Contributing Paper

Biotechnology in the Semi-Arid Tropics

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Prepared for Thematic Review IV.2:
Assessment of Irrigation Options

For further information see http://www.dams.org/

Potential for improving agricultural production through biotechnology in the semi-arid tropics

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Acronyms

Bt	*Bacillus thuringiensis*
CGIAR	Consultative Group on International Agricultural Research
DBT	Department of Biotechnology
DNA	Deoxyribonucleic acid
GMOs	Genetically modified organisms
GUS	ß-glucuronidase
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IPM	Integrated pest management
MTA	Material transfer agreement
NARS	National Agricultural Research Systems
NPT II	Neomycin phosphotransferase
PCV	Peanut clump virus
PGIP	Polygalacturinase inhibiting protein
PCR	Polymerase chain reaction

QTL Quantitative trait loci

RAPD Random amplified polymorphic DNA

RFLP Restriction fragment length polymorphism

RNA Ribonucleic acid

SSR Simple sequence repeats

Contents

This review provides an update about biotechnology research for the genetic enhancement of the most important crops grown by farmers in the semi-arid tropics. Advances in biotechnology applications to develop new crop cultivars with improved yield, drought tolerance, and pest resistance are also given in this article. This review also discusses examples of both national private and public sector in promoting new crop cultivars ensuing from applications of genetic research.

1. The semi-arid tropics

The semi-arid tropics include parts of 48 countries in the developing world: most of India, locations in south east Asia, a swathe across sub-Saharan Africa, much of southern and eastern Africa, and a few locations in Latin America, which are characterised by unpredictable weather, long dry seasons, inconsistent rainfall, and soils that are poor in nutrients. These environments provide home to one-sixth of the world's population, who are the poorest among the poor. Half of them live on less than US $ 1, and "work hard to sustain a living through daily and seasonal struggle to protect poorly endowed natural resources, conserve scarce water, improve soil fertility, and diversify crops choices" (Barghouti, 1999b). Sorghum, millet, cowpea, chickpea, pigeonpea and groundnut are among the vital crops that feed the poor people living in the semi-arid tropics, or the "home of the hungry" due to the expanding population and associated demand for food. These crops are affected by many biotic and abiotic stress factors contributing to crop losses.

Salinity and drought still remain as major abiotic stresses that pose a threat to agricultural production in many parts of the world (Altmann, 1999). Arable lands are lost annually due to desertification and salination, toxication, and mismanagement of the natural resource base for agriculture (Evans, 1998). Water is becoming a scarce resource that requires careful economic and environmental management (Barghouti, 1999a). Expansion of irrigation does not seem feasible in many countries in Asia, the Middle

East, and North Africa, where most of the available and easily accessible water resources have been already developed. Furthermore, public irrigation systems need substantial investments for rehabilitation, modernisation, operation and maintenance. Likewise, irrigated soils are affected by salting, although the yield loss estimates vary widely. Desertification may be aggravated by both over exploitation by native populations and regional climatic changes. Hence, breeding programs must rank high the development of crops with tolerance to both drought and salinity stress. The genetically complex control of these stresses in the plant genome may be facilitated through the manipulation of specific genes governing the component characteristics needed to achieve tolerance to salt or drought in plant crops.

2. The International Crops Research Institute for the Semi-Arid Tropics

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) was created in 1972 to address the challenges associated with "growing marginal crops on marginal lands with marginal resources". The challenges for agriculture in the semi-arid tropics are to: (1) improve the productivity of the subsistence crops that include coarse grains such as sorghum and millet, and food legumes such as cowpea, groundnut, and pigeonpea; (2) improve and protect the assets and resources of the poor farmers living in this agro-ecological zone; and (3) build partnerships with local organisations to enhance and protect natural resources (Barghouti, 1999b). Hence, the mission of ICRISAT is to help the developing world in the semi-arid tropics to increase farm productivity and food security, reduce poverty, and protect the environment through optimum use of natural resource endowment, through partnership-based international agricultural research, particularly with national agricultural research systems (NARS) and advanced research institutes.

ICRISAT and its partners expect that their "research for development" will lead to enhanced production of these crops and to improved management of the limited natural resources of the semi-arid tropics. To achieve its mission, ICRISAT has focused its work following a four-point mandate to (1) improve the most important crops of the semi-arid tropics and safeguard their seeds, (2) develop improved farming systems, (3) find ways to overcome agricultural constraints, and (4) collaborate with local organizations in participatory research and technology exchange with farmers.

3. Genetic enhancement for crops of the semi-arid tropics

The genetic resources available in the crops grown in the semi-arid tropics has provided the source of genes for their betterment. A few genes from wild species have been also transferred to the cultivated gene pool by interspecific hybridisation followed by embryo culture (Mallikarjuna, 1999), which overcomes the cross incompatibility that often occurs after pollination in the so-called "wide-crosses" between species. In some interspecific incompatible crosses, fertilization occurs but the embryo aborts a few days later. Hybrid plants may be obtained by preventing pod abscission and saving aborting hybrid embryos with "rescue" techniques.

An international crop improvement program, such as the one at ICRISAT, includes research projects that deal with (1) rescuing and preserving endangered crop biodiversity, (2) identifying important characteristics for resistance to biological and environmental stresses, (3) improving breeding populations with these new characteristics as a vehicle for sharing them with NARS, and last but not least (4) introducing and applying biotechnology protocols to overcome constraints unable to be tackled by conventional cross-breeding methods. These four research areas advocated above are a logical progression from an appropriate conservation, management and utilisation of plant genetic resources and the genes available in the different gene pools. This co-ordinated research should culminate into sharing of products with the stakeholders and leading generations of impact, and will contribute to the alleviation of poverty.

