Sivakumar MVJ, Kenard C, Bationo A, Powell JM, Weber J and Goli AE. 1994. Inter-center collaboration at the ICRISAT Sahelian Center. Research Bulletin no. 18. Patancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

Thompson A and Metz M. 1997. Implications for economic-policy for food security. Training Manuals for Agricultural Planning No. 40. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO).

Pearl Millet Molecular Marker Research

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Accumulating Stores of Knowledge: Disease Resistance QTLs in Pearl Millet

The slow magic of agricultural research relies on accumulation of knowledge that future generations of scientists can use. New technologies, such as molecular markers, are expensive to develop, and returns from the research take a long time. However, once the knowledge reaches a critical level, gains accelerate and provide a deeper, more flexible, resource. Research on molecular markers to aid in breeding pearl millet [*Pennisetum glaucum* (L.) R. Br.] for resistance to the downy mildew pathogen *Sclerospora graminicola* (Sacc.) J. Schrot., which has been largely funded by the Plant Sciences Research Programme of the UK's Department for International Development (DFID), provides a classic example.

In 1990, there were no pearl millet molecular markers, no marker-based genetic linkage map, and, of course, traits could not be linked to marker locus positions on a non-existent map. By 2001, hundreds of pearl millet molecular markers had been created (Liu et al. 1994; Allouis et al. 2001; Qi et al. 2001), detailed marker-based genetic linkage maps produced (Liu et al. 1994 and 1996; Devos et al. 2000), and using those maps, genomic positions of quantitative trait loci (QTLs) for pearl millet downy mildew resistance flagged (Jones et al. 1995 and 2002; Azhaguvel 2001; Kolesnikova-Allen 2001; Breese et al. 2002; Hash et al. unpublished). What is surprising, even to the researchers involved, is how many genomic regions contributing to downy mildew resistance have already been identified (Fig. 1, see pages 9-12).

Not only have QTLs for downy mildew resistance been mapped, but genotypes produced or tested in the mapping studies have provided a valuable genetic resource. They are donors of naturally occurring host-plant resistance genes, and a well-chosen set of lines can differentiate among many different populations of the causal agent of pearl millet downy mildew. All of this new information allows breeders, both now and in future decades of pearl millet breeding, to incorporate and pyramid resistance genes into cultivars grown by resource-poor farmers. The information is widely available and there is little risk of its loss. The genetic resources that have been generated are more fragile, but by appropriate storage and distribution this crop germplasm can also be a long-term resource for plant breeding.

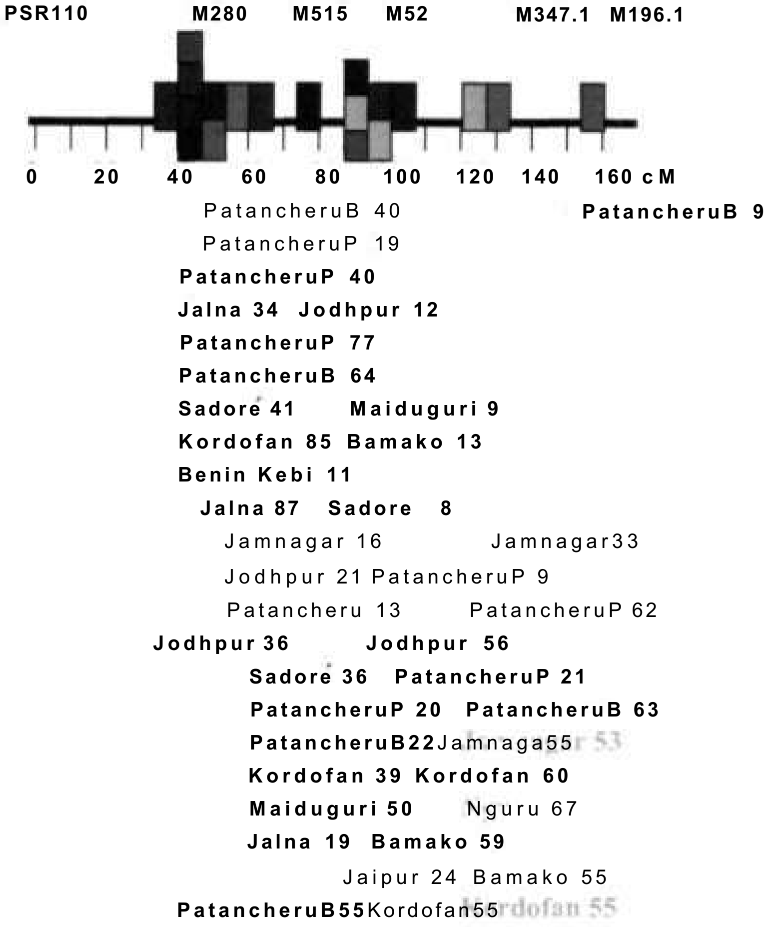
Such accumulated knowledge contributes to the speed of cumulative gains that plant breeding can make. The extent of this knowledge provides flexibility (different gene deployment strategies), depth (many genes for potential pyramiding), and breadth (many geographical targets).

