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Pearl Millet Molecular Marker Research

CT Hash[1,*] and JR Witcombe[2] (1. ICRISAT, Patancheru 502 324, Andhra Pradesh, India; 2. Centre for Arid Zone Studies, University of Wales, Bangor, Gwynedd LL57 2UW, UK)
*Corresponding author: c.hash@cgiar.org

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 unpublished).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).
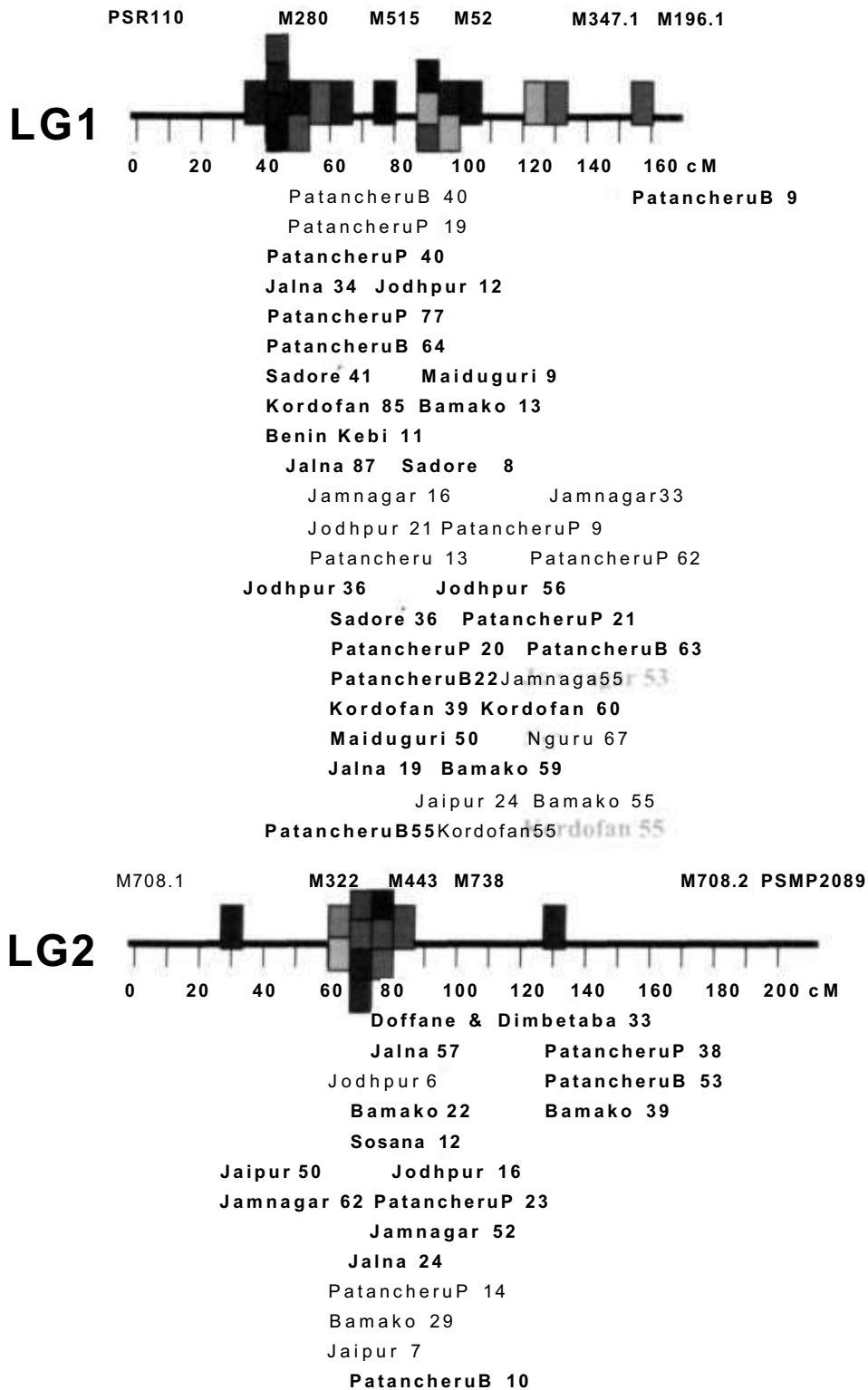