The crops grown in the semi-arid tropics are affected by several constraints, e.g. diseases (bacteria, fungi, viruses), insect pests, weeds such as *Striga*, and drought. The development of stress-resistant or tolerant crops has been shown to be a feasible strategy to achieve high and stable productivity in the semi-arid tropics. Biotechnology complements conventional breeding approaches to incorporate a more effective and durable resistance to these stresses, which will lead to substantial production of food crops of the poor in the semi-arid environments. Likewise, an enhanced ability to manipulate genes may help in the improvement of the crop nutritional quality, contributing, therefore, to human health and rural household food security.

4. Biotechnology and conventional cross-breeding

The new tools of molecular genetics enable researchers to understand better and faster the full potential of the genetic resources endowment of crops, to preserve this genetic heritage, and to develop improved plant materials. The identification, isolation and cloning of new genes controlling specific characteristics will also facilitate the development of a more stable, diversified germplasm with improved resistance to diseases and pests, stress tolerance, better food quality, and higher productivity. For example genes allowing a reduced crop cycle or modified plant structure will provide pathways for new cropping systems. Nonetheless, conventional cross-breeding will be still required for an appropriate testing and further transfer of these genes to the advanced breeding pools of the crop. Furthermore, seed delivery systems of improved genotypes should be in place to promote the utilisation of new cultivars, which will enhance and stabilise the agricultural production, farm income, and farm-family welfare. In brief, the new tools of biotechnology alone cannot provide the answer to genetic improvement, but they are facilitating and accelerating the pace in the development of new cultivars.

Specific characteristics to be improved and breeding materials to be targeted depend on both the productivity per unit area and inputs. **Fig. 1** shows the smallholder development trajectory from subsistence to commercial stages. We assume that (a) farmers are not homogeneous, (b) research products should help them to move along the trajectory, and (c) plant breeders should have an array of products to offer. Low input environments require a yield stabilizing technology, whereas a matching technology to achieve high yield potential should be developed for high input environments. Hence, such a moving target needs to be addressed by a heterogeneous, but dynamic moving strategy, which often changes at a given point of time. Both conventional cross-breeding and biotechnology may be used by plant breeders along this trajectory.

At ICRISAT, genetic transformation complements conventional cross-breeding, which has been a worthwhile investment as demonstrated by the results so far obtained. Nearly 300 improved plant cultivars have been released worldwide among the mandate crops (millet, sorghum, chickpea, groundnut, and pigeonpea) since the inception of ICRISAT in 1972. The impact analysis for this genetic improvement and cultivar development by ICRISAT and its partners worldwide suggests an economic benefit worth more than ten times ICRISAT's annual budget. Similarly, new cowpea cultivars developed by the International Institute of Tropical Agriculture have enhanced significantly the national yield of this crop in Nigeria (Ortiz 1998a). Despite this impressive achievement, international agricultural research centres are adopting a new paradigm in strategic germplasm research, i.e., "integrated gene management", which uses all relevant disciplines and "new science" to exploit more systematically and fully, the genetic endowment in gene banks. Such an effort will lead to intermediate genetic products that will be shared with partners worldwide.

5. The role of biotechnology in crop improvement

The management of pests and diseases affecting crops can be achieved by exploiting host plant resistance, biological control or improved crop husbandry. Genetic enhancement appears as one of the best options because it offers an easy, cheap and sustainable technology to farmers, i.e., improved cultivars. However, there are some pests and diseases for which resistance genes are lacking in the primary gene pool of the host plant for an easy gene transfer through conventional cross-breeding. Hence, genetic transformation provides a complementary means to crop breeding, especially for traits that are rare or not available in the investigated gene pool. Some transformation systems, especially for legumes have been developed in recent years. These protocols are now being adapted or improved for some of the legume crops of the semi-arid tropics (Sharma and Ortiz, 2000).

6. The controversy about transgenic crops in genetic improvement

Irrespective of the recent advances in plant biotechnology and its potential for crop improvement (Altman, 1999), some doubts and fears have been dominating the news recently. These ethical dilemmas associated to the incorporation of transgenic crops in the farming systems have divided both public and private researchers worldwide (Robinson, 1999). This was not unexpected because the adoption of a new technology has been always subjected to different views and ethical perspectives (Shiva & Moser, 1996). Nonetheless, banning of transgenic crops does not appear as a scientifically sound option because of the potential benefits derived from their utilisation by farmers, e.g. resistant or tolerant crops to abiotic and biotic stresses obtained through genetic engineering. Furthermore, biotechnology, as suggested by Serageldin (1999) “can contribute to future food security if it benefits sustainable small-farm agriculture in developing countries”.

Perhaps, public awareness of the new biotechnology for crop improvement may allow a proper discussion of its risks and benefits in the farming systems and its effects on biodiversity. As suggested earlier (Ortiz, 1998b), scientists, farmers, consumers and policy-makers should objectively assess the potential hazards of crop biotechnology in farming and food systems regarding the current situation and the likelihood that such hazards may occur. Of course, scientific honesty will be the best argument to convince people about the advantages of transgenic crops.

Researchers and their managers should be fully aware of the consequences of dealing with the concepts of genetically modified organisms. National and international standards must be followed in the development of these crops and dealing with such organisms. Researchers who are doing experiments with transgenic plants must follow appropriate recombinant-DNA safety guidelines (for example DBT, 1998a) as well as guidelines for research in transgenic plants, including other aspects such as toxicity and allergenicity of transgenic seeds, plants, and plant parts (DBT, 1998b). They may need to be adjusted to specific environments, and the modifications approved by a duly constituted Biosafety Committee with the participation of senior government officials. All dealings with import, development and use of genetically modified organisms (GMOs) must be cleared officially by the respective governments. The facilities where transgenic research takes place should comply to international standards and approved regulations, and at all stages highest safety measures should be adopted.