We describe below the application of these stores of knowledge to the specific improvement of pearl millet hybrid HHB 67, which is only grown in India. Although HHB 67 is, in practice, a public good for India, the knowledge generated in its improvement is an international public good.

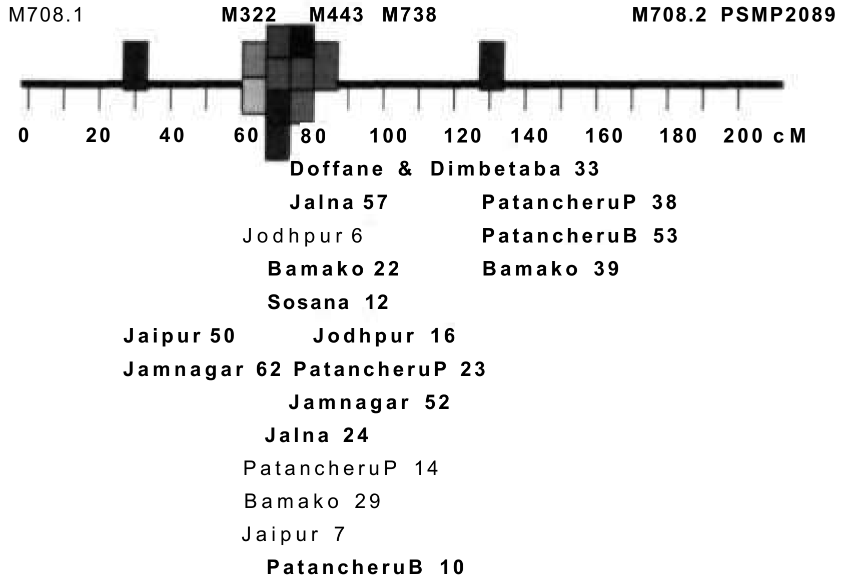
Figure 1 (see pages 9-12 of this issue). Distribution of pearl millet downy mildew resistance QTLs across the seven linkage groups of a consensus skeleton linkage map of the pearl millet genome. The letter/number combinations above each linkage group are abbreviated names of anchor loci on the RFLP-based pearl millet consensus map. The approximate length of each linkage group is indicated in Haldanc centiMorgans. Each linkage group corresponds to a pearl millet chromosome pair. QTLs for downy mildew resistance have been mapped to all seven pearl millet linkage groups. Colored blocks indicate approximate genomic positions of downy mildew resistance QTLs; the color of the block indicates the parental line mat contributed resistance mapping that position in the pearl millet genome. Pearl millet downy mildew pathogen populations (named after the locations from which they were collected) against which the resistance QTL is effective are listed in the same color directly below the QTL position. The number that follows each pathogen population name in the portion of disease incidence variation in the particular pearl millet mapping population x pathogen population combination that is accounted for by the host plant resistance QTL mapping to this position.

