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Molecular Markers in Plant Breeding-III: Practical Applications and Difficulties Encountered

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Abstract: Some of the empirical results obtained through the use of RFLP, AFