SUCCESSFUL MARKER ASSISTED SELECTION FOR DROUGHT TOLERANCE AND DISEASE RESISTANCE IN PEARL MILLET

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The Problem

For many millions of resource-poor farmers in agriculturally marginal areas of the world, pearl millet provides basic sustenance. It is a crop that is able to produce nourishment from the poorest soils in the driest regions in the hottest climates, where no other cereal can grow. However, not even millet can grow when there is no water, and the low and very unpredictable rainfall of the areas in which it is grown results in extremely unstable yields (Figure 3.1). The major disease of pearl millet is downy mildew (caused by *Sclerospora graminicola*) and can result in up to an 80% yield loss as the grain is replaced by leaf-like structures (Figure 3.2). Success in improving tolerance to drought and resistance to mildew would improve food security for many of the world's poorest people.

Figure 3.1 Drought susceptible and drought tolerant pearl millet under assessment in the field.

Figure 3.2 Effect of infection with downy mildew on grain production in pear millet (right). An uninfected panicle is shown on the left hand side.

Until now, progress in producing new drought tolerant varieties of millet has been very slow and difficult. In 1990, the Plant Sciences Research Programme of the UK Overseas Development Agency (ODA - now known as the Department for International Development (DFID)), began funding a collaborative research project on pearl millet involving scientists at IGER, at the University of Wales Bangor, at the John Innes Centre and at the International Crops Research Institute for the Semi-Arid tropics in India. The objective of the collaboration was to use molecular plant breeding methods to address these long-standing problems of pearl millet production.

The Solution

(a) Assess genetic variation for disease resistance and drought tolerance, particularly in landraces, elite

breeding lines and other potential donors.

(b) Identify molecular markers linked to specific components of disease resistance and drought tolerance.

(c) Use the markers directly in the breeding programme to follow the introgression of desirable regions of the genome.

This would enable us to breed directly for improved drought tolerance in this crop rather than depending on unreliable field screens and also to incorporate multiple resistance genes along with accumulating favourable alleles for other agronomically important traits (gene stacking).

How is this done?

Firstly, the creation of a molecular marker-based genetic linkage map of the pearl millet genome

To do this we used polymorphic DNA markers (as described in Chapter 2). Since 1990, more than 600 pearl millet markers have been identified and mapped. Genetic mapping is based on the statistical likelihood that markers that are linked will be inherited together and this is used to order markers on the chromosome. The closer the markers, the more likely the linked markers will be inherited together. Mapping also indicates relative distances between markers and assigns them to linkage groups. Pearl millet has seven linkage groups that correspond to its seven chromosomes (2n = 2 x 7 = 14).

Table 3.1	Lines of pearl millet used in crosses to develop genetic maps
Line	Characteristics
ICMP 451	downy mildew resistant; male parent of grain and fodder hybrids
H 77/833-2	elite male parent of grain hybrids in India, susceptible to downy mildew but with seedling thermotolerance, high tillering capacity and earliness
PRLT 2/89-33	inbred, based mainly on Iniadi landrace germplasm from West Africa; low tillering, large seeds, drought tolerant

Secondly, once the genetic map is established it can be searched for associations with traits of interest

Both drought tolerance and downy mildew resistance are quantitative traits. An individual's phenotype is a composite that depends on the segregation of several to many naturally occurring polymorphic genes. Areas or regions on the genome that affect a target trait are known as a quantitative trait loci or QTL.

To map QTL for a particular trait, highly inbred homozygous parents are chosen that differ in their response to that trait, whether it is disease resistance or drought tolerance. These are crossed to produce heterozygous F1 seed. An F1 plant is grown from a single seed, self-fertilised, and the resultant F2 seed is sown to produce a segregating F2 population. A tissue sample is taken from each F2 plant for analysis using DNA markers (genotyping) and the individual segregating plants are self-fertilised and bulked to produce progenies.

For each individual cross, a skeleton genetic map is developed with anchor loci that are polymorphic between the two parents of the cross. The bulk progenies are screened for drought tolerance in a wide range of environments across India, and for downy mildew resistance either in a greenhouse or in the field.

Using this approach, we have developed genetic maps for a number of crosses including between the lines H 77/833-2 and both ICMP 451 and PRLT 2/89-33 (for details see Table 3.1).