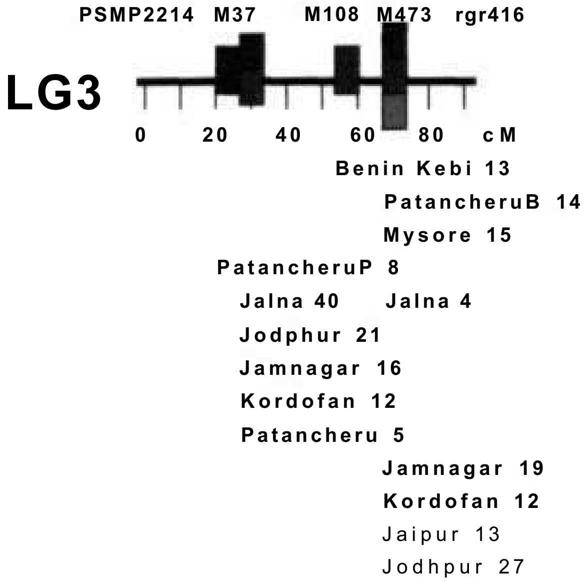
Pearl millet downy mildew resistance QTLs

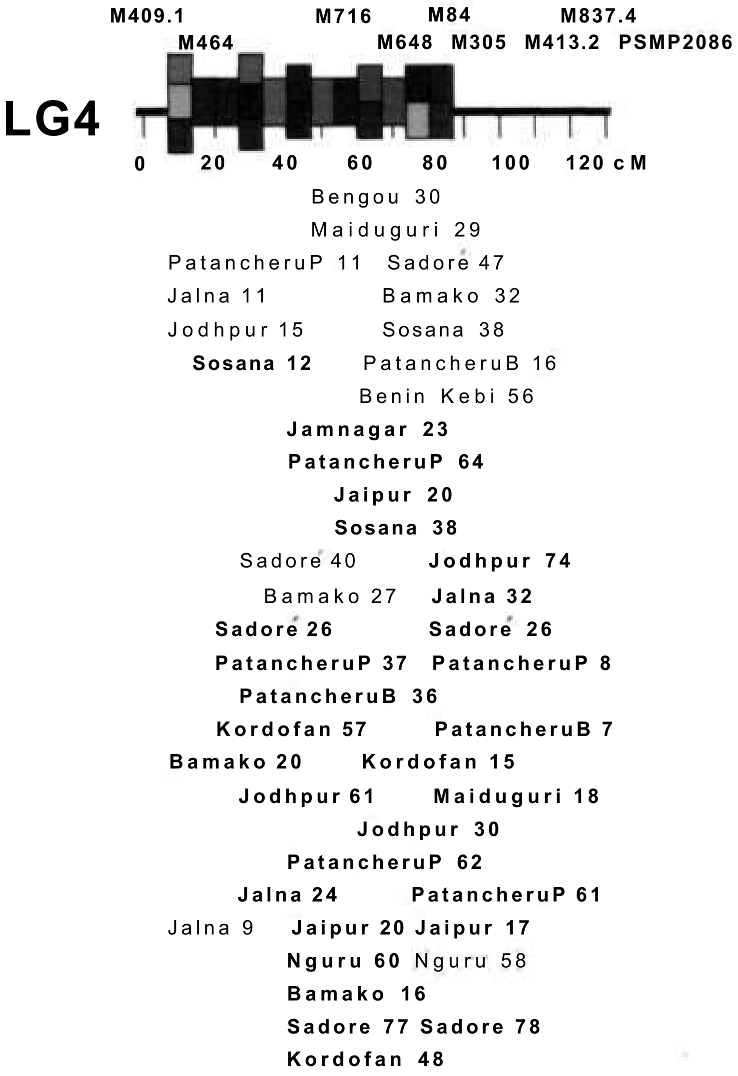
LG1

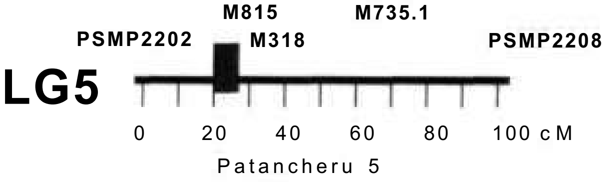


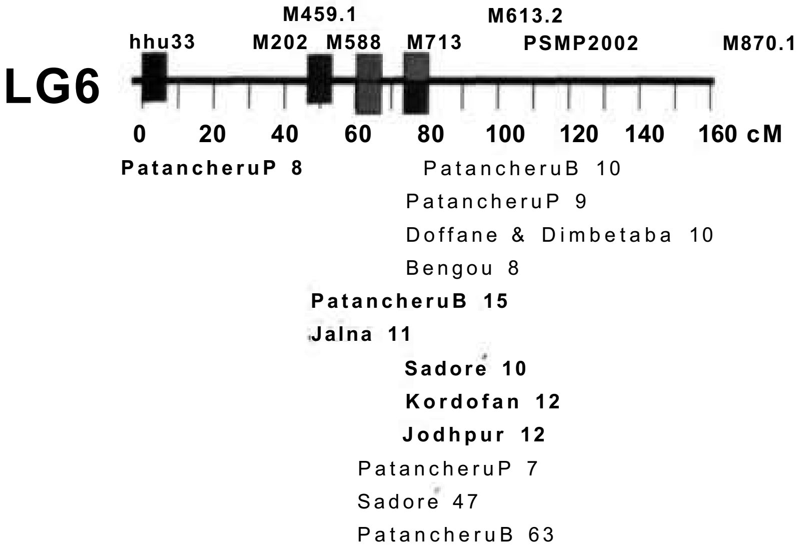
LG2

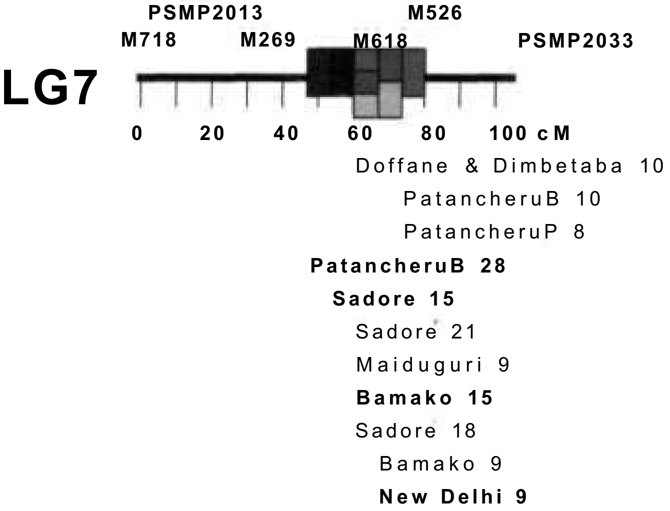



PSMP2214
M37
M108
M473
rgr416
LG3
0
20
40
60
80
cM
Benin Kebi 13
PatancheruB 14
Mysore 15
PatancheruP 8
Jalna 40
Jalna 4
Jodphur 21
Jamnagar 16
Kordofan 12
Patancheru 5
Jamnagar 19
Kordofan 12
Jaipur 13
Jodhpur 27


M409.1
M716
M84
M837.4
M464
M648
M305
M413.2
PSMP2086
LG4
0
20
40
60
80
100
120 cM
Bengou 30
Maiduguri 29
PatancheruP 11
Sadore 47
Jalna 11
Bamako 32
Jodhpur 15
Sosana 38
Sosana 12
PatancheruB 16
Benin Kebi 56
Jamnagar 23
PatancheruP 64
Jaipur 20
Sosana 38
Sadore 40
Jodhpur 74
Bamako 27
Jalna 32
Sadore 26
Sadore 26
PatancheruP 37
PatancheruP 8
PatancheruB 36
Kordofan 57
PatancheruB 7
Bamako 20
Kordofan 15
Jodhpur 61
Maiduguri 18
Jodhpur 30
PatancheruP 62
Jalna 24
PatancheruP 61
Jalna 9
Jaipur 20
Jaipur 17
Nguru 60
Nguru 58
Bamako 16
Sadore 77
Sadore 78
Kordofan 48