Not only have QTLs for downy mildew resistance been mapped, but genotypes produced or tested in the mapping studies have provided a valuable genetic resource. They are donors of naturally occurring host-plant resistance genes, and a well-chosen set of lines can differentiate among many different populations 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.
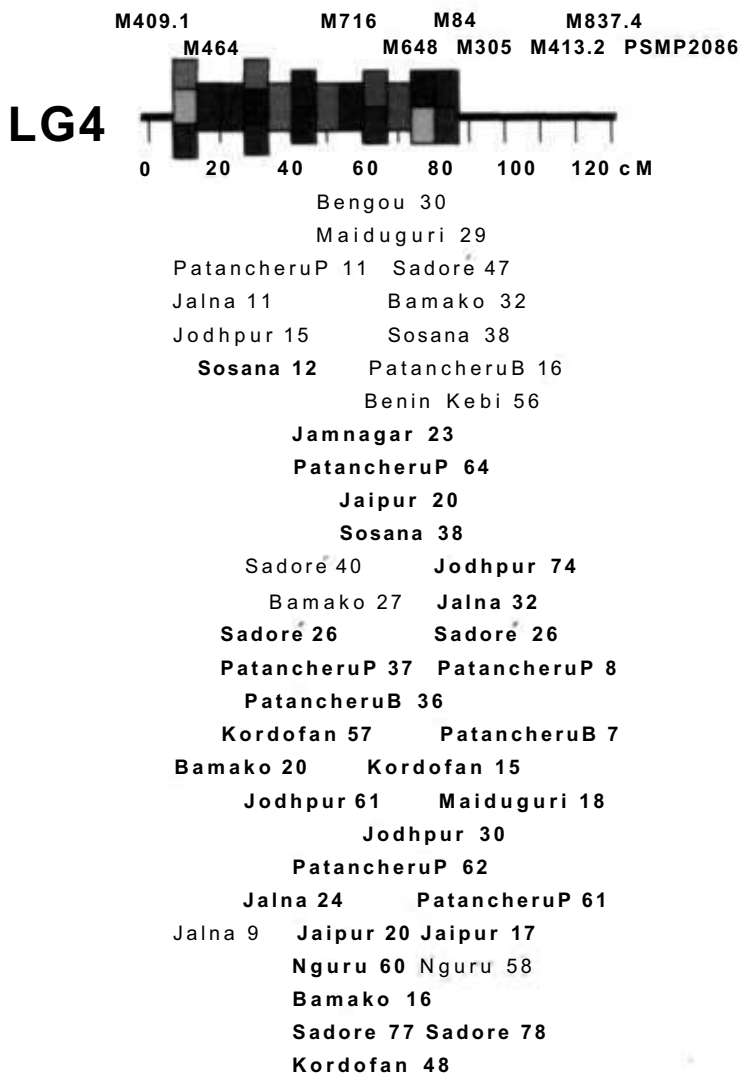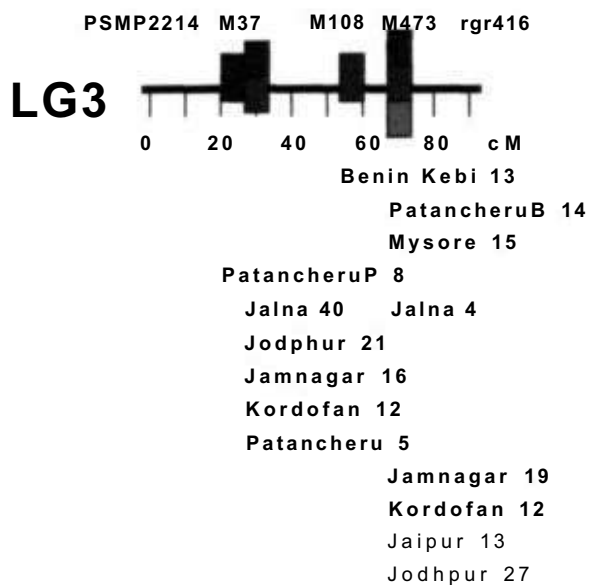
Such accumulated knowledge contributes to the speed of cumulative gains that plant breeding can make. The extent of this knowledge provides flexibility (different gene deployment strategies), depth (many genes for potential pyramiding), and breadth (many geographical targets).
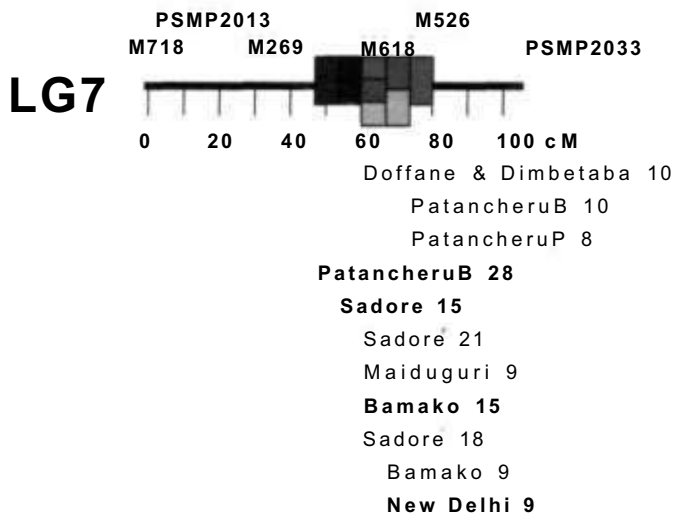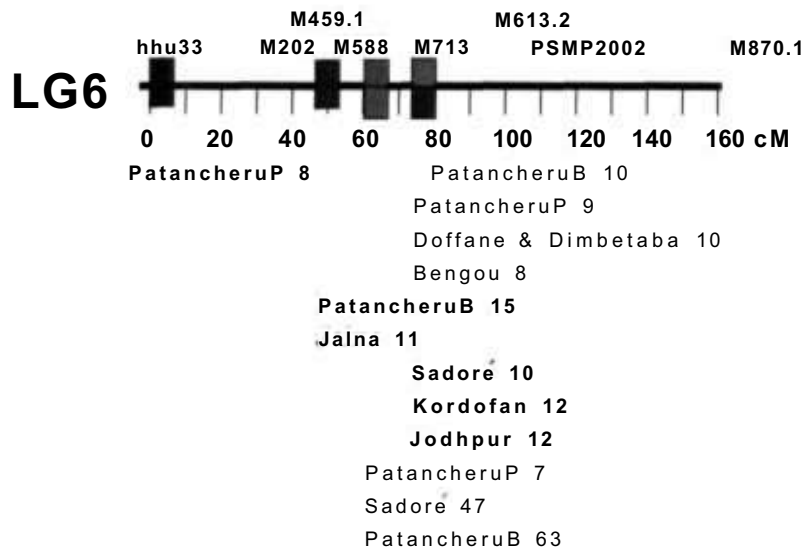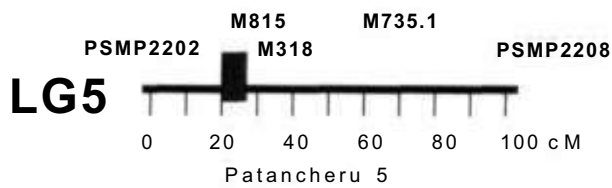
We describe below the application of these stores of knowledge to the specific improvement of pearl millet hybrid HHB 67, which is only grown in India. Although HHB 67 is, in practice, a public good for India, the knowledge generated in its improvement is an international public good.

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

PSR110   M280   M515   M52   M347.1 M196.1

LG1

0   20   40   60   80   100   120   140   160 cM

PatancheruB 40   **PatancheruB 9**

PatancheruP 19

PatancheruP 40

Jalna 34 Jodhpur 12

PatancheruP 77

PatancheruB 64

Sadore 41   Maiduguri 9

Kordofan 85 Bamako 13

Benin Kebi 11

Jalna 87 Sadore 8

Jamnagar 16   Jamnagar33

Jodhpur 21 PatancheruP 9

Patancheru 13   PatancheruP 62

Jodhpur 36   Jodhpur 56

Sadore 36 PatancheruP 21

PatancheruP 20 PatancheruB 63

PatancheruB22 Jamnaga55**r 53**

Kordofan 39 Kordofan 60

Maiduguri 50   Nguru 67

Jalna 19 Bamako 59

Jaipur 24 Bamako 55

PatancheruB55 Kordofan55 **Kordofan 55**

M708.1   **M322   M443 M738**   **M708.2 PSMP2089**

LG2

0   20   40   60   80   100   120   140   160   180   200 cM

Doffane & Dimbetaba 33

Jalna 57   **PatancheruP 38**

Jodhpur 6   **PatancheruB 53**

Bamako 22   **Bamako 39**

Sosana 12

Jaipur 50   **Jodhpur 16**

Jamnagar 62 PatancheruP 23

Jamnagar 52

Jalna 24

PatancheruP 14

Bamako 29

Jaipur 7

PatancheruB 10

PSMP2214 M37   M108 M473 rgr416

LG3

0   20   40   60   80   c M

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 c M

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

LG5

M815
PSMP2202   M318   M735.1   PSMP2208

0   20   40   60   80   100 cM

Patancheru 5

LG6

M459.1
hhu33   M202   M588   M713   M613.2   M870.1
PSMP2002

0   20   40   60   80   100   120   140   160 cM

PatancheruP 8   Patancheru B 10
PatancheruP 9
Doffane & Dimbetaba 10
Bengou 8
PatancheruB 15
Jalna 11
Sadore 10
Kordofan 12
Jodhpur 12
PatancheruP 7
Sadore 47
PatancheruB 63

LG7

PSMP2013   M526
M718   M269   M618   PSMP2033

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   Jones et al. (1995)
Blue = LGD 1-B-10

Orange = 81B-P6   Hash et al. (unpublished)
Green = ICMP 451-P8

Sky blue = ICMP 451-P6   Breese et al. (2002)
Plum = H 77/833-2-P5(NT)   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 = P3I0-17B   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 *per se*. 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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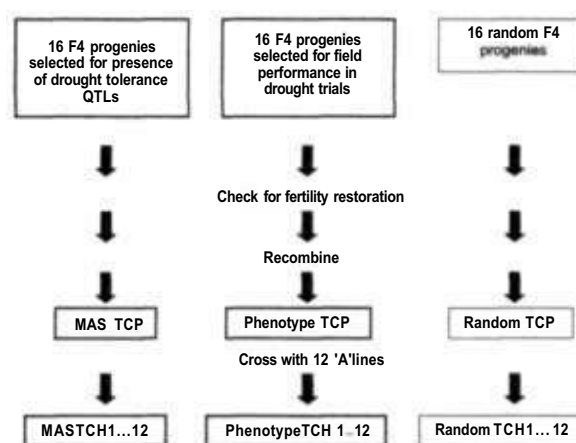
Improving Pearl Millet Drought Tolerance

RS Yadav[1]*, FR Bidinger[2], CT Hash[2], GP Cavan[1], R Serraj[2] and CJ Howarth[1] (1. IGER, Aberystwyth, SY23 3EB, UK; 2. ICRISAT, Patancheru 502 324, Andhra Pradesh, India)

*Corresponding author: rattan.yadav@bbsrc.ac.uk

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.