6.1 Private seed sector, crop breeding and plant biotechnology

The search for profit, as in any other business, attracts the interest of the private sector for investing in the seed industry. They also look for new ways of protecting these investments through intellectual property rights, patents or the like, especially if biotechnology applications are used for accelerating the genetic enhancement of crops. The private seed sector considers that plant variety protection encourages and ensures return of research investments, and serves as an economic incentive for private and public sector investments in research and development (Grewal, pers. comm. 1999). Likewise plant variety protection, as indicated by agri-business managers, helps to facilitate the transfer of technology and knowledge, and provides encouragement and assurance for plant breeders to introduce their best varieties for production and propagation. In short, the private seed sector considers that any kind of plant variety protection would attract new and improved genetic material and technology, thereby enhancing the quality and yield of breeding materials. As a consequence of the introduction of intellectual property protection, material transfer agreement (MTA) has become a routine document for exchange of genetic material.

The MTA has been adopted not only by the private seed sector but also by public research organisations. For example, the international agricultural research centres of the Consultative Group on International Agricultural Research (CGIAR), ICRISAT among them, are using MTAs for exchanging breeding materials, which are recognised by these centres and their development investors as international public goods. Likewise, MTAs are needed for the "designated" germplasm accessions held by the CGIAR gene banks in trust for the international community, as per the agreement with the Food and Agriculture Organization of the United Nations. The CGIAR's commitment to fairness gives emphasis to the needs of the rural poor and to disadvantage member of the society. Therefore, CGIAR centres recognize "the contribution of many different communities and individuals, especially of women, to the conservation and enhancement of genetic diversity of potential use for food and agriculture, and will strive to ensure they benefit from such contributions" (CGIAR, 1999).

The main objectives of these MTAs of the CGIAR are to protect the germplasm or breeding lines and associated information from ownership or intellectual property claims by the recipients of this material. Hence, MTAs ensure continued and free availability of the genetic materials from the CGIAR centres. The MTA may evolve and become part of a multilateral system that facilitates access, exchange and use of genetic resources, as well as ensures a fair and equitable share of the benefits ensuing from the commercialisation or further utilisation of these genetic materials in the development of improved cultivars.

As a part of the policy of maximising the utilisation of genetic materials for any agricultural research or breeding purposes, the centres of the CGIAR have facilitated in the last four decades the development of both public and private seed sector in many parts of the developing world. Sorghum provides a good example of such support for the seed sector in India. In 1986, 89% of the demand for sorghum breeding materials developed at ICRISAT was from the public sector, while this demand shifted to 64% for the emerging private seed sector in 1997. Nowadays, about 60% of the proprietary sorghum hybrids in India are derived from lines or segregating material that were provided by ICRISAT (Chopra, pers. comm. 1999).

It has been argued that the private sector plays a key role in, and therefore has been investing in the development of hybrid seeds for cereal crops. However, research for development in the public sector may promote the utilisation of so-called "orphan" or neglected crops. ICRISAT research on pigeonpea provides an example of this situation. Innovative research at ICRISAT challenged the assumption that hybrid seeds in farmers' fields were not achievable for food legumes, in which cross-pollination systems are difficult to develop (Saxena et al. 1996). Leguminous species exhibit low natural out-crossing and seed multiplication rates, thereby resulting costs of hybrid seed are un-economic. Nonetheless, the first pigeonpea hybrid ICPH 8 reached Indian farmers in 1991 as a result of a research partnership between the ICRISAT and Indian Council of Agricultural Research (ICRISAT, 1998). An Indian private seed company became involved in 1992 and started marketing hybrid pigeonpea seeds. The pigeonpea hybrids show a yield advantage of 25 to 35% over open-pollinated cultivars, which partially explains the high demand by farmers for hybrid seed, which has been so far exceeding the supply. After this initial success a new wave of research was launched by a public research consortium led by ICRISAT that won the funding support from a private seed company to identify and incorporate cytoplasmic male sterility, which facilitates the production of hybrid seeds.

Are hybrid crops the only option for the development of a dynamic national seed sector in the developing world? The case of the pearl millet Okashana 1 in Namibia suggests otherwise. Okashana 1 was promoted jointly by ICRISAT and Namibian NARS since the second half of the 1980s, and nowadays farmers grow this open-pollinated cultivar on almost 50% of the national pearl millet area. A 50% internal rate of return to public investment (by both ICRISAT and Government of Namibia) has been calculated (Rohrbarch et al., 1999). The net return exceeded US $ 11 million in 1998. Such a success resulted from the introduction of genetic material improved by ICRISAT breeders, early involvement of farmers in participatory selection, rapid cultivar release responding to farmers' preferences, and the commitment of the national government for high-quality seed multiplication and dissemination.

Above examples clearly show how international agricultural research and its strong links with both private and public sector lead to economic impact ensuing from the development of new cultivars, even for "orphan" crops. The establishment of public-public or private-public partnerships will enhance the potential for agricultural production enhancement through on going developments in plant biotechnology. We expect that applications of biotechnology may attract new investments in other crops grown in the semi-arid tropics, but yet research-neglected.

6.2. "Terminator" gene technology in seeds

There has been considerable publicity and controversy about "terminator" seeds since a patent was announced for this genetic protection technology in 1998. This transgenic seed technology prevents farmers from saving seeds from their harvest for subsequent utilisation as next season propagules. However, "terminator" gene technology that affects the seed germination is only one of a class of gene control or protection methods, which it is still under research and at different stages of development by private and public organisations worldwide. Patents for this gene protection technology are being sought to protect the investment required to develop these seeds. However, the potential impact of such a technology on traditional farming system s is under scrutiny by both public and private organisations. On going discussion focuses on the farmers' concerns of this gene protection technology versus the potential benefits for the seed industry, though none of these protection methods have yet moved beyond the development phase. They are not expected to be ready for commercialisation for at least within the next five years. Nonetheless, one of the largest plant biotech company, which owns a patent for this technology, has announced that it will not commercialise "terminator" seeds until an open and comprehensive debate takes place to examine the environmental, economic and social implications of this gene protection technology.

7. Advances by ICRISAT and its research partners in applied crop biotechnology for the semi-arid tropics

The staple crops of the semi-arid tropics feed tens of millions of poor people daily. However, they receive relatively little attention from the biotechnology industry, because they are not major cash commodities and grown mostly for home consumption. As suggested by Serageldin (1999), “public investment will be needed, and new and imaginative collaboration can make the gene revolution beneficial to developing countries”. ICRISAT has been, therefore, bringing and applying biotechnology developed by the advanced research institutions around the world to developing countries, thereby helping them to share the benefits of biotechnology.

7.1 Tissue culture in cereals

A new technique to develop homozygous plants of pearl millet in a single generation, known as dihaploids, has been recently adapted (ICRISAT 1999). Cultured spikelets generate double haploids from female gametes. The plants derived by using this method can set seed normally, survive, and reproduce outside the laboratory, thus shortening the breeding cycle to produce uniform lines, and accelerating the development of new cultivars. Likewise, geneticists of this crop have a new tool for identifying and isolating genes that govern specific characteristics, thereby enhancing their ability to manipulate genes and develop new cultivar with high yield, enhanced resistance to pests and diseases, and improved adaptation to targeted agro-ecozones.

More recently, plant regenerations from mesophyll-derived protoplasts of sorghum (Sairam et al., 1999), and from embryonic cell suspensions of wild sorghum (Mythilli et al., 1999) have been reported. These results may enhance the utilization of sorghum protoplasts for gene transfer.

7.2 Genetic transformation in legumes

This novel approach aims to incorporate genes conferring resistance to various constraints for which no or very low levels of resistance are available in the existing gene pools. This method overcomes the problems associated to interspecific, intergeneric or interkingdom barriers. At ICRISAT, the main efforts are on incorporating resistance genes not available in primary gene pools into existing genotypes to overcome major biotic constraints. These include viruses, insect pests and fungal pathogens. On going research includes the development of shoot regeneration by tissue culture that will be used for genetic transformation with improved efficiency. Protocols are being fine tuned to introduce cloned foreign genes through selected tissue culture systems and biolistics- or *Agrobacterium tumefaciens* -mediated gene transfer. Some examples are given below of the research in transgenic crops being undertaken at ICRISAT. As indicated earlier, transgenic plants are being grown under containment conditions at this time, and any field-testing of these transgenic plants will be attempted after obtaining permission from the concerned authority of the national government.

Transgenic plants with resistance to major biotic constraints are being developed and tested by ICRISAT and its research partners, especially for legume crops (Sharma & Ortiz, 2000). For example, they are investigating, in collaboration with scientists from Scotland (UK), the enhancement of resistance to *Botrytis* gray mold of chickpea using polygalacturinase inhibiting protein (PGIP) genes. This collaborative project has developed a preliminary system for regeneration and transformation of chickpea using *Agrobacterium tumefaciens* based binary vectors. In another collaborative project with Belgian scientists, regeneration and transformation of chickpea and pigeonpea are being investigated. This research aims to improve the nutritional status of these pulse crops by introducing genes for lysine and threonine, as well as insect and pathogen resistance using lectin genes.

In groundnut several scientists worldwide are engaged in developing tissue culture and transformation systems but they have limited success rates in terms of obtaining large numbers of transgenic plants and their progeny. ICRISAT researchers, by manipulating various factors affecting tissue culture such as culture media constituents and explants, were able to develop an efficient tissue culture system based on cotyledon explants from mature seeds where over 95% of the explants produce multiple shoots within four to six weeks. These can be recovered, rooted and transplanted to the glasshouse with high success rates (over 90%). By *using Agrobacterium tumefaciens*-based binary vectors (Sharma et al., 1993), this system can produce a large number of putative transgenic plants carrying marker genes like ß-glucuronidase (GUS) and neomycin phosphotransferase (NPT II). This system so far has worked with most of the Spanish type cultivars (http://198.93.234.24/grep/gtl.htm), and over 100 putative transformants of groundnut have been produced that express the coat protein gene of peanut clump virus (PCV-cp). Polymerase chain reaction (PCR) and Southern blot hybridisation has revealed the integration of PCV-cp in over 50% of the putative transformants. While 70% of the transformants carry a single gene, the rest carry two to in excess of eight copies of the gene when analysed in the first generation of transformed plants (or T_1). Work on their further characterisation, including resistance to PCV, is on going. This appears to be one of the first reports where such large numbers of transgenic peanut plants can be recovered. Similarly, resistance to the groundnut rosette assister luteovirus may be achieved by introducing coat protein gene and putative polymerase gene of the luteovirus.

7.3 Integrated pest management and transgenic crops

Integrated pest management (IPM) has historically placed great hopes on host plant resistance. However, conventional host plant resistance involves many genes at several chromosome sites, which makes progress slow and difficult to achieve. With the advent of genetic transformation techniques, it has become possible to insert genes into the plant genome that confer resistance to insects. Among the biological pesticides, bacteria such as *Bacillus thurigiensis* (Bt) and *B. sphearicus* have been the most successful group of organisms identified for use in pest control on a commercial scale. Transgenic plants with insecticidal genes will have a prominent role in pest management in the near future. Bt genes have now been incorporated into crops such as cotton, soybean, maize, potato, rice, broccoli, lettuce, walnut, apple, and alfalfa. Insecticidal genes such as Bt, trypsin inhibitors, lectins, ribosome inactivating proteins, secondary plant metabolites, vegetative insecticidal proteins, and small RNA viruses can also be used alone or in combination with Bt genes for pest control. At ICRISAT, such genes are presently being evaluated for their biological efficacy against sorghum shoot fly, *Atherigona soccata*, spotted stem borer, *Chilo partellus*, tobacco caterpillar, *Spodoptera litura*, and cotton bollworm/legume pod borer, *Helicoverpa armigera* (http://grep.icrisat.cgiar.org/grep/transgen.htm). Insecticidal genes as those derived from Bt and plant genes such as protease inhibitor from soybean and pigeonpea are being considered by ICRISAT researchers for transformation of pigeonpeas and chickpeas. Preliminary experiments with tobacco as a model system has already demonstrated the usefulness of Bt and soybean trypsin inhibitor in reducing the leaf feeding in transgenic plants by *Helycoverpa armigera* and *Spodoptera litura*.

The deployment of transgenic crops with insecticidal genes for pest control will lead to the reduction in insecticide sprays, an increased activity of natural enemies, and IPM of secondary pests. However, as with any other technology, the following are some problems associated with the utilization of transgenic pest resistant crops. For example, secondary pests are not controlled in the absence of sprays for the major pests, and control of secondary pests requires chemical sprays that will kill the natural enemies, and thus offsetting one of the advantages of transgenic crops. Furthermore, transgenic crops may have very high costs, and the proximity to sprayed fields will reduce the benefits of transgenic crops. Insect migration may also reduce the effectiveness of transgenic crops, and last but not the least, insects and other crop pathogens may develop resistance to the transgenes because of persistent exposure. Therefore, deployment of transgenic crops with pest resistance must be integrated into an IPM strategy, because the management of pests needs to be seen as an integral aspect of farming systems. Entomologists, breeders, and the molecular biologists need to determine how to deploy this technology for pest management, and at the same time reducing possible environmental hazards. An appropriate understanding will be needed about the insect biology and behaviour, its response to the insecticidal proteins, and temporal and spatial expression of insecticidal proteins in plants. Such knowledge will help to deploy an environmentally sound strategy for resistance management, and perhaps to determine the impact of insecticidal proteins on natural enemies and non-target organisms. Likewise, an adequate mechanism to deliver the technology to the resource poor farmers should be developed.

7.4 Diagnostic tools for, and DNA fingerprinting of pathogens

The rural poor and their livestock often consume cheap, mold-infected nuts, cereals and spices containing carcinogenic and immuno-suppressive aflatoxins, which are produced by the fungi *Aspergillus flavus* and *A. parasiticus*. In 1994, in excess of 200,000 chickens died in Andhra Pradesh (India) after eating feeds contaminated with aflatoxin. Besides endangering human health, aflatoxin contamination effectively blocks groundnut exports, a lost income opportunity for farmers of the semi-arid tropics. Environment, crop handling and genetic factors play a role in fungal development and consequent toxin production. Therefore an integrated approach is required to tackle this problem. One of the major problems has been the availability of tools for cost effective estimation of aflatoxins in food and feed because analytical protocols are expensive. Immunochemical methods offer cost effective ways to estimate aflatoxins. However, these methods are mostly available in kits from developed countries, which are prohibitively expensive at US $ 12 per sample. With the aid of biotechnology, a simple, cost-effective method was developed by ICRISAT scientists for the quantitative estimation of aflatoxin in food and feed (Thirumala Devi et al., in press). In pilot studies polyclonal and monoclonal antibodies have been produced, and they are the first step in designing practical kits to ensure safer food and feed. The indirect competitive penicillase-based ELISA test developed by ICRISAT in India can detect carcinogenic aflatoxins for a mere US $ 1 per sample, versus US $ 12 for imported test kits.

DNA fingerprinting of pathogens reveals their genetic variability (Sastry et al., 1995), so appropriate resistance can be deployed in advance. Micro-satellite markers have detected variability in host-specific pathotypes of *Sclerospora graminicola*, the pearl millet downy mildew pathogen (Thakur et al., 1999). Likewise, DNA fingerprints show variability among sexual and asexual progenies of this fungus, whose genome was recently characterised (Sastry et al., 1997). Similar research with random amplified polymorphic DNA (RAPD) has shown promise in the sorghum anthracnose pathogen (Thakur et al., 1998). This understanding of "the enemy" permits pre-empting breakdowns of plant resistance to diseases.

7.5 DNA markers and gene cloning

Molecular markers help unravel patterns of diversity in crops and their wild relatives. DNA markers are used to evaluate the genetic variation in gene banks, as well as to identify phylogenetic and molecular structure of crops and their associated wild species. One of the early methods for molecular marking is called restriction fragment length polymorphisms (RFLP) that are obtained after digesting DNA with special “cutters” called restriction enzymes. Micro-satellites or simple sequence repeats (SSR) are tandemly repeated DNA with a very short repeat unit, which are new powerful genetic markers in plants because they seem to be more variable than early known DNA markers.

These SSR have assisted in the assessment of genetic diversity within and among a sample of germplasm accessions included in the world collection of cultivated accessions held at ICRISAT's gene bank (Dje et al., 1999). The results suggest that sorghum races are not substantially distinct genetically, which challenges the system of racial classification of Harlan and De Wet (1972) based on morphological descriptors. Similarly, SSR analysis confirmed the lack of correlation between geographical origin of accessions and their genetic distance. Hence, the germplasm collection of sorghum is highly structured genetically with about 70% of the genetic diversity occurring among accessions (Dje et al., 1999). Such information provides a means to optimise the sampling strategy, and save resources for preservation of genetic resources. For example, accessions from a geographical area or race showing the highest genetic diversity will receive priority for conservation.

Mitochondrial DNA-RFLP analysis has also distinguished new cytoplasmic male sterility sources in pearl millet (Chhabra et al., 1998; Rajeshwari et al., 1994; Sujata et al., 1994), sorghum (Sivaramakrishnan et al., 1997a), and pigeonpea (Sivaramakrishnan et al., 1997b). As a consequence of these advances in molecular biology broad-base gene pools with valuable new characteristics will be developed and shared for the benefit of farmers in modern agriculture. Likewise, DNA markers are useful tools to confirm gene introgression after interspecific hybridisation facilitated by embryo rescue techniques.

Molecular markers are also important for investigating gene flow between crops and their wild species. ICRISAT has initiated the development of a model for gene flow in pigeonpea, using DNA markers, to understand the risk of genetic erosion as well as to have a tool for risk assessment when transgenic plants of this crop become available for field-testing.

Molecular-assisted genetic analysis provides a means to locate and select genes controlling important agronomic, disease-resistance, and food quality traits. An RFLP-based map of pearl millet was developed together by scientists from UK and ICRISAT (Liu et al., 1994). This new tool was applied to enhance the genetic knowledge about this crop (Busso et al. 1995) for further improvement (McGaw, 1998). ICRISAT scientists with the collaboration of their research partners worldwide are also developing molecular marker maps for other crops such as chickpea (Kumar & Muehlbauer, 1999), groundnut, and sorghum.

One of the first characteristics to be mapped by ICRISAT scientists was downy mildew resistance in pearl millet (Jones et al., 1995). As a result of this endeavour, quantitative trait loci (QTL) for resistance to this disease were transferred, using marker-assisted selection, into an elite line, which is a parent to pearl millet hybrids grown over one million hectares in India. The declining resistance to the dreaded downy mildew disease in the parent was boosted by adding new complementary resistance using molecular marker-aided backcross. The incorporation of this resistance does not only extend the useful life of the line, thereby stabilising pearl millet production, but also adds significant economic returns to past investments in national and international breeding research. Likewise, DNA marker-assisted breeding allows a new gene resistance deployment strategy by gene pyramiding in 3-way hybrids and synthetic parent populations of cereal crops such as pearl millet (Witcombe & Hash, in press).

Plant defence responses to a range of pest attacks are correspondingly diverse. ICRISAT researchers are learning the "genetic language" of this pest resistance and putting it to use. For example, sections of distinct resistance genes are structurally and functionally similar, with nearly identical chunks of protein sequences. ICRISAT scientists have been investigating whether a particular DNA segment contains the so far elusive resistance gene to downy mildew resistance in pearl millet. A new synthetic gene may be developed by stitching together sequences from different naturally occurring genes, thereby obtaining multiple resistance to the pathogen. This approach may lead to improved disease management and more durable broad-base resistance. Furthermore, similarity of resistance gene sequences among species allows the identification of resistance gene candidates, which may eventually be transferred to other crops.

DNA markers are also playing an important role in the genetic manipulation to improve digestibility and feed quality of sorghum and millet crop residues. Ex-ante impact assessment shows that a mere increase of 1% in stover digestibility results in US $ 42 to 208 million, depending on adoption rates increasing from 10% upwards (Kristjanson & Zerbini, 1999). Predicted rates of return to research investment vary from 28 to 43%, with corresponding benefit:cost ratios of 15:1 to 69:1, respectively. Researchers from ICRISAT and the International Livestock Research Institute, and together with other research partners are making significant progress to map QTL controlling the characteristics for stover quality in pearl millet. After these QTL are mapped, pearl millet breeders will apply marker-assisted selection to develop new populations with the desired stover characteristics.

Positions of useful genes in target crops can be inferred by cross-referencing to the maps of other popular crops -raising the possibility of eventual inter-generic transfers (Lee, 1998). Owing to this gene synteny, the extreme drought resistance of pearl millet may contribute some day to achieve more hardy and water efficient sorghum, maize, rice and wheat. ICRISAT and Indian scientists are exploring the synteny among cereal crops to identify QTL governing crop response to drought, which are common to rice and sorghum. Syntenic regions between rice and sorghum genomes were determined with RFLP markers previously mapped in rice (Nagamura et al., 1999). The resulting map of sorghum consists of 607 DNA markers covering 1285 cM. Comparative mapping revealed a high degree of colinearity between the 12 rice chromosomes and 10 sorghum chromosomes.

ICRISAT scientists and their research partners have designed experiments across a range of environments to simulate different types of drought-stress encountered by the crops. These researchers expect in the mid-term to identify and characterise useful genomic regions conferring drought tolerance to cereals. The common regions relevant to drought tolerance will be further saturated and annotated. Additionally, appropriate test materials will be chosen to assess the relevance of these genomic regions in each of the targeted cereal crops in managed stress environments. In the long-term, this approach should lead to the isolation and characterisation of candidate genes for drought tolerance because the ordering of DNA loci between rice and sorghum chromosomes corresponds well (Nagamura et al., 1999). This conservation of gene order between both genomes permits that genes located in sorghum are isolated by map-based cloning at the homologue in rice, and then by homology in sorghum.

8. Conclusion

Gains in crop productivity through research advances in genetic enhancement will help to achieve sustainable food security, poverty alleviation, and environmental protection in the semi-arid tropics. Hence, ICRISAT has a commitment to transfer the benefits of plant biotechnology to the developing world, as genes, both relevant to crop improvement and environmentally safe, become available. Furthermore, the application of the tools of biotechnology offers a new means to achieve ICRISAT's mission, i.e., applying science to improve agriculture in areas of the world where rainfall and biotic stresses are the major constraints for increasing and stabilising crop productivity.

9. Acknowledgements

Biotechnology research at ICRISAT has benefited from research partnerships with scientists from advanced research institutes and NARS, and it is made possible through the funding support provided by the development investors of the CGIAR.

10. References

Altman, A. 1999. "Plant biotechnology in the 21st century: the challenges ahead", in Electronic Journal of Biotechnology Vol. 2 (2): http://www.ejb.org

Barghouti, S.M. 1999a. "Growing challenge to water resources management", in Sustainable Development International Launch Edition: 43-52.

Barghouti, S.M. 1999b. "Enhancing natural assets in less favourable areas - the case of the semi-arid tropics", in Sustainable Development International Launch Edition: 127-130.

Busso, C.S., Liu, C.J., Hash, C.T., Witcombe, J.R., Devos, K.M., de Wet, J.M.J. & Gale, M.D. 1995. "Analysis of recombination rate in female and male gametogenesis in pearl millet (*Pennisetum glaucum*) using RFLP markers", in Theoretical and Applied Genetics 90: 242-6.

CGIAR (Consultative Group on International Agricultural Research). 1999. CGIAR Center Statements on Genetic Resources, Intellectual Property Rights, and Biotechnology, Mexico: CIMMYT for the CGIAR Centers Directors Committee and Center Board Chairs Committee.

Chhabra, A.K., Rai, K.N., Khalrwal, I.S., Sivaramakrishnan, S. & Hash C.T. 1999. "Mitochondrial DNA-RFLP analysis distinguishes new CMS sources in pearl millet (*Pennisetum glaucum* (L.) R. Br.)", in J. Plant Biochemistry & Biotechnology Vol. 7: 85-92.

Chopra, K.R. Director Research and Production, Mahendra Hybrid Seeds Co. Ltd., Jalna, India. Personal communication with senior author. 15 September 1999.

DBT (Department of Biotechnology). 1998a. Recombinant DNA safety guidelines, New Delhi: Department of Biotechnology, Ministry of Science and Technology, Government of India.

DBT. 1998b. Revised guidelines for research in transgenic plants & guidelines for toxicity and allergenicity evaluation of transgenic seeds, plants and plant parts, New Delhi: Department of Biotechnology, Ministry of Science and Technology, Government of India.

Dje, Y., Hoertz, M., Lefebre, C. & Vekemans, X. 1999. “Assessment of genetic diversity within and among germplasm accessions in cultivated sorghum using microsatellite markers”, in Theoretical and Applied Genetics Vol. 99: in press.

Evans, L.T. 1999. Feeding the ten billion, Cambridge, UK: Cambridge University Press.

Grewal, H.S. SEA Regional Director, Pioneer Hi-Bred International, USA. Personal communication with senior author. 15 September 1999.

Harlan, J.R. & De Wet, J.M.J. 1972. “A simplified classification of cultivated sorghum” in Crop Science Vol. 12:172-6.

ICRISAT. 1998. From Orphan Crop to Pacesetter: Pigeonpea Improvement at ICRISAT - Winner of the 1998 CGIAR King Baudouin Award, Pathancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

ICRISAT. 1999. Building Tomorrow Together, Pathancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Jones, E.S., Liu, C.J., Gale, M.D., Hash, C.T. & Witcombe, J.R. 1995. "Mapping quantitative trait loci for downy mildew resistance in pearl millet", in Theoretical and Applied Genetics Vol. 91: 448-456.

Kristjanson, P.M. & Zerbini, E. 1999. “Genetic enhancement of sorghum and millet residues fed to ruminants – an ex ante assessment of returns to research”, in International Livestock Research Institute Impact Assessment Series Vol. 3.

Kumar, J. & Muelhbauer, F.J. 1999 "A proposal for a directory of recombinant inbred lines for chickpea genomic mapping", in International Chickpea and Pigeonpea Newsletter Vol. 6:3.

Lee, M. 1998. "Genome projects and gene pools: new germplasm for plant breeding?", in Proceedings of the National Academy of Sciences USA Vol. 95: 2001-4.

Liu, C.J., Witcombe, J.R., Pittaway, T.S., Nash, M., Hash, C.T., Busso, C.S., Gale, M.D. 1994. "An RFLP-based genetic map of pearl millet (*Pennisetum glaucum*)", in Theoretical and Applied Genetics Vol. 89: 481-7.

Mallikarjuna, N. 1999. "Ovule and embryo culture to obtain hybrids from interspecific incompatible pollinations in chickpea", in Euphytica Vol. 110: 1-6.

McGaw, E.M. 1998. Tempest in a test tube - making miracles with millet molecules, Patancheru, Andhra Pradesh, India: ICRISAT-DFID (Department for International Development, UK).

Mythilli, P.K., Seetharama, N., Reddy, V.D. 1999. "Plant regeneration from embryonic cell suspension cultures of wild sorghum (*Sorghum dimidiatum*)", in Theoretical and Applied Genetics Vol. 18: 424-8.

Nagamura, Y., Tanaka, T., Nozawa, H., Kidai, H., Kasuga, S. & Sasaki, T. 1999. “Syntenic regions between rice and sorghum genomes”, in Japan Natural Grassland Research Institute Working Report 1998 Vol. 9: 97-103.

Ortiz, R. 1998a. "Cowpeas from Nigeria: a silent food revolution", in Outlook on Agriculture Vol. 27: 125-8.

Ortiz, R. 1998b. "Critical role of plant biotechnology for the genetic improvement of food crops: perspectives for the next millennium", in Electronic Journal of Biotechnology Vol. 1 (3): http://www.ejb.org

Rajeshwari, R., Sivaramakrishnan, S., Smith, R.L. & Subrahmanyam, N.C. 1994. "RFLP analysis of mitochondrial DNA from cytoplasmic male-sterile lines of pearl millet", in Theoretical and Applied Genetics Vol. 88: 441-8.

Robinson, J. 1999. "Ethics and transgenic crops: a review", in Electronic Journal of Biotechnology Vol. 2 (2): http://www.ejb.org

Rohrbach, D.D., Lechner, W.R., Ipinge, S.A. & Monyo, E.S. 1999. "Impact from investments in crop breeding: the case of Okashana 1 in Namibia", in International Crops Research Institute for the Semi-Arid Tropics Impact Series Vol. 4.

Sairam. R.V., Seetharama, N., Devi, P.S., Verma, A., Murthy, U.R. & Potrykus, I. 1999. "Culture and regeneration of mesophyll-derived protoplasts of sorghum [*Sorghum bicolorI* (L.) Moench.]", in Plant Cell Reports Vol. 18: 972-7.

Sastry, J.G., Ramakrishna, W., Sivaramakrishnan, S., Thakur, R.P., Gupta, V.S. & Ranjekar, P.K. 1995. "DNA fingerprinting detects genetic variability in the pearl millet downy mildew pathogen (*Sclerospora graminicola*)", in Theoretical and Applied Genetics Vol. 91: 856-861.

