QTLs for deeper and thicker roots on drought resistance. Results from this experiment will provide breeders with understanding on how to successfully breed drought resistance in rice.

## Methods

A total of 55 near isogenic lines (NILs) of IR64 introgressed with four QTLs for deeper and thicker roots (Shen et al., 2001) and their parents, IR64 and Azucena, were grown in three replications in randomized complete block design to examine genotypic variation for grain yield and physiological traits under lowland. Different water regimes were used in order to allow pants to expose to water stress at flowering to grain filling stage. An experiment was conducted under line-source sprinkler irrigation system (LSS) in dry season 2002 at Chum Phae Rice Experimental Station in northeast Thailand. Plants were direct seeded in December 2001. Four staggered seeding dates, 8, 17, 22, and 27 December 2001, were used to synchronize the flowering time of the NILs. Seeds were sown in plot size of 0.90 ´ 14 m at a 15 ´ 20 cm spacing and only one seedling was kept at each hill after thinning about one month after sowing. Surface irrigation was applied to ensure a 5-10 cm of standing water during tillering stage before water was drained from the field on 10 March 2002, around panicle initiation stage. Further irrigation was then applied using LSS to create verying levels of water deficit until the crop matured on 24 April 2002. Uniformity in water application was achieved by appropriate sprinkler spacing and pressure distribution, which had been evaluated previously at the experimental site. Water application rates varied from the highest level at W0 (approximately 4 cm day$^{-1}$) to the lowest level at W4 (0 cm day$^{-1}$). W0 was a well-watered treatment and W1, W2, W3, and W4 were very mild, mild, moderate, and severe drought stressed treatments, respectively.

## Results

Azucena genomic segments harboring QTLs for deeper and thicker roots affected agro-physiological performance of IR64 genetic background. The effects might due to linkage of QTLs or pleiotropy. The NILs of IR64 carrying target QTLs on chromosome 2 and 9 have the same potential grain yield as IR64. Grain yield was linearly reduced as intensity of water stress increased. When water stress was not severe where yield loss less than 50%, grain yield obtained from this water stress treatments were associated with potential yield and days to flower under non-stress treatment (Figure.1). Under mild to severe stress, the NILs of IR64 carrying target QTLs on chromosome 2 produced the same amount of grain yield as the recurrent parent 'IR64' while the NILs of IR64 carrying target QTLs on chromosome 9 produced higher grain yield than IR64 (Table 1). The NILs of IR64 carrying target QTLs on chromosome 9 were able to maintain higher LWP under drought than the NILs of IR64 carrying other target QTLs (Table 2). Those NILs that have higher LWP also have increased panicle exertion rate and decreased leaves rolling and drought score. Drought response index (DRI) was not significant difference among the NILs and recurrent parent 'IR64' under drought stress.

## Conclusions

The genomic segments carrying QTLs for deeper and thicker roots are linked with QTLs for plant productivity and associated with ability to maintain LWP. Introgression of root QTLs into an elite rice variety 'IR64' may not improve grain yield under drought stress because of linkage drag. Better ability to maintain higher LWP under drought stress at reproductive stage clearly helped to maintain grain yield. Marker assisted introgression of QTLs for drought related traits alone may not help breeders to improve grain yield under drought stresses. New strategy by integration of MAS and conventional backcross breeding may be an answer.

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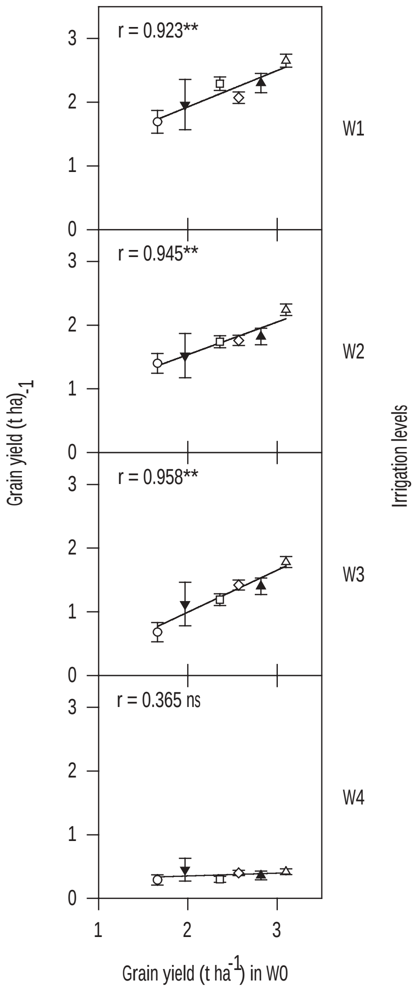

**Figure 1. Relationship between potential grain yield (t ha$^{-1}$) under well watered condition (W0) and grain yield under different intensity of water stress conditions (W1, W2, W3 and W4; very mild to severe stress) of 55 IR64 near isogenic lines separated with QTLs in target 1* (%), target 2 (◊), target 7 (%), and target 9 (Δ) and their parents, IR64 (%) and Azucena (%) at Chum Phae Rice Experiment Station in 2002 dry season. Vertical bars are the standard error of the mean.**

** Target 1; chromosome 1 conferring root thickness, total root weight, deep root weight (root weight below 30 cm),* deep root weight per shoot, deep root weight per tiller;

Target 2, chromosome 2 conferring maximum root length;

Target 7, chromosome 7 conferring deep root weight, deep root weight per shoot, deep root weight per tiller;

Target 9, chromosome 9 conferring maximum root length, total root weight.

**Table 1. Mean grain yield (t ha$^{-1}$),plant height (cm), tiller number hill$^{-1}$, grain number panicle$^{-1}$ and 1000 grain weight (g) of 55 IR64 near isogenic lines separated with QTLs on target 1*, 2, 7, and 9 and their parents (IR64 and Azucena) determined under a) well watered, b) very mild stress, c) mild stress, d) moderate stress and e) severe stress at Chum Phae Rice Experiment Station in 2002 dry season.**

|  | Grain yield (t ha$^{-1}$) | Plant height (cm) | Tiller number hill$^{-1}$ | Grain number panicle$^{-1}$ |  |  |  | 1000 grain weight (g) |
|---|---|---|---|---|---|---|---|---|
|  |  |  |  | Filled | Patially filled** | Unfilled | Total grain |  |
| a) Well water (W0) |  |  |  |  |  |  |  |  |
| Target 1* | $1.66^{d}$ | $76^{cd}$ | $12^{a}$ | $29^{c}$ | $10^{b}$ | $11^{b}$ | $49^{c}$ | $23.47^{b}$ |
| Target 2 | $2.57^{bc}$ | $73^{d}$ | $12^{a}$ | $34^{c}$ | $10^{b}$ | $9^{b}$ | $53^{c}$ | $26.77^{a}$ |
| Target 7 | $2.36^{c}$ | $95^{b}$ | $9^{b}$ | $39^{b}$ | $10^{b}$ | $17^{a}$ | $66^{b}$ | $25.60^{ab}$ |
| Target 9 | $3.10^{a}$ | $79^{c}$ | $10^{ab}$ | $46^{a}$ | $11^{b}$ | $11^{b}$ | $67^{b}$ | $23.71^{b}$ |
| IR64 | $2.82^{ab}$ | $74^{cd}$ | $11^{a}$ | $42^{ab}$ | $11^{b}$ | $11^{b}$ | $63^{b}$ | $25.29^{ab}$ |
| Azucena | $1.97^{cd}$ | $136^{a}$ | $5^{c}$ | $43^{ab}$ | $19^{a}$ | $18^{a}$ | $79^{a}$ | $26.87^{a}$ |
| Grand mean | 2.52 | 89 | 11 | 40 | 12 | 14 | 65 | 26.11 |
| b) Very mild stress (W1) |  |  |  |  |  |  |  |  |
| Target 1 | $1.69^{d}$ | $73^{c}$ | $12^{a}$ | $30^{d}$ | 8 | $12^{b}$ | $50^{cd}$ |  |
| Target 2 | $2.07^{cd}$ | $71^{c}$ | $11^{a}$ | $35^{cd}$ | 6 | $8^{c}$ | $49^{d}$ |  |
| Target 7 | $2.29^{b}$ | $96^{b}$ | $10^{b}$ | $38^{c}$ | 8 | $17^{a}$ | $63^{b}$ |  |
| Target 9 | $2.65^{a}$ | $75^{c}$ | $12^{a}$ | $49^{b}$ | 8 | $10^{bc}$ | $67^{b}$ |  |
| IR64 | $2.30^{ab}$ | $71^{c}$ | $12^{a}$ | $41^{c}$ | 8 | $10^{bc}$ | $59^{bc}$ |  |
| Azucena | $1.96^{abc}$ | $138^{a}$ | $4^{c}$ | $55^{a}$ | 9 | $17^{a}$ | $81^{a}$ |  |
| Grand mean | 2.30 | 87 | 11 | 41 | 9 | 14 | 63 | Not available |
| c) Mild stress (W2) |  |  |  |  |  |  |  |  |
| Target 1 | $1.40^{c}$ | $71^{cd}$ | $12^{a}$ | $24^{b}$ | $10^{c}$ | $12^{b}$ | $46^{de}$ |  |
| Target 2 | $1.76^{b}$ | $68^{d}$ | $12^{a}$ | $25^{b}$ | $11^{c}$ | $9^{c}$ | $45^{e}$ |  |
| Target 7 | $1.74^{bc}$ | $90^{b}$ | $10^{b}$ | $25^{b}$ | $14^{b}$ | $19^{a}$ | $58^{bc}$ |  |
| Target 9 | $2.24^{a}$ | $72^{c}$ | $12^{a}$ | $37^{a}$ | $14^{b}$ | $11^{bc}$ | $62^{b}$ |  |
| IR64 | $1.82^{b}$ | $67^{cd}$ | $12^{a}$ | $30^{b}$ | $12^{bc}$ | $11^{bc}$ | $53^{cd}$ |  |
| Azucena | $1.52^{bc}$ | $126^{a}$ | $5^{c}$ | $26^{b}$ | $37^{a}$ | $16^{ab}$ | $79^{a}$ |  |
| Grand mean | 1.87 | 82 | 11 | 29 | 14 | 15 | 58 | Not available |
| d) Moderate stress (W3) |  |  |  |  |  |  |  |  |
| Target 1 | $0.68^{d}$ | $68^{cd}$ | $12^{a}$ | $19^{c}$ | $7^{b}$ | $18^{c}$ | $43^{e}$ | $23.01^{c}$ |
| Target 2 | $1.42^{b}$ | $66^{d}$ | $10^{b}$ | $28^{b}$ | $7^{b}$ | $15^{c}$ | $51^{d}$ | $24.62^{b}$ |
| Target 7 | $1.19^{c}$ | $85^{b}$ | $8^{c}$ | $28^{b}$ | $9^{ab}$ | $31^{b}$ | $69^{b}$ | $24.54^{b}$ |
| Target 9 | $1.78^{a}$ | $70^{c}$ | $11^{b}$ | $37^{a}$ | $11^{a}$ | $17^{c}$ | $64^{bc}$ | $23.55^{c}$ |
| IR64 | $1.40^{bc}$ | $67^{d}$ | $11^{b}$ | $32^{ab}$ | $10^{ab}$ | $17^{c}$ | $59^{c}$ | $25.25^{c}$ |
| Azucena | $1.12^{abcd}$ | $125^{a}$ | $4^{d}$ | $30^{ab}$ | $14^{a}$ | $35^{a}$ | $79^{a}$ | $22.32^{a}$ |
| Grand mean | 1.40 | 80 | 10 | 31 | 10 | 23 | 63 | 24.41 |
| e) Severe stress (W4) |  |  |  |  |  |  |  |  |
| Target 1 | 0.29 | $64^{c}$ | $10^{a}$ | $9^{d}$ | $5^{a}$ | $46^{c}$ | $60^{d}$ | 22.92 |
| Target 2 | 0.40 | $61^{cd}$ | $9^{b}$ | $12^{c}$ | $4^{b}$ | $47^{c}$ | $63^{cd}$ | 22.89 |
| Target 7 | 0.30 | $79^{b}$ | $7^{c}$ | $9^{d}$ | $3^{c}$ | $58^{a}$ | $70^{ab}$ | 23.42 |
| Target 9 | 0.42 | $64^{c}$ | $8^{bc}$ | $15^{b}$ | $5^{a}$ | $49^{bc}$ | $68^{bc}$ | 22.46 |
| IR64 | 0.36 | $59^{d}$ | $9^{b}$ | $13^{bc}$ | $4^{b}$ | $53^{ab}$ | $70^{ab}$ | 22.32 |
| Azucena | 0.45 | $104^{a}$ | $4^{d}$ | $23^{a}$ | $5^{a}$ | $51^{abc}$ | $79^{a}$ | 22.71 |
| Grand mean | 0.39 | 72 | 8 | 13 | 5 | 52 | 69 | 23.05 |

* Target 1; chromosome 1 conferring root thickness, total root weight, deep root weight (root weight below 30 cm), deep root weight per shoot, deep root weight per tiller; Target 2, chromosome 2 conferring maximum root length; Target 7, chromosome 7 conferring deep root weight, deep root weight per shoot, deep root weight per tiller; Target 9, chromosome 9 conferring maximum root length, total root weight

** Patial filled grain is a spikelet in which there is some grain development but the size is less than half of the hull, Unfilled grain is a spikelet with no evidence of grain development.

[a] Mean followed by different letters in the same column differ significantly at $P = 0.05$.

Table 2. Mean predawn and midday LWP, drought score, leaf rolling score, panicle exertion rate (cm day$^{-1}$), day from sowing to 50% flowering, delay in flowering time (days) and drought response index (DRI) of the 55 IR64 near isogenic lines separated with OTLs on target 1*, 2, 7, and 9 and their parents (IR64 and Azucena) determined under a) well watered, b) very mild stress, c) mild stress, d) moderate stress and e) severe stress at Chum Phae Rice Experiment Station in 2002 dry season.

| | LWP (MPa) | | Drought score | Leaf rolling rate (cm day$^{-1}$) | Panicle exertion to 50% flowering | Day from sowing time (days) | Delay in flowering DRI | |
|---|---|---|---|---|---|---|---|---|
| Predawn | Midday | score | | | | | | |
| a) Well water (W0) | | | | | | | | |
| Target 1* | -0.46 | -1.58 | 3.22 | $1.61^{bc}$ | 3.63 | $78^{d}$ | – | – |
| Target 2 | -0.46 | -1.65 | 3.02 | $1.54^{c}$ | 5.15 | $82^{c}$ | – | – |
| Target 7 | -0.48 | -1.62 | 3.05 | $1.85^{ab}$ | 5.43 | $83^{b}$ | – | – |
| Target 9 | -0.45 | -1.66 | 2.75 | $1.51^{c}$ | 5.11 | $83^{b}$ | – | – |
| IR64 | -0.46 | -1.62 | 2.79 | $1.53^{c}$ | 4.86 | $83^{bc}$ | – | – |
| Azucena | -0.46 | -1.51 | 3.05 | $1.94^{a}$ | 6.22 | $91^{a}$ | – | – |
| Grand mean | -0.47 | -1.63 | 2.98 | 1.66 | 5.07 | 83 | – | – |
| b) Very mild stress (W1) | | | | | | | | |
| Target 1 | | | 2.82 | $1.90^{bc}$ | | $78^{d}$ | 1 | $-0.12^{abc}$ |
| Target 2 | | | 2.63 | $1.85^{c}$ | | $82^{c}$ | 1 | $-0.37^{c}$ |
| Target 7 | | | 2.75 | $2.11^{ab}$ | | $83^{b}$ | 1 | $0.16^{a}$ |
| Target 9 | | | 2.41 | $1.74^{c}$ | | $84^{b}$ | 1 | $0.07^{ab}$ |
| IR64 | | | 2.4 | $1.87^{c}$ | | $83^{bc}$ | 0 | $-0.41^{bc}$ |
| Azucena | | | 2.5 | $2.33^{a}$ | | $91^{a}$ | 1 | $-0.19^{abc}$ |
| Grand mean | Not available | Not available | 2.59 | 1.97 | Not available | 84 | 1 | -0.14 |
| c) Mild stress (W2) | | | | | | | | |
| Target 1 | | | $3.22^{ab}$ | $1.69^{bc}$ | $4.10^{d}$ | $79^{d}$ | 2 | -0.22 |
| Target 2 | | | $3.04^{ab}$ | $1.62^{c}$ | $4.44^{cd}$ | $83^{c}$ | 1 | -0.23 |
| Target 7 | | | $3.28^{a}$ | $2.10^{a}$ | $5.70^{ab}$ | $84^{b}$ | 1 | -0.08 |
| Target 9 | | | $2.88^{b}$ | $1.55^{c}$ | $5.14^{bc}$ | $85^{b}$ | 1 | 0.23 |
| IR64 | | | $3.00^{ab}$ | $1.64^{c}$ | $4.33^{cd}$ | $83^{bc}$ | 1 | -0.35 |
| Azucena | | | $2.78^{b}$ | $2.05^{ab}$ | $6.98^{a}$ | $92^{a}$ | 1 | -0.01 |
| Grand mean | Not available | Not available | 3.03 | 1.77 | 5.12 | 84 | 1 | -0.11 |
| d) Moderate stress (W3) | | | | | | | | |
| Target 1 | $-0.68^{bc}$ | -1.93 | 3.29 | $1.87^{c}$ | $3.79^{d}$ | $79^{d}$ | 2 | -0.53c |
| Target 2 | $-0.61^{ab}$ | -1.94 | 3.49 | $1.88^{c}$ | $4.70^{bc}$ | $83^{c}$ | 2 | $0.00^{ab}$ |
| Target 7 | $-0.71^{c}$ | -2.01 | 3.39 | $2.33^{ab}$ | $5.26^{ab}$ | $84^{b}$ | 2 | $-0.20^{bc}$ |
| Target 9 | $-0.60^{a}$ | -1.97 | 3.14 | $1.86^{c}$ | $4.85^{b}$ | $85^{b}$ | 1 | $0.19^{a}$ |
| IR64 | $-0.62^{ab}$ | -1.96 | 3.41 | $1.94^{bc}$ | $3.98^{cd}$ | $84^{bc}$ | 1 | $-0.26^{abc}$ |
| Azucena | $-0.78^{c}$ | -1.99 | 3.5 | $2.44^{a}$ | $6.58^{a}$ | $93^{a}$ | 2 | $0.14^{abc}$ |
| Grand mean | -0.65 | -1.98 | 3.37 | 2.05 | 4.86 | 85 | 2 | -0.11 |
| e) Severe stress (W4) | | | | | | | | |
| Target 1 | $-0.97^{bc}$ | $-2.40^{b}$ | $6.02^{a}$ | $3.11^{ab}$ | 3.6 | $79^{d}$ | 2 | -0.22 |
| Target 2 | $-0.92^{ab}$ | $-2.29^{ab}$ | $5.45^{b}$ | $2.76^{c}$ | 3.94 | $83^{c}$ | 2 | -0.06 |
| Target 7 | $-1.03^{c}$ | $-2.32^{ab}$ | $5.70^{ab}$ | $3.24^{a}$ | 4.86 | $85^{b}$ | 2 | -0.24 |
| Target 9 | $-0.89^{a}$ | $-2.24^{a}$ | $5.04^{c}$ | $2.52^{d}$ | 4.12 | $85^{b}$ | 2 | -0.13 |
| IR64 | $-0.96^{b}$ | $-2.31^{ab}$ | $5.46^{b}$ | $2.93^{bc}$ | 3.74 | $84^{bc}$ | 1 | -0.43 |
| Azucena | $-0.90^{abc}$ | $-2.10^{a}$ | $4.44^{c}$ | $2.83^{abcd}$ | 5.22 | $95^{a}$ | 3 | 0.92 |
| Grand mean | -0.95 | -2.28 | 5.35 | 2.90 | 4.26 | 85 | 2 | -0.03 |

* Target 1; chromosome 1 conferring root thickness, total root weight, deep root weight (root weight below 30 cm), deep root weight per shoot, deep root weight per tiller; Target 2, chromosome 2 conferring maximum root length; Target 7, chromosome 7 conferring deep root weight, deep root weight per shoot, deep root weight per tiller; Target 9, chromosome 9 conferring maximum root length, total root weight

[a] Mean followed by different letters in the same column differ significantly at $P = 0.05$.

# High resolution mapping of a genomic region harboring several QTLs and *sd1* in rice

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The Green Revolution that resulted in a dramatic increase of rice productivity was attributed largely to the wide use of the semidwarf gene, *sd1*, in breeding programs worldwide. However, recent studies on mapping QTLs affecting traits related to drought tolerance (DT) and breeding for DT in rice often reveal a close relationship between *sd1* and DT. To resolve and characterize the pleiotropic effects of the *sd1* on a number of grain yield traits and DT, a large population of 720 $BC_4F_2$ individuals derived from a cross between two near isogenic lines (NILs) differing at the *sd1* region and their $BC_4F_3$ progeny were genotyped with 11 SSR (simple sequence repeats) markers polymorphic between the NILs, and phenotyped for a number of grain yield related traits in replicated experiments under the controlled greenhouse, water stress (upland) and normal lowland field conditions.

Results in table 1 indicate that *sd1* does appear to have pleiotropic effects on a number of traits in addition to plant height, since the LOD peaks of all these QTLs matched the peak of plant height. Under the normal irrigated lowland conditions, *sd1* was associated with early heading, slightly increased tiller number per plant, and panicle fertility, but also with slightly reduced grain yield per plant. Under the stressful upland conditions, the associations of *sd1* with these traits were much stronger as these QTLs were detected with much higher LOD scores. Figure 1 shows that expression of *sd1* increases as rice plants develop and reaches the peak right before panicle initiation, and then drops. Thus, it can be concluded that the association of increased plant height and the drought tolerance observed in many breeding populations is due to linkage, rather than pleiotrpy and development of semidwarf and drought tolerant rice lines is possible. In addition, a QTL affecting grain weight was detected in the region more upstream of *sd1*.

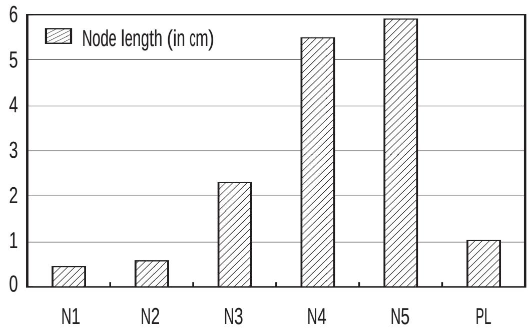

**Figure 1. Effects of *sd1* on the five different nodes of rice plants and panicle length.**

**Table 1. QTLs associated with grain yield related traits at the *sd1* region detected in 720 F2 $BC_4F_2$ individuals and $BC_4F_3$ progeny derived from a cross between two near isogenic lines (NILs) differing at the *sd1* region**

| | $BC_4F_2$ in lowland | | | $BC_4F_3$ in lowland | | | $BC_4F_3$ in upland | | |
|---|---|---|---|---|---|---|---|---|---|
| Trait | LOD | A | D | LOD | A | D | LOD | A | D |
| Plant height (cm) | 160.30 | -15.92 | 10.99 | 197.58 | -14.85 | 9.32 | 131.49 | -8.12 | 15.58 |
| Heading date (days) | | | | 24.38 | -1.69 | 2.22 | 35.59 | -2.65 | 2.50 |
| Tiller number per plant | 3.36 | 0.93 | -0.69 | 3.83 | 0.89 | -0.93 | | | |
| Fertility (%) | | | | 4.67 | 1.36 | -2.03 | 21.64 | 4.73 | -12.06 |
| Yield (g) per plant | | | | 2.02 | -0.37 | 0.36 | 15.15 | -0.33 | 0.76 |
| Filled grains per panicle | 2.72 | 5.47 | 7.91 | 12.35 | 3.82 | 11.45 | 11.07 | 0.09 | -0.22 |
| Roots per tiller [a] | | | | 2.43 | 0.52 | 0.18 | | | |

[a] Roots per tiller was detected in the greenhouse experiment.

# Mapping quantitative trait loci (QTL) for important agronomic traits in wheat under rainfed and well irrigated conditions

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## Introduction

Wheat is the most widely grown and consumed food crop. It is the staple food of nearly 35% of the world population and demand for wheat will grow faster than for any other major crop (Braun et al., 1998). Drought is one of the important factors limiting wheat production in arid and semiarid regions of the world. Breeding for drought tolerance is a challenging task because of the complexity of drought responses in crops, environmental factors, and their interactions. Although the conventional breeding approach has been successful for drought tolerance in crops, it is still unable to select the genotype of drought tolerance precisely in conventional breeding programs (Pellegrineschi et al., 2002). The objective of our research is to reveal the genetic basis of important agronomic traits in wheat under different water regimes by the use of molecular markers and QTL mapping.

## Methods

Quantitative trait loci (QTLs) controlling yield components and plant height under rainfed (T) and well irrigated (CK) conditions were mapped using a double haploid (DH) population derived from two common Chinese varieties: Hanxuan 10, a drought tolerant variety grown under rainfed condition in semiarid regions; and Lumai 14, a high yield potential variety grown under irrigated conditions (Jing et al., 1999). The phenotypes were scored at six locations over four years, i.e., at Beijing in 1999, 2000, and 2001, at Luoyang, Henan Province, and Fuping, Shaanxi Province in 2000, and at Fenyang, Shanxi Province in 2002, respectively. QTL mapping was conducted with software Mapmaker / QTL1.1 (Lincoln et al., 1993) based on the method of interval mapping by choosing Kasombi mapping function. The threshold for QTL detection was fixed at a LOD value of 2.5.

## Results

QTLs controlling spike number per plant and grain yield per plant were dispersively located in all seven homoeologous chromosome groups. Otherwise, QTLs for other traits were mainly located on genome B and A, with high repetition under different conditions. Among these, QTLs controlling spike length were mapped in the same region as chromosomes 1A, 2D, 3D, 5A, 5B, and 7A. QTLs associated with total spikelet were linked with 1A, 4B, 5A, 6A, and 6B. QTLs for plant height were on 1B, 2B, 3B, 4D, and 7B, while QTLs controlling sterile spikelet, kernel number per spike and 1000 kernels weight were associated with 2D, 5A, 6B, and 7B, respectively. Hence the genomic regions located on chromosomes 2D, 5A, 6B, and 7B played a key role in important agronomic characteristics under both rainfed and irrigated conditions.

## Conclusions

The DH population is useful for identifying QTLs for important agronomic characteristics. There are consistent results between six different locations and different water regimes. Future work will involve further testing, validating and using these molecular markers to select breeding germplasm.

Table 1. QTLs for agronomic traits identified under rainfed (T) and irrigated (CK) conditions

| Trait | Chromosome Site | Flanking Marker Loci | Water | Regime |
|---|---|---|---|---|
| **Spike Length** | 1A | WMC93 – WMC304 | T | |
| | 2D | Xgwm261 – WMC112 | T | CK |
| | 2D | WMC144 – Xgwm157 | T | CK |
| | 3D | Xgdm8 – Xgwm645 | T | CK |
| | 5A | Xgwm415 – Xgwm304 | T | CK |
| | 5B | P5166-275 – P8143-349 | | CK |
| | 7A | CWM462.2 – Xgwm635.2 | T | |
| **Total Spikelet** | 1A | WMC336 – P2478-280 | T | CK |
| | 4B | Xgwm495 – Xgwm149 | T | CK |
| | 5A | Xgwm291 – Xgwm410 | T | CK |
| | 6A | CWM306 – Xpsp3071 | T | CK |
| | 6B | Xgwm219 – WMC341 | T | CK |
| | 6B | P1142-155 – P8166-160 | T | CK |
| **Sterile Spikelet** | 5A | Xgwm291 – Xgwm410 | T | |
| **Kernel per Spiker** | 2D | WMC453.1 – WMC18 | | CK |
| | 7B | Xgwm68.1 – WMC269.1 | T | |
| **1000 Kernel Weight** | 2D | Wmc181 – P3176-120 | T | |
| | 6B | Xgwm219 – Xgwm193 | T | |
| **Plant Height** | 1B | WMC156 – P3446-183 | T | CK |
| | 2B | P5322-238 – P4233-160 | T | CK |
| | 3B | P3622-400 – P2076-147 | | CK |
| | 4D | Xgwm192 – WMC331 | | CK |
| | 7B | Xpsp3033 – Xgwm297 | T | CK |

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# Mapping QTLs and ESTs associated with drought tolerance in rice towards discovery of candidate genes

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## Introduction

The genetic and molecular dissection of stress tolerance has led to the identification of either genomic regions involved in stress tolerance (major loci or QTLs), or DNA sequences known to play a role in molecular stress responses (stress related genes, *cis*-acting elements and transcription factors). Although QTL analysis and gene cloning have been used to investigate the same stress responses, the relationship between QTLs and stress related sequences is still far from understood and remains a challenge. In the last couple of years, our understanding of the processes underlying plant response to drought, at the molecular and whole-plant levels (rainfed rice ecosystem), has rapidly progressed. The application of molecular markers and genetic linkage maps has allowed specific regions of the genome controlling drought-related polygenic traits, called quantitative trait loci (QTLs), to be identified. Key QTLs have been mapped for root morphology, root distribution, drought avoidance, root penetration ability, osmotic adjustment, dehydration tolerance, stomatal conductance, leaf rolling, heading date, cell membrane stability, abscisic acid accumulation, and several other phenological traits (Price et al., 1999; Katiyar et al., 2003). Attention was also drawn to the perception and signaling processes of water deficits using the sophisticated cDNA microarrays for global gene expression profiling of thousands of rice genes. Knowledge of these processes is essential for a holistic understanding of plant resistance to stress, which is needed to improve crop management and breeding techniques. Hundreds of genes that are induced under drought have been identified. A new challenge in breeding programs is to integrate information from functional genomics and that from QTL analysis, in order to identify sequences controlling the variation of important traits. Thus, discovering co-locations between candidate genes and QTLs is an essential step.