M815
M735.1
PSMP2202
M318
PSMP2208
LG5
0
20
40
60
80
100 cM
Patancheru 5


M459.1
M613.2
hhu33
M202
M588
M713
PSMP2002
M870.1
LG6
0
20
40
60
80
100
120
140
160 cM
PatancheruP 8
PatancheruB 10
PatancheruP 9
Doffane & Dimbetaba 10
Bengou 8
PatancheruB 15
Jalna 11
Sadore 10
Kordofan 12
Jodhpur 12
PatancheruP 7
Sadore 47
PatancheruB 63


PSMP2013
M526
M718
M269
M618
PSMP2033
LG7
0
20
40
60
80
100 cM
Doffane & Dimbetaba 10
PatancheruB 10
PatancheruP 8
PatancheruB 28
Sadore 15
Sadore 21
Maiduguri 9
Bamako 15
Sadore 18
Bamako 9
New Delhi 9

Pearl millet mapping populations contributing the more resistant allele:

■ Red = ICMP 85410-P7 ■ Blue = LGD 1-B-10	Jones et al. (1995)
■ Orange = 81B-P6 ■ Green = ICMP 451-P8	Hash et al. (unpublished)
■ Sky blue = ICMP 451-P6 ■ Plum = H 77/833-2-P5(NT)	Breese et al. (2002) and Cavan et al. (unpublished)
■ Pink = 841B-P3 ■ Purple = 863B-P2	Hash et al. (unpublished)
■ Grey = PT 732B-P2 ■ Brown = P 1449-2-P1	Hash et al. (unpublished)
■ Teal - W 504-1-P1 ■ Gold = P310-17B	Kolesnikova (2001)
■ Turquoise = IP 18293-P152	Azhaguvel (2001)
■ Bright green = P 7-3	Jones et al. (2002)

Sources of pearl millet downy mildew populations (and years of collection) used in greenhouse disease screens of pearl millet mapping populations used to detect QTLs for host plant resistance to *Sclerospora graminicola:*

Screens conducted at the University of Wales, Bangor:

- Sosana, Barentu, Eritrea (2000)
- Patacheru, Andhra Pradesh, India (1989 and 1994) = PatancheruB
- Bamako, Mali (1996)
- Sadore, Niger (1996)
- Bengou, Niger (1992)
- Benin Kebi, Nigeria (1996)
- Maiduguri, Nigeria (1991 and 1994)
- Nguru, Nigeria (1994)
- Doffane & Dimbetaba, Senegal (1992)
- Kordofan, Sudan (1997)

Screens conducted at ICRISAT-Patancheru, India:

- Jaipur, Rajasthan, India
- Jalna, Maharashtra, India
- Jamnagar, Gujarat, India
- Jodhpur, Rajasthan, India
- Mysore, Karnataka, India
- New Delhi, Delhi, India
- Patancheru, Andhra Pradesh, India = PatancheruP

Improving Pro-poor Public Goods

Pearl millet seeds are tiny - in one kilogram there are typically over 100,000 seeds - so farmers need only a small amount to sow their crop. This makes the more expensive hybrid seed a profitable option, even for poor farmers, so it is unsurprising that single-cross hybrids are now the most widely grown pearl millet cultivar type in India and they are grown by all categories of farmers. There are many pearl millet hybrids from private-sector breeding programs, but hybrids bred in the public-sector, such as HHB 67 from CCS Haryana Agricultural University, are also widely grown. This particular hybrid was released in 1989 (Kapoor et al. 1989) and has many traits that farmers appreciate, including early maturity that allows it to escape end-of-season drought stress. It is probably the most popular public-sector pearl millet hybrid in India and occupies over half of the pearl millet area in Haryana (over 300,000 hectares during the rainy season of 2001). It is grown in rainfed farming systems where its short duration allows farmers ample time to prepare land for any following crop grown largely on residual moisture, such as chickpea (*Cicer arietinum* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), or oilseed mustard (*Brassica* sp.).