To go from disease scores to QTL mapping, the genotyping data and disease screening data are combined and analysed using computer algorithms to determine the likely associations of traits with particular markers on the chromosome map. Different pearl millet cultivars contain different downy mildew resistance QTL effective against

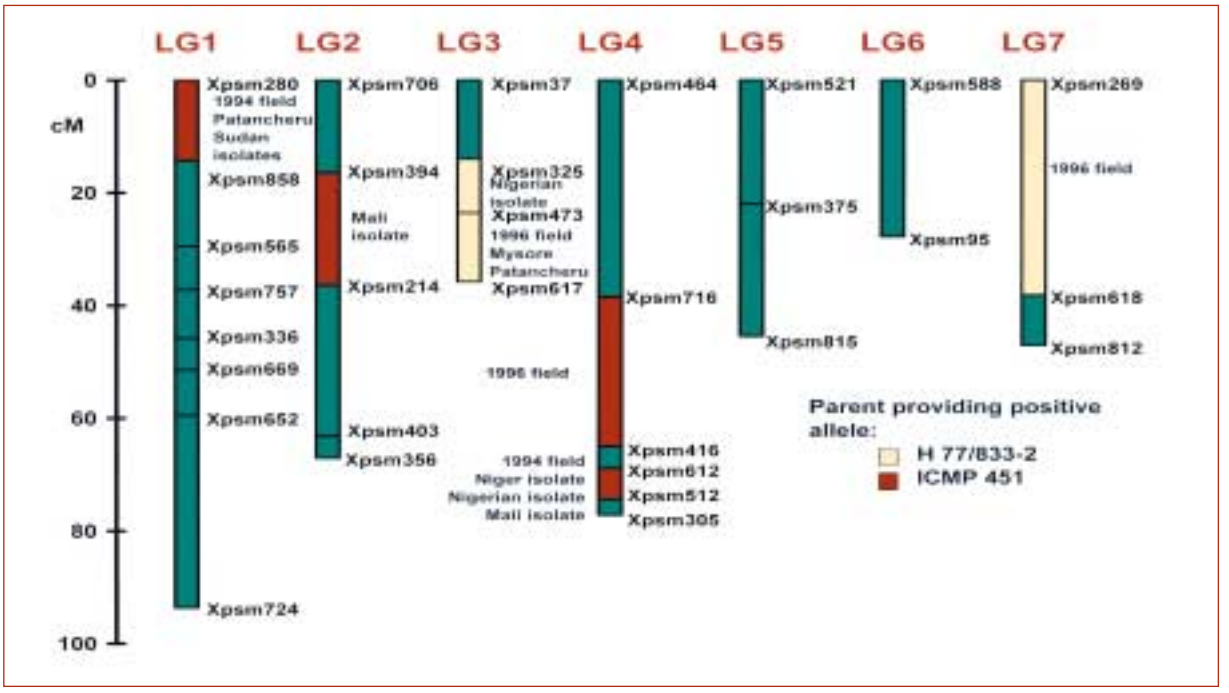

Figure 3.3 Skeleton genetic linkage map of the pearl millet cross ICMP 451 (resistant) with H 77/833-2 (susceptible) indicating positions of QTL associated with the response to downy mildew, indicating the countries in which the resistance is effective.

various isolates of the pathogen. For the cross of ICMP 451 x H 77/833-2, resistance QTL from the resistant parent map to linkage groups 1, 2 and 4 (Figure 3.3). These QTL are effective against pathogen isolates from India, Sudan, Mali, Nigeria and Niger. The susceptible parent also carries resistance QTL on linkage groups 3 and 7 that are effective against isolates from Nigeria and India. A similar approach has been used to identify QTL for the maintenance of grain yield during drought stress.

Table 3.2 Downy mildew incidence (against 3 isolates) of parental lines and of the products of marker assisted selection (ICMR 01007 and ICMR 01004) after 4 generations of marker assisted backcrossing of resistance QTL on linkage groups 1 and 4 from ICMP 451 to H 77/833-2

	Delhi Isolate	Patancheru Isolate	Jodhpur isolate
H77/833-2	94.2%	92.7%	99.2%
ICMP 451	4.1%	50.0%	45.8%
ICMR 01007	3.5%	50.1%	49.9%
ICMR 01004	9.1%	39.4%	32.6%

Finally, and most importantly, the incorporation of marker-assisted selection (MAS) into the ongoing breeding programme.

Once the QTL are mapped, MAS can be used to breed for resistance (Figure 3.4). Markers flanking the QTL are used as tags to identify pieces of the donor parent linkage group containing the putative resistance QTL. The F1 is back-crossed several times to an agriculturally important variety. At each stage, molecular markers are used to identify individual plants that contain as much of the recurrent parent genome as possible, but that are heterozygous for the region carrying the resistance QTL. Selected plants are then finally self-fertilised to obtain homozygous progeny.