## Materials and methods

Two double haploid mapping populations; derived from the indica X japonica crosses CT9993 X IR62266 and IR64 X Azucena were used to identify QTLs for a set of phenological traits under two sets of environmental conditions: transplanted with water stress and transplanted with no stress (over years). The parental lines of these crosses, i.e., CT9993, IR62266, IR64, and Azucena, were also used to study the global gene expression profiles using 14K cDNA microarrays (Katiyar et al., 2004). We are now trying to co-localize the drought-responsive genes with the QTLs.

## Results

A total of 39 putative QTLs for 18 different phenological traits were identified, which were found to explain a minimum of 9.8% to a maximum of 21.3% of phenotypic variation, individually. These QTLs were mapped on the rice genome and linked SSR markers have been validated for use in the marker-assisted breeding program. Our microarray experiment (carried out at UIUC, USA) used a set of four rice varieties, which were the parents of the DH mapping populations, as discovered when exposed to different level of dehydration stress in a time course experiment. The transcript profiling experiment provided the expression profiles of ~14000 rice unigenes genes (cDNA clones). Several hundred rice genes that have shown significant induction or repression due to drought stress were identified. These include several transcription factors, signal transduction, and defense-related transcripts. We are mapping these genes on rice chromosomes using different approaches.

**Table 1. QTLs for some drought related component traits and their chromosomal locations**

| Chr# | Trait | Position of QTL between | Linked SSR markers |
|---|---|---|---|
| Chr#1 | Plant Height | RZ801 - RZ 19 | RM-302, RM-212, RM-315 |
| | Spikelet Fertility | RZ810 - RG690 | RM-302, RM-212, RM-315 |
| Chr#2 | Total No. Of Tiller | Amy1A/C - RM221 | RM-318 |
| | No. Of Effective Tiller | RM240 - RM221 | RM-318 |
| | 100 Grain Weight | RM221 - Pai_1<br>RG171 -RG437 | RM-263, RM-29, RM-324, RM-290, RM-191 |
| Chr#3 | Days To Flowering | RG418A - Pgi-1 | RM-85, RM-130 |
| | No. Of Effective Tiller | RM168 - pRD10A | RM-16 |
| Chr#4 | Yield / Plant | RG-620 - RG163 | RM-349 |
| Chr#5 | Plant Height | RZ649 - RG313 | RM-194, RM-13, RM-39, RM-146 |
| Chr#6 | Yield / Plant | RM50 - RM204 | RM-276 |
| | Yield / Plot | RG162 - Pai_2<br>Amp-3 - RM-204 | RM-276 |
| Chr#8 | Grain Breadth | RM25 - A181A1120<br>A10K250 - RM-223 | RM-223, RM-342A, RM-32 |
| Chr#9 | Plant Height | G103 - CDO590 | RM-296, RM-316 |
| | No. Of Effective Tiller | G103 - RG757 | RM-296, RM-316 |
| | Panicle Length | RM205 - RM667 | RM-205 |

## Conclusions

The combination of sequence information and RNA expression profiling is powerful tool for providing correlative evidence on gene functions, although direct demonstration can only be achieved by genetic approaches. This new approach that operates at molecular, plant, and ecosystem levels will revolutionize our understanding of rice response to drought, and our ability to apply molecular tools for enhance tolerance of drought and related abiotic stresses to accelerate breeding.

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# Identifying quantitative trait loci (QTL) for drought tolerance in bread wheat (*Triticum aestivum* L.)

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## Introduction

Production of wheat is constrained by drought in many regions of the world, with about 60% of the acreage suffering from periodic drought. Breeding for tolerance to drought is difficult because of tremendous variability in the intensity and timing of drought events and a diversity of drought tolerance mechanisms used by the plant. Consequently, progress in cultivar yields in drought-prone environments is estimated at only about half of the gains realized under well-watered environments. The advent of DNA markers has facilitated the study of quantitative traits and made possible their dissection into quantitative trait loci (Tanksley, 1993). Individual genetic factors as well as their function can de identified that determine complex phenotypes. Molecular markers can then be designed to aid in selection for superior genotypes. The objective of this research was to identify QTLs associated with increased grain production under drought-stress conditions using a recombinant inbred wheat population.

## Materials and methods

A recombinant inbred wheat population between cultivar Dharwar Dry (drought tolerant) and Sitta (drought susceptible) was chosen for the study. One hundred and forty (140) lines were grown in the Yaqui Valley, Sonora, Mexico during 1998-99 and 1999-00 seasons. The materials were grown under irrigated and drought-stressed conditions. There was negligible rainfall during the growing season, creating conducive conditions for the drought treatments. Seven hundred (700) microsatellite markers were amplified (Röder et al., 1998.)

## Results and discussion

High phenotypic correlations were recorded for grain yield and days to heading and for duration of grain filling during the stress period for cv. Dharwar Dry in contrast to cv. Sitta. Earliness to heading and the duration of grain filling were not correlated with grain yield under stress conditions for cv. Sitta. More than 700 microsatellite and STS markers have been tested and 91 have been found polymorphic and have been genotyped on the population. This represents a low level of polymorphism that was not expected since the two parents used, Dharwar Dry (from India), and Sitta (Mexico) have different origins.

The microsatellite *Xwmc48* was found to be associated with a reduction in days to heading, increased grain fill duration, increased grain filling rate, a low drought susceptibility index (DSI), and a high grain yield under drought stress. *Xwmc48* was mapped near the centromere on chromosome 4AS in the ITMI population (Figure 1). Low polymorphism has resulted in low marker density in this region. Preliminary physical mapping placed microsatellite Xwmc48 on deletion bin 4AS-4-0.63-0.71 using Chinese Spring wheat deletion stocks (Endo and Gill, 1996).

## Future prospects

Given the low polymorphism observed with these parents, the results provide an initiating point to start fine mapping by designing and mapping EST-STS markers mapped in the 4AS-4-0.63-0.71 bin by the NSF EST mapping project with the aim of saturating this region with markers. We plan to combine this technique with a search for AFLP markers using the bulk segregant analysis approach, wherein individual parents, one bulk from resistant and one from susceptible offspring and deletions 4AS-4 and 4AS-2 which delimit the target bin, will be used.

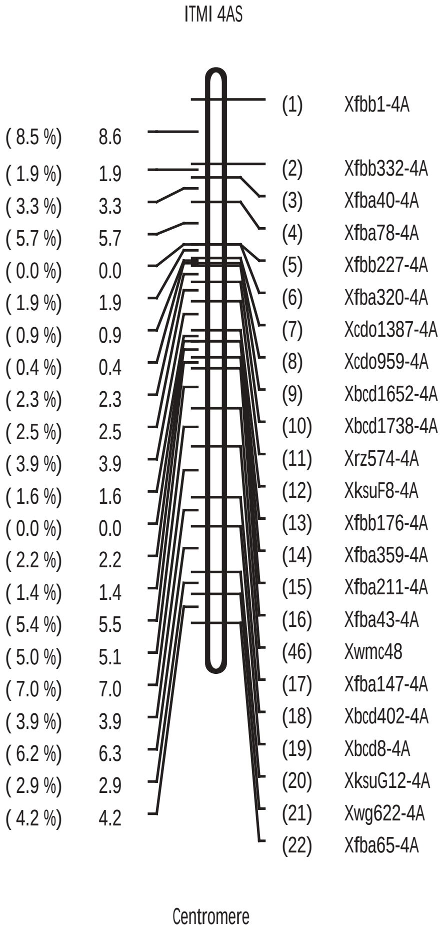

**Figure 1. We genotyped the ITMI (W7984 x Opata85 ) reference population using the microsatellite marker Xwmc48. Additional data on this population were downloaded from the website http://wheat.pw.usda.gov/ggpages/maps/Triticum/Wheat and used together with our data to map the Xwmc48 143 bp amplicon (Arrow) on the short arm of chromosome 4A.**

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# Developing high yield and drought tolerant rice cultivars and discovering the complex genetic network underlying drought tolerance in rice

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Drought is the largest constraint to rice production in the rainfed and upland systems. Rice acreage in the traditional irrigated system is also decreasing resulting from emerging water shortages as urbanization proceeds in many parts of Asia. Breeding for drought tolerant (DT) or water-saving rice cultivars has not been a major target in most breeding programs in Asia. Low-yielding traditional varieties are still grown in about 50% of the rainfed area of Asia, largely because of their better adaptation to different stresses and favored grain quality. Progress in developing high-yielding, DT rice cultivars by conventional breeding has been slow, largely because of difficulties in precisely defining the target environment, complex interactions of DT with environments, and lack of appropriate screening methodology. Considerable work has recently been undertaken to understand the genetic basis of putative drought-adaptive traits in rice, but it has been difficult to identify genetic segments with clear and repeatable effects on yield under stress.

We report here a large-scale backcross breeding effort, initiated in 1998 at the International Rice Research Institute, to develop large numbers of introgression lines (ILs) of elite genetic backgrounds with significantly improved DT and to discover large numbers of genes / QTLs for DT in rice using the ILs. Two broadly adapted, high-yielding lowland *indica* cultivars (IR64 and Teqing) and a tropical *japonica* new plant type (NPT) breeding line, IR68552-55-3-2, were used as the recurrent parents. A total of 163 varieties from 34 countries were used as donors. Together, these parents represent a significant sample of the genetic diversity in the primary gene pool of *O. sativa.* A total of 375 crosses were made between the recurrent parents and donors. These were advanced to BC$_2$F$_2$ generation, including 124 IR64 BC$_2$F$_2$ populations, 75 Teqing BC$_2$F$_2$ populations and 123 NPT BC$_2$F$_2$ populations. The 375 BC$_2$F$_2$ populations were subjected severe water stress under both lowland (at the reproductive stage) and upland conditions that killed the recurrent parents. A total of 4721 BC$_2$F$_2$ plants survived the stresses and were advanced to BC$_2$F$_4$ generation. The selected DT B$_2$C$_4$ introgression lines (ILs) were confirmed in progeny testing under the stress conditions and over 50% of the selected ILs outperformed the parents in yield under drought, from which 43 promising DT lines were identified and sent to the yield trials in several NARES.

Significant progresses have been made in large-scale discovery of DT gene / QTL by genotyping the DT ILs using DNA markers. Two types of ILs were used for mapping genes / QTLs associated with DT. The first type included 254 Teqing ILs containing randomly introgressed Lemont (*japonica*) segments, and 120 IR64 ILs selected for quality parameters. These ILs were genotyped with more than 160 well-distributed SSR markers and assayed for grain yield and related traits in replicated trials under both irrigated and water stress before anthesis conditions. Many QTLs associated with differential responses of the ILs to water stress in flowering time, height, and grain yield were identified and mapped. In addition, 742 ILs from more than 29 BC populations selected for their DT were assayed using SSR markers. Based on $X^2$ tests of the introgression frequencies in the DT ILs from each BC population against the expectation, we were able to identify large numbers of QTLs across the rice genome for DT. Our results indicated that using selected ILs for identifying genes / QTLs for DT is highly efficient and powerful (Table 1). Several important results were obtained. First, many DT QTLs from a wide range of donors were detected. Second, some QTLs appeared to be more important, as they were detected in more BC populations with very frequencies in the DT ILs. Third, multiple functional alleles at some important QTLs were revealed. Fourth, several important DT QTLs of large effects were defined to small genomic regions < 5 cM using overlapping ILs and closely linked markers (Figure 1). All of these provide useful information for marker-assisted pyramiding of multiple non-allelic DT QTLs.

A total 33 additional crosses were simultaneously made between the promising DT sister ILs to pyramid DT QTL from different sources. Screening of the $F_2$ populations from theses crosses under very severe drought of the lowland condition resulted in 560 progeny with much improved DT from 10 crosses. These DT F4 lines are being progeny tested for their DT under the replicated trials in the 2003-2004 dry-season at IRRI and genotyped by SSR markers. Our results have led us to several conclusions. First, there is tremendous hidden diversity for DT in the primary gene pool of rice and most lines in the germplasm collection could be good donors for desirable alleles (traits) for DT regardless of their own performance in DT. Second, BC breeding combined with DNA markers are a powerful way to exploiting this diversity for simultaneous development of DT rice cultivars and discovery of genes / QTLs for DT. The implications of our results to plant breeding and the strategies using DT ILs for dissecting the genetic network underlying DT and the development of superior DT rice cultivars by marker assisted gene / QTL pyramiding, will be discussed.

**Table 1. Power of QTL detection using 442 ILs from 19 BC populations selected for their drought tolerance under the lowland (at the reproductive stage) and upland (the whole growth period) stress conditions**

| Selection environment | Test type[a] | Number QTLs[b] | N[c] | *n* | Number of significant cases P ≤ | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0.05 | 0.01 | 0.001 | 0.0001 | $X^2$ |
| Lowland | Single | 314 | 1.0 | 16 | 69 | 65 | 41 | 139 | 26.3 |
| | Pool | 76 | 4.0 | 68 | 0 | 7 | 0 | 69 | 72.3 |
| Upland | Single | 194 | 2.6 | 17 | 51 | 39 | 21 | 87 | 28.8 |
| | Pool | 76 | 2.6 | 45 | 4 | 6 | 6 | 60 | 59.1 |
| | | 79 | 5.4 | 114 | 1 | 0 | 1 | 77 | 117.8 |
| Negative selection [d] | | | | | | | | | |
| | Pool | 64 | 2.8 | 85 | 10 | 16 | 6 | 32 | 19.6 |

[a] Test type indicates that $X^2$ tests for genotypic frequencies were performed based on ILs selected from single BC populations (Single), or from ILs from more than one BC population where tests based on single populations were significant. For example, when a QTL (marker) was detected at in ILs from 3 BC populations, then, all ILs from the 3 populations were pooled (increased population size) together, subjecting to the statistical tests.

[b] The number of cases (putative QTLs for drought tolerance) where significant excess of donor alleles (genotypes) was detected.

[c] N and ***n*** are the numbers of the BC populations and the selected ILs used in the $X^2$ tests.

[d] Negative selection indicates cases in which the donor introgression was zero (selection favored the recipient allele).

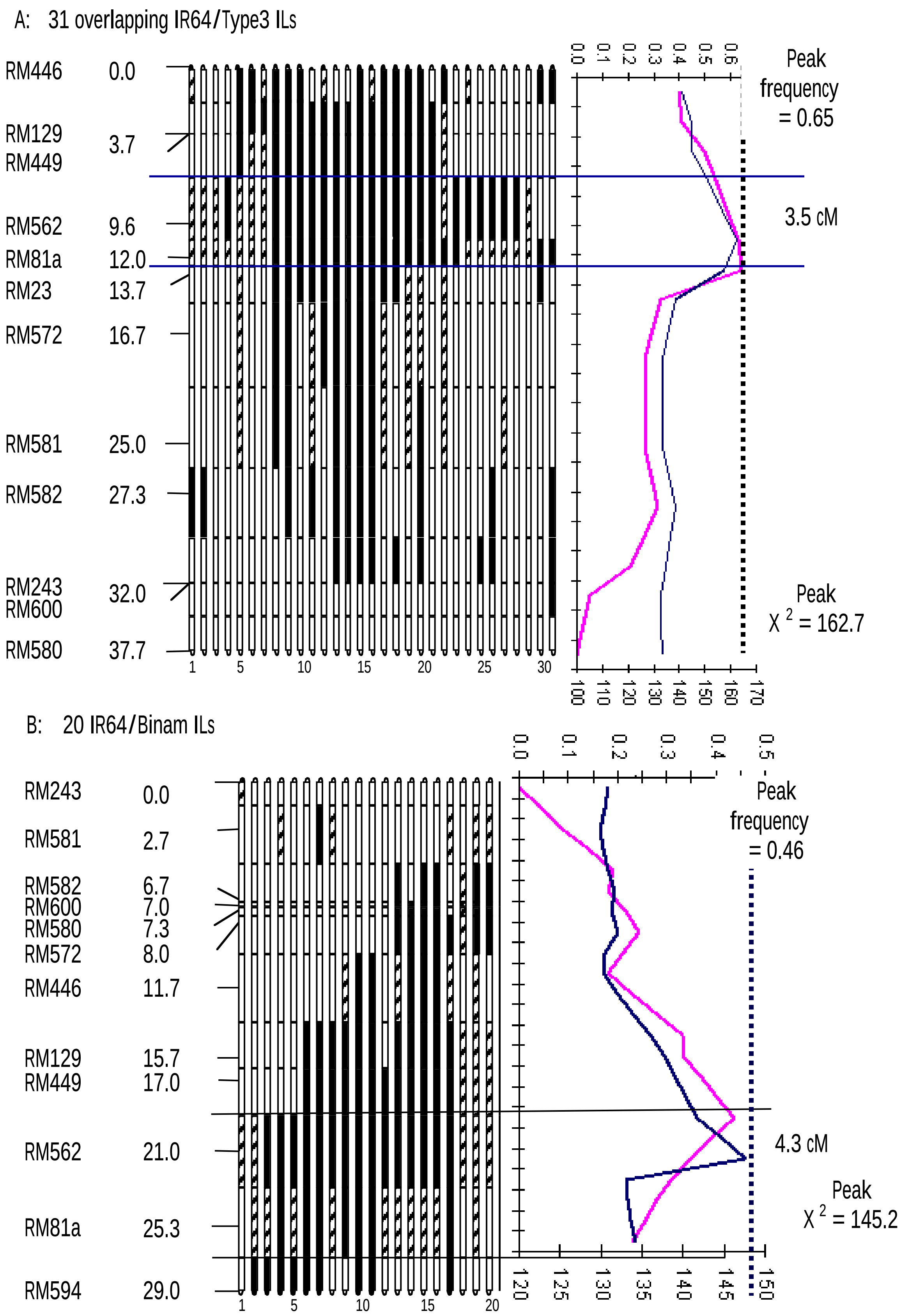

**Figure 1. Fine-mapping of a QTL for drought tolerance on chromosome 1 using 51 introgression lines from two BC populations.**

# Discovery of drought tolerant (DT) gene / QTLs and development of DT rice cultivars

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## Introduction

Rice is the staple food for most of the Chinese people. However, rice is a big water consumer as rice production uses 70% of the fresh water used in agriculture, which, in turn, consumes 80% of the total fresh water resources of China (Padolina, 1996). Because of water shortages, the likelihood of drought damage has become more and more serious. The annual economic loss from water shortages alone reaches more than US$25 billion in China (Deng, 1999). Developing drought tolerant (DT) or water saving rice cultivars is considered to be one of the most effective and economic approaches to ensuring China's food security (Luo and Zhang, 2001). Nevertheless, the drought tolerance of rice is extremely complex and little is known about its genetic mechanisms (Blum, 1999). To better understand the DT of plants and improving the DT of rice, several efforts have been made and some progress achieved in recent years under support mainly from The Rockefeller Foundation.

## Methods

1. Field screening facility establishment and DT germplasm evaluation. The first priority was setting up a "scientific based" field screen facility. A rainproof screen house was constructed with water control facilities, including sprinkler and drip irrigation systems, channel drainage system, and TDR to measure the soil water status. By draining the underground water, the gradient of soil water content can be controlled and the materials can be screened in different drought conditions. A total of 820 rice accessions from different origins were evaluated in the facility during the last two years.
2. Near isogenic introgression lines (NIIL) / recombination inbred line (RIL) population development and DT gene / QTL discovery. A commercial rice restorer, Zhong 413, was used as recurrent parent to cross with 100 diverse donor parents including 20 DT upland rice germplasm. Through continued backcrossing, several hundred introgression lines were developed. In addition, a set of 195 RIL population derived by single seed descent from a cross between an upland rice cultivar IRAT 109 and paddy rice Zhenshan 97 was constructed. More than 200 SSR markers were used to genotype the mapping populations above. The phenotype was conducted in the field screening facility. Several morphological and physiological traits as well as yield related traits were investigated under stress and non-stress conditions. The QTLs were detected using Windows QTL Cartographer V2.0 (Bastern et al., 2001). An attempt to discover DT related genes by different display and electronic cloning technologies was also made.
3. DT rice cultivar development. Usually, the upland rice with high-levels of DT was used as DT parents to cross with the commercial paddy rice to develop DT rice cultivars for the breeding program. A CMS line, Zhenshan 97A, was used as the tester to screen the maintainer in DT rice germplasm. A maintainer, H3034, was identified and used to develop a DT CMS line. The comprehensive evaluation on promising breeding lines includes agronomic performance, yield potential, important pest and disease resistances, and drought tolerance.

## Results

1. A primary methodology and standard system for evaluating the drought tolerance of rice was established. Canopy temperature and panicle neck diameter was selected as the easier and more effective index to evaluate the drought tolerant rice accessions in the reproductive stage. A total of 60 high DT germplasm was obtained through this system. This germplasm was distributed to various rice breeding programs in China.

2. Several approaches were applied to discover DT related genes / QTLs. A molecular linkage map was constructed with 188 SSR markers. The map spanned 1773.2 cM and covered 12 rice chromosomes with an average interval of 10.19 cM. A total of 58 main-effect QTLs related with 17 performance traits were identified and mapped in 12 rice chromosomes. Several marker intervals were found to affect multi-traits under stress conditions (Figure 1). A major QTL in chromosome 12 affects relative water content as well as percentage of exserted stigma and transpiration rate, which is consistent with the significant relationship between these traits and the actual water status of the plant. This QTL was also detected to affect the two yield components: 1000-grains weight and spikelet number per panicle.
3. Several promising DT cultivars have been released and perform well in water-limited environments. In addition, a DT CMS line with excellent grain quality and agronomic performance (Table 1) was developed and named Huhan 1A. Two promising DT hybrids using Huhan 1A as a parent were selected and will be demonstrated next year.

## Conclusion

To better understanding the drought tolerance of rice, a field screen facility was established and used to evaluate the DT rice germplasm and for phenotyping a mapping population. More than 20 high DT germplasm and 58 putative QTL affecting DT related traits were identified. Several promising DT rice cultvars were released.

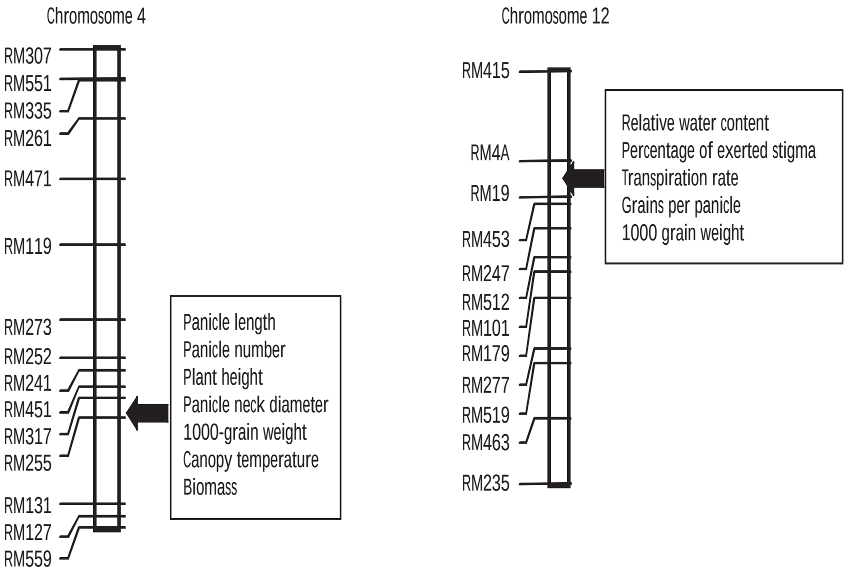

**Figure 1. The significant putative QTLs associated with multi-traits.**

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**Table 1. The agronomic performance and grain quality of Huhan 1A**

| Characteristics | | Characteristics | | Characteristics | |
|---|---|---|---|---|---|
| Maturity | 121 d. | Brown rice recovery | 80.3% | Chalkness degree | 1.2% |
| Plant height | 85 cm | Milled rice recovery | 72.1% | Gel consistency | 85mm |
| Panicles /plant | 10 | Grain length | 6.1mm | Translucency | 2 |
| Grains / panicle | 92 | Length/width | 3 | Amylose content | 14.5% |
| 1000-grain weight | 26.5g | Chalkness rate | 8% | Protein content | 10.6% |

# Molecular dissection of drought tolerance (DT) related quantitative trait loci (QTL) in ZhenshanB/IRAT109 RIL population

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## Introduction

Recently, a series of studies on drought tolerance (DT) resulted in the identification of many QTLs associated with the DT related traits (Courtois et al., 2000; Luo and Zhang, 2001) As drought tolerance is an integrated response of rice plants to water stress together with other environmental factors, it is extremely complex and attained within three major physiological domains (Blum, 1999). Work in this area requires standard assays of drought related traits under effective water controlled conditions with whole-plant testing in the field. The investigation of most traits under different water stress conditions requires separate fields and usually ignores the affects generated by soil and other environmental factors. In this study, a RI population was evaluated for drought toleranceunder a gradient of soil water content conditions in a field screen facility.

## Methods

A set of 195 $F_{10}$ recombinant inbred lines were developed from Zhanshan 97B (drought sensitive paddy rice) and IRAT 109 (drought tolerance upland rice) and its parents were evaluated for drought tolerance (DT) at reproductive stage under gradient water stress conditions. Each line was seeded directly in two-rows plots with 15 plants per row in a random block design with three replications. To synchronize the heading date, the RI population was separated into three subsets and seeded on three different dates with seven days interval. In each row of plants, individuals near the channel were under most severe stress while ones near the drip were well watered. After giving stress for four weeks, measurements were performed on three groups of plants, i.e., Group 1 (3rd and 4th plant, counting from the row end beside the channel) under severe stress (denoted as *Stress*); Group 2 (8th and 9th plants) under moderate stress (*Half Stress*); and Group 3 (13th and 14th plants) under normal water conditions with wet soil surface (*Normal*).

A total of 12 traits were investigated: plant height (PH), panicle length (PL), relative water content (RWC), leaf water potential (LWP), canopy temperature (CT), panicle neck diameter (PND), biomass (BMS), grain yield (GY) and its components, panicle number (PN), spikelets per panicle (SN), spikelet fertility (SF), and 1000-grain weight (GW). Standard analyses of variance were performed to check the genetic variance among the RI lines for the investigated traits.

The genotyping was conducted according to published procedures (Luo et al., 2001). An integrated genetic linkage map with 188 SSR markers was constructed using Mapmaker vision 3.0 (Lincoln and Lander, 1992). The putative quantitative trait loci linked to the traits were identified using Windows QTL Cartographer V2.0 (Basten et al., 2001) with a threshold LOD score of 2.0.