Pearl millet farmers in India have no public-sector alternative to HHB 67 in this maturity class, and all private-sector hybrids mature later than HHB 67. However, its popularity makes it vulnerable to an epidemic of downy mildew. In the past, every single highly popular pearl millet hybrid in India has ultimately succumbed to this disease. When this happens, farmers not only suffer the direct losses caused by the epidemic, but they lose the management options associated with growing their most preferred hybrid. The hybrid seed industry also faces economic losses as it takes time to gear up seed production of the next best alternatives, and for farmers to then identify which of these best match their needs.

Marker-assisted backcrossing (MABC) has been used to improve the disease resistance of the parental lines of HHB 67 (Sharma 2001). MABC is rapid, and more effective than conventional breeding where it is often impracticable to deliberately add several resistance genes (so-called gene pyramiding) into a particular crop genotype. In conventional breeding, once a single effective resistance gene is included it is often impossible to detect the presence of a second without expensive and time-consuming progeny testing every backcross generation. MABC also allows the introduction of the resistance genes while, because of the inherent properties of the backcross breeding method, ensuring that other genetic changes are minimal. Indeed, marker-assisted selection can be not just for the resistance gene from the donor, but to select for the original parent genotype over other regions of the genome that are not tightly linked to the targeted resistance gene(s). These minimal genetic changes greatly facilitate adoption by the seed industry and farmers of the improved products. All that is needed is the replacement of new lines for old in the seed multiplication chain. This can be done once it is confirmed that: the new parental lines have better disease resistance; are otherwise identical to the old lines as far as seed certification is concerned; and that the new version of the hybrid performs at least as well as the old. This may sound complicated but it is much faster and easier than releasing a new hybrid that requires more extensive trials and complex and uncertain release procedures.

MABC provides a new model for hybrid breeding - incremental breeding - rather than relying on the chance results of random re-assortment of genes from crosses. The process of building on a successful public-sector hybrid need not end by simply ensuring it does not succumb to a downy mildew epidemic. MABC can be applied for other traits to produce further incremental improvements in a popular hybrid such as HHB 67. Over time, more and more desirable traits can be added, and the hybrid becomes closer and closer to the farmers' ideal. The crucial advantage of building on an already popular hybrid is that the process is participatory. Farmers have demonstrated demand by voting with their cash when they buy the hybrid seed, and incremental gains can be made for any weaknesses identified by farmers or the seed industry.

Table 1. The pearl millet hybrid seed multiplication chain (after Khairwal et al. 1990).

Stage	Year	Season	Responsibility
1. Parental line Breeder Seed	1	Summer season	Breeder
2. Parental line Foundation Seed	1	Rainy season	Seed producer
3. Certified Hybrid Seed production	2	Summer season	Seed producer
4. Certified Hybrid Seed use	2	Rainy season	Input suppliers & farmers

What Is Needed to Now Deliver Improved Versions of HHB 67 to Farmers?

The simplest option for getting seed of improved versions of HHB 67 to farmers would be to replace the seed without following release procedures. Local seed laws permitting, all that is required is to simply replace the seed lots that are used for Breeder Seed production of the hybrid parental line(s), and the improved version of the hybrid will reach farmers through the seed multiplication chain 18 months later (Table 1).

This strategy has been possible in India for private-sector seed companies that produce 'truthfully labeled' seed, the quality of which is marketed on the strength of the companies' reputations rather than by employing government-supported seed certification. Following positive perceptions of farmers in on-station and on-farm trials in 2001, this replacement process could begin within the private sector in 2002. Seed of the new versions would then first reach farmers in the rainy season of 2003.

However, for hybrid seed multiplied and marketed by private and public seed production agencies that rely on government-supported seed certification to ensure seed quality, this substitution of the improved version for the original is delayed for at least one year for field trials. The field trial data are then used to support the official release of the new version of the hybrid. The release documents include revised descriptions of the parental lines for seed certification purposes. In this case, the shortest possible path for the new hybrid to reach farmers would involve rainy season trials in 2001 and 2002 followed by a state release proposal in early 2003. By simultaneously producing Breeder Seed of the new versions of the hybrid parental lines during the summer season of 2003, Certified Hybrid Seed of the new versions of HHB 67 could first reach farmers in the rainy season of 2004.