Sastry, J.G., Sivaramakrishnan, S., Thakur, R.P., Gupta, V.S. & Ranjekar, P.K. 1997. "Characterization of the genome of *Sclerospora graminicola*, the causal fungus of downy mildew of pearl millet", in World Journal of Microbiology & Biotechnology Vol. 13: 693-7.

Saxena, K.B., Chauhan, Y.S., Laxman Singh, Kumar, R.V. & Johansen, C. 1996. "Research and development of hybrid pigeonpea", in International Crops Research Institute for the Semi-Arid Tropics Research Bulletin Vol. 19.

Serageldin, I. 1999. "Biotechnology and food security in the 21st century", in Science Vol. 285: 387-389.

Sharma, K.K., Anjaiah, V. & Moss, J.P. 1993. "Production of transgenic plants of groundnuts by *Agrobacterium*-mediated genetic transformation", in International Arachis Newsletter Vol. 13: 23-25.

Sharma, K.K. & Ortiz, R. 2000. "Program for the genetic transformation for crop improvement in the semi-arid tropics", in In Vitro Cellular and Development Biology - Plant Vol. 36: in press.

Shiva, V. & Moser, I. (eds.). 1996. Biopolitics, Madras: Orient Longman.

Sivaramakrishnan, S., Seetha, K. & Reddy B.V.S. 1997a. "Characterization of the A_4 cytoplasmic male-sterile lines of sorghum using RFLP of mtDNA", in Euphytica Vol. 93:301-5.

Sivaramakrishnan, S., Seetha K., Nageshwar Rao, A. & Laxman Singh. 1997b. "RFLP analysis of cytoplasmic male-sterile lines of pigeonpea [*Cajanus cajan* (L.) Millsp.] developed by interspecific crosses", in Euphytica Vol. 93: 307-312.

Sujata, V., Sivaramakrishnan, S., Rai, K.N. & Seetha, K. 1994. "A new source of cytoplasmic male sterility in pearl millet: RFLP analysis of mitochondrial DNA". In Genome Vol. 37: 482-486.

Thakur, R.P., Mathur, K., Rao, V.P., Chandra, S., Shivramkrishnan, S., Kannan S., Hiremath, R.V., Tailor, H.C., Kushwaha, U.S., Dwivedi, R.R., and Indira, S. 1998. "Pathogenic and genetic characterization of six Indian populations of *Colletotrichum sublineolum*, the causal agent of sorghum anthractnose", in Indian Phytopathology Vol. 51: 338-348.

Thakur, R.P., Rao, V.P., Sastry, J.G., Sivaramakrishnan S., Amruthesh, K.N. & Barbind, L.D. 1999. "Evidence for a new virulent pathotype of *Sclerospora graminicola* on pearl millet", in J. Mycol. Pl. Pathol. Vol. 29: 61-9.

Thirumala Devi, K., Mayo, M.A., Reddy, K.L.N., Delfosse, P., Gopal Reddy, Reddy, S.V. & Reddy, D.V.R. In press. "Production and characterization of monoclonal antibodies for aflatoxin B1", in Letters of Applied Microbiology.

Witcombe, J.R. & Hash, C.T. In press. "Resistance gene deployment strategies in cereal hybrids using marker-assisted selection: gene pyramiding, three-way hybrids, and synthetic parent populations", in Euphytica.

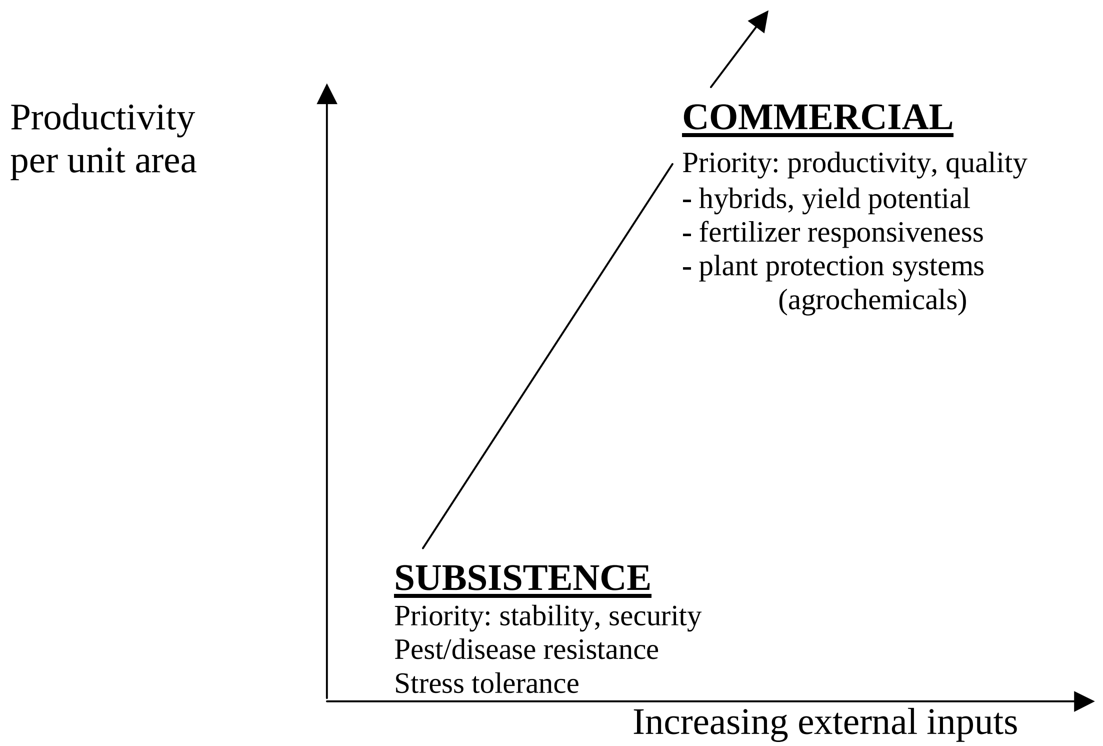

Fig. 1. Smallholder development trajectory