**Table 1. QTLs affecting 12 DT related traits detected under three different water conditions in rice**

| Trait | Loci | Chr | Treat* | Interval | LOD | *a* | $R^2$ (%) |
|---|---|---|---|---|---|---|---|
| Plant height (PH, cm) | *QPh1* | 1 | *Normal* | RM472-RM104 | 3.87 | -4.56 | 11.90 |
| | | | *Half Stress* | | 5.62 | -4.52 | 15.30 |
| | | | *Stress* | | 2.89 | -3.67 | 9.17 |
| | *QPh4* | 4 | *Normal* | RM451-RM317 | 2.72 | -3.11 | 5.46 |
| | | | *Stress* | | 3.73 | -3.38 | 7.73 |
| | *QPh8* | 8 | *Half Stress* | RM531-RM80 | 2.08 | 2.73 | 5.39 |
| Canopy temperature (CT, ! ) | *QCt3a* | 3 | *Normal* | RM426-RM203 | 4.13 | 0.27 | 11.34 |
| | *QCt3b* | 3 | *Normal* | RM130-RM514 | 2.39 | -0.20 | 6.28 |
| | *QCt4a* | 4 | *Stress* | RM273-RM252 | 3.54 | 0.23 | 9.55 |
| | *QCt4b* | 4 | *Stress* | RM317-RM255 | 2.32 | -0.17 | 5.47 |
| | *QCt5* | 5 | *Stress* | RM459-RM161 | 2.07 | 0.14 | 4.34 |
| | *QCt11* | 11 | *Normal* | RM20B-RM167 | 3.69 | 0.25 | 11.83 |
| Relative water content (RWC, %) | *QRwc7* | 7 | *Stress* | RM11-RM346 | 2.32 | 0.84 | 5.20 |
| | *QRwc12* | 12 | *Stress* | RM19-RM453 | 4.33 | 1.15 | 9.87 |
| Leaf water potential (LWP, bar) | *QLwp1a* | 1 | *Normal* | RM302-RM476B | 2.35 | -0.39 | 5.41 |
| | *QLwp1b* | 1 | *Normal* | RM472-RM104 | 2.75 | 0.54 | 9.85 |
| | *QLwp11* | 11 | *Normal* | RM287-RM209 | 2.02 | -0.45 | 4.59 |
| Panicle number (PN) | *QPn4a* | 4 | *Normal* | RM471-RM119 | 3.05 | 0.51 | 6.23 |
| | *QPn4b* | 4 | *Half Stress* | RM317-RM255 | 4.46 | 0.49 | 10.56 |
| | | | *Stress* | | 3.94 | 0.48 | 8.26 |
| | *QPn6* | 6 | *Normal* | RM30-RM340 | 2.20 | 0.46 | 5.11 |
| | *QPn8a* | 8 | *Half Stress* | RM25-RM544 | 2.36 | 0.35 | 5.07 |
| | *QPn8b* | 8 | *Stress* | RM404-RM339 | 3.86 | 0.49 | 8.13 |
| | *QPn9* | 9 | *Normal* | RM105-RM321 | 2.16 | 0.42 | 4.24 |
| | *QPn11* | 11 | *Half Stress* | RM536-RM287 | 2.39 | 0.39 | 6.48 |
| Panicle length (PL, cm) | *QPl1* | 1 | *Normal* | RM472-RM104 | 2.79 | -0.75 | 7.39 |
| | *QPl4* | 4 | *Normal* | RM451-RM317 | 7.16 | -1.00 | 13.35 |
| | | | *Half Stress* | | 7.84 | -1.13 | 15.55 |
| | | | *Stress* | | 8.15 | -1.07 | 15.9 |
| | *QPl8* | 8 | *Normal* | RM531-RM80 | 4.92 | 0.93 | 11.05 |
| | | | *Half Stress* | | 4.31 | 0.94 | 9.94 |
| | | | *Stress* | | 3.32 | 0.67 | 6.04 |
| | *QPl9* | 9 | *Half Stress* | RM553-RM160 | 2.69 | 0.65 | 5.01 |
| | | | *Stress* | | 2.00 | 0.51 | 3.62 |
| Panicle neck diamater (PND, mm) | *QPnd4* | 4 | *Normal* | RM255-RM131 | 2.93 | -0.09 | 9.28 |
| | | | *Half Stress* | | 3.55 | -0.08 | 7.51 |
| | | | *Stress* | | 3.70 | -0.08 | 9.1 |
| | *QPnd6* | 6 | *Normal* | RM111-RM549 | 2.72 | 0.08 | 8.01 |
| | | | *Half Stress* | | 2.53 | 0.07 | 7 |
| | *QPnd10* | 10 | *Normal* | RM311-RM467 | 2.19 | 0.07 | 5.6 |
| | | | *Half Stress* | | 3.46 | 0.09 | 9.58 |
| | *QPnd11* | 11 | *Stress* | RM144-RM224 | 2.69 | 0.07 | 5.68 |
| Spikelet number (SN) | *QSn2* | 2 | *Stress* | RM424-RM341 | 2.16 | -46.49 | 6.54 |
| | *QSn3* | 3 | *Half Stress* | RM282-RM411 | 2.25 | -41.55 | 6.28 |
| | | | *Stress* | RM7-RM282 | 2.17 | -46.52 | 6.78 |
| | *QSn11* | 11 | *Normal* | RM206-RM144 | 2.20 | 61.51 | 4.69 |
| | *QSn12* | 12 | *Stress* | RM4A-RM19 | 2.05 | 38.04 | 4.79 |
| Spikelet fertility (SF, %) | *QSf2a* | 2 | *Stress* | RM555-RM492 | 3.27 | -4.21 | 7.14 |
| | | | *Half Stress* | | 2.86 | -3.98 | 6.39 |
| | *QSf2b* | 2 | *Normal* | RM526-RM525 | 2.63 | -3.65 | 5.88 |
| | *QSf5* | 5 | *Half Stress* | RM122-RM507 | 2.87 | 4.26 | 7.01 |
| | *QSf7* | 7 | *Normal* | RM134-RM28 | 5.22 | -4.87 | 11.78 |
| | *QSf8* | 8 | *Normal* | RM544-RM547 | 3.36 | 3.78 | 6.62 |
| | | | *Stress* | | 2.28 | 3.39 | 4.66 |
| | *QSf9a* | 9 | *Stress* | RM285-RM444 | 2.32 | -3.41 | 4.75 |
| | *QSf9b* | 9 | *Half Stress* | RM257-RM242 | 2.13 | -3.34 | 4.51 |
| | *QSf10a* | 10 | *Half Stress* | RM596-RM258 | 3.27 | 4.76 | 9.26 |
| | *QSf10b* | 10 | *Half Stress* | RM269-RM294A | 4.77 | 5.91 | 13.77 |
| | | | *Stress* | | 3.11 | 4.46 | 8.14 |
| | *QSf10c* | 10 | *Half Stress* | RM591-RM333 | 2.15 | 3.39 | 4.28 |
| 1000-grain weight (GW, g) | *QGw1* | 1 | *Normal* | RM476B-RM315 | 3.25 | -0.07 | 6.72 |
| | *QGw2* | 2 | *Normal* | RM526-RM525 | 2.83 | -0.07 | 6.58 |
| | | | *Stress* | | 3.17 | -0.08 | 7.18 |
| | *QGw3* | 3 | *Half Stress* | RM520-RM571 | 2.94 | -0.07 | 6.95 |
| | | | *Stress* | | 3.40 | -0.08 | 8.56 |
| | *QGw4a* | 4 | *Normal* | RM119-RM273 | 3.67 | -0.10 | 13.32 |
| | | | *Half Stress* | | 2.49 | -0.07 | 7.33 |
| | | | *Stress* | | 4.06 | -0.11 | 14.07 |
| | *QGw4b* | 4 | *Stress* | RM255-RM131 | 2.56 | 0.08 | 7.27 |
| | *QGw12* | 12 | *Normal* | RM4A-RM19 | 2.33 | -0.05 | 4.44 |
| | | | *Half Stress* | | 5.46 | -0.09 | 10.48 |
| | | | *Stress* | | 2.64 | -0.06 | 5.28 |
| Grain yield per plant (GY, g) | *QGy2* | 2 | *Normal* | RM526-RM525 | 2.40 | -0.87 | 5.72 |
| | | | *Stress* | | 2.06 | -0.51 | 4.53 |
| | *QGy7* | 7 | *Normal* | RM134-RM28 | 4.88 | -1.13 | 10.78 |
| | *QGy10a* | 10 | *Normal* | RM311-RM467 | 2.16 | 0.78 | 5.05 |
| | | | *Half Stress* | | 3.00 | 0.69 | 7.76 |
| | *QGy10b* | 10 | *Normal* | RM228-RM591 | 2.23 | 0.72 | 4.23 |
| Biomass (BMS, g) | *QBms3* | 3 | *Half Stress* | RM22-RM231 | 2.16 | 1.36 | 4.61 |
| | *QBms4* | 4 | *Half Stress* | | 2.07 | -1.33 | 4.39 |
| | | | *Stress* | RM451-RM317 | 2.28 | -1.31 | 4.9 |
| | *QBms7a* | 7 | *Stress* | RM11-RM346 | 4.31 | -1.91 | 9.8 |
| | *QBms7b* | 7 | *Normal* | RM134-RM248 | 2.43 | -1.99 | 5.57 |
| | *QBms9* | 9 | *Stress* | RM321-RM566 | 2.16 | -1.51 | 6.09 |

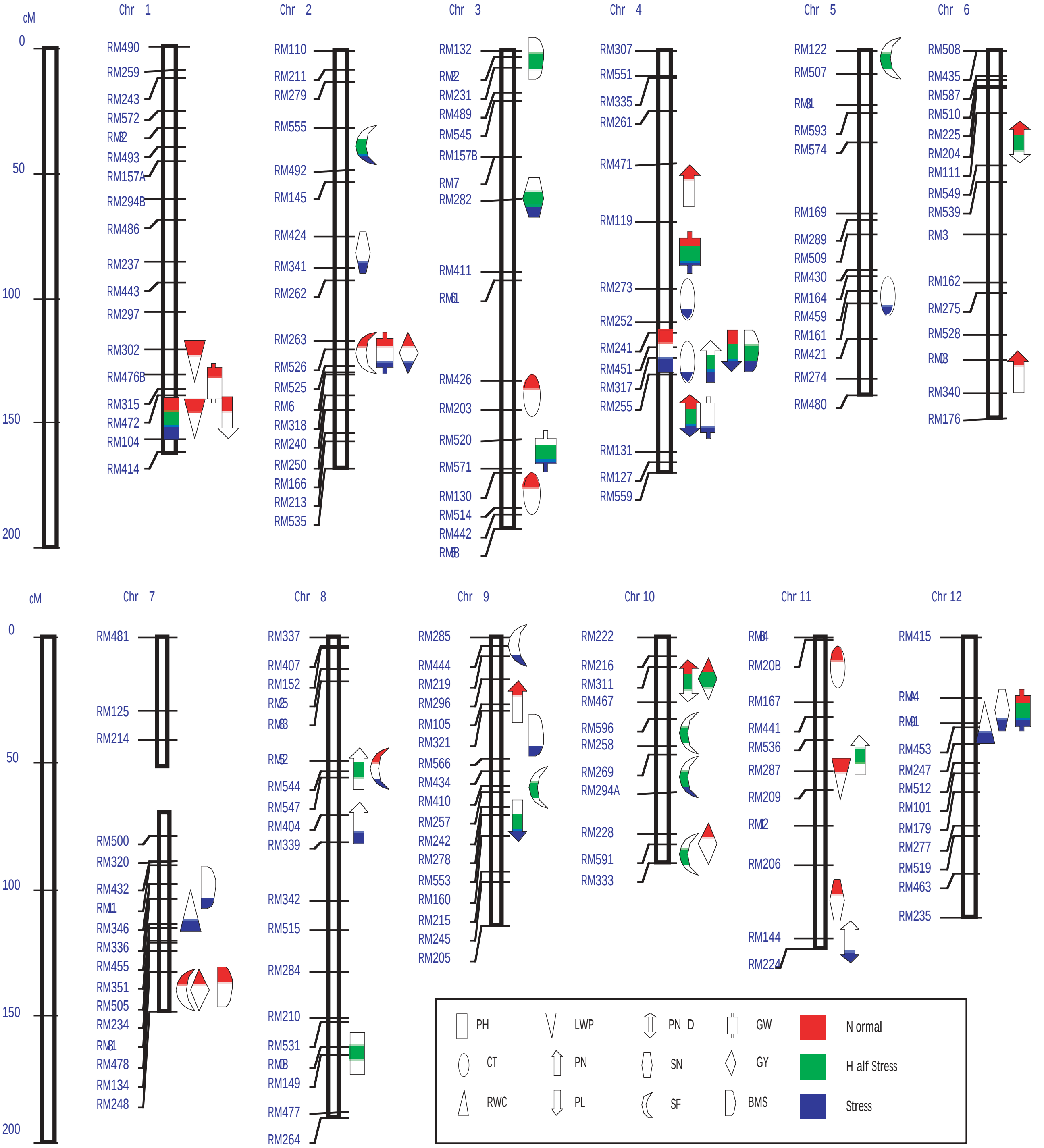
cM
0
50
100
150
200
Chr 1
RM490
RM259
RM243
RM572
RM493
RM157A
RM294B
RM486
RM237
RM443
RM297
RM302
RM476B
RM315
RM472
RM104
RM414
Chr 2
RM110
RM211
RM279
RM555
RM492
RM145
RM424
RM341
RM262
RM263
RM526
RM525
RM6
RM318
RM240
RM250
RM166
RM213
RM535
Chr 3
RM132
RM231
RM489
RM545
RM157B
RM7
RM282
RM411
RM426
RM203
RM520
RM571
RM130
RM514
RM442
Chr 4
RM307
RM551
RM335
RM261
RM471
RM119
RM273
RM252
RM241
RM451
RM317
RM255
RM131
RM127
RM559
Chr 5
RM122
RM507
RM593
RM574
RM169
RM289
RM509
RM430
RM164
RM459
RM161
RM421
RM274
RM480
Chr 6
RM508
RM435
RM587
RM510
RM225
RM204
RM111
RM549
RM539
RM3
RM162
RM275
RM528
RM340
RM176
Chr 7
RM481
RM125
RM214
RM500
RM320
RM432
RM346
RM336
RM455
RM351
RM505
RM234
RM478
RM134
RM248
Chr 8
RM337
RM407
RM152
RM544
RM547
RM404
RM339
RM342
RM515
RM284
RM210
RM531
RM149
RM477
RM264
Chr 9
RM285
RM444
RM219
RM296
RM105
RM321
RM566
RM434
RM410
RM257
RM242
RM278
RM553
RM160
RM215
RM245
RM205
Chr 10
RM222
RM216
RM311
RM467
RM596
RM258
RM269
RM294A
RM228
RM591
RM333
Chr 11
RM20B
RM167
RM441
RM536
RM287
RM209
RM206
RM144
RM224
Chr 12
RM415
RM453
RM247
RM512
RM101
RM179
RM277
RM519
RM463
RM235
PH
LWP
PN D
GW
N ormal
CT
PN
SN
GY
H alf Stress
RWC
PL
SF
BMS
Stress

**Figure 1.**

## Results

1. The SSR linkage map construction: A complete linkage map spanned 1773.2 cM and covered 12 rice chromosomes, with average interval of 10.2 cM. There is a single gap between RM214 and RM500 on chromosome 7. All SSR makers were selected from the published papers.
2. Morphological traits: Three QTLs were detected for PH on rice chromosomes 1, 4, and 8. Of these loci, *QPh1* and *QPh4* had consistent additive effects under *Normal* and *Stress* while the 3rd one was only detected in *Half Stress*. These three QTLs were also found to be associated with panicle length. An additional QTL affecting PL was located on chromosome 9; four QTLs on chromosomes 4, 6, 10, and 11 were associated with PND. *QPnd4*, *QPnd6* and *QPnd10* were detectable under three conditions or under *Normal* and *Half Stress*.
3. Physiological traits: Six QTLs had significant effect on CT. Half of them (*QCt3a*, *QCt3b*, and *QCt11*) were detected under *Normal* and other three (*QCt4a*, *QCt4b*, and *QCt5*) under *Stress*. Two loci were located on chromosomes 7 and 12, affecting RWC under *Stress* only. Three QTLs for LWP were only detectable under *Normal* and were located on chromosomes 1 and 11, respectively.
4. Yield and its components. Five QTLs for biomass were detected under single treatment, but *QBms4* under both *Stress* and *Half Stress*. These QTLs were mapped on chromosomes 3, 4, 7, and 9, respectively. Four QTLs affecting grain GY were mapped on chromosomes 2, 7, and 10; all of them were detectable under *Normal* conditions, but only two (*QDgw2* and *QDgw10a*) could be detected under *Stress* or *Half Stress*. Seven QTLs related to PN were mapped to chromosomes 4, 6, 8, 9, and 10 under one of three water conditions, except *QPn4b* under both *Half Stress* and *Stress*. Four QTLs influencing SN (*QSn2*, *QSn12*, *QSn3* under *Stress*, and *QSn11* under *Normal)* were located on chromosomes 2, 3, 11, and 12, respectively. A total of 10 QTLs had significant effects on spikelet fertility (SF). Among these loci, seven (*QSf2b*, *QSf5*, *QSf7*, *QSf9a*, *QSf9b*, *QSf10a* and *QSf10c*) were detectable under only one treatment while the other three were detectable under two conditions. Six QTLs on chromosomes 1, 2, 3, 4, and 12 were associated with 100-grain weight (HGW), including *QHgw4a* and *QHgw12*, detectable under all treatments, *QHgw2* and *QHgw3* under two conditions.

## Conclusion

A RI population was phenotyped in the water control screen facility for 12 traits and genotyped with 188 SSR markers. A total of 58 putative QTLs were detected under different water status and mapped on 12 rice chromosomes.

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# Genetic analysis of drought tolerance in tropical maize

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## Introduction

Drought stress is evenly distributed across the world's major maize production regions and is a particularly severe problem for slightly more than one-fifth of the tropical and subtropical maize planted in developing countries (Pingali and Pandey, 2000). Maize is unusually susceptible to drought around the flowering period. Drought tolerance is closely associated with a short anthesis-silking interval (ASI), reduced kernel barrenness (Chapman and Edmeades, 1999), increased growth rates of ovaries (Bertrán et al., 2003) and the stay-green characteristics (Sanchez et al., 2002).

## Materials and methods

"CML444" is a drought tolerant maize line developed at CIMMYT. "SC-Malawi" is a local line used in Africa and is very susceptible to water limited conditions. By crossing these two lines, a segregating population of recombinant inbred lines (RILs) has been developed. Individual DNA samples of the $F_5S_6$ plants have been used to construct a genetic linkage map, which consists of the allelic information of 236 RILs at 160 marker loci.

The 236 RILs were used for the phenotypic evaluation in the field. To date, three field experiments have been performed in Tlaltizapán, Morelos, Mexico. All of them were repeated twice and designed as an alpha (0, 1) lattice (Patterson and Williams, 1976) with 10 plots per block and 12 plants per plot. Standard traits like male flowering, plant height, ear height, leaf chlorophyll content, leaf senescence, and root conductivity were measured in all the experiments. Two experiments were performed under drought stress conditions (DS), one to record female flowering (which allows calculating ASI) and grain yield related parameters, and one to measure dry weight of the ears and silks at pollen shedding and seven days after pollen shedding. (It is not possible to collect data on female flowering and ear and silk growth in together in one trial, since the last measurement is destructive.) The third field experiment was performed under well-watered conditions (WW), and was used as a control for female flowering, ASI and grain yield.

Phenotypic data in combination with genetic marker data allowed us to calculate quantitative trait loci (QTLs) to identify genomic regions involved in the expression of the traits. QTLs were considered significant if the corresponding LOD-score was higher than 2.5.

## Results and discussion

The RIL population segregated well for plant height and leaf chlorophyll content. The segregation for ASI was less pronounced. However, there was a negative correlation between ASI and grain yield, both under well-watered and drought stress conditions. The QTL "A" (Figure 1 and Table 1) on chromosome 1 has been detected for ASI, ear number, and grain yield in the well-watered trial, indicating that ASI is closely related to kernel set and grain yield. The QTL "F" was involved in the expression of ASI under stress and influenced ear and silk weight measured seven days after pollen shedding. These two parameters reflect how fast the ears and the silks grow during the flowering period. They were under a strong genetic control (Carcova, Andrieu et al., 2003), and good secondary traits for drought tolerance (Edmeades et al. 2000). Unsurprisingly, ASI was negatively correlated with ear and silk growth during stress.

The objective of the QTL study was not to present entire series of all the QTLs identified for individual traits, but we rather focused on target regions where multiple QTLs were detected.

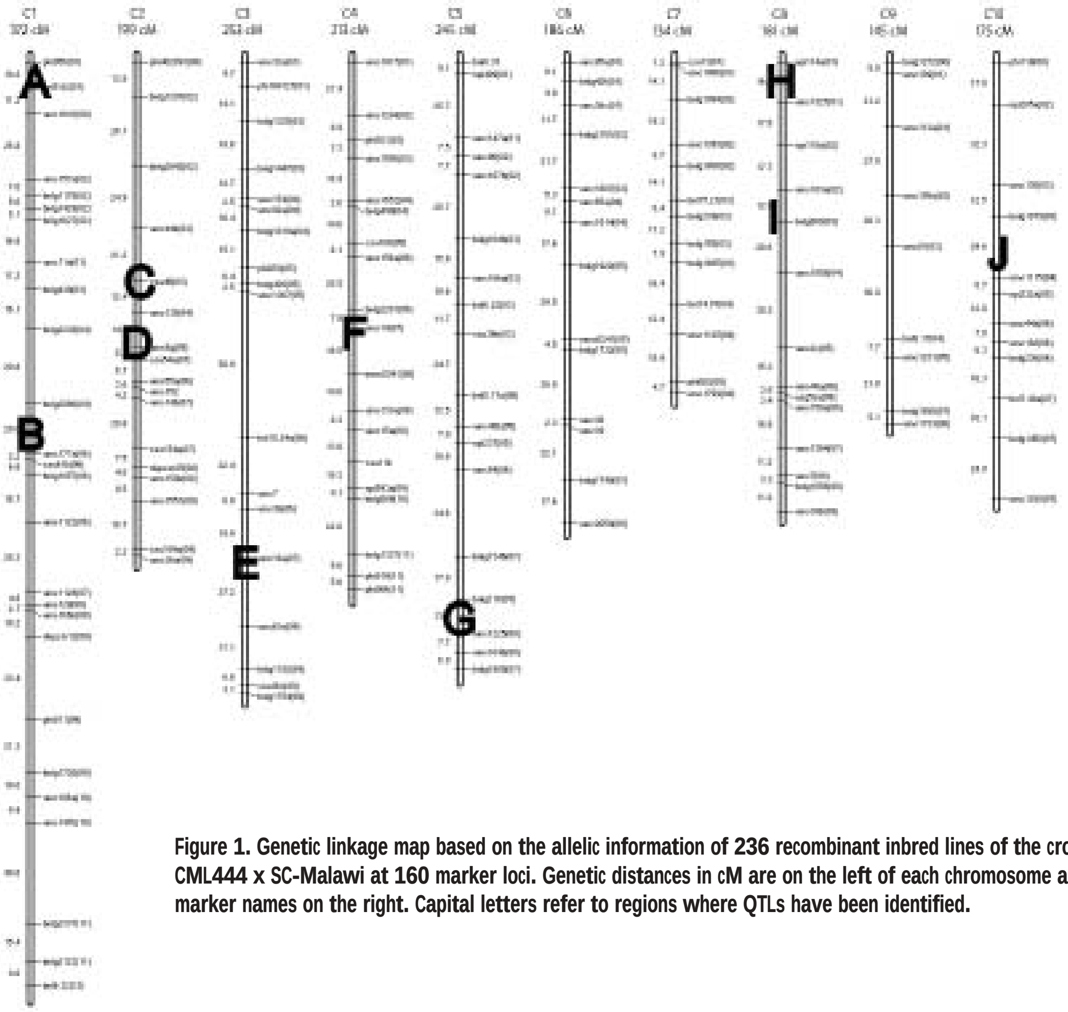

**Figure 1.** Genetic linkage map based on the allelic information of 236 recombinant inbred lines of the cross CML444 x SC-Malawi at 160 marker loci. Genetic distances in cM are on the left of each chromosome and marker names on the right. Capital letters refer to regions where QTLs have been identified.

**Table 1.** QTL results based on phenotypic data of two drought stress and one well-watered field experiment. Capital letters in the column "map", indicate the location of the QTL on the genetic map in figure 1

| map | cond | trait | chr | cM | marker | LOD | add | % | map | cond | trait | chr | cM | marker | LOD | add | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | WW | ENO | 1 | 5 | 2 | 5.2 | 0.5 | 13.2 | G | DS | KNO | 5 | 225 | 16 | 4.2 | -21.1 | 7.8 |
| A | WW | GY | 1 | 6 | 2 | 3.6 | 24.0 | 6.0 | G | DS | GY | 5 | 235 | 17 | 3.8 | -3.7 | 6.8 |
| A | WW | ASI | 1 | 7 | 2 | 2.8 | -0.4 | 6.9 | H | WW | EHT | 8 | 0 | 1 | 3.2 | -2.7 | 6.7 |
| B | WW | PHT | 1 | 131 | 11 | 12.4 | -8.0 | 18.7 | H | DS | EHT | 8 | 9 | 2 | 5.4 | -4.4 | 14.5 |
| B | DS | PHT | 1 | 137 | 11 | 9.3 | -5.8 | 20.4 | H | DS | PHT | 8 | 17 | 2 | 5.6 | -4.5 | 13.4 |
| B | DS | PHT | 1 | 138 | 11 | 12.4 | -6.6 | 22.2 | H | DS | EHT | 8 | 19 | 2 | 5.4 | -3.6 | 12.4 |
| B | WW | EHT | 1 | 142 | 11 | 8.3 | -5.2 | 19.2 | H | DS | PHT | 8 | 21 | 2 | 4 | -3.9 | 10.5 |
| B | DS | EHT | 1 | 150 | 12 | 11.1 | -4.9 | 19.7 | I | DS | EHT | 8 | 64 | 5 | 5.2 | -3.2 | 7.0 |
| B | DS | EHT | 1 | 152 | 12 | 8.1 | -4.9 | 15.5 | I | DS | EHT | 8 | 67 | 5 | 5 | -4.1 | 9.9 |
| C | DS | CHYL | 2 | 83 | 5 | 7.3 | 0.9 | 13.7 | I | WW | EHT | 8 | 73 | 5 | 4.4 | -3.9 | 6.4 |
| C | DS | CHEL | 2 | 83 | 5 | 6.6 | 0.8 | 12.9 | J | DS | CHYL | 10 | 60 | 4 | 3.3 | 0.6 | 5.4 |
| D | WW | CHYL | 2 | 113 | 7 | 7.2 | 1.3 | 12.6 | J | DS | EHT | 10 | 69 | 4 | 3.7 | -3.6 | 6.0 |
| D | WW | CHEL | 2 | 115 | 7 | 2.9 | 0.7 | 5.7 | J | DS | CHEL | 10 | 74 | 5 | 8.4 | 0.8 | 11.8 |
| E | DS | EHT | 3 | 199 | 14 | 4.2 | 3.0 | 4.0 | J | WW | EHT | 10 | 74 | 5 | 2.3 | -2.9 | 1.4 |
| E | DS | EHT | 3 | 200 | 14 | 3.3 | 3.1 | 4.9 | J | DS | PHT | 10 | 75 | 5 | 4 | -4.2 | 4.3 |
| E | WW | EHT | 3 | 200 | 14 | 3.7 | 3.3 | 3.5 | J | WW | PHT | 10 | 76 | 5 | 4.7 | -5.2 | 5.0 |
| F | DS | EW7D | 4 | 105 | 10 | 3.1 | 0.1 | 4.9 | J | WW | LODG | 10 | 77 | 5 | 9.7 | -0.7 | 16.2 |
| F | DS | SW7D | 4 | 105 | 10 | 2.6 | 0.0 | 3.3 | J | DS | CHYL | 10 | 78 | 5 | 7.1 | 0.5 | 16.1 |
| F | DS | ASI | 4 | 108 | 10 | 3 | -0.6 | 5.3 | | | | | | | | | |

**Abbreviations**

| | |
|---|---|
| ASI | anthesis-silking interval |
| CHEL | chlorophyll content ear leaf |
| CHYL | chlorophyll content youngest leaf |
| DS | drought stress |
| EHT | ear height |
| ENO | ear number |
| EW7D | ear weight 7 days after pollen shedding |
| GY | grain yield |

There was a QTL at the end of chromosome 5 (“G”) for grain yield and kernel number under stress conditions, which could reflect a genetic response to drought, since this QTL was not detected under control conditions.

Plant height and ear height were highly correlated in all environments. Ear position, however, did not depend on plant height. There was a small positive correlation between plant height and grain yield under well-watered conditions. Under drought stress, however, these two traits were not correlated, and there was also no correlation between plant height and ASI. QTLs for plant and ear height (“B”, “E”, “H”, “I”, “J”) showed high LOD values and were consistently detected across environments.

Chlorophyll content of the youngest leaf and the ear leaf were negatively correlated with ASI, and positively correlated with grain yield both, under stress and non-stress growing conditions. Sanchez et al. (2002) and Xu et al. (2000) reported that leaf chlorophyll content in sorghum was significantly correlated to the stay-green trait, which improves resistance to premature senescence under soil moisture stress during grain filling. Three main QTLs involved in the expression of the leaf chlorophyll content have been identified: QTL “C” on chromosome 2 under drought stress and QTL “D” under well-watered conditions, reflecting a shift in gene expression under drought stress. The third QTL for leaf chlorophyll content was present on chromosome 10 (“J”) and was also involved in the expression of other traits like plant height, ear height, progress of senescence and lodging, which is in accordance to Sanchez et al. (2002).

## Outlook

There will be more field evaluations under well-watered conditions and drought stress in Mexico as well as in Zimbabwe in order to improve the physiological understandings of drought tolerance and to enhance the stability of the QTL results. Additionally, seedlings of the RILs are currently being analyzed under high temperature conditions in the growth chamber, since drought and heat often occur together. The gene expression analysis of the two parental lines, which will be done in a second phase of the project, will hopefully allow to better understand the genetic responses to drought, particularly in this tropical maize population.

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# Mapping QTLs associated with root traits related to drought resistance in Vietnamese upland rice

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## Introduction

In upland rice, root thickness, deep rooting, and root/shoot ratio are considered as the most important root traits for drought resistance (Yoshida and Hasegawa, 1982). Mapping genes controlling root morphology and root distribution in upland rice have been reported in a doubled-haploid population derived from an *indica* x *japonica* cross using RFLP markers (Yadav et al., 1997). QTLs for root growth and root penetration ability in upland rice were also mapped in a F2 population derived from a cross between drought resistant varieties Bala and Azucena using RFLP and AFLP markers (Price and Tomos, 1997; Price et al., 2000). In this report, we present results on mapping QTLs for root traits related to drought resistance in upland rice using a RI population derived from a cross between Vietnamese upland rice accessions (one of which belong to the *japonica* subspecies, the other is *indica*), and SSR and AFLP markers.

## Materials and methods

### *Materials*

A population of 135 RI lines derived from F6 progenies of a cross between two upland rices, one belonging to the *japonica* subspecies RDB09 and the other belonging to *indica* subspecies R2021, was used in this study. Both parents of the cross are Vietnamese local accessions.

### *Evaluation of root traits*

Maximum root length (MRL), root thickness (RT), root numbers (RN), root weight to shoot ratio (R/SR), and deep root weight to shoot ratio (DR/SR) were analyzed at the Institute of Biotechnology, Hanoi. The upland rices were grown in plastic tubes (one plant/tube), 16 cm in diameter and 1 m in height, with a hole for drainage. The RI lines were evaluated in a completely randomized design, with three replication of one plant per line. The sampling was taken at 45 days after sowing. In addition, the measurement of shoot traits like tiller number and plant height were also taken for investigating the relationship between root and shoot parameters.

### *DNA isolation*

Genomic DNA was isolated from 0.5 - 1g of leaf of the upland rice lines using the CTAB method described by Saghai Maroof et al. (1994) with minor modification.

### *SSR analysis*

SSR analysis was follow using the procedure of Panaud et al. (1996).

### *AFLP analysis*

AFLP analysis was conducted at the Plant Cell Genetics Laboratory, Institute of Biotechnology (IBT) according to Vos et al. (1995).

### *Map construction*

The linked map was constructed using Mapmaker 3.0 (Lander et al., 1987; Lincoln et al., 1990) with LOD score of 3.0 and 8.0, and recombination fraction of 0.4. The QTL map was constructed using Mapmaker/QTL 1.1 Program (Lincoln et al., 1992).

### *Statistical and QTL analysis*

Analyses of variance were performed to determine the genetic variation between lines of the RI population for all investigated traits using SAS program (SAS Institute, North California).

## Results

The present map consists of 239 markers (36 SSR and 203 AFLP markers) mapped to all 12 chromosomes. This map covers 3973.1 CM of the rice genome. The average distance between markers is 16.62 CM (Fig. 1). Of 203 AFLP markers, 36 were mapped on chromosome 1, 12 on chromosome 2, 23 on chromosome 3, 9 on chromosome 4, 15 on chromosome 5, 23 on chromosome 6, 2 on chromosome 7, 33 on chromosome 8, 10 on chromosome 9, 13 on chromosome 10, 5 on chromosome 11, and 22 on chromosome 12. The QTL analysis was done for five root traits and two shoot traits using Mapmaker / QTL (version 1.1) software. A total 16 QTLs for root and 7 QTLs for shoot traits were identified (Table 1).

Comparison of QTLs for maximum root length and deep root per shoot ratio detected in RDB09 x R2021 and IR64 x Azucena (Yadav et al., 1997) revealed the following:

- Three common regions for MRL were identified on chromosomes 3, 9, and 12.
- The common regions on chromosome 3 were flanked by CDO87 marker (in IR64 x Azucena population), and AVM56.2-AVM8 (in RDB09xR2021 population), and located below marker RM16.
- On chromosome 9, the common QTL regions for MRL were located near RZ12 marker (in IR64 x Azucena population ), and AVM62.5 (in RDB09xR2021), flanked by RM242 and RM288 markers.

**Table 1. QTLs for root and shoot traits as identified by interval mapping (MapMarker / QTL)**

| Trait | Interval | Chrom # | Length[a] | Position[b] | Variance (%)[c] | Additive effect | LOD[e] |
|---|---|---|---|---|---|---|---|
| *Plant height* | AVM26.9-AVM26.4 | 1 | 25.4 | 25.0 | 9.7 | 11.423 | 2.12 |
| | AVM9.18-AVM3.7 | 1 | 10.7 | 5.0 | 19.2 | 11.025 | 5.02 |
| Tiller number | AVM84.19-AVM65.3 | 3 | 12.0 | 5.0 | 11.3 | 1.0119 | 2.85 |
| | AVM10.26-AVM22.6 | 4 | 30.0 | 25.0 | 23.3 | -1.8511 | 3.43 |
| | AVM87.13-RM131 | 4 | 56.5 | 10.0 | 14.8 | -1.3587 | 2.29 |
| | RM50-AVM29.2 | 6 | 25.2 | 10.0 | 34.7 | -2.5765 | 3.28 |
| | AVM3.5-AVM86.16 | 12 | 15.7 | 0.0 | 9.8 | 0.9658 | 3.01 |
| Root thickness | AVM65.11-AVM10.26 | 4 | 25.6 | 15.0 | 13.5 | 0.0702 | 2.28 |
| | AVM75.2-AVM87.13 | 4 | 24.5 | 15.0 | 14.8 | 0.0738 | 2.76 |
| Root number | AVM22.7-AVM8.12 | 1 | 87.2 | 5.0 | 9.6 | 10.966 | 2.26 |
| | AVM84.19-AVM65.3 | 3 | 12.0 | 5.0 | 13.0 | 11.709 | 3.29 |
| Maximum root length | AVM43.1-RM250 | 2 | 32.1 | 30.0 | 8.5 | -4.2222 | 2.20 |
| | AVM56.2-AVM8.6 | 3 | 13.8 | 5.0 | 9.1 | 4.4586 | 2.28 |
| | AVM62.5-AVM77.7 | 9 | 30.2 | 0.0 | 12.1 | -5.0608 | 3.72 |
| | RM270-AVM28.17 | 12 | 64.5 | 0.0 | 7.2 | 3.7784 | 2.17 |
| Root weight to shoot ratio | AVM8.1-RM263 | 2 | 14.1 | 10.0 | 13.4 | -2.3027 | 3.24 |
| | AVM42.10-AVM43.18 | 8 | 21.8 | 10.0 | 18.0 | 3.2824 | 2.78 |
| | AVM12.3-AVM7.4 | 9 | 14.9 | 0.0 | 10.5 | -2.0362 | 3.17 |
| | RM242-RM288 | 9 | 3.16 | 0.0 | 10.2 | -2.0394 | 3.12 |
| Deep root weight to shoot ratio | AVM38.9-RM221 | 2 | 7.4 | 5.0 | 16.1 | -0.4871 | 4.17 |
| | AVM12.3-AVM7.4 | 9 | 14.9 | 0.0 | 8.9 | -0.3622 | 2.67 |
| | AVM62.5-AVM77.7 | 9 | 30.2 | 0.0 | 11.3 | -0.4192 | 3.44 |
| | AVM65.9-RM242 | 9 | 29.5 | 25.0 | 12.5 | -0.4331 | 3.13 |

[a] **Interval between the two flanking markers (cM) where QTL is located**

[b] QTL position from the first marker (cM)

[c] Phenotypic variation explained by each QTL (%)

[d] Additive genetic effect and the negative sign meant that P2 allele reduced the trait

[e] Maximum likelihood LOD score for the individual QTL

- On chromosome 12, the regions between RG341 and RG958 (in IR64 x Azucena population ) and near RM270 (in RDB09xR2021) were common QTL regions for MRL.
- For DR/SR, there is one common QTL region on chromosome 2, flanked by PALI-RZ58 and AVM38.9-RM221 markers, respectively. (Table 2, Figure 1).
- The percentage of phenotypic variance explained by the common QTLs was rather high (12.1 to 16.1). Thus, these common QTLs observed in different genetic background and ecological locations could be very useful for precisely locating drought resistant gene(s) and for marker-assisted selection.

**Table 2. Common QTLs in RDB09 x R2021 and IR64 x Azucena**

| | | Chromosome Location of common QTL | | | | |
|---|---|---|---|---|---|---|
| Trait | Common QTL | Chr. | RDB09 x R2021 | % Var. | IR64 x Azucena | % Var. |
| MRL | 3 | 3 | AVM56.2-AVM8.6 | 9.1 | PGI-1-CDO87 | ? |
| | | 9 | AVM62.5-AVM77.7 | 12.1 | RZ12-RG667 | 9.0 |
| | | 12 | RM270-AVM28.17 | 7.2 | RG958-RG341 | ? |
| DR/SR | 1 | 2 | AVM38.9-RM221 | 16.1 | PALI-RZ58 | ? |

## Conclusion

The map consists of 239 markers (36 SSR and 203 AFLP markers) mapped to all 12 chromosomes. This map covers 3973.1 CM of the rice genome. The average distance between markers is 16.62 CM. A total 16 QTLs for root and 7 QTLs for shoot traits were identified. Three common regions for MRL on chromosomes 3, 9, and 12; and one common QTL region for DR/SR ratio on chromosome 2 were found in RDB09xR2021 and IR64 x Azucena populations. In our map, there are several root QTLs flanked by SSR markers such as RM250, RM270 (for MRL), RM263, RM242, RM288 (R/SR), and RM221, RM242 (for DR/RS). These SSR makers could be useful for marker-assisted drought tolerance selection.

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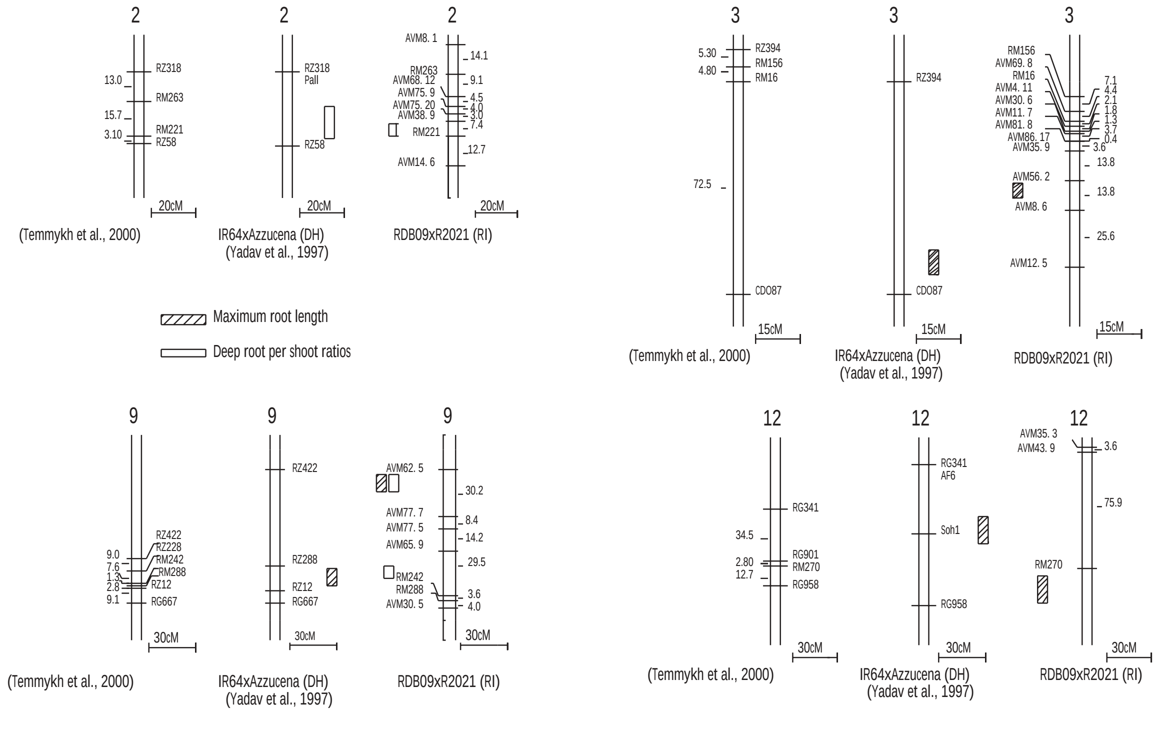

▨ Maximum root length

▭ Deep root per shoot ratios

**Figure 1. Comparison of common QTL for maximum root length and deep root to root ratio with IR64xAzuzucena DH population.**

# Integrating molecular approaches in breeding for drought tolerance of maize in India

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Drought is a major factor limiting the production and productivity of maize in India, where nearly 70% of the maize is grown under rainfed conditions. Only a limited number of inbred lines and cultivars with drought stress tolerance have been identified or developed so far in India through the conventional breeding approaches. Increasing emphasis is, therefore, being laid by the NARS on integrating various technological options for enhancing the resilience of maize to drought stress.

Genetic and molecular dissection of a complex trait like drought tolerance continues to be a highly challenging endeavor. International research efforts during the last two decades led to the identification of QTLs for various traits associated with drought tolerance at the flowering stage in maize. Under the Asian Maize Biotechnology Network (AMBIONET) program in India, we are making intensive efforts to optimize drought phenotypic assays, identify tolerant lines among indigenous and exotic maize germplasm, and map QTLs conferring drought stress tolerance (Prasanna and Hoisington, 2003).

We have evaluated more than 100 inbred lines (from India and CIMMYT) at two locations (Hyderabad and Karimnagar) during the Rabi (winter / dry season) under controlled irrigation regimes during experiments carried out over four years (2000-2003). The materials included 28 CM lines (from the Indian maize program), 60 inbreds developed at Maize Breeding Unit, IARI, and 28 CIMMYT lines. The entries were categorized into two maturity groups: early and full season. The characters recorded were ASI, leaf senescence, ears per plant, ear length, kernel number per ear, 100-kernel weight and grain yield, based on the guidelines provided by Bänziger et al. (2000). The study led to the identification of six promising inbred lines, including three lines from CIMMYT (derived from Pool 26 Sequia) and three Indian maize inbred lines, including CM140 (male parent of a popular hybrid ‘Parkash’). Mapping populations are being developed using these drought tolerant lines in combination with selected elite, drought susceptible Indian maize inbred lines.

The AMBIONET-India team has also initiated experiments on mapping QTL for drought stress tolerance of maize. For this purpose, a mapping population comprising 206 recombinant inbred lines (RILs), derived from a cross between Ac7643 (drought tolerant) and Ac7729 / TZSRW (drought susceptible), were evaluated under controlled irrigation regimes (well-watered and water stress, commencing two weeks before flowering until completion of flowering) at the Maize Research Station, Karimnagar during Rabi (winter / dry season 2002-03). The test entries were planted in an alpha lattice design with two replications per treatment (WW and stress). Each field plot included 15 plants grown in a single 3 m row, with 0.75 m between rows. Observations were recorded on 13 traits, including male flowering (MFLW), female flowering (FFLW), anthesis-silking interval (ASI), plant height (PH), ear position (EPO), ear number per plant (ENO), grain yield (GY), HKW (100-kernel weight), ear length (EL), ear diameter (ED), kernel number per ear (KNO), kernel rows per ear (KR), and kernel number per row (KNR).

QTL analysis was conducted by combining the phenotypic dataset with the genotypic dataset for the RILs available at CIMMYT, Mexico. Composite interval mapping (CIM) (Zeng, 1994) was used to map the QTL and estimate their genetic effects using a series of four models. Model 1 (simple interval mapping) was used for the selection of cofactors; Model 2 (CIM with unlinked markers as cofactors) to maximize QTL detection; and Models 3 and 4 (CIM with selected markers as cofactors). Model 3 was with a minimum map distance of 30 cM (window size = 30 cM) and Model 4 with a window size = 20 cM. The threshold used for QTL detection set at a likelihood ratio (LR) value greater than the threshold values for LOD of 2.5 or 3.0. Multiple regression analysis was performed to estimate the proportion of phenotypic variation due to the additive effects (R2).

QTL analysis revealed a number of genomic regions that influenced yield and yield components under well-watered (WW) and drought stress conditions. A total of 33 and 49 putative QTLs were identified for a total of 13 different traits analyzed under WW and drought stress conditions in the field, respectively. The majority of the QTLs expressed 2-11% of the phenotypic variance and the total phenotypic variance expressed by combining all of the significant QTLs ranged from 4.53-24.62% in case of WW conditions, and 3.89-37.14% under stress conditions. Most of the QTLs for various yield related characters under WW conditions were found on chromosomes 2, 4, and 10, and a few on chromosomes 1, 5, and 9. Most of the QTLs related to the drought tolerant traits were identified on chromosomes 1, 2, 3, 5, 10, and a few on chromosomes 4, 7, 8, and 9 under stress conditions.

The recent focus of CIMMYT with respect to molecular breeding for drought stress tolerance in maize has been on “universal drought genomic regions” identified on a maize consensus map (Ribaut et al., 2004). The first important step towards this goal is construction of a unique linkage map using a set of ‘anchor markers’ common to different segregating populations, different environments, or different target traits (same cross and / or different crosses or environments). The strategy has led to the detection of some key chromosomal regions associated with drought tolerance in maize. The QTL information being generated by the AMBIONET-India team shall contribute towards this important goal. In this regard, analysis based on the Karimnagar experiment confirmed the significance of the QTL on chromosomes 2 and 10 (for ASI), chromosome 2 (for ENO), and chromosomes 2 and 10 (for KNO) under WW conditions, and the QTL on chromosome 8 (for FFLW), chromosome 10 (for ASI), chromosomes 5 and 10 (ENO), chromosomes 2 and 5 (for GY), chromosome 2 (for HKW), and chromosome 10 (for KNO).

We are also currently phenotyping two mapping populations (RILs and F3s derived from the cross between Ac7643 [drought tolerant] and Ac7729 [drought susceptible]) in a field experiment at Hyderabad in India (during winter / dry season 2003-04). QTL analysis of this dataset, coupled with the dataset and information from Karimnagar (2002-03) shall further aid in ascertaining the significance and stability of the QTL for drought tolerance across environments.

An integrated strategy of QTL mapping, MAS and functional genomics (Ribaut et al., 2002) shall provide useful information and tools to effectively complement conventional selection for drought tolerance in maize. Accurate and large-scale field phenotyping of germplasm, including mapping populations, for drought stress, is currently the most important limiting factor in many countries, including India. Deployment of an integrated strategy for drought selection would not only warrant development of reliable phenotypic assays for drought but also effective utilization of the emerging knowledge from physiology, breeding and molecular genetics. Synergistic partnerships between various national and international institutions will be vital for enhancing drought tolerance in different crop plants, including maize.

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# Quantitative trait loci identification under drought conditions in maize

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## Introduction

A lot of effort has been given to QTL location of drought tolerance in maize. Several hundred loci throughout the genome were identified as drought tolerance QTLs, with different criteria and mapping populations under different conditions. In this study, based on a practical evaluation system for drought tolerance we developed two years ago, we attempted to use a mapping population between a highly tolerant and a highly sensitive inbred line to locate several criteria that were identified to be significantly correlated to drought tolerant coefficient of kernel yield.

## Materials and methods

An F2 mapping population was made between inbred line N87-1 (highly tolerant) and 9526 (highly sensitive) and F2:3 families from F2 individuals were reproduced. SSR primers that had codominant markers between the two parental inbred lines were screened and used to detect DNA polymorphism among the F2 individuals. The F2:3 families were phenotyped with random block design under drought conditions at three ecological environments. Anthesis-silking interval (ASI), root number, root biomass, and kernel yield were investigated for each family. An SSR linkage map was constructed and QTLs of the criteria were located through composite interval mapping (LOD>2.5). All the analysis was conducted using MAPMAKER/EXP3.0 and Windows QTL Cartographer (2.0).

## Results and analysis

One hundred and three (103) pairs of primers were found codominant between the two parental inbred lines and used to detect polymorphism among 183 F2 individuals of the mapping population. An SSR linkage map was constructed with average distance of 16.27 cM (Figure 1) and span of 1512.9 cM. The biggest gap between bnlg666 and umc1141 on chromosome 8 was 34.4 cM. The QTLs and their gene effect are shown in table 1. Three QTLs for ASI were located between bnlg1564 and bnlg1811 on chromosome 1 (27.0%, over dominance), between bnlg1879 and mmc0282 on chromosome 5 (10.1%, additive), and between bnlg1305 and bnlg1792 on chromosome 7 (5.3%, over dominance). These three QTLs made up 42.4% of total phenotypic variation.

Five QTLs for root number were found near bnlg1347 (6.9%, partial dominant) on chromosome 1, umc1294 on chromosome 4 (39.2%, over dominant), near bnlg2162 on chromosome 4 (19.3%, additive), near bnlg1885 on chromosome 5 (6.0%, additive), and near umc1037 on chromosome 9 (7.7%, over dominant). These five QTLs made up 79.1% of total phenotypic variation. Three QTLs for root biomass were located near bnlg1055 on chromosome 1 (10.5%, over dominance), near bnlg1126 on chromosome 4 (26.2%, over dominant), and near bnlg1051 on chromosome 4 (16.6%, partial dominance). These three QTLs made up 53.3% of total phenotypic variation.

Three QTLs for kernel yield were located near umc1117 on chromosome 4 (9.4%, partial dominance), near bnlg1863 on chromosome 8 (7.3%, additive), and near bnlg1714 on chromosome 8 (10.9%, over dominance). These three QTLs made up 27.6% of total phenotypic variation.

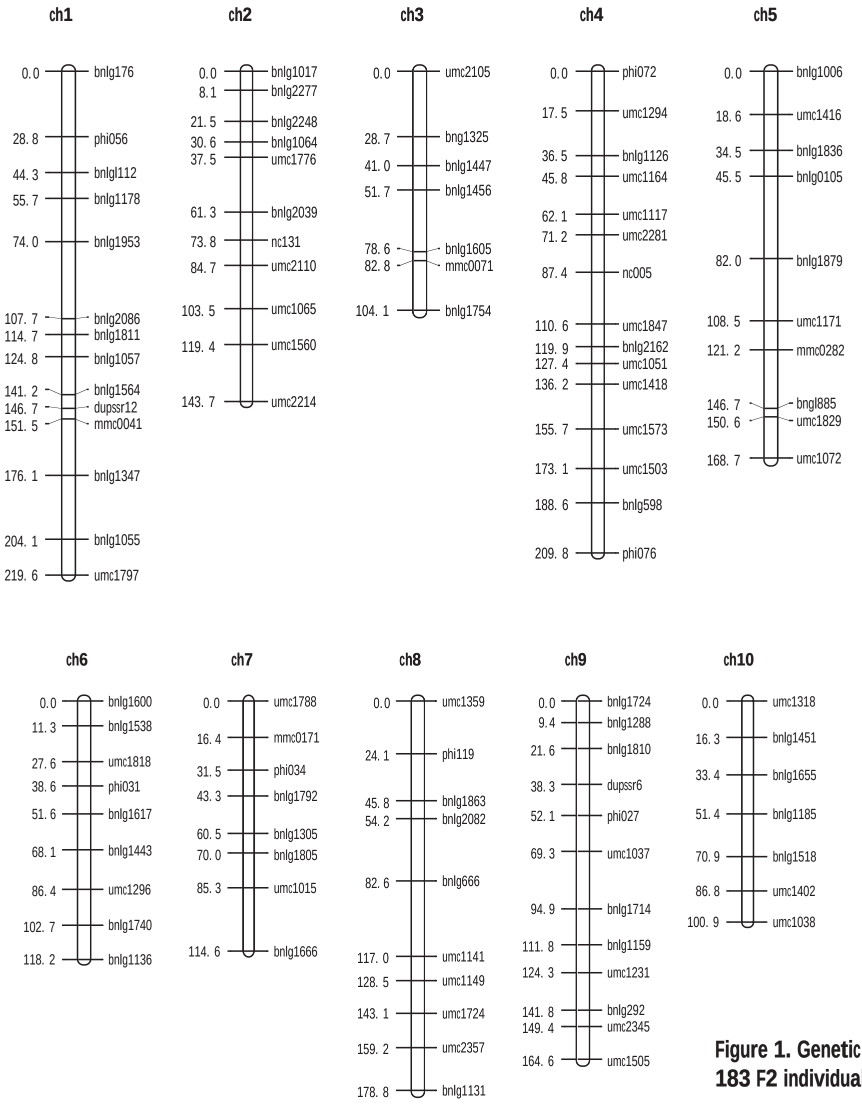

**Figure 1. Genetic linkage map of 103 pairs of SSR primers among 183 F2 individual plants.**

**Table 1. QTLs of ASI, root number, root biomass and kernel yield under water-stressed conditons**

| Trait | Chromosome | Nearestmarker | Position (cM) | Additive | Dominant | Gene action | Contribution percentage to Phenotypic Variance |
|---|---|---|---|---|---|---|---|
| ASI | 1 | bnlg1564 | 108.8 | 3.9 | -7.8 | OD | 27.0 |
| | 5 | bnlg1879 | 87.3 | -4.0 | -0.8 | A | 10.1 |
| | 7 | bnlg1305 | 68.2 | 1.06 | -1.69 | OD | 5.3 |
| Root number | 1 | bnlg1347 | 176.1 | 3 | 1.5 | PD | 6.9 |
| | 4 | umc1294 | 47.5 | 4.1 | -8.5 | OD | 39.2 |
| | 4 | bnlg2162 | 135.9 | -4.2 | -0.6 | A | 19.3 |
| | 5 | bnlg1885 | 146.7 | -2.1 | -3.1 | A | 6.0 |
| | 9 | umc1037 | 75.31 | 2.1 | 4.2 | OD | 7.7 |
| Root biomass | 1 | bnlg1055 | 214.6 | 0.32 | 1.08 | OD | 10.5 |
| | 4 | bnlg1126 | 49.5 | 1.15 | -1.81 | OD | 26.2 |
| | 4 | umc1051 | 137.9 | 2.12 | 1.4 | PD | 16.6 |
| Kernel yield | 4 | umc1117 | 97.9 | 6.3 | 4.6 | PD | 9.4 |
| | 8 | bnlg1863 | 45.8 | 8.5 | 1.6 | A | 7.3 |
| | 8 | bnlg1714 | 96.6 | 4.8 | 13.4 | OD | 10.9 |

*A: additive (dominant degree=0-0.2), PD: partial dominant (dominant degree=0.21-0.80), D: dominant (dominant degree=0.81-1.20), OD: over-dominant (dominant degree>1.20)

# Quantitative trait loci (QTL) associated with drought tolerance at the reproductive stage in rice

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## Introduction

Drought is a major constraint to rice (*Oryza sativa* L.) yield and its stability in rainfed and poorly irrigated environments. More than 40% of the areas in north and northeast Thailand are drought-prone. The increasing shortage of water in irrigated areas means that drought is also a problem for irrigated rice (Pandey 2002). The effect of drought in rainfed lowland ecosystems is multidimensional. It can result in loss in output, due to yield reduction (up to the approximately 50%), and / or a reduction in area planted or harvested. The yield loss is often used as a measure of the cost of drought and is a part of its economic cost. The production failure by drought will not only result in hunger or worse for poor farmers, but also has many consequences for the community. Studies in India by IRRI (Pandey 2002) showed that there were additional economic costs of drought such as changes in farmers' assets. Farmers often sell productive assets such as farm implements, bullocks, and even land when drought leads to failed harvests; this also frequently occurs in Thailand. The year after drought, farmers, who are continue to farm, often have incurred significant debt. Identifying genomic regions influencing the response of primary traits—yield and its components—and secondary traits, such as root mass density (RMD), canopy temperature (CT), osmotic adjustment (OA), leaf water potential (LWP), and drought score (DS) to water deficits, will aid in our understanding of the genetics of drought tolerance and development of more drought tolerant cultivars (Zhang et al., 2001).

## Methods

Quantitative trait loci (QTL) for yield and its components and secondary traits such as root mass density (RMD), canopy temperature (CT), osmotic adjustment (OA), leaf water potential (LWP), and drought score (DS) to water deficits were studied using a subset of 154 doubled haploid lines derived from a cross between two rice cultivars, CT9993-510-1-M and IR62266-42-6-2. Drought stress treatments were managed by use of a "line source sprinkler" irrigation system, that provided a linearly decreasing level of irrigation coinciding with the sensitive reproductive growth stages (Hanks et al., 1976). The research was conducted at the Ubon Rice Research Center, Ubon, Thailand in 2000-2002.

## Results

A total of 77 QTLs were identified for primary traits under varying levels of water stress. Of the total of 77 QTLs, the number of QTL per trait were 7-grain yield (GY); 8-biological yield (BY); 6-harvest index (HI); 5-days to flowering after initiation of irrigation gradient (DFAIG); 10-total spikelet number (TSN); 7-percent spikelet sterility (PSS); 23-panicle number (PN); and 11-plant height (PH). Genomic regions associated with secondary traits were mainly located on chromosomes 1, 2, 3, 4, 5, 8, 9, and 11 (Figure 1). The phenotypic variation explained by individual QTLs ranged from 6.9 to 55.7%. Under well-watered conditions, we observed a high genetic association for BY, HI, DFAIG, PSS, TSN, PH, and GY. However, only BY and HI were found to be significantly associated with GY under drought treatments. QTL flanked by markers RG104 – RM231, EMP2_2 – RM127 and G2132 - RZ598 on chromosomes 3, 4, and 8 were associated with GY, HI, DFAIG, BY, PSS, and PN under drought treatments and also coincided with QTLs for root thickness, root penetration index, OA, and LWP.

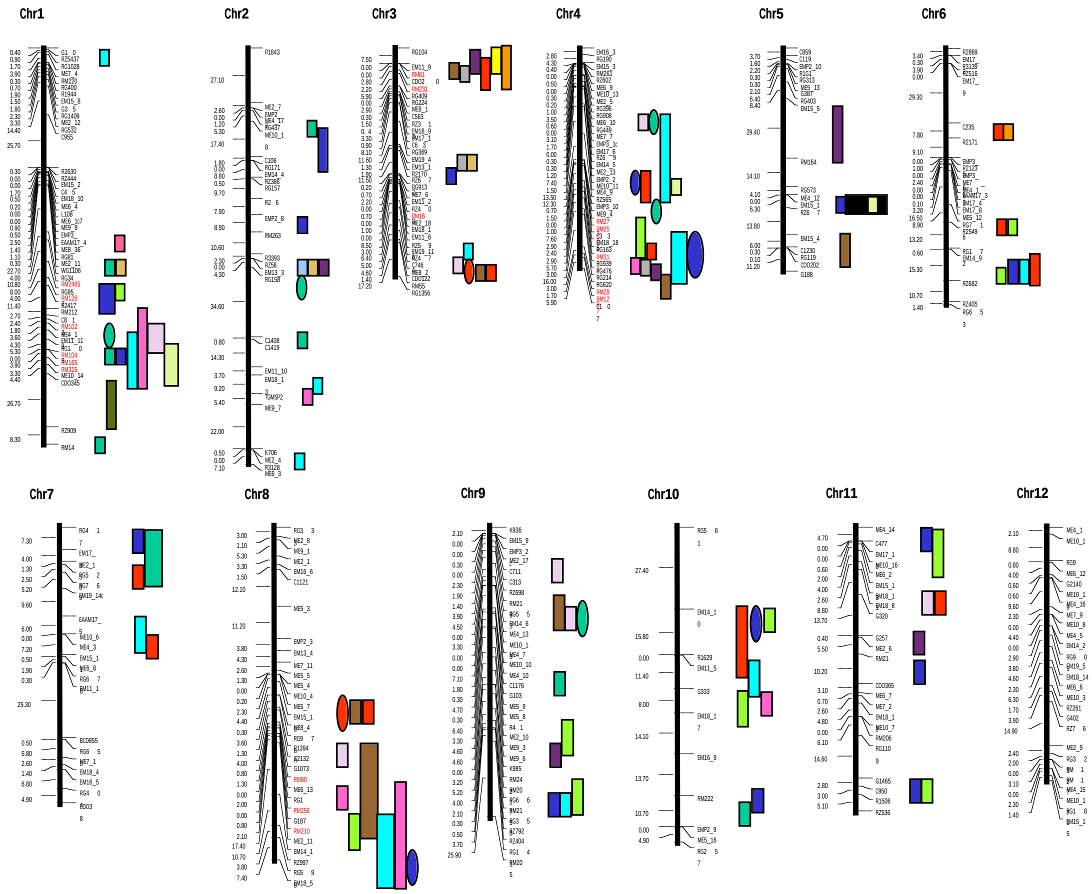

**Figure 1. Chromosomal locations of QTL for yield and yield components and secondary traits identified in the CT9993 x IR62266 population under 'line source sprinkler' irrigation system in Thailand between 1996-2002.**

## Conclusions

The number of QTLs detected for a drought tolerance trait generally varied between 1 and 5. Comparisons of multiple-season and multiple-site field drought screens in the DHL population have identified several genomic regions (on chromosomes 1, 3, 4, 8, and 9) of potential value for the improvement of drought tolerance in rice. The aggregate effects of identified QTL resulted in higher grain yield. Repulsion linkages between the QTL for root traits or adaptive traits and the QTL for yield and yield components were observed. Although most of the QTLs associated with yield potential was linked in repulsion phase with the QTLs associated with root traits or adaptive traits, transgressive segregants were observed in the DHL population. This indicates that these traits can be combined into one genotype. QTL mapping provides a powerful tool for conducting physiological and genetic research to understand drought tolerance.

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# Progress in developing drought tolerant rice cultivars for eastern India

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Rainfed lowland rice is grown mainly in South and Southeast Asia, without access to irrigation water. The area occupied by this ecosystem is ~46 M ha (Huke and Huke, 1997). This area accounts for about 39% of the total cultivated area for rice, and supports about 700 million people (Wade, 2002). Throughout the rainfed rice ecosystem, the amount and timing of water supply are considered to be the most severe constraints to productivity (Widawsky and O'Toole, 1990; Zeigler and Puckridge, 1995), with more than 50% of the rainfed lowland being drought prone. A perusal of last 15 years of meteorological data reveals that rice crop at Raipur and adjoining areas experiences water stress at terminal stages, and drought at this stage is believed to be more detrimental (Sarkarung and Pantuwan, 1999). Because of the rapid cessation of rains in this region, IGAU, Raipur is a key site for the screening and evaluation of breeding materials for adoption to terminal stage drought conditions. Therefore, at IGAU, Raipur, developing drought tolerant rice cultivars has been one of the major objectives of rice breeders. Conventional breeding efforts during the last 15 years have resulted in development of several cultivars such as Kranti, Mahamaya, Poornima, etc., that exhibit good drought tolerance, however, progress has been slow. Recently, a more targeted approach has been followed, by integrating the conventional breeding, molecular techniques, and improved field screening protocol, to increase the efficiency of selection under well-defined water stress. The major activities under The Rockefeller Foundation funded project on drought follows.

## Identification of suitable donors from local germplasm

The exploitation of a narrow genetic base in breeding program has resulted in reduced gain in improvement. The Indira Gandhi Agricultural University, Raipur has a large collection of germplasm (~21,000 accession) from rainfed lowland agroecosystems of southeastern and eastern India, which frequently experience drought. An effort was undertaken to explore new donors exhibiting stable performance under water stress with good yield potential, having different mechanisms for drought resistance that can bring potential new genes / QTLs into the breeding pool. A set of 75 lines was tested in wet and dry seasons for two consecutive years under water stress and irrigated conditions. Simultaneously, these lines were evaluated in pipe for root traits. Genotypes having specific traits are being identified and are presented in table 1. These lines are being used in the crossing program.

## Development of new breeding and mapping populations

Various breeding and mapping populations with different objectives have been prepared: (i) to transfer drought tolerance into agronomically adopted and extensively grown cultivars, crosses were made using these lines as one parent with known donors for drought tolerance. These populations are also being used for the genetic analysis; (ii) use of local germplasm. We identified a number of lines from the local germplasm and are using these lines for the development of segregating populations; (iii) transfer of QTLs. For this purpose, a population involving CT9993, IR62266, and

**Table 1. Genotypes with specific traits**

| Trait | Genotype |
|---|---|
| Deep root | Bakal, Safri-17, Dagad Deshi |
| High RWC (under stress) | Dagad Deshi, Luchai, Cross 116 |
| Photosensitive | Safri-17 |
| Good grain quality | Laloo-14 |
| Early Maturing | Poornima |

selected DH lines has been used for the development of our own breeding populations for MAS based transfer of QTLs; and (iv) combining resistance of two known drought tolerant cultivars. Crosses were made to develop the transgressive sergeants for drought tolerance. At present, more than 40 populations are at different stages, and advanced in different ways.

## Standardization of the field screening protocol and screening of the segregating population under defined stress conditions

As we have already developed a number of breeding populations, these populations are being screened under terminal drought. The field screening protocol has been standardized to some extent to increase the heritability of drought related traits and repeatability of the results. The sowing and transplanting are being delayed by 15 days to increase the probability of exposing test material for terminal drought. Physical, biological, and statistical tools are being used to overcome the problem of within field heterogeneity. The field screening is mainly dependent on cessation of rain. The wet season 2000 and 2002 were drought years. In these two years, we managed the water stress such that it resulted in reduction of yield to the extent of ~50% compared to irrigated control. However, the performance of the top 25 entries in 2000 and 2002 was not good in wet season 2003 (no water stress), except Mahamaya, Safri, Kranti, and IR42253. From the segregating populations, a number of lines were also selected, which will be tested again in the subsequent years.

## Development of genotypic data of Safri X Kranti RIL population using SSR markers

Using SSR markers, we started developing genotypic data of one RIL population (Safri-17 X Kranti). This population is in the $F_8$ generation. The genotypic data will be used for the identification of QTLs for drought tolerance, and particularly for the identification of candidate genes for various root traits, as this population exhibits segregation for these traits.

## Identification of stable QTLs

A double haploid mapping population of a cross between CT 9993-5-10-1-M and IR 62266-42-6-2 was evaluated in the wet season 2000/01 under water stress as well as irrigated conditions. Water stress was imposed at flowering. The observations were recorded for phenological and morphological traits. MAPMAKER/QTL 1.1 was used for interval mapping and to estimate the percentage of total phenotypic variation (Lander et al., 1987). A total of 146 QTLs were detected for 15 quantitative traits. These 146 QTLs were mapped between 109 marker intervals on 11 rice chromosomes. The detected QTLs explained a minimum of 7% to a maximum of 39.6% phenotypic variance individually. Different numbers of QTLs were detected under different environmental conditions, signifying the QTL x E interaction. Of 146 QTLs detected, 98.6% were classified as minor QTLs. The QTLs for traits that exhibited phenotypic correlations tended to be mapped at the same location. Spikelet fertility, harvest index, and biological yield exhibited significant positive correlation with yield under stress. None of the stable QTLs was identified for yield per se under different sets of conditions. Some of the QTLs identified in this study for various traits match with the report of Li (1999) and Redona and Mackill (1999). For MAS to be effective, only a limited number of QTLs with large effects should be identified. The breeding population involving CT9993, IR62266 and selected DH lines has already been used for the development of our own breeding populations for MAS.

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# Identification of QTLs for flowering time in maize under water-stressed conditions

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## Introduction

In China, 70% of maize acreage is of rainfed crops; drought stress causes about 30% yield losses annually. With development of molecular markers, it is possible to identify major QTLs regulating specific drought responses, and further to establish a marker-assisted selection (MAS) approach for improving drought tolerance of maize (Veldboom et al., 1996). Many QTLs for flowering time in maize have been identified under drought-stressed conditions (Ribaut et al., 1996; Sari-Gorla et al., 1999), while the comprehensive genetic basis of drought tolerance during flowering time can be obtained by analyzing populations with different genetic background in maize. The objective of this study was to locate QTLs of three flowering traits under water-stressed conditions by SSR linkage map and to analyze the genetic effects in Chinese maize germplasm.

## Materials and methods

An $F_2$ population of 234 plants derived from the cross, X178xB73, was used to construct the SSR genetic linkage map. The corresponding $F_{2:3}$ families were evaluated for drought response in Shanxi province during dry summer season in 2002. The phenotyping experiment was arranged in a complete random design with two replicates. The plot consisted of one 4 m-row with 0.60 m spacing between rows. The regime obtained irrigation at 20, 65, and 90 days after planting, respectively. Male flowering time (MFT) and female flowering time (FFT) were recorded as the number of days from emergence to anther extrusion from the tassel or to visible silks of 50% of the plants per plot. Anthesis-silking interval (ASI) was calculated as the difference between FFT and MFT of each plot. Leaf tissue of each $F_2$ plant was collected and ground to fine powder in liquid nitrogen. DNA extraction, PCR reaction, gel electrophoresis, and the silver staining method were performed following the SSR protocol adopted by CIMMYT Applied Molecular Genetics Laboratory (2001). A linkage map was constructed using Mapmaker 3.0 with the LOD threshold of 3.0. The procedure of composite interval mapping was used to identify QTLs and estimate their effects. QTLs for each trait were searched using WinqtlCart2.0 with the LOD threshold of 2.5 (Wang et al., 2003).

## Results

### *SSR marker linkage map*

One hundred and thirty-seven SSR markers (36.9%) from total 371 markers were polymorphic between two parental lines, and were further used to genotype the 234 $F_2$ individuals. At 137 loci, 39 markers (28.5%) showed distorted segregation from the expected ratio (1:2:1), and the parental genomes were 49.1% from X178 and 50.9% from B73. After running Mapmaker software at LOD 3.0, 121 SSR markers were assigned to 10 linkage groups, while 16 markers remained unlinked. The linkage map covered 1379.5 cM on a total of 10 chromosomes of maize with an average interval of 11.4cM. The marker order in the map was in agreement with their bins on the chromosomes (Maize DB, 2002).

### *QTL mapping*

QTLs detected with an LOD threshold of 2.5 for three flowering traits were presented in table 1 and figure1. For MFT, 8 QTLs were detected, which accounted for 67.2% of phenotypic variation. The QMFT9 on chromosome 9, adjacent to nc134, was identified with the highest $R^2$, explaining 26.9% of the phenotypic variation, and expressed additive gene effects. Five (5) QTLs for FFT were identified, and could explain 53.2% of phenotypic variation. The QFFT6 on chromosome 9, adjacent to nc134, was found to be the major QTL, which was also identified in expression of MFT. Six (6) QTLs for ASI was found on chromosomes 1, 3, and 9, explaining 50.9% of phenotypic variation.

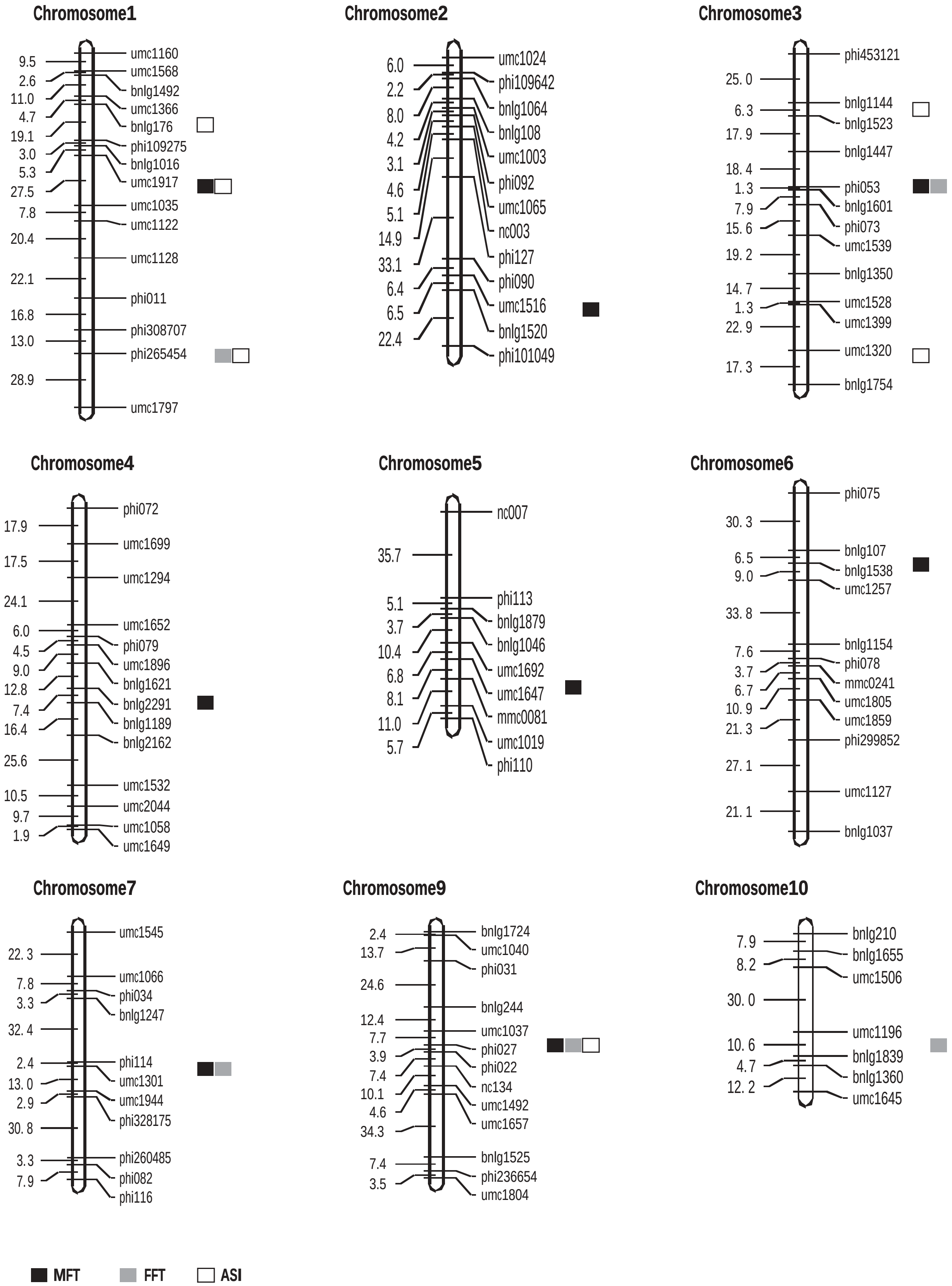

**Figure 1 The linkage map with 121 SSR marker loci based on the $F_2$ population derived from the cross X178xB73**

**Table 1. QTLs involved in expression of flowering traits under water-stressed regime**

| Trait | Locus | Chr. | Marker | Position | LOD | d / a | Gene action | $R^2$ (%) |
|---|---|---|---|---|---|---|---|---|
| MFT | QMFT1 | 1 | umc1917 | 0.69 | 2.5 | 1.37 | OD | 5.54 |
| | QMFT2 | 2 | umc1516 | 0.90 | 4.1 | 0.47 | PD | 5.15 |
| | QMFT3 | 3 | phi053 | 0.68 | 6.8 | 0.31 | PD | 8.49 |
| | QMFT4 | 4 | bnlg2291 | 0.96 | 4.8 | 0.29 | PD | 6.35 |
| | QMFT5 | 5 | umc1647 | 0.64 | 2.6 | 0.79 | PD | 3.33 |
| | QMFT6 | 6 | bnlg1538 | 0.37 | 2.6 | 0.23 | PD | 2.99 |
| | QMFT7 | 7 | phi328175 | 1.04 | 4.0 | 0.34 | PD | 8.42 |
| | QMFT8 | 9 | nc134 | 0.71 | 18.4 | 0.18 | A | 26.89 |
| FFT | QFFT1 | 1 | phi265454 | 1.91 | 4.9 | 0.30 | PD | 5.80 |
| | QFFT2 | 3 | phi053 | 0.68 | 3.5 | 0.13 | A | 4.04 |
| | QFFT3 | 7 | phi328175 | 0.96 | 4.8 | 0.28 | PD | 8.85 |
| | QFFT4 | 9 | nc134 | 0.69 | 22.1 | 0.36 | PD | 31.65 |
| | QFFT5 | 10 | bnlg1360 | 0.61 | 2.5 | 0.36 | PD | 2.88 |
| ASI | QASI1 | 1 | bnlg176 | 0.42 | 4.9 | 0.29 | PD | 8.96 |
| | QASI2 | 1 | umc1917 | 0.63 | 5.8 | 0.36 | PD | 11.81 |
| | QASI3 | 1 | phi265454 | 1.83 | 5.0 | 0.18 | A | 9.67 |
| | QASI4 | 3 | bnlg1144 | 0.25 | 3.5 | 0.53 | PD | 5.03 |
| | QASI5 | 3 | umc1320 | 1.65 | 2.9 | 0.35 | PD | 4.82 |
| | QASI6 | 9 | nc134 | 0.72 | 7.1 | 0.61 | PD | 10.63 |

Some QTLs located in the same region of chromosomes were found conferring different traits. The region close to nc134 on chromosome 9 consistently mapped QTLs for MFT, FFT, and ASI with an allele from B73 contributing to an increase of the trait values. The region adjacent to phi053 on chromosome 3 mapped QTLs of both MFT and FFT with allele from X178 increasing the trait value. And the region located in the interval phi328175-phi260485 on chromosome 7 mapped QTLs for MFT and FFT with an allele from B73 increasing the trait value. The molecular markers linked to these QTLs could be further investigated and validated to establish an MAS approach for germplasm improvement for drought tolerance of maize.

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# Identification of QTLs for drought tolerance in a set of random introgression lines of rice

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## Introduction

Rice is sensitive to drought at different developmental stages, particularly during the reproductive stage when varied degrees of sterility can arise under drought stress (Widawsky and O'Toole, 1990). Tolerance of rice plants to drought is both genetically and physiologically complex. The difficulty of accurately timing and managing water stress means that secondary traits and genetic markers are very attractive selection criteria for tolerance to reproductive stage stress. Quantitative trait loci (QTL) mapping and gene tagging have enabled dissection of drought tolerance in a manner that is easier to study and comprehend. A wide range of genetic and phenotypic variability for several components of DT have been well documented in most major crops over the past few decades (Price and Tomos, 1997; Zhang et al., 2001). Our efforts at improving DT in rice are first focusing mainly on dissecting DT into components and identifying their main-effect QTLs for each component.

## Methods

A set of Teqing near-isogenic introgression lines (TQ-NIILs), consisting of 254 lines (133 $BC_2F_5$, 96 $BC_3F_4$ and 25 $BC_4F_3$), were subjected to phenotypic evaluation and QTL mapping in fully irrigated (non-stress) control and water stress conditions, following a randomized block design with three replications at the International Rice Research Institute (IRRI). Under irrigated conditions, seeds were sown in the seedbed and 30-day seedlings were transplanted into 3-row plots (36 plants per plot or entry) with a spacing of 25 x 20 cm. In water stress conditions, seeds were dibbed in the rows at a rate of 80 kg / ha. The plots consisted of three 3 m long rows at 0.25 m spacing. Plants were spaced at 10 cm intervals along the row. The stress treatment consisted of no irrigation for 10 days with the stress starting at anthesis of most of the lines (80 days), followed by one hour of irrigation every three days until the end of the season. Heading date (HD, days) was recorded when 50% plants in each plot was flowering. Plant height (PH, cm) was measured at maturity and grain yield (YLD, $g/m^2$) was measured from the same area for each plot. A total of 160 SSR markers were used to construct a complete linkage map for the TQ-NIILs, as described previously (Xu et al., 2004). Analysis of variance and correlation between the traits and the environments were performed (SAS Institute, 1996). A mixed linear model was used to detect QTLs and digenic interactions without genetic control using QTLMapper 1.0 (Wang et al., 1999).

## Results

Significant differences were detected among the NIILs for their responses to the water stress, reflected as the differentiated trait expressions in the normal and water-stress conditions. Under the water stress, the NIILs showed tremendous variation in heading time, ranging from 20 days of early heading to 16.2 days of delayed heading. Compared with the non-stress control, most lines (>99%) showed a decreased height by an average of 20.1 cm (ranging from 0.4 to 41.7 cm) and reduced yield by 800.3 $g/m^2$ (ranging from 120 to 1807.2 $g/m^2$) under stress. Only a single line showed increased height and yield under stress. A total of 35 putative QTL were identified for various traits under the control and stress conditions, and individually explained 3.1–35.7% of the phenotypic variation. Several major

QTLs affecting PH, HD, and YLD under drought were identified and mapped to chromosomes 1, 5, 7, and 8. Two QTLs affecting HD on chromosomes 3 and 7 were detected under both the control and stress conditions, which had less effect under stress than the normal condition. There were three regions harboring common QTLs affecting both PH and YLD under the stress condition, while two other regions harbored QTL affecting HD and YLD.

**Table 1. Summary statistics of phenotypic performance of theTeqing near-isogenic introgression lines (TQ-NIILs) for heading date (HD), plant height (PH) and grain yield (YLD)**

| Condition | Trait | LT | TQ | TQ-NIILs Mean±SD | CV% | Range |
|---|---|---|---|---|---|---|
| Control | HD(days) | 89.5 | 90.7 | 92.83 ± 5.01 | 5.40 | 73.839 - 104.5 |
|  | PH(cm) | 81.2 | 84.3 | 90.99 ± 5.19 | 5.71 | 69.526 - 103.017 |
|  | YLD(g/m$^2$) | 800.2 | 872.4 | 867.88 ± 288.75 | 33.27 | 188.08 - 1895.76 |
| Stress | HD(days) | 85.5 | 96.9 | 92.91 ± 4.81 | 5.18 | 70.976 - 105.976 |
|  | PH(cm) | 68.4 | 73.5 | 70.96 ± 5.23 | 7.37 | 56.5 - 89.1333 |
|  | YLD(g/m$^2$) | 46.6 | 88.3 | 73.10 ± 57.25 | 78.31 | 0 - 274.65 |
| Control-Stress | HD(days) | -4 | 6.2 | 0.03 ± 5.37 | 15499.35 | -19.863 - 16.161 |
|  | PH(cm) | -12.8 | -10.8 | -20.03 ± 7.10 | -35.43 | -41.6465 - 2.7535 |
|  | YLD(g/m$^2$) | -735.6 | -784.1 | -796.34 ± 283.18 | -35.56 | -1807.2033 - 26.91 |

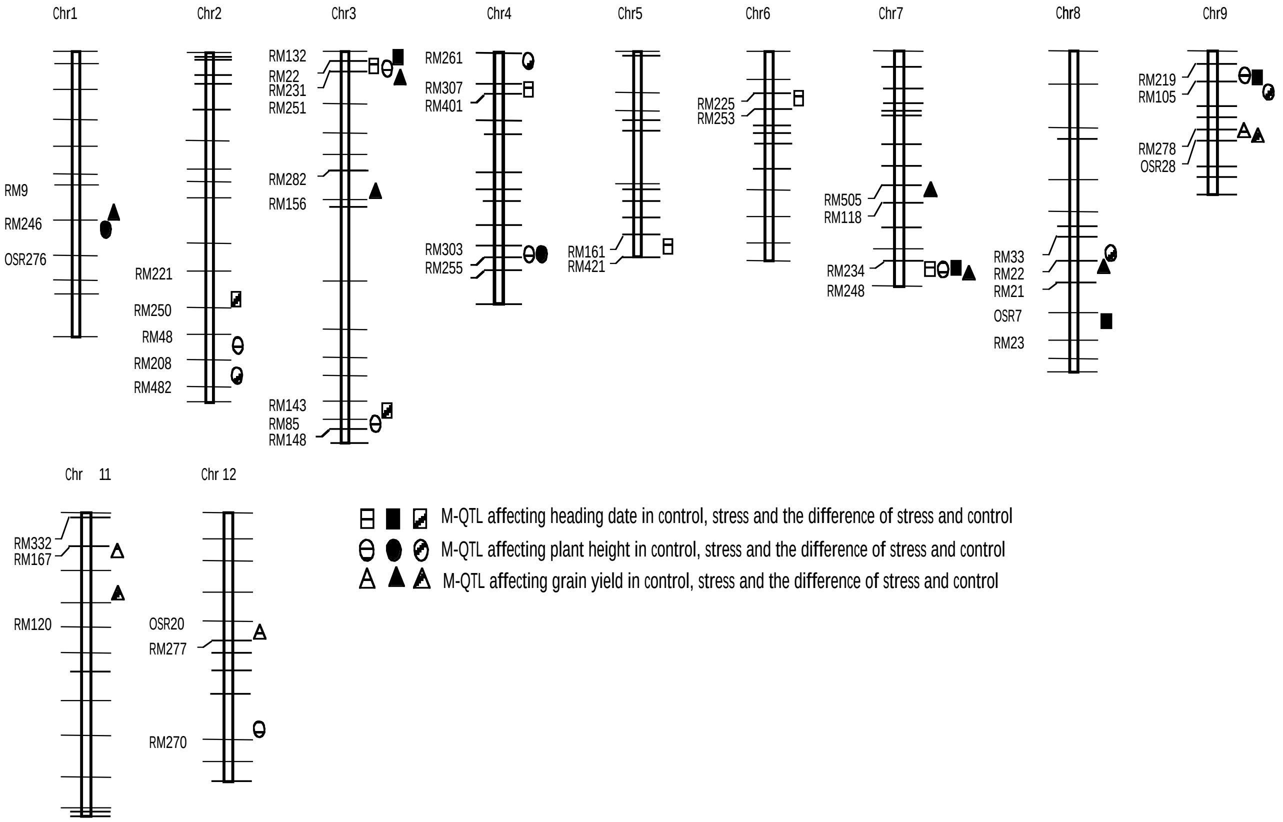

**Figure 1. Putative QTL associated with heading date, plant height and grain yield detected in two different moisture conditions in an near-isogenic introgression mapping population derived from Lemont / Teqing in Teqing genetic background.**

## Discussion

Although the QTL regulating grain yield under stress are scattered throughout the genome, certain chromosomal locations assumed significance. For example, the regions RM22-RM231 on chromosome 3 and RM234-RM248 on chromosome 7 was found to be important in terms of drought tolerance in rice, since they regulate PH, HD, and YLD under stress. When QTL for different traits were linked, the favorable alleles for both the component traits and yield had opposite additive effects, suggesting the presence of genes with pleiotropic effects / linkage on the investigated traits under drought stress. The possibility of improving grain yield under drought stress by incorporating favorable alleles for these linked loci into useful cultivars looks promising.