Indeed, initial field trials have documented that some of the improved versions of HHB 67 actually are superior to the original for traits other than downy mildew resistance - the original target of our marker-assisted backcrossing program. For example, multilocational trials of hybrids produced with two new versions of the pollinator suggest that grain yield gains of 15% may have been achieved while simultaneously making significant improvements in downy mildew resistance without adversely affecting the early maturity of HHB 67 that farmers appreciate so much. This is a yield gain of 3% per annum, over and above the returns from improved disease resistance, in a breeding program that did not target grain yield improvement *perse.* This is remarkable, because yield gains from conventional yield-focused hybrid breeding are typically on the order of 1-2% per year.

This is a novel and systematic way for plant breeders to generate positive variation for economic yield in very elite, farmer-accepted genetic backgrounds. The genetic integrity of the hybrid parental lines of HHB 67 has largely been maintained by the backcrossing process, and the donor parents have contributed genomic segments conferring improved downy mildew resistance, along with genomic regions tightly linked to those conferring improved downy mildew resistance. In at least some cases, resistance donor parent alleles in genomic regions tightly linked to the downy mildew resistance genes contribute positively to grain yield, even in the absence of disease, and these favorable variants have been detected and selected from conventional line x tester experiments conducted as multilocational yield trials.

Now we are set to start targeting QTLs that are expected to confer increased grain and stover yields. For example, we have already used a morphological marker that is tightly linked to a gene for a flowering-date-indcpendent increase in plant height to increase the straw yield of HHB 67, which is identified by farmers as one of the few weaknesses of this popular hybrid. In addition, we have just completed transfer to the male parent of this hybrid a genomic region expected to confer enhanced tolerance to terminal drought stress. Hybrids produced with these new versions of the male parent will be screened under a range of drought stress regimes during 2002.

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References

Allouis S, Qi X, Lindup S, Gale MD and Devos KM. 2001. Construction of a BAC library of pearl millet. *Pennisetum glaucum.* Theoretical and Applied Genetics 102:1200-1205.

Azhaguvel P. 2001. Linkage map construction and identification of QTLs for downy mildew (*Sclerospora graminicola*) resistance in pearl millet (*Pennisetum glaucum* (L..) R. Br.). PhD thesis, Department of Agricultural Botany, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, Tamil Nadu, India. 204 pp.

Breese WA, Hash CT, Devos KM and Howarth CJ. 2002. Pearl millet genomics - an overview with respect to breeding for resistance to downy mildew. Pages 243-246 *in* Sorghum and Millets Pathology 2000 (Leslie JF, ed.). Ames, Iowa, USA: Iowa State Press.

Devos KM, Pittaway TS, Reynolds A and Gale MD. 2000. Comparative mapping reveals a complex relationship between the pearl millet genome and those of foxtail millet and rice. Theoretical and Applied Genetics 100:190-198.

Jones ES, Liu CJ, Gale MD, Hash CT and Witcombe JR. 1995. Mapping quantitative trait loci for downy mildew resistance in pearl millet. Theoretical and Applied Genetics 91: 448-456.

Jones ES, Breese WA, Liu CJ, Singh SD, Shaw DS and Witcombe JR. 2002. Mapping quantitative trait loci for resistance to downy mildew in pearl millet: Field and glasshouse screens detect the same QTL. Crop Science 42:1316-1323.

Kapoor RL, Kakkar PS, Khairwal IS, Bainiwal CR, Nijhawan DC and Yadav HP. 1989. Bajra hybrid HHB 67 - a major break through. Haryana Farming 18:17, 21.

Khairwal IS, Ram C and Chhabra AK. 1990. Pearl millet seed production & technology. New Delhi, India: Ramesh Jain Manohar Publications. 224 pp.