MAS has a number of important advantages in terms of transferring desirable genes, but the main one is that the breeding process is more rapid, selection being based on the test for the appropriate markers rather than on unreliable testing for phenotype in the field. We have not only succeeded in transferring downy mildew resistance into H77/833-2 from ICMP 451 (Table 3.2), but more recently have completed transfer of the major region of the genome controlling grain yield during

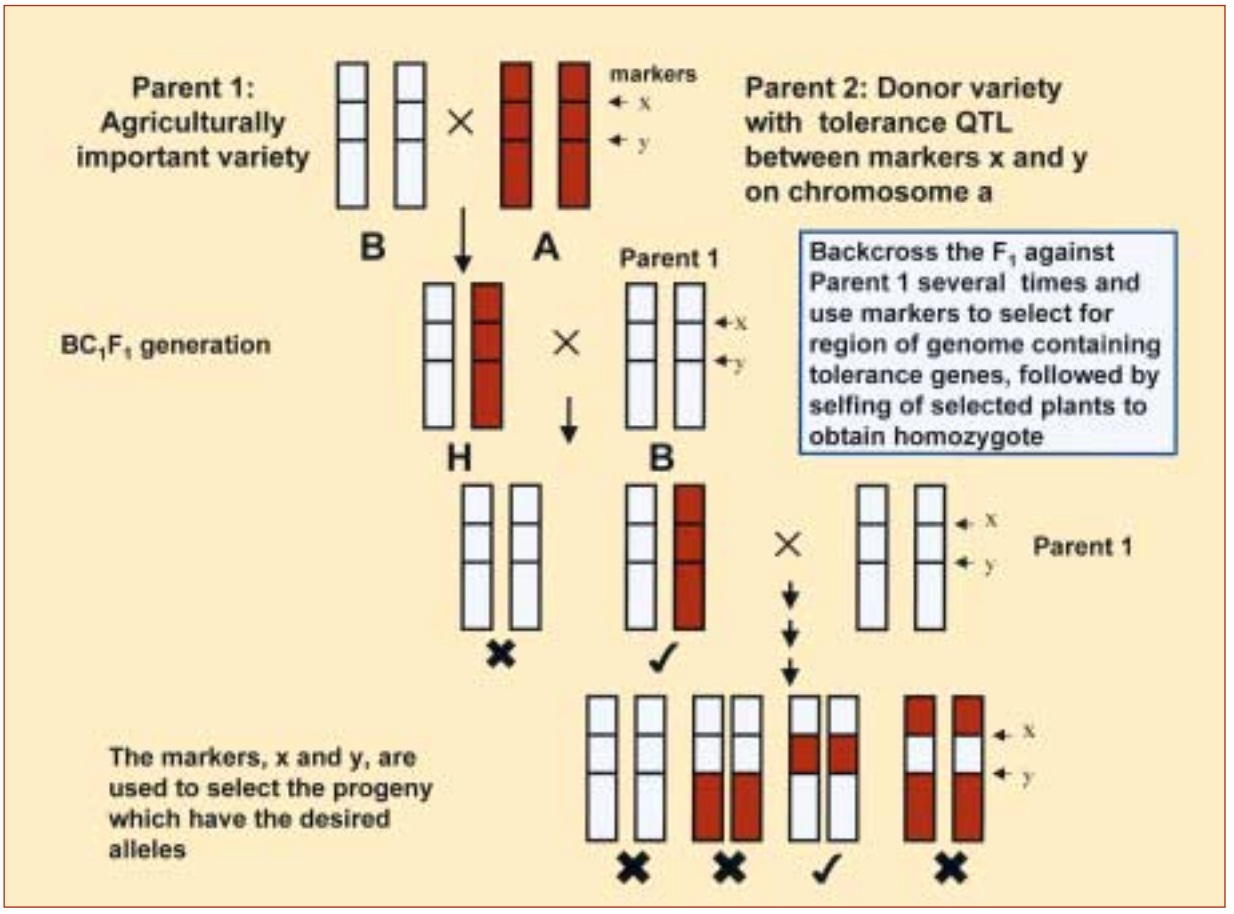

Figure 3.4 Schematic diagram for the marker-assisted selection of desirable traits, such as drought tolerance

drought stress again into H77/833-2, but this time from PRLT 2/89-33. As H77/833-2 is the male parent of one of the most popular pearl millet hybrids in India (HHB67), the outputs from this project have direct use by farmers.

New technologies, such as molecular markers, are expensive to develop, and returns from the initial research can take time. However, once the knowledge reaches a critical level gains accelerate enormously. All the information and accumulation of knowledge gained since 1990 will allow breeders, for future decades of plant breeding, to incorporate and stack resistance genes into pearl millet, to benefit resource-poor farmers. Products of breeding supplemented with MAS are just now beginning to become available in India, and work is continuing to transfer downy mildew resistance and drought tolerance QTL into other economically important hybrid parental lines at ICRISAT. The next decade will hopefully see this technology spreading to benefit pearl millet farmers worldwide. It is now practical to use marker-assisted methods for step-wise maintenance and enhancement in pearl millet hybrid programmes to extend the economically useful lifespan of important pearl millet hybrids such as HHB 67 and to more efficiently target step-wise improvement that best meets farmers' needs.

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