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# Genetic and molecular understanding of drought tolerance for the improvement of irrigated rice under drought stressed conditions in central and southern China

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## Introduction

Drought frequently occurs in late summer and early fall in rice producing areas of central and southern China, hitting the rice crop at late stages of growth and development, which causes significant yield loss. We have been taking a comprehensive approach to studying the genetic and molecular bases of drought tolerance in rice, emphasizing the late stages of the rice crop, with the goal to improve the cultivars and hybrids. The project has been supported by The Rockefeller Foundation and consists of the following components: (i) drought tolerant germplasm screening and identification, (ii) genetic analysis and mapping QTLs for drought tolerance, (iii) expression profiling of drought induced genes, (iv) root morphology related to drought tolerance, and (v) molecular breeding.

## Methods

Representative rice germplasms, including upland and lowland, both indica and japonica varieties, and cultivars, hybrids, and mutant lines by T-DNA insertion were screened under severe drought stress at 30 d after transplanting and mild drought stress at grain filling stage by using the rain-out facility. The extent of grain yield loss and phenotypic changes by drought were used to evaluate the drought tolerance performance. A RIL population, derived from a cross between irrigated rice Zhenshan 97 and upland rice IRAT109, were planted in polyvinyl chloride (PVC) tubes as well as in the field for drought testing at anthesis stage. Various above-ground and root traits, directly or indirectly related to drought tolerance, were investigated under both normal and stressed growth conditions. Meanwhile, genetic map was constructed for this population by using SSR markers. The two parents were also subjected to expression profiling under drought stress by using a cDNA microarray containing 9,216 unique rice genes. Drought stress was applied at both seedling and anthesis stages. The relative water content (RWC) in the leaves was measured daily to monitor the process of drought stress. Drought-induced or suppressed genes were confirmed by Northern blotting and some of the most promising genes were chosen for genetic transformation to test their functions in drought tolerance.

## Results

### *Germplasm screening and identification*

A total of 735 representative germplasms and nearly 3,000 mutant lines were screened for drought tolerance. Most upland germplasms showed significantly less yield loss by drought than paddy hybrids or cultivars, and a few of them have been selected as parents in breeding for drought tolerance. Interestingly, three mutant lines were found to be drought resistant but molecular analysis (copy number, flanking sequence, co-segregation of T-DNA insertion, and the phenotype) suggested that the drought tolerance was irrelevant to T-DNA insertion.

### *QTL mapping*

A genetic map containing 228 SSR markers was constructed using the RIL populations, which were tested in two years under fully irrigated and controlled drought conditions, respectively. More than 20 QTLs were detected for various drought-tolerance related traits including drought resistant index, leaf rolling, days to leaf rolling, canopy temperature, yield ,and yield component traits. Meanwhile, 17 QTLs were also detected for various root traits (maximum root depth, deep root rate, drought induced deep root depth, drought induced root volume, etc.) under normal growth and drought stressed conditions. Five repeatedly detected QTLs in different populations and with large genetic effect (LOD >5.0) were chosen for constructing near-isogenic lines (NIL) and for marker-assisted selection.

***Expression profiling***

From the expression profiling of 9,216 unique cDNAs under drought stress at seedling stage, a total of 462 and 404 genes were up- and down-regulated, respectively, in the upland rice IRAT109. However, in the irrigated rice Zhenshan 97, only 264 and 240 genes were up- and down-regulated, respectively. A total of 74 differentially expressed genes were detected at early stage of drought stress (RWC 90%). Among them, 40 genes are up-regulated and 34 genes are down-regulated. Examples of these genes are MAPKK, MADS box protein, protein kinase, EF-hand $Ca^{2+}$-binding protein, ATP-dependent Clp protease, EREBP-like protein, calmodulin, heat shock protein 70, zinc finger-like protein, etc. Nearly 8% of the genes arrayed on the chips were differentially expressed at the late stage of drought stress (RWC 70%). Among them, 465 and 406 genes were up- and down-regulated, respectively. The differentially expressed genes at this time point were involved in a wide spectrum of functions, including osmoprotectant synthases, LEA-like proteins, membrane proteins, detoxification enzymes, proteinase inhibitors, protein kinases, and transcription factors. The results of Northern hybridization for 90% clones agreed with the DNA chip data very well. A few drought inducible genes, including a Myb transcription factor, a GATA-zinc finger, a PGPD-like gene, acidic protein phosphotase, and a protein kinase, were under transformation with a drought sensitive rice genotype to test the function of these regulatory genes on drought tolerance.

## Conclusions

Drought resistance is one of the most complex and also economically important quantitative traits in rice. In this study, we have collected many valuable germplasms or mutants for improving drought tolerance in rice. A broad scope of data has been collected to dissect the genetic and molecular bases of drought resistance in the RIL population, in which both drought tolerance and drought escape, the two major mechanisms for drought resistance, were detected. The preliminary analysis of these data has provided a basic structure for the genetic and molecular bases (i.e., genetic loci and genes involved) of drought tolerance in rice. Further integration of these data and functional characterization of those critical regulatory components, which is in progress, will shed more light on the molecular basis of drought resistance and may suggest new strategies on drought tolerance breeding.

# Mapping QTLs affecting rice stigma exsertion in a RIL population with and without water stress conditions

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## Introduction

The yield potential of a CMS line and hybrid F1 seed production is one of most important factors in hybrid rice development. It was determined mainly by the percentage of exerted stigma (PES) of CMS lines. The CMS lines with higher PES usually have high fertility resulting in high yield in seed production. There is great genetic diversity of PES among rice varieties. Several studies indicated that the PES of paddy rice was controlled by a few quantitative traits loci(QTL) in irrigated conditions (Chen et al., 2001; Uga et al., 2003; Li et al.,2003). In this study, a major effort was given to detecting the genetic factors influencing stigma exsertion in a recombinant inbred population (RIL), in both irrigated and drought conditions. The results have special significance in DT hybrid rice development, as water shortages and drought status become increasingly serious in China.

## Methods

A set of 187 F10 recombinant inbred lines were developed from a cross between a commercial CMS maintainer, “Zhanshan 97B,” (with the largest growing area in China) and a DT upland rice cultivar “IRAT 109.” This RILs population and its parents were planted in paddy field with normal irrigation and in the drought screen facility with water stress at the reproductive stage. All the materials were planted in random block design with three replications. Three panicles from threeplants in each plot were investigated on the following traits: spikelet number per panicle (SN), single, and dual exserted stigma number. The percentage of single, dual, and total exserted stigma was calculated, respectively.

Standard analyses of variance were performed to check the genetic variance among the RI Lines for the investigated traits. The genotyping was conducted at Shanghai Agrobiological Gene Center following the published procedures (Luo et al., 2001). An integrated genetic linkage map with 186 SSR markers was constructed using Mapmaker vision 3.0 (Lincoln and Lander, 1992). The putative quantitative trait loci linked to the traits were identified using Windows QTL Cartographer V2.0 (Bastern et al., 1994, 2001) with a threshold LOD score of 2.0.

## Results

1. As a CMS maintainer, the female parent Zhanshan 97 had a significantly higher percentage of exserted stigma than the upland parent IRAT109, in both irrigated and stress conditions. There was significant variation for all the investigated traits in the population. No morphological correlation was found between spikelet number and the percentage of exserted stigma.
2. Three QTLs related with spikelet number under stress conditions were located on chromosomes 2, 3, and 12, respectively (Table 1). Only one significant QTL was found under normal conditions and mapped in the marker interval RM206-RM144 on chromosome 11.

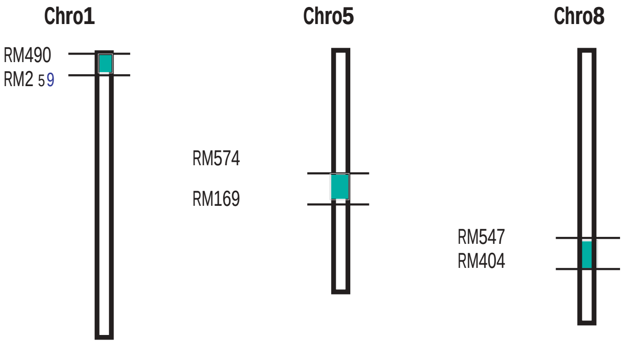

**Figure 1.** The chromosome location of putative QTLs related with PES in stress condition.

**Table 1. QTLs influencing stigma exsertion under irrigated and water stress condition in rice**

| Trait | Loci | Treat | Chr | Interval | LOD | *a* | $R^2$ (%) |
|---|---|---|---|---|---|---|---|
| Percentage of single exserted stigma (%) | *QSpes1* | Stress | 1 | RM490-RM259 | 3.78 | 1.8396 | 7.74 |
| | *QSpes4* | CK | 4 | RM127-RM559 | 2.06 | 1.8296 | 4.47 |
| | *QSpes5a* | Stress | 5 | RM574-RM169 | 2.76 | -2.2860 | 12.03 |
| | *QSpes5b* | Stress | 5 | RM289-RM509 | 2.13 | -1.4155 | 4.28 |
| | *QSpes6* | Stress | 6 | RM528-Rm30 | 2.40 | -1.5278 | 5.23 |
| | *QSpes8* | Stress | 8 | RM547-RM404 | 2.49 | 1.6650 | 6.35 |
| | *QSpes12* | CK | 12 | RM4A-RM19 | 2.32 | 1.9223 | 4.86 |
| Percentage of dual exserted stigma (%) | *QDpes7a* | Stress | 7 | RM320-RM432 | 2.01 | 0.3824 | 4.25 |
| | *QDpes7b* | Stress | 7 | RM11-RM346 | 2.09 | 0.3912 | 4.46 |
| | *QDpes7c* | Stress | 7 | RM336-RM455 | 2.07 | 0.3829 | 4.44 |
| | *QDpes9* | CK | 9 | RM285-RM444 | 2.17 | 0.7784 | 5.92 |
| | *QDpes11* | CK | 11 | RM167-RM441 | 2.97 | 0.6452 | 6.04 |
| Percentage of exserted stigma (%) | *QPes1* | Stress | 1 | RM490-RM259 | 2.23 | 1.7560 | 4.54 |
| | *QPes5* | Stress | 5 | RM574-RM169 | 2.45 | -2.5558 | 10.02 |
| | *QPes8* | Stress | 8 | RM547-RM404 | 2.27 | 1.8495 | 5.27 |
| | *QPes12* | CK | 12 | RM4A-RM19 | 2.28 | 2.2934 | 4.84 |

3. Seven QTLs related with percentage of single exserted stigma (PSES) were located on chromosomes 1, 4, 5, 6, 8, and 12, respectively. Among these QTLs, two (Qpses4 and Qpses12) were detected under normal water irrigation while five (QPses1, QPses5a, QPses5b, QPses6 and QPses8) were found under the water stress conditions. All QTLs except 3 (QPses5a, QPses5b, QPses6) have positive additive effects, which means the substitution of allele from Zhenshan 97B can increase stigma exsertion. Three negative QTLs were only detected under stress, implying the alleles from IRAT109 can increase the PSES. This is because the allele from IRAT109 had some recovery effect from the depressed sigma exsertion in the stress condition. Five QTLs associated with percentage of dual exerted stigma (PSDS) were located on chromosomes 7, 9, and 11, respectively. All QTLs have positive effects.
4. Four QTLs underlying the percentage of exerted stigma (PES) were mapped on chromosomes 1, 5, 8, and 12 respectively. Among them, three (QPes1, QPes5, QPes8) were detected under stress and one (QPes8) was detected under normal conditions. All above QTLs also affect the percentage of single exerted stigma. It is consistent with the correlation study results showing significant correlation between the PSES and PES(r=0.7480**).

## Conclusions

There is great diversity on percentage of exserted stigma among the rice germplasm. Drought usually decreases the stigma exsertion. Twelve putative QTLs were found to associate with the percentage of exserted stigma and mapped on 9 of 12 rice chromosomes. Most of the alleles from the CMS maintainer can increase the stigma exsertion. The allele from IRAT109 in marker interval RM574-RM169 on chromosome 5 could increase the drought effects on stigma exsertion.

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# QTL mapping of drought-resistance at late growing stage in rice

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## Introduction

Rice is one of the staple food crops in Asian countries. In recent years, drought has become the main source of losses for rice production due to increasing water shortages and decreasing and uneven distribution of rainfall. The large amounts of water consumption in rice production makes the research of drought-resistance in rice especially important. The mechanisms of drought-resistance in rice include drought escape, drought avoidance, drought tolerance, and drought recovery (Zhang et al., 2001). Although drought escape is very important in rice production, it is not an actual cellular drought resistance mechanism within the plant, but rather achieves its effects through a short life cycle, small plant size, or developmental plasticity. Drought recovery does not contribute very much to drought resistance in the late growing stage in rice. Therefore, drought avoidance and drought tolerance are the main targets for research on late season drought resistance of rice.

## Methods

To investigate the genetic basis of late season drought resistance in rice, a recombinant inbred line (RIL) population was generated from a cross between Zhengshan 97, an elite parental line for hybrid, and an upland rice, IRAT109. To avoid possible confounding effects of the segregation of heading date in the population, the sowing dates of the lines were adjusted according to the observation made in previous years to synchronize heading time. Drought stress was applied at booting to anthesis stage, and the experiments were conducted in both field (2002) and PVC tubes (2003). Yield and yield-related traits under both normal and stressed conditions were investigated. The population was genotyped using SSR markers for genetic analysis and QTL mapping of drought tolerance.

## Results

1. A genetic linkage map was constructed. The map includes 240 SSR markers covering a total length of 1733.2 cM with five gaps of less than 40 cM each.
2. Six QTLs were detected for the traits of relative yield (yield under drought and yield under unstressed conditions), relative fertility (seed set rate under drought and seed set rate under unstressed conditions) and drought resistance index (DRI, DRIi=(Yi-Yesti) / SE, Yi: yield under drought; Yesti: estimate yield under drought, the values were normalized by potential yield, heading date, plant dry mass and seed set rate; SE: standard error of the regressive equation to calculate Yesti). The LOD values ranged from 2.47 to 5.60, explaining 6.43 to 14.87% of the variation.
3. The traits related to water status of the shoots, investigated in 2002, included leaf rolling score, leaf drying score, flowering time delay, and canopy temperature; those investigated in 2003 included leaf rolling time, leaf drying score, flowering time delay, and the degree of panicle dehydration. In total, 30 QTLs were detected for these traits; two QTLs controlling leaf rolling were detected in both 2002 and 2003. The LOD values ranged from 2.40 to 11.17, and the variation explained by these QTLs ranged from 4.76 to 47.18%.
4. Root traits investigated for QTL mapping included maximum root depth, basal root thickness, root distribution, root volume, dry mass, and root to shoot rate under both normal and drought stress conditions. Ten QTLs were detected for the traits of maximum root depth and relative maximum root depth, but only one QTL was detected in the two environmental conditions. This suggests that the genetic bases controlling maximum root depth under well-watered and drought stress conditions are very different. The LOD values of these QTLs ranged from 2.88 to 5.63, explaining from 8.30 to

14.03% of the variation. Seven QTLs associated with root thickness were detected under normal or stressed conditions and two QTLs were detected in both environmental conditions. The LOD values of the QTLs ranged from 2.52 to 5.79, and explained from 6.76 to 22.19% of the variation. More than 100 QTLs were detected for other root traits such as root volume, root dry weight, deep root rate, and root/shoot ratio. The LOD values for these QTLs varied from 2.40 to 11.60, and explained from 4.32 to 28.18% of the variation.

## Conclusions

In general, more QTLs were detected for root traits and water status related traits of the shoots than the traits of relative yield and DRI. This suggests that drought avoidance may play a critical role in the late season drought resistance of rice. Further and more detailed analyses are in progress. In order to further the understanding of the contributions of the QTLs to yield under stress and their mechanisms in drought tolerance, we have selected five QTL regions controlling maximum root depth, deep root rate, relative yield, and relative fertility for developing near isogenic lines.

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# Comparison of conventional and molecular-based techniques in cassava mosaic disease resistance screening of new cassava genotypes tested in dry and humid zones of Nigeria

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Cassava (*Manihot esculenta* Crantz) production is hampered usually by both biotic (mostly cassava mosaic disease, CMD) and abiotic (poor soils and drought) factors. Thus, breeding for resistance to the disease and drought tolerance is important. However, screening for resistance to CMD is tedious, relying on natural infection conditions and on virus types at a given time and location.

In this study, resistance to CMD in a range of cassava genotypes was assessed in different agroecologies. The yield performances in terms of fresh root weights were also obtained to ascertain genotypes with stable performance across locations. Six selected genotypes with ascertained field resistance to CMD were further screened in two trial experiments using molecular techniques.

Field and graft transmission studies revealed clone 96/1039 easily infectible to CMD as Isunikankiyan (susceptible checks), while 96/0160, 96/1087 and 96/1089A showed significant ($p < 0.01$) resistance to all virus species in the field. Mild infection symptoms induced by homologous DNA-A and B components of *East African cassava mosaic virus* (Uganda variant) Kakamega isolates, EACMV-UG2[Ka] were delayed (6 weeks post inoculation, w.p.i). However, biolistic inoculation of DNA extracts containing EACMV[KE-Kilifi] and *Sri-Lanka cassava mosaic virus* and heterologous DNA-A and B components of EACMV[KE-Kilifi] and EACMV-UG2[Ka], respectively, resulted in earlier (10 – 12 days p.i.) and more severe infection symptoms ($p < 0.01$). Virus concentration (ELISA titres) increased during an initial phase of virus replication and decreased as infection symptoms aborted in highly resistant (HR) genotypes, 96/1089A, TME 4. Negative PCR results were also obtained in plants that had recovered from the virus infections. Genotypes 96/1089A, 96/0160, and TME 4 proved HR to all begomoviruses tested in single and mixed infections. These genotypes were also with commendable fresh root yield as tested in dry and humid areas of Nigeria.

This study emphasized the importance of molecular-based screening techniques in differentiating between field resistance response and immunity to infection. Some disease resistant genotypes with appreciable yield performances observed in this study are suggested for further testing for tolerance to drought. This will ensure pyramiding gene(s) in cassava lines conferring combining abilities of CMD resistance and drought tolerance. Therefore, exploiting combining abilities of resistance to CMD and drought tolerance in a range of cassava genotypes is suggested.

# Marker-aided breeding for development of drought tolerant IR64 lines

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Drought has been the single largest factor limiting the rice yield in the rainfed areas of Asia. Rice is sensitive to drought at different developmental stages, particularly at the reproductive stage (Widawsky and O'Toole, 1990). Because of its genetic complexity, drought tolerance (DT) in rice is probably the most difficult trait to improve through conventional plant breeding. Recently, large numbers of introgression lines (ILs) in the IR64 genetic background with improved DT have been developed in a large backcross breeding program at the International Rice Research Institute (Lafitte et al., 2004). QTLs for DT were also identified using these DT ILs and simple sequence repeat (SSR) markers (Li et al., 2004). In this study, we conducted a large marker-assisted breeding experiments to confirm the DT QTLs identified in the ILs; to pyramid multiple non-allelic DT QTLs of different origins; to remove the possible linkage drag in some promising IR64 ILs; and to develop IR64 lines with significantly improved DT for the rainfed areas of South and Southeast Asia.

From 227 DT IR64 $BC_2F_3$ ILs, 14 promising DT ILs with good yield potential from BC populations of seven donors (6 countries) were selected as the parental lines used in this study (Table 1). These ILs were genotyped with 200 SSR markers and more than 30 putative non-allelic DT QTLs showing excessive introgression in the ILs were identified (Li et al., 2004).

Ten crosses were made between the DT ILs and advanced to the $F_2$ generation, and they were single-seedling transplanted in the lowland field at the IRRI experimental farm with population sizes ranging from 135 to 305 during the dry season of 2002-2003 (Table 2). Two rows of the parental ILs were planted next to each of the $F_2$ populations as checks. The $F_2$ populations were then subjected to severe drought at the reproductive stage, which killed all parental ILs. A total of 560 progeny from the 10 $F_2$ populations survived the stress with good seed set (Table 2). These progeny were selected for genotyping analyses.

**Table 1. Origin and important characteristics of the donor parents for the DT introgression lines**

| Donor | Origin | Group | Plant height (cm) | Particular characteristics |
|---|---|---|---|---|
| IR64 | IRRI | Indica | 83 | High yielding used as the recurrent parent |
| Shwe Thwe Yin Hyv (STYH) | Myanmar | Indica | 98 | Long grain |
| BR 24 | Bangladesh | Indica | 137 | Upland variety |
| OM 1723 | Vietnam | Indica | 132 | Long panicle |
| Binam | Iran | Indica | 140 | Good quality grain |
| Type 3 | India | Indica | 142 | Basmati type |
| Haoannong (HAN) | China | Japonica | 177 | Long panicle |
| Zhihui 100 | China | Indica | 125 | Long panicle, thick stem, non- photosensitive |

**Table 2. Parental DT IR64 ILs ($BC_2F_3$ lines), their pedigrees, and crosses made between them and the number of selected DT plants during the dry season of 2002-2003**

| Female IL (donor) | Male IL (donor) | $F_2$ population size | Plant selected |
|---|---|---|---|
| DGI21 (STYH) | DGI60 (BR24) | 137 | 25 |
| DGI21 (STYH) | DGI62 (BR24) | 190 | 55 |
| DGI29 (STYH) | DGI353 (Zihui100) | 299 | 30 |
| DGI74 (BR24) | DGI187 (Binam) | 218 | 90 |
| DGI74 (BR24) | DGI238 (OM1723) | 305 | 105 |
| DGI76 (BR24) | DGI238 (OM1723) | 248 | 55 |
| DGI75 (BR24) | DGI187 (Binam) | 154 | 30 |
| DGI142 (Type3) | DGI373 (HAN) | 255 | 70 |
| DGI146 (Type3) | DGI353 (Zihui100) | 135 | 70 |
| DGI353 (Zihui100) | DGI374 (HAN) | 219 | 30 |
| Total | | | 560 |

The selected DT $F_3$ progeny are being phenotyped in replicated experiments under both stress and non-stress conditions during the dry season of 2003-2004 and genotyped by differentiating SSR markers. From these experiments, we expect to be able to confirm previously identified DT QTLs and to understand how DT QTLs from two different donors were working together to create a high level of DT in rice.

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# Marker-assisted selection for drought tolerance in maize

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## Introduction.

Maize is the staple food crop in Malawi and is grown on more than 60% of all the arable land. It is mostly grown by small-scale farmers who have 0.2 to 5.0 ha of land comprising over 90% of the farming community. Productivity of this crop is limited by low soil fertility, and little or no use of inorganic fertilizers, especially nitrogen and drought.

Drought is one of the major factors limiting maize production, with sub-Saharan Africa suffering the greatest impact of meteorological drought in the world (FAO, 1990). Effects of drought are known to have the greatest impact on maize yield when they occur within the period around flowering (Bänziger et al., 2000; Ribaut et al., 2002). For the past decade, CIMMYT has conducted research on developing maize varieties for drought tolerance using molecular markers. Four mapping populations were used to map quantitative trait loci (QTLs) associated with drought tolerance, following selection across several environments and locations in Mexico and Africa. The resultant selected genotypes were test crossed to two testers and are being evaluated in Zimbabwe and Malawi. The objective of this work is to assess the efficiency of marker-assisted selection (MAS) in selecting drought tolerant maize genotypes.

## Methods

About 200 genotypes, each selected from four mapping populations, were test crossed to two testers. The MAS selected test crosses and non-selected genotypes are being evaluated under drought and optimal conditions in Malawi and Zimbabwe at six locations. The trials were laid out using alpha lattice design and will be analyzed using Random Estimate of Multiplicative Index (REML tool) and stability of genotype performance across locations will be analyzed using the IRRISTAT computer package (principle component analysis). In order to assess the efficiency of MAS, the performance of the MAS selected genotypes will be compared with the non-selected genotypes.

## Results and discussions

The results of this trial will be presented following the final trial because harvesting will be undertaken in April.

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# Genetic response to strong directional selection on rice tolerance to critical stress

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Genetic response to strong directional selection was studied by simulation and selection experiment of rice tolerance to critical stress. In the simulation, a balanced $BC_2F_2$ population with 200 individuals was constructed for randomly sampling with 5, 10, 20, and 30 individuals that covered all cases in the selection experiment. Two elite *indica* varieties of rice, IR64 from IRRI and Teqing from China, were crossed with seven donors from different countries, and backcrossed two times with IR64 and Teqing as recurrent parents. Three selection experiments with 200 individuals in the original $BC_2F_2$ were conducted for evaluating on genetic response: experiment I, selection on non fitness-related trait, kernel elongation (non fitness-related trait for comparison); experiment II, selection on submergence tolerance (fitness-related trait); and experiment III, selection on drought tolerance (fitness-related trait). The selected plants at both directions in experiment I, the survived plants in experiment II and III, were used to analyze the variation of haplotype and diplotype frequencies and linkage disequilibrium with 140 anchor SSR markers representing 79 well-distributed bins.

The average frequencies of allele and genotype of 72 markers in 1,000 simulations by sampling 5, 10, 20, and 30 individuals were very close to those of the original population constructed, which did not show significant differences to the expected segregation in $BC_2F_2$ population. The standard deviations (SD) of allele frequencies in random sampling populations were much higher than in the original population, and the smaller the sample size, the larger the SD. The significantly distorted markers were more than expected, the trends were more obvious at the critical significance level and the larger sample size. The frequencies of significant linkage disequilibrium (LD) were higher than the expected and increased with the sample size.

In experiment I, the frequencies of two marker alleles and three genotypes showed obvious differences to the theoretical segregation. All standard deviations were larger than those in simulation with similar sample size. The extreme selection in different direction could result in the change in allele and genotype frequencies with the genetic background. Increasing sample size and merging the data of two directional extreme selections could centralize the allele frequency to the expectation and lessen the SD. The marker distortion showed differences between two directional selections and two genetic backgrounds. The larger the sample, the more marker distortion could be observed, especially at the critical significance level. In almost all cases, except sample size 5, the proportions of significant allele and genotype distortion were much higher than the average in the simulation with randomly sampling. In experiment I, frequencies of significant LD were much lower than the average of randomly sampling with similar sample size.

Selection on submergence (experiment II) showed diverse response between two genetic background and three donors. The donor alleles seemed to be more easily introgressed into the Teqing background than into the IR64 background. The much higher proportions of marker allele and genotype distortion in experiment II than in simulations were demonstrated in all crosses except IR64/Khazar. The frequencies of significant distortion of marker alleles and genotypes changed between two recurrent parents and three donors. The frequencies of significant LD were much higher than those in simulation. The fluctuations could be observed between two genetic backgrounds and three donors, and the results showed that most of significant LDs did not come from linkage.

In experiment III, different donors and different genetic backgrounds showed divergent responses under different drought stresses. The frequencies of marker alleles and genotypes fluctuated between crosses with donors, genetic backgrounds, and drought stresses. The significantly distorted markers and the significant LDs occurred more frequently than in simulation.