Kolesnikova MA. 2001. Mapping new quantitative trait loci (QTL) for downy mildew resistance in pearl millet. PhD thesis, Russian National Academy of Sciences, Moscow, Russia. (English and Russian). 266 pp.

Liu CJ, Witcombe JR, Pittaway TS, Nash M, Hash CT, Busso CS and Gale MD. 1994. An RFLP-based genetic map of pearl millet *(Pennisetum glaucum).* Theoretical and Applied Genetics 89:481-487.

Liu CJ, Devos KM, Witcombe JR, Pittaway TS and Gale MD. 1996. The effect of genome and sex on recombination rates in *Pennisetum* species. Theoretical and Applied Genetics 93:902-908.

Qi X, Lindup S, Pittaway TS, Allouis S, Gale MD and Devos KM. 2001. Development of simple sequence repeat markers from bacterial artificial chromosomes without subcloning. BioTechniques 31:355-361.

Sharma A. 2001. Marker-assisted improvement of pearl millet (*Pennisetum glaucum*) downy mildew resistance in elite hybrid parental line H 77/833-2. PhD thesis, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India. 131 pp.

Yadav RS, Hash CT, Bidinger FR and Howarth CJ. 1999 QTL analysis and marker-assisted breeding of traits associated with drought tolerance in pearl millet. Pages 211-223 *in* Genetic improvement of rice for water-limited environments (Ito O, O'Toole J and Hardy B, eds.). Los Banos, Philippines: International Rice Research Institute (IRRI).

Yadav RS, Hash CT, Bidinger FR, Cavan GP and Howarth CJ. 2002. Quantitative trait loci associated with traits determining grain and stover yield in pearl millet under terminal drought-stress conditions. Theoretical and Applied Genetics 104:67-83.

Improving Pearl Millet Drought Tolerance

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Quantitative trait loci (QTL) have been identified for drought tolerance of grain yield in pearl millet (Yadav et al. 1999 and 2002). Marker-assisted selection (MAS) is being used to develop improved parental lines by introgression of QTLs into a homozygous inbred line background for the subsequent production of improved hybrids (marker-assisted back crossing), and by transforming them into topcross pollinator populations that are more heterogeneous than inbred lines. Until - and unless - it is clearly demonstrated that the incorporation of these QTLs into elite breeding lines will significantly enhance the performance of cultivars based on those lines, the benefits of these QTLs are unlikely to ever reach farmers' fields.

Three topcross pollinator populations (TCPs) were developed by selecting and inter-mating individual genotypes from within the $F_{2:4}$ mapping families of a pearl millet population used for mapping QTLs for drought tolerance (Fig. 1). The three TCPs produced were selected according to the following methods and criteria:

- Marker-assisted selection: genetic composition at the drought tolerance QTL (to constitute a MAS-based TCP);
- Phenotypic selection: field performance (best 16) in the drought trials used to identify QTLs (to constitute a phenotype-based TCP); and

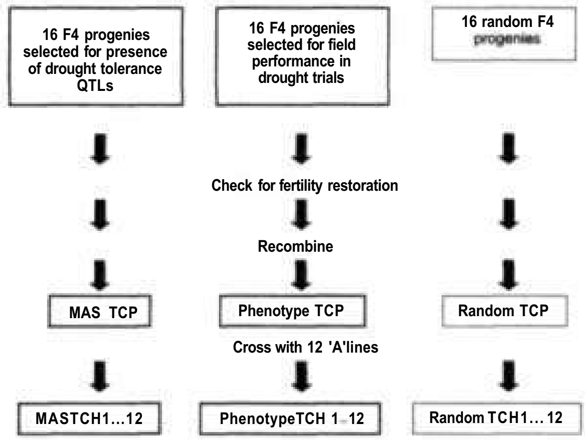

Figure 1. The scheme used to test marker-assisted selection for pearl millet QTLs controlling drought tolerance using topcross pollinators. Topcross pollinators based on phenotypic and random selections are controls for the pollinator based on marker-assisted selection.