Our results suggest that selection for complex traits, especially on fitness-related traits, could produce much stronger genetic response than genetic drift, which might be controlled by both single loci and epistatic loci. We found diverse genetic responses among experiments, genetic backgrounds, donors, and stresses. The loci closely related with target traits in selection experiments could be found and located by proposed method.

# Impact of moisture stress at different reproductive stages of plant growth on grain yield parameters and marker assisted QTL pyramiding for root length in rice

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Rice is subjected to low moisture stress at two main growth stages: vegetative and reproductive. Of the two, reproductive stress is critical as it can drastically reduce the grain yield. An experiment was carried out at GKVK, University of Agricultural Sciences, Bangalore, India during wet season 2002, in pots with three replications, maintaining two seedlings per pot with two genotypes, IR50 (*indica*) and Moroberekan (*japonica*); the two parents of the 220 RILs we generated. Analysis of variance and the mean for ten traits was determined in IR50 and Moroberekan subjected to moisture stress at different reproductive stages of plant growth, and estimated on various traits including panicle length and single panicle weight (Figure 1).

Moisture stress at different stages of growth reduced plant height significantly in both varieties, but had no effect on number of tillers and panicle length. Single panicle weight was greatly reduced when stress was induced after 10 days of 50% flowering. The negative impact of moisture stress was higher on IR50 than on Moroberekan, except when stress was induced 10 days after 50% flowering, where both varieties were equally affected. Significant reduction in test weight was observed when stress was induced at 50% flowering and 10 days after 50% flowering. The negative impact of moisture stress on test weight was more on IR50 than on Moroberekan when stress was induced at 50% flowering.

The impact of moisture stress on number of fertile spikelets per panicle was more on IR50 than on Moroberekan except when induced during 50% flowering and 10 days after 50% flowering, wherein both varieties were equally affected. However, in neither genotype was there a differential effect of stress on number of sterile spikelets per panicle. Total number of grains per panicle and spikelet density was greatly reduced when stress was induced 10 days after 50% flowering. No differential effect of stress on total number of grains per panicle and spikelet density in the varieties was observed.(Girish, 2004).

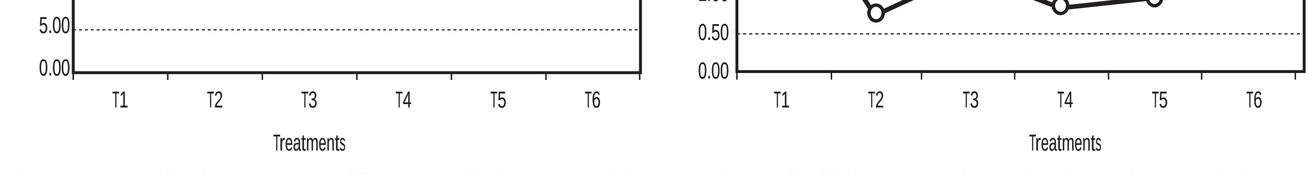

**Figure 1. Impact of moisture stress at different reproductive stages of plant growth on grain yield parameters in Moroberekan and IR50 varieties.**

In another experiment, newly developed recombinant inbred lines of IR50 and Moroberekan were phenotyped for low moisture stress at vegetative and reproductive stages, root morphology-related traits under contrasting moisture regimes, and for rice blast disease at seedling stage. Highly significant variation was observed among the RILs for all the traits observed in both well watered (WW) and low moisture stress (LMS) conditions at vegetative and reproductive stage stress, root morphological traits, and for blast resistance.

Under vegetative stress, number of tillers, panicles per plant, number of fertile spikelets per panicle, above ground biomass, and harvest index showed significant positive association with grain yield under both WW and LMS conditions. Panicle length and test weight were found to be positively associated with yield only under WW as in earlier study (Hemamalini, 2000) where as under LMS, yield was negatively associated with plant height. RI, IRMO-119 and IRMO-230 were found to be high yielding and drought tolerant. They had deep roots and were resistant to rice blast at seedling stage. RIL, IRMO-119 was also found to be high yielding under reproductive stage stress. A similar observation was reported by Venuprasad et al. (2002).

For reproductive stage stress, under LMS, there was high reduction in mean values for plant height, number of panicles per plant, panicle exertion, spikelet fertility, grain yield per plant, above ground biomass, test weight, harvest index, and pollen fertility. Grain yield per plant was positively associated with number of panicles per plant, spikelet fertility per panicle, above-ground biomass, pollen fertility, and harvest index under both WW and LMS conditions. In addition, it showed positive association with plant height and number of tillers per plant and negative association with days to maturity under WW condition. IRMO-166 was found to produce the highest yield.

Maximum root length was positively and significantly associated with root volume, root dry weight, root to shoot length ratio, plant height, tiller number, and shoot dry weight in both WW and LMS conditions. Root volume, total root number, root length, root dry weight, shoot dry weight, and total dry weight were found to be interrelated, positively and significantly.

Five QTLs for root parameters introgressed into IR64 genotype (Shen et al, 2001) from Azucena were crossed and an effort was made to combine the five QTLs present on four different chromosomes. Four crosses were made: MAS 11(Chr.7) X MAS18 (Chr.(1+7), MAS11 (Chr.7) X MAS19(Chr.1+7 ), MAS24 (Chr.9) X MAS11(Chr.7) and MAS7(Chr.2) X MAS11 (Chr.7). Closely associated SSR markers associated with the five QTLs were used for detecting the QTLs in the crossed progeny. The $F_2$ plants were raised in PVC cylinders until maturity and then observed in comparison with the near isogenic line parents, as well as IR64 and Azucena under stress. The results indicated an interaction effect of the QTLs.

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# Molecular marker assisted selection for drought tolerance in a maize population bred for semi-arid Kenya

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## Introduction

Drought is one of the major causes of reduced crop production in the tropics. (Edmeades et al., 1992). Grain yield of maize grown under severe stress at flowering and during grain filling is highly correlated with kernel number per plant (Bolaños and Edmeades, 1996). Edmeades et al. (1998), showed that decreasing the interval between anthesis and silking in maize increases yields in drought prone environments and this characteristic can be screened in large populations. In water limited environments, conventional breeding for improved yields has been slow due to year-to-year variation in rainfall and within season variation in rainfall distribution in dry lands. (Turner, 2001). This study used molecular markers to select for agronomic traits linked to performance under drought conditions.

## Materials and methods

A wide range of germplasm consisting of lines and their hybrids was screened under well-watered, intermediate drought stress, and severe drought stress conditions in June 1997 at the Kenya Agricultural Research Institute (KARI), substation Kiboko, in order to identify suitable parental lines. Plots were sown with three seeds per hill, which were later thinned to one plant per hill. Each plot consisted of a 2.5 m row of 12 plants spaced at 20 cm between plants and 0.75 m between rows.

Mapping populations were generated from a cross between a CIMMYT drought tolerant line (H16) and a drought susceptible high yielding Zimbabwe line (K64R) at Tlaltizapan, Mexico. From an F2 population of 2,000 plants sown in August 1998, 400 randomly selected ears were selfed to produce F3 families. For mapping purposes, DNA was isolated from about 350 individual F3 plants. In November 1998, each of the 400 plants was crossed to one of two plants of testers, CML 202 and CML 311. In February 1999, 250 test-crosses were evaluated under three watering regimes: well-watered, intermediate stress, and severe stress, at the KARI Kiboko substation. In all trials, male flowering (MFLW) and female flowering (FFLW) were measured on individual plants. Anthesis to silking (ASI) was calculated as the difference between FFLW and MFLW family means. Grain yield (GRWT) and other yield components such as plant height (PLHT) and ears per plant (EPP) were measured per plot basis.

One hundred and fifty (150) RFLP markers were screened on genomic DNA isolated from the two parental lines K64R and H16 and also from 311 F3 plants. The DNA samples were digested with one of the two restriction enzymes (*EcoR1* and *HindIII*). In addition, another 60 polymorphic SSR markers were used to saturate the map. Map positions of polymorphic loci were established by multi-point analysis using the computer programme “MAPMAKER”(version 3.0 (Lander et al., 1987). Composite interval mapping mixed model method of Jiang and Zeng (1995), which performs joint analysis of multiple environments and multiple traits, was employed to estimate QTL and their genetic effects. Markers closely linked to the two major QTLs on chromosome 1 and 2 were used to screen 2,000 F3 individuals. Of these, 45 F5 families were selected and sown in the field at Kiboko substation in August 2001. Individual plants were selfed and crossed to six testers.

## Results

Although the degree of drought stress applied was not sufficiently strong to allow for the finer analysis of genetic variance under water limiting conditions, two major QTLs for grain yield and ears per plant were identified on chromosomes 1 and 2, across the intermediate and severe stress environments at 190 cM and 124 cM, respectively. One QTL for ASI was identified in the same location as that of grain yield and ears per plant. Another QTL for grain yield and ASI was found on chromosome 4 whereas minor QTL, whose log of odds (LOD) were below the threshold, were detected on chromosomes 1, 3, 5, and 10. ASI, grain yield, and ears per plant showed strong phenotypic and genotypic correlations, suggesting they may have linked QTL. The QTL for grain yield on chromosomes 1 and 2 and that of ASI on chromosome 2 gave non-significant QTL X Environment interactions, indicating that these QTL are stable across environments.

## Conclusion

Several QTL for yield components associated with performance under drought conditions were located, although they accounted for less than 10% of the total phenotypic variance. Molecular marker assisted selection using markers linked to these QTL, identified 45 F5 families. In this respect, the best molecular marker strategy would be one that would combine selection of grain yield QTL with that of QTL for such key traits as ears per plant and ASI.

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# Marker-assisted selection in tropical maize based on consensus map, perspectives, and limitations

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## Introduction

For approximately 15 years, genetic dissection of polygenic traits has been hailed as a promising application of DNA markers, resulting in extensive mapping experiments aimed toward the development of marker-assisted selection (MAS). Few concrete MAS results have been published that would justify the initial enthusiasm (Ribaut and Hoisington, 1998; Young, 1999; Dekkers and Hospital, 2002). The difficulty of manipulating quantitative traits is related to their genetic complexity, principally the number of genes involved in their expression and interactions between genes (epistasis). Since several genes are involved in the expression of polygenic traits, they generally have smaller individual effects on the plant phenotype and are cross dependent. This implies that several regions, or QTL, must be manipulated simultaneously to have a significant impact, and that the effect of individual regions is not easily identified. Marker-assisted backross (BC-MAS) conducted at CIMMYT has proven successful for improving drought tolerance of a recipient line, thanks to the introgression of five chromosome segments from a drought tolerant donor line (Ribaut et al., 2002). However, the real challenge remains the application of MAS to broad genetic bases to diversify both the sources of favorable alleles and the final material available for cultivation. A point of key importance would be to better anticipate the phenotype of a given combination of alleles at several QTL, through relevant physiological modeling.

## Materials and methods

To construct the consensus map, anchor molecular markers that are common to the four segregating crosses developed at CIMMYT have been used to position all the markers on a single map through linear regression. In a second phase, CIMMYT has synthesized results obtained from about 35 drought trials, using six populations (a total of about 3,500 QTL) and the QTL information generated for each cross (QTL for yield components, morphological traits, and physiological parameters), which has been compiled on the consensus map using the Comparative Map and Trait viewer (CMTV) tool. The CMTV allows a user to combine multiple linkage maps into a consensus map and displays in a unique visual manner output files produced by generic QTL analysis in various combinations, e.g., for particular traits, environments, crosses, or any combination of these. This tool allows the import and display of expression data on the consensus map. The original idea was to assign a “weight” for each QTL set taking into account (i) the nature of the trait and (ii) the threshold value of the QTL identified under a specific environment (likelihood ratio, LR). However, due mainly to time constraints, only the number of QTLs and their LR value have been considered in identifying the most significant regions, and equal weight was attributed to all for the current MAS experiment. To validate pyramiding of favorable alleles at those target regions, four F2:3 “good x good” segregating populations (ca. 400 families per population) have been developed by CIMMYT from the pair-wise crosses of eight drought tolerant lines with different genetic backgrounds. To identify favorable alleles for the trait of interest at selected loci, 60 progenies per population were evaluated in Africa over two cycles under water limited conditions. It should be noted that this number would be low for QTL detection with no a priori information, but it is justified in this instance as the target regions are known a priori. The entire segregating populations were screened with neutral markers that mapped within the target regions, and genotypes were ranked within each population by taking into consideration the allelic composition at selected loci. The best and worst 50 genotypes, based on MAS ranking, were evaluated in Zimbabwe during the summer dry season 2003 under water-limited conditions (randomized design), and are currently being evaluated during the winter dry cycle in Mexico.

## Results and discussion

By compiling the genetic information generated at CIMMYT onto a single consensus map we have been able to identify 11 genomic regions of key importance for drought tolerance in tropical maize. Examining the regions we have identified on the consensus map, it is clear that there is a large interaction of QTL by population. Within a given segregating population, QTL results seem quite stable across years, especially for secondary morphological traits, and even across countries (Mexico and Zimbabwe). However, when one examines the results across genetic backgrounds there is little QTL colocalisation for a given trait, with some exceptions. For example, with traits such as chlorophyll content, genomic regions on chromosome 6 and 10 are fairly consistent across environments and populations. In addition to this, a number of candidate genes related to a particular metabolic pathway also mapped with high frequency within the QTL identified for this given pathway. For example, in the carbohydrate regulation pathway, some key genes involved in this pathway—sucrose invertase, sucrose synthase, and sucrose phosphatase (ivr1 2.02, sus1 9.04, sps1 8.06)—all map to a QTL for glucose or sucrose content in one or several of our three target tissues (ear-leaf, ear, and silk). Most of the map locations for QTL identified for our target pathways (sugars, ABA, and proline) overlap with QTLs for secondary traits and / or yield components. This underlines the fact that the genetic dissection of the physiological parameters is key to identifying genetic regions involved in plant response under stress conditions. Co-localization of QTLs for morphological traits, related physiological parameters, and candidate genes is a strong indication that a particular region is of interest (Ribaut et al., 2004). Examining these results, the current conclusion is that although a large amount of information has been generated through genetic studies, this is still “raw” data that needs to be analyzed in a suitable manner before it can be made useful for making genetic gains. This assessment has been confirmed by the results we obtained from our first set of trials conducted in Zimbabwe last year. Results were not as expected as there was no significant phenotypic difference observed between the two contrasting groups identified through MAS selection, except for one population. As we are still expecting further results from the trials in Mexico, no definite conclusion can be made at this point. However, before developing a new selection index, a number of issues require serious consideration : (i) QTL locations need to be refined through meta QTL analysis, (ii) the candidate genes showing changes in expression through profiling experiment need to be better integrated, and (iii) the nature of the traits (e.g., correlation with yield components and heritability) and the environment for which they have been identified. A further issue is to test and compare the relevance of conducting selection using neutral markers or polymorphism correlated with favorable haplotypes identified through association mapping.

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# Marker-assisted farmer participatory breeding for drought resistance in rice (*Oryza sativa* l.)

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## Introduction

High yielding rice varieties for rainfed lowlands and aerobic mode of cultivation are urgently needed to augment food and social security for farmers and communities inhabiting parched habitats. Marker-assisted selection for root morphological traits to enhance drought tolerance is widely perceived as a viable strategy (O'Toole, 1979; O'Toole and Chang, 1982) as conventional breeding (for drought tolerance) has hit roadblocks and no simple solutions are in sight (Serageldin and Presley, 2000).

## Methods

Mapping quantitative trait loci (QTLs) associated with desirable root morphological traits, phenology, and productivity in different mapping populations, across moisture regimes (Champoux et al., 1995; Price and Tomos, 1997; Hemamalini et al., 2000; Venuprasad et al., 2002), and development of a "comprehensive map" of all published QTLs has been done. EST-SSR (www.graingenes.org) and SSRs (www.gramene.org) within and in the vicinity of five 'chromosomal hot-spots' and deep root allele specific SCAR (and SSR) markers (Shashidhar et al., 2001; Sharma et al., 2002) are being used for marker-assisted selection. An exhaustive and comprehensive list of trait-specific markers guides us to determine multiple trait associations at target loci.

$F_3$ segregants from Moroberekan / IR20 and Budda / IR64 were grown in the target habitat during 2003 wet season. Natural infestation of rice blast disease offered scope for selection. At maturity, 48 farmers (45 male and 3 female) were asked to choose five "best segregants" among 20,000 plants. Farmers' selection criteria(on) were documented with a 'structured' questionnaire. Interesting segregants have been immortalized for further backcrossing, retrospect replicated analysis of root morphology, and molecular marker assisted investigations. Single seed descent is being adopted to develop a mapping population in the Moroberekan / IR20 cross.

## Results

The progeny of Moroberekan / IR20 was highly resistant to the leaf and neck blast compared to Budda / IR64. Majority of the segregants selected by both farmers and breeders belonged to only five families (Blast resistant) in Budda / IR64 and 30 families in Moroberekan / IR20. Segregants manifested pollen and spikelet sterility ranging from 5 to 100 %. Segregants manifested phenomenal growth rates and leaf greenness, width, length, and shoot sturdiness. Several lines were significantly early maturing compared to both parents. Near-isogenic lines have been identified for root morphological traits in the CT9993 / IR62266 mapping population by assessing genomic similarity based on marker data and in IR64 /

Azucena by way of continued backcrossing (Venuprasad unpublished). Some of the $BC_2F_1$ plants exhibit short shoot and long rooting habit. Following single seed descent, a mapping population (1000 lines) in the *japonica / indica* cross is at the $F_4$ stage.

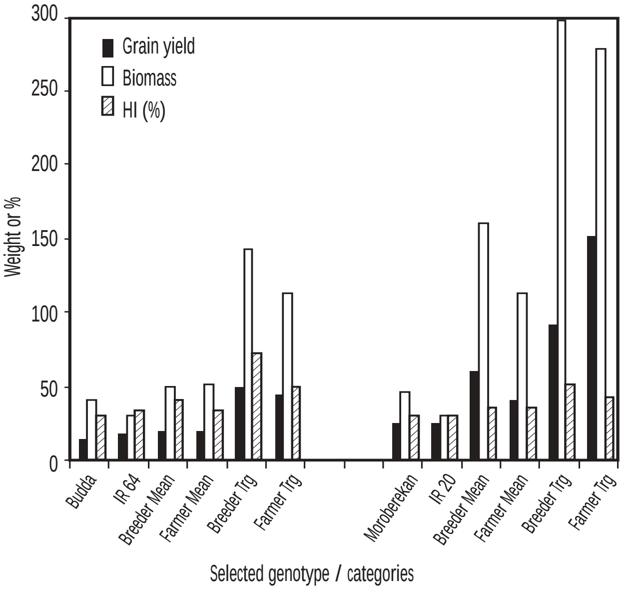

**Figure 1. Three traits from the F3 generation progeny comprising means of parents of two crosses, and selections made by either farmer or breeders. Means of selected plants and transgressants are presented.**

## Conclusions

Two hundred selections (made by farmers and breeders) have been forwarded to $F_4$ during the 2004 dry season (Jan–May). Modified bulk, pedigree, and SSD are being followed. We (breeders) selected based on phenology, number of productive tillers per plant, panicle length, spikelet fertility, grain yield, biomass, and harvest index. Farmers selected based on panicle characters, grain type, and perceived yield. Farmers' selection and breeders' selection overlapped by only 41.73%. Intermating between interesting segregants will help reduce height, induce disease. Candidate gene markers can be used when association is established and / or map position known.

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# Stress protein (SDS-PAGE) for MAS-breeding: Seed characteristics and vigour to detect stable QTLs using seed protein markers in developing drought tolerance in rice *(O. sativa* L.)

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## Introduction

To date, verification of yield QTLs prior to molecular MAS breeding has been found to be an essential step towards cultivar improvement. Therefore, without a thorough understanding of the physiological and agronomic particulars of any QTL and the target-environment, MAS for QTL showing qualitative interactions should be firstly minimized, because it is regarded as non-random association between genetic markers and trait values for drought tolerance. In that, root characters and other growth parameters involved in regeneration potential (after release of drought) are vital components. Conceptually, it is similar to recombination mapping of several loci spread over the rice genome. Seed vigour, root morphology (length, volumes, regeneration capacity of root after drought, grain yield (fertility vs. sterility), affect on the seedling tillering, and apparent water translocation rate from roots to shoot and shoot to leaves should be responsible for nutritional translocation in scoring drought injury index.

## Methods

The studied genotypes that have differential characters for leaf rolling, shoot growth (stunted), prolonged roots, higher tillering, and grain filling under water stressed conditions and seed storage protein profiles of eight top drought donors (NDR-97, OS-6, Azucena, Ananda, Laloo-14, etc.) were analysed for drought induced protein. These donors have already been identified for other morpho-physiological traits (see Table 1). The methodology of seed storage protein (Singh and Singh, 1996) has been followed for seed *oryzenins* (SDS-PAGE) for protein profile analysis. Other growth parameters were observed as per the IRRI scale.

## Results

Amongst the eight top donors identified for combined traits for drought tolerance, it was indicated that under normal rainfed condition, the low molecular weight (LMW) stress proteins tend to produce low in comparison to HMW (high molecular weight) stressed proteins (seed *oryzenins*). In contrast, genotyes like *Azucena* and *Annanda* produced higher levels of both LMW-and HMW-stressed protein (at -10 bar osmotic potential), which also had moderate leaf rolling and a high ratio of radicle / plumulc. Likewise, OS-6 and Gaurav showed similar protein profile patterns under stress conditions. As seed, storage protein is a product of gene expression, however DNA-based markers will confirm the above findings more precisely.

The location of genes control and physiological traits related to drought resistance is spread over the various chromosomes in rice. A total of 146 QTLs were detected for 15 quantitative traits, mapped between 109 marker intervals, and spread over 11 rice chromosomes. These QTLs explained a minimum of 7% to a maximum of 39.6% phenotypic variance, individually showing a different number of QTLs x E interactions, which resulted in 98.6% QTLs with minor effects (Verulkar, 2003).

Therefore, under different environmental conditions signifying the QTLs x E interactions, 98.6% of QTLs are classified as minor.. Such putative QTLs for these traits with large effects could be identified for yield per se. Therefore, only a limited number of QTLs with large effects should be identified for favourable alleles viz. reduced plant height, total root length, high regenerative tiller, least leaf rolling, and differential protein expression pattern, both under normal and stressed-conditions by modulating expression of gene products.

**Table.1 Combined morphometric traits selected for top donors and level of expressed seed storage proteins (SDS-PAGE) in *O.* sativa L. proteins**

| Donor | Combined traits for drought tolerance | Protein Expression level (PEG-6000; -10 bar) | | Total No. of expressed bands | |
|---|---|---|---|---|---|
| 1. NDR-97 | 1. Low degree of leaf rolling<br>2. High drought tolerant lines<br>3. Low sterility: 1.10%<br>4. Root/shoot ratio: 0-3-0.5<br>5. Yield base: 21-30g/ plant<br>6. Earliness to flowering | **Normal**<br>1. Low lavel of LMW protein expression<br>2. High level of HMW protein expression. | **Drought**<br>1. Moderate level of LMW expression of protein<br>2. Low level of HMW –protein expression. | **Normal**<br>13 | **Stressed**<br>16 |
| 2. Azucena | 1. Germination range under osmotic stress (-10 bar PEG 6000) after 96 hr: 80-90%<br>2. Root/Shoot ratio at high temp: 0.3-0.5<br>3. Radicle/plumule ratio at –10 bar: 0.4-0.7<br>4. Coleoptile length (cm) as –10 bar: 3-4 cm<br>5. Moderate degree of leaf rolling<br>6. High tolerance during recovery phase<br>7. Grain yield/ plant: 21-40g | 1. Low level of LMW protein profile. | 1. High level of both LMW and HMW proteins. | **Normal**<br>13 | **Stressed**<br>19 |
| 3. Annanda | 1. Germination at –10bar: 80-90%<br>2. Coleoptile length (cm) at –10 bar: 3-4 cm<br>3. Root/shoot ratio under at high temp: 0.4-0.7<br>4. Radicle / Plumule ratio at -10 bar: 0.4-0.7<br>5. Moderate degree of leaf rolling<br>6. High degree of drought tolerance | 1. Moderate level of HMW - stress protein and absence of LMW- stress protein. | 1. High level of both HMW- & LMW stress protein. | **Normal**<br>15 | **Stressed**<br>18 |
| 4. Laloo-14 | 1. Long flag leaf: 41-50 cm<br>2. Grain yield / plant: 21-40g<br>3. Germination at–10 bar after 96 hr: 80-90%<br>4. Coleoptile length -10 bar: 3-4 cm<br>5. Root shoot ratio at high temp: 0.3-0.3<br>6. Radicle/ Plumule ratio at –10 bar: 0.4-0.7<br>7. Moderate degree of leaf rolling<br>8. Yield base: 31.40g/plant | 1. Low level of LMW - stress protein. | 1. High level of stress protein synthesis of both HMW &LMW. | **Normal**<br>18 | **Stressed**<br>16 |
| 5. Ratna | 1. Grain no./panicle: 30-11<br>2. Germination percent at –10 bar: 80-90%<br>3. Radicle /plumule ratio -10 bar: 0.4-0.7<br>4. Root/shoot ratio at high temp: 0.3-0.5<br>5. Low degree of leaf rolling<br>6. High degree of drought tolerance<br>7. Yield base: 18.9 g/plant | 1. Moderate level of LMW protein is. | 1. Moderate level of LMW-stress protein<br>2. Overall level of protein profile is *at par* with normal plants. | **Normal**<br>19 | **Stressed**<br>10 |
| 6. N-22 | 1. High Germination percent: 80-90%<br>2. Coleoptile length at – 10 bar: 3-4 cm<br>3. Radicle/Plumule ratio: 0.4-0.7<br>4. Root/shoot ratio at high temp: 0.3-0.5<br>5. High regeneration ability.<br>6.High drought tolerance.<br>7. Earliness to flowering. | 1. Very low level of LMW proteins. | 1. Moderate level of LMW protein. | **Normal**<br>17 | **Stressed**<br>14 |
| 7. OS-6 | 1. High germination percent at-10bar: 80-90<br>2. Moderate coleoptile length 2cm<br>3. High radicel/ Plumule ratio at –10 bar: 0.4-0.4<br>4. High level of root/shoot ratio 0.3-0.5<br>5. High regenerations after recovery phase | 1. LMW protein level is very low. | 1. LMW stressed protein level is high. | **Normal**<br>12 | **Stressed**<br>14 |
| 8. Gaurav | 1. High root/shoot ratio at high temp: 0.3-0.5<br>2. High regeneration capacity<br>3. Yield base: 19.6g/plant | 1. LMW protein level is very low. | 1. LMW stressed protein level is high. | **Normal**<br>12 | **Stressed**<br>14 |

## Findings

1. Storage seed protein profile (SSPP) in top donors seemed similar to stressed proteins (LEA: late embryogenesis protein) or like that of RAB (responsive to ABA) dehydrin protein/osmotin protein, heat shock proteins (HSP), etc.
2. It should be analyzed at an early stage to mark "stress protein indicators" under laboratory conditions.
3. Synthesis and accumulation of *osmolytes, aquaporins* (channel-protein) for leaf-desiccation must be examined for finding strong correlation with stress protein expression and field based phenotypic observations.

# Improving drought resistance in rainfed lowland rice for the Mekong region: Concept and practical use of MAS to develop cultivars with high quality and drought tolerance

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## Introduction

The Mekong region of the Southeast Asia comprises Myanmar, Thailand, Cambodia, and Laos. It is known as the primary origin of domesticated rice and also as one of best areas for high quality rice production. More than 60% of total rice growing areas in the region depends on rainfall. Drought is a major constraint limiting yield and grain quality in these regions (Fukai, 2002). In the past, genetic improvement for drought tolerance has been hampered because genetics and incidence of genetic by environment (G x E) interactions are not unclearly understood. In addition, the improved cultivars most often were not well adapted to the rainfed environments, proved susceptible to diseases and insect pests, and produced unacceptable grain qualities (Rerkasem). In general, a conventional breeding program takes at least 15 years to release a new cultivar. Recently, biotechnology has been a powerful tool to discover genes governing these traits and understand their function. Establishing association between such traits and gene or molecular markers would facilitate genetic manipulation via marker assisted selection (MAS). Therefore, new breeding strategies can be established to the shorten period for cultivar improvement by using MAS, rapid generation advance (RGA), and early generation testing in multi-locations for grain yield and other qualities.

## Methodology

Approximately 15 populations based on crosses between donor lines selected for drought tolerance and recipient lines selected for yield and quality traits of acceptance to farmer / markets are being developed in Thailand, Laos, and Cambodia (for details, see other papers on the Mekong Regional Project for developing drought tolerant rice for the rainfed lowlands). These populations will not only be used for molecular analysis focusing on the drought tolerant traits of the drought response index (DRI), leaf water potential (LWP), flower delay, and spikelet sterility, but also will be used for cultivar improvement. Drought related markers, as well as markers for grain quality, will be used for genotypic selection to increase the chance of selecting lines containing favorable alleles of QTL harboring genes for drought tolerance. Backcross breeding will be implemented as a breeding strategy, which will help maintain grain qualities of recipients and also synchronizing of flowering time in later backcross generations in the breeding programs. When flowering time is synchronized, field management for drought stress will be easier and phenotypic selection will not be biased by plant phenology. At the Rice Gene Discovery Unit, a library of QTL has been assembled for drought. It includes 9 QTL for shoot traits, 19 for root traits and 26 for yield and yield components (Lanceras et al.). In many cases, the coincidences of QTL for shoot and root traits with agronomic traits (Figure 1) frequently occur. It makes using MAS alone as selection strategy for drought tolerance more difficult. QTL for shoot and root traits frequently showed repulsion linkage with QTL for agronomic traits. Selection for shoot and root traits by MAS might lose yield potential and other good agronomic characteristics. Therefore, phenotypic selection, in later generations of a population that segregates in the genomic region where QTL coincide, is required to increase the opportunity for selecting lines that combine all good alleles of QTL for target traits. A modified breeding strategy is purposed as shown in figure 2.

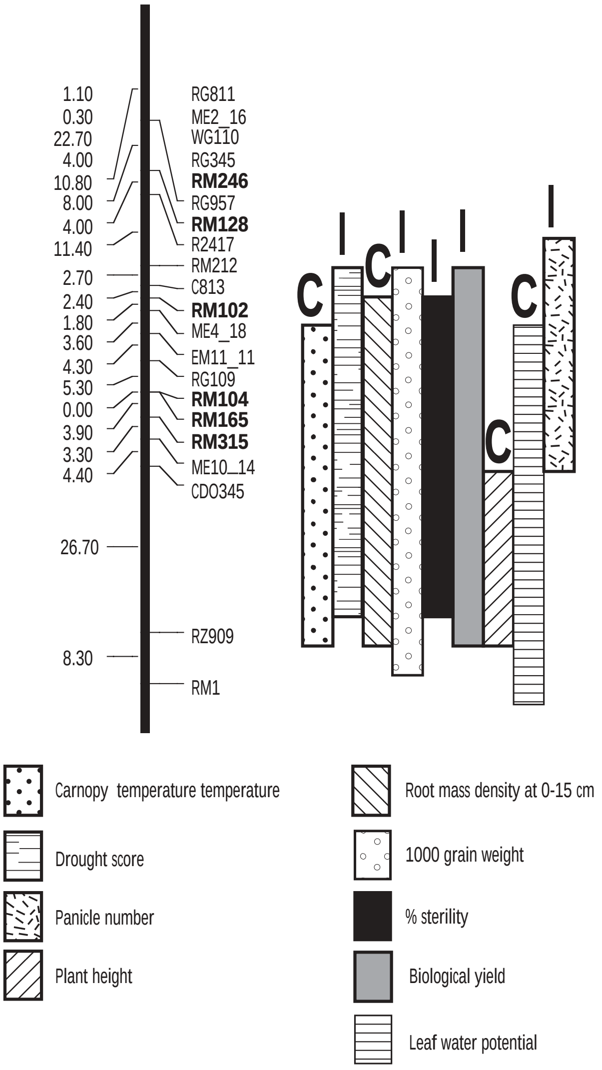

Figure 1. Portion of chromosome 1 showing the coincidence of QTL for traits associated with good performance under drought. C represents CT9993 allele and I for IR62266 allele.

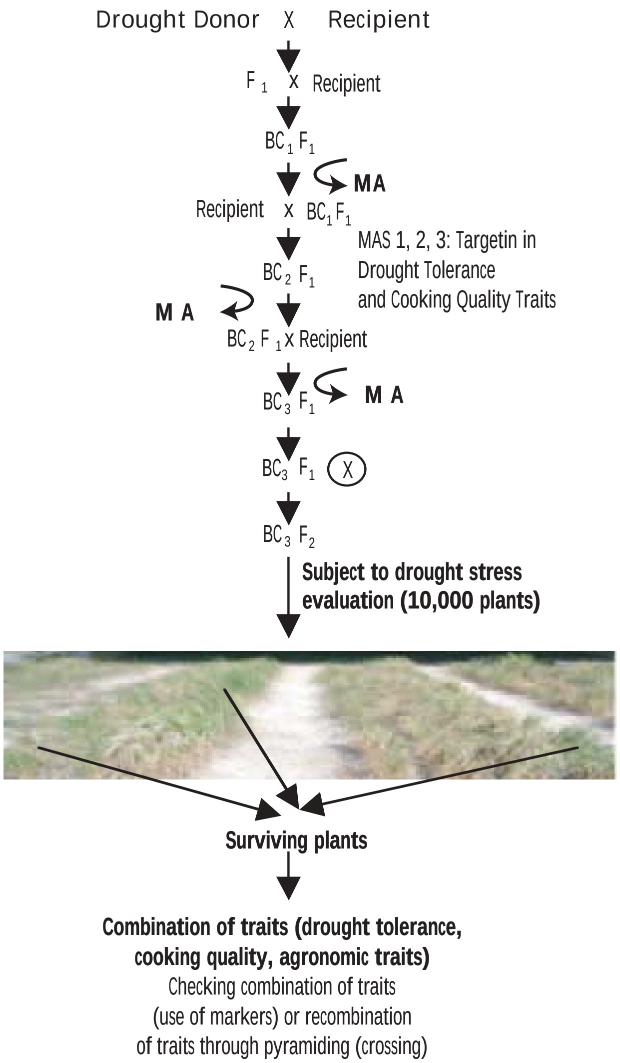

Figure 2. Breeding scheme in improving lines with drought resistance and good cooking and eating qualities.

## Results

In Thailand, work related to MAS for biotic and abiotic stresses is already being used to screen large numbers of lines in selecting genotypes containing target QTL and the cooking quality profile of recipient cultivar ' KDML105' before their yield testing and screening for adaptation to rainfed lowland environments. Here we demonstrated the breeding program to improve blast resistance in the traditional cultivar 'KDML105' by using MAS and phenotypic selection. Table 1 shows the performance of new rice lines, which were selected, based on DNA markers, for blast resistance and cooking quality in an early backcross generation, and phenotypic selection for agronomic traits in later generation. This work is being used as a "proof of concept' to implement MAS for the Mekong Regional Project. The approach will use MAS for quality and drought screening and phenotyping for drought tolerant traits.

**Table 1. Neck blast and agronomic performance of backcross introgressed lines (BC3F2) selected for blast resistance and cooking quality by meant of marker asiisted selection in an early generation**

| Pedigree | Blast Chr1 | QTL Chr2 | allele Chr4 | Neck Blast infection (%) | Amylose content (%) | score Aroma | Day of flo (days) | Plant height (cm) | No. of Tiller / plant | No. of Panicles / plant | Sterility (%) | Grain Weight (kg / ha) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IR77955-32-66 | IR | KD | IR | 0 | 17.18 | 2 | 70.67 | 119.67 | 9 | 9 | 9.29 | 4,35 |
| IR77955-25-19 | IR | HI | R2 | 2 | 15.54 | 3 | 71.33 | 72.27 | 19 | 18 | 12.00 | 3,43 |
| IR77955-27-12 | IR | KD | IR | 3 | 18.65 | 3 | 68.33 | 115.07 | 14 | 13 | 9.36 | 4,58 |
| R7I7955-12-8 | IR | HI | R | 3 | 18.46 | 2 | 70.00 | 108.20 | 13 | 13 | 8.62 | 3,78 |
| IR77955-18-11 | IR | HI | R | 3 | 16.73 | 2 | 68.33 | 104.40 | 14 | 14 | 11.10 | 3,55 |
| R7I7955-5-89 | IR | HI | R | 3 | 18.01 | 2 | 67.50 | 110.27 | 14 | 14 | 9.43 | 3,75 |
| KDML105 | KD | KD | KD | 33 | 16.00 | 3 | 66.00 | 111.47 | 11 | 10 | 14.61 | 3,74 |
| PTT (check1) | - | - | - | 0 | -- | -- | - | 83.13 | 21 | 20 | 11.20 | 4,52 |
| SP1 (check2) | - | - | - | 0 | -- | -- | - | 104.27 | 11 | 11.00 | 14.45 | 4,52 |

IR, H and KD stands for IR68835, heterozygous and KDML alleles respectively

[a]— fragrance score (2- moderate fragrance and 3- strong fragrance).

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Rerkasem, B. www.rockmekong.org/pubs/social/11_benjavan.PDF=

# Association mapping of loci for drought tolerance and non-target traits using introgression lines in rice

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Drought is the single largest factor limiting rice yield in some 19 million hectares of upland rice and 14 million hectares of rainfed lowland areas of Asia. To develop drought tolerant (DT) rice cultivars, a large backcross (BC) breeding program was initiated at the International Rice Research Institute (IRRI), which has led to development of large numbers of DT introgression lines (ILs). To identify DT QTLs and characterize the linkage drag associated with introgressed donor segments in the selected ILs, more than 300 $BC_2F_5$ ILs in two genetic backgrounds (IR64 and Teqing) were phenotyped for several non-targeted traits (grain yield and components, sheath blight tolerance, and seedling anaerobic tolerance), and genotyped with more than 200 well-distributed simple repeat sequence (SSR) markers (Svetlana Temnykh, et al., 2001). Both linkage and linkage disequilibrium (LD) analyses were performed to identify QTLs associated with DT and non-target traits.

Chi-square tests on individual markers indicated that frequencies of the introgressed donor segments in the upland selected ILs fit well to the expectations, but those selected from the lowland stress showed an excessive introgression (Table 1). A large number of loci showed excessive introgression in the selected DT ILs, 17 of which were highly significant (Table 2), revealed by their strong associations with drought tolerance. Regression analyses also detected many loci associated with non-targeted traits.

Our results indicated that LD is a powerful and efficient method to identify genes / QTL for DT in the selected Ils, and genetic drag, arising from either linkage or epistasis, were quite common, although in most cases, the introgressed donor segments appeared to be randomly associated with non-target traits. The advantages of using selective introgression for development of DT rice cultivars and in detecting QTLs for the target trait (DT) will be discussed.

**Table 1. Introgression of donor segments (based on SSR markers) of the selected (surviving) plants from 7 $BC_2F_2$ populations under the lowland (LL) and upland (UL) drought conditions**

| Cross Name | Stress | Number of surviving plants | Introgression Freq. Mean | SD | Max |
|---|---|---|---|---|---|
| Babaomi x IR64 | LL | 8 | 0.1691 | 0.1887 | 0.7143 |
| Babaomi x IR64 | UL | 10 | 0.1014 | 0.1592 | 0.7000 |
| Babaomi x TQ | LL | 10 | 0.1327 | 0.1709 | 0.8000 |
| Tarom Molaii x IR64 | LL | 8 | 0.2311 | 0.2115 | 0.8750 |
| Tarom Molaii x IR64 | UL | 19 | 0.1732 | 0.1628 | 0.9737 |
| Tarom Molaii x TQ | LL | 8 | 0.2416 | 0.1877 | 1.0000 |
| Tarom Molaii x TQ | UL | 3 | 0.0775 | 0.1905 | 1.0000 |

* The population size of the $BC_2F_2$ progeny from each of the crosses was 200.

**Table 2. Seventeen important loci associated with drought tolerance (excessive introgression) detected in 206 introgression lines from 6 $BC_2F_2$ populations selected under the lowland (LL) and upland (UL) drought conditions**

| Marker | Chromosome | Cross Name | Stress* | N | Intro. freq. | $X^2$ |
|---|---|---|---|---|---|---|
| RM283 | 1 | Basmati x Teqing | LL | 12 | 0.75 | 37.5 |
| RM274 | 5 | Basmati x Teqing | LL | 12 | 0.79 | 42.7 |
| RM505 | 7 | Basmati x Teqing | LL | 12 | 0.83 | 48.2 |
| RM332 | 11 | Basmati x Teqing | LL | 12 | 0.75 | 37.5 |
| RM472 | 1 | Bg 300 x IR64 | LL | 16 | 1.00 | 98.0 |
| RM208 | 2 | Bg 300 x IR64 | LL | 16 | 1.00 | 98.0 |
| RM293 | 3 | Bg 300 x IR64 | LL | 16 | 0.94 | 84.5 |
| RM227 | 3 | Bg 300 x IR64 | LL | 16 | 0.66 | 36.1 |
| RM248 | 7 | Bg 300 x IR64 | LL | 16 | 0.84 | 66.1 |
| RM479 | 11 | Bg 300 x IR64 | LL | 16 | 1.00 | 98.0 |
| RM512 | 12 | Bg 300 x IR64 | LL | 16 | 0.94 | 84.5 |
| RM230 | 8 | Bg 304 x IR64 | LL | 6 | 1.00 | 36.8 |
| RM286 | 11 | Bg 304 x IR64 | UL | 12 | 0.75 | 37.5 |
| RM7 | 3 | Tarom Molaii x Teqing | LL | 10 | 0.80 | 36.5 |
| RM314 | 6 | Tarom Molaii x IR64 | LL | 8 | 0.88 | 36.0 |
| RM242 | 9 | Bg 300 x Teqing | LL | 10 | 0.90 | 48.1 |

* N is the number of introgression lines selected. $X^2_{0.05} = 3.84$, $X^2_{0.01} = 6.76$, $X^2_{0.001} = 10.86$, and $X^2_{0.0001} = 15.50$.

# Annex A: Program

## SUNDAY, MAY 23

All Participants arriving by air to the Workshop will be met at the Mexico City airport by World Travel-BTI representatives

| | | |
|---|---|---|
| **08:00+** | Check-In at Hotel Camino Real Suymiya, Cuernavaca | Lobby |
| **12:30-14:00** | Lunch (pre-set menu) | Jardin Laureles |
| **14:00-18:30** | Registration | Jardin Ave Fenix |
| **18:30-19:30** | Welcome Cocktails | Jardin Bugambilias |
| **19:30-21:00** | Dinner | Jardin Laureles |

## MONDAY, MAY 24

| | | |
|---|---|---|
| **07:00-08:30** | Breakfast | Jardin Laureles |
| ***Opening Session - J. O’Toole, Chair*** | | Salon Crisatemos |
| **08:30-09:30** | Welcome and Workshop Objectives, J. O’Toole<br>Opening Remarks: The Rockefeller Foundation, G. Conway, President<br>Opening Remarks: CIMMYT, M. Iwanaga, Director General<br>Workshop Structure and Logistics, D. Hoisington | |
| **09:30-10:15** | Selected Lecture (M. Bänziger) Breeding for improved drought tolerance in maize adapted to Southern Africa | |
| **10:15-10:45** | Group Photograph | Kabuki Theater |
| **10:45-11:00** | Coffee Break | OutsideTerrace |
| **11:00-11:45** | Selected Lecture (Q. Zhang) Genetic and molecular understanding of drought tolerance for the improvement of irrigated rice under drought stressed conditions in central and southern China | |

***Session I. Farmer participatory breeding and economic studies - M. Bänziger, Chair***

| | | |
|---|---|---|
| **11:45-12:15** | Key Lecture (D. Virk) An international partnership for the breeding and delivery of drought tolerant rice varieties by market oriented plant breeding and marker assisted selection | |
| **12:15-12:45** | Key Lecture (S. Pandey) Economic costs of drought and rice farmers coping mechanisms: A synthesis of cross country comparative analysis | |
| **12:45-13:00** | Questions and Discussion | |
| **13:00-14:00** | Lunch | Arboledas, Sumiya |
| **14:00-14:20** | (S. Ding) Rice seed systems in Southern China: views from institutions and farmers | |

| | | |
|---|---|---|
| **14:20-14:40** | (S. Mahendran) Farmers participatory plant breeding technique - effective tool for the early selection and adoption of rice varieties in rainfed rice ecosystem | |
| **14:40-15:00** | (A. Prasad) Development and dissemination of drought tolerant rice varieties through on-farm, farmer-oriented approaches | |
| **15:00-15:20** | (C. Ramasamy) Drought and cropping pattern change in Tamil Nadu, India - needed technological transformation in rice farming | |
| **15:20-15:40** | (K. Selvaraj) Varietal adoption and farmers coping strategies in rainfed rice ecosystems of Tamil Nadu, India | |
| **15:40-16:00** | Questions and Discussion | |
| **16:00-16:30** | Refreshment Break | Outside Terrrace |

***Session II. Gene discovery and novel approaches - Q. Zhang, Chair***

| | | |
|---|---|---|
| **16:30-17:00** | Key Lecture (A. Reddy) Development of functional markers for drought tolerance in rice: Identification and validation of candidate genes and SNPs | |
| **17:00-17:20** | (R. Bruskiewich) Empowering rice drought gene discovery activities through bioinformatics | |
| **17:20-17.40** | (L Comai) Tilling and ecotilling: mutation and polymorphism discovery for crop improvement | |
| **17:40-18:00** | (F. Feltus) Rice SNP Map between Indica and Japonica subspecies: DNA marker resolution on the kilobase scale | |
| **18:00-18:30** | Questions and Discussion | |
| **19:00-20:30** | Dinner | Jardin Laureles |
| **20:30-22:00** | Workshops I<br>1) A. Farmer and M. Sawkins: Comparative Map and Trait Viewer<br>2) D. Hodson: Geographic Information Systems (GIS) | <br>Salon Bugamvilias<br>Salon Crisantemos |

## TUESDAY, MAY 25

| | | |
|---|---|---|
| **07:00-08:30** | Breakfast | Jardin Laureles |

***Session II (continued)***

| | | |
|---|---|---|
| **08:30-08:50** | (S. Mundree) Towards the improvement of abiotic stress tolerance in maize using genes isolated from the monocotyledonous ressurection plant Xerophyta viscosa | |
| **08:50-09:10** | (B. Xiao) Genetic transformation and testing of stress responsible genes on improving drought tolerance in rice | |
| **09:10-09:30** | (A. Covarrubias) Functional analysis of plant hydrophilins | |
| **09:30-09:50** | (J. Nieto-Sotelo) Role of maize Hsp101 in thermotolerance and plant growth | |
| **09:50-10:30** | Questions and Discussion | |
| **10:30-11:00** | Coffee Break | Outside Terrace |

***Session III. Germplasm characterization and improvement - K. Fischer, Chair***

| | | |
|---|---|---|
| **11:00-11:30** | Key Lecture (J. Crossa) The importance of experiment designs and statistical analysis for genetic studies under water-limited conditions | |
| **11:30-11:50** | (G. Atlin) Heritability of mean grain yeild under reproductive-stage drought stress and correlations across stress levels in sets of selected and unselected rice lines in the Philippines, Thailand, and India: Implications for drought tolerance breeding | |
| **11:50-12:10** | (J. Betran) Breeding approaches to develop drought tolerant maize hybrids | |
| **12:10-12:30** | (P. Jeyaprakash) Screening and selection of rice lines for drought tolerance in target production environment | |
| **12:30-13:00** | Questions and Discussion | |
| **13:00-14:00** | Lunch | Arboleda, Sumiya |
| **14:00-14:20** | (K. Kaonga) Evaluation of early maturing maize hybrids for the low altitude areas of Malawi | |
| **14:20-14:40** | (K. Kitenge) Advanced evaluation yield trials of drought and low N tolerant maize varieties for mid-altitude areas of Tanzania | |
| **14:40-15:00** | (R. Lafitte) Response to direct selection for yield under reproductive stage stress in rice backcross populations | |
| **15:00-15:20** | (D. Makumbi) Diallel analysis of tropical maize inbreds under stress and optimal conditions | |
| **15:20-15:40** | (C. Muntinda) Meeting challenges of breeding for improved drought tolerance and other traits in maize in Kenya | |
| **15:40-16:00** | Questions and Discussion | |
| **16:00-16:30** | Refreshment Break | Outside Terrace |
| **16:30-18:30** | Poster Session I | Salon Orquideas<br>Salon Crisantemos<br>Outside Terrace |
| **19:00-20:30** | Dinner | Jardin Laureles |
| **20:30-22:00** | Workshops II | |
| | 1) R. Lafitte, M. Bänziger: Phenotyping under managed stress conditions | Salon Bugamvilias |
| | 2) R. Bruskiewich: Bioinformatics for drought gene discovery | Salon Crisantemos |

## WEDNESDAY, MAY 26

| | | |
|---|---|---|
| **07:00-08:30** | Breakfast | Jardin Laureles |

***Session III (continued)***

| | | |
|---|---|---|
| **08:30-08:50** | (S. Robin) Breeding for reproductive stage drought tolerance in rice - prospects and problems from experiments under natural stress conditions | |
| **08:50-09:10** | (D.N. Singh) Breeding for drought tolerance varieties of rice through participatory plant breeding for the rainfed uplands | |
| **09:10-09:30** | (F. Sito) Role of drought tolerant germplasm in increasing maize productivity in dry-prone areas of Angola | |

| | | |
|---|---|---|
| **09:30-09:50** | (M. Semon) Population structure of O. glaberrima Steud and its implications for breeding drought tolerance in cultivated rice | |
| **09:50-10:30** | Questions and Discussion | |
| **10:30-11:00** | Coffee Break | Outside Terrace |

### *Session IV. Physiological approaches - R. Lafitte, Chair*

| | | |
|---|---|---|
| **11:00-11:30** | Key Lecture (F. Tardieu) Control of leaf growth and stomatal conductance: combining genetic and ecophysiological analyses | |
| **11:30-11:50** | (W. de Milliano) A potentially new screening method for tolerance of plants against limiting growing conditions | |
| **11:50-12:10** | (S. Fukai) Improving drought resistance in rainfed rice for the Mekong region: defining target population of environments (TPE), characterizing the available water and breeding for better adaptation to the variable water supply including an overview of the project “Improving drought resistance in rainfed lowland rice for the Mekong region” | |
| **12:10-12:30** | (B. Jongdee) Improving drought resistance in rainfed rice for the Mekong region: the experience from Thailand with a focus on the use of leaf water potential (LWP) and spikelet sterility as indirect drought tolerant traits | |
| **12:30-13:00** | Questions and Discussion | |
| **13:00-14:00** | Lunch | Arboleda, Sumiya |
| **14:15-18:00** | Tours (free)<br>1) Cuernavaca historical center, Hacienda de Cortes, handicrafts market<br>2) Tepoztlan, visit convent, museum, market, stores | Buses Parking Area |
| **19:00- 23:00+** | Noche Mexicana | Jardin Laureles |

## THURSDAY, MAY 27

| | | |
|---|---|---|
| **07:00-08:30** | Breakfast | Jardin Laureles |

### *Session IV (continued)*

| | | |
|---|---|---|
| **08:30-08:50** | (R. Kumar) Evaluation of rapid drought stress protocol to predict field performance of rice under drought stress conditions | |
| **08:50-09:10** | (C. Magorokosho) Phenotypic diversity of southern Africa maize landraces | |
| **09:10-09:30** | (O. Makara) Improving drought resistance in rainfed lowland rice for the Mekong region: the experience from Cambodia and on the use of Drought Resistance Index (DRI) as an integrative drought tolerance trait | |
| **09:30-09:50** | (B. Mishra) Breeding for aerobic rice adapted to non-flood, irrigated conditions | |
| **09:50-10:10** | (G. Pantuwan) Screening method and phenotypic evaluation of rice genotypes resistance to drought in rainfed drought prone environments in north and northeast Thailand. | |
| **10:10-10:30** | Questions and Discussion | |
| **10:30-11:00** | Coffee Break | Outside Terrace |

### *Session V. QTL Identification - F. Tardieu, Chair*

| | | |
|---|---|---|
| **11:00-11:30** | Key Lecture (Z. Li) Developing high yield and drought tolerant rice cultivars and discovering the complex genetic network underlying drought tolerance in rice | |
| **11:30-11:50** | (P. Boddupalli) Integrating molecular approaches in breeding for drought tolerance in maize in India | |
| **11:50-12:10** | (R. Babu) Mapping QTL for drought tolerance in rice: comparison across environments, genetic backgrounds and validation | |
| **12:10-12:30** | (Y. Jiang) Highly efficient fine mapping of QTLs for drought tolerance using overlapping introgression lines of rice | |
| **12:30-13:00** | Questions and Discussion | |
| **13:00-14:00** | Lunch | Arboledas, Sumiya |
| **14:00-14:20** | (S. Jearakongman) Validation of QTLs for drought resistance in NILs of rice | |
| **14:20-14:40** | (L. Luo) Discovery of drought tolerant (DT) gene / QTLs and development of DT rice cultivars | |
| **14:40-15:00** | (T. Toojinda) Associated with drought tolerance at reproductive stage in rice | |
| **15:00-15:20** | (R. Tuberosa) Isogenization and characterization of root-ABA1, a major QTL affecting root traits and leaf ABA concentration in maize | |
| **15:20-15:40** | (J. Xu) Identification of QTLs for drought tolerance in a set of random introgression lines of rice | |
| **15:40-16:00** | Questions and Discussion | |
| **16:00-16:30** | Refreshment Break | Outside Terrace |
| **16:30-18:30** | Poster Session II | Salon Orquideas<br>Salon Crisantemos<br>Outside Terrace |
| **19:00-20:30** | Dinner | Jardin Laureles |
| **20:30-22:00** | Workshops III | |
| | 1) G. Atlin: Synthesis of results from screening of the CT9993 / IR62266 rice QTL mapping population | Salon Crisantemos |
| | 2) S. Hittalmani, H. Shashidhar: Bridging the gap between QTL mapping and marker-assisted selection | Salon Tulipanes |

## FRIDAY, MAY 28

| | | |
|---|---|---|
| **07:00-08:30** | Breakfast | Jardin Laureles |

### *Session VI. Marker-assisted selection - Z. Li, Chair*

| | |
|---|---|
| **08:30-09:00** | Key Lecture (J-M Ribaut) Marker-assisted selection in tropical maize based on consensus map, perspectives and limitations |
| **09:00-09:20** | (O. Ariyo) Comparison conventional and molecular-based techniques in Cassava mosaic virus resistance screening of new cassava genotypes tested in dry and humid zones of Nigeria. |

| | | |
|---|---|---|
| **09:20-09:40** | (Y. Gao) Genetic responses to strong directional selection on rice tolerance to critical stress | |
| **09:40:10:00** | (S. Hittalmani) Impact of moisture stress at different reproductive stages of plant growth on grain yeild parameters and marker assisted QTL pyramiding for root length in rice | |
| **10:00-10:30** | Questions and Discussion | |
| **10:30-11:00** | Coffee Break | Outside Terrace |
| **11:00-11:20** | (H. Shashidhar) Marker assisted farmer participatory breeding for drought resistance in rice (Oryza sativa L.) | |
| **11:20-11:40** | (T. Zheng) Association mapping of loci for drought tolerance and non target traits using introgression lines | |
| **11:40-12:00** | Questions and Discussion | |
| **12:00-13:00** | Closing Remarks | |
| **13:00-14:00** | Lunch | Arboledas, Sumiya |
| **14:00-19:00** | Posters available for viewing<br>Tours available on a personal / optional basis | Salon Orquideas |
| **19:00-20:30** | Dinner | Jardin Laureles |

**SATURDAY, MAY 29**

| | | |
|---|---|---|
| **07:00-08:30** | Breakfast | Arboledas, Sumiya |
| **07:00-12:00** | Checkout from Hotel<br>Transportation to the airport or elsewhere will be provided by World Travel-BTI | |
| **12:30-14:00** | Lunch | Arboledas, Sumiya |

## Summary of the daily program

| | **May** | **Morning Session 1 (08:30-10:30)** | **Morning Session 2 (11:00-13:00)** | | **Afternoon Session 1 (14:00-16:00)** | **Afternoon Session 2 (16:30-18:30)** | | **Evening Session (20:30-22:00)** |
|---|---|---|---|---|---|---|---|---|
| **Sat** | 22 | ARRIVALS | | | | | | |
| **Sun** | 23 | ARRIVALS / REGISTRATION | | | | | | |
| **Mon** | 24 | Opening Remarks | I | **LUNCH (13:00-14:00)** | I | II | **DINNER (19:00-20:30** | Workshop I |
| **Tue** | 25 | II | III | | III | Posters I | | Workshop II |
| **Wed** | 26 | III | IV | | **Tours** | | **Noche Mexicana 18:30-23:00)** | |
| **Thu** | 27 | IV | V | | V | Posters II | **DINNER (19:00-20:30** | Workshop III |
| **Fri** | 28 | VI | VI | | Poster Viewing | | | |
| **Sat** | 29 | DEPARTURES / SIGHTSEEING | | | | | | |

# Annex B: List of Participants

| Form of Address | Part Last Name | Part First Name | PositionTitle | Department | Organisation | Mailing Address | City | Postal Code-Aft | Stateor Province | Phone | Country | PartEmail1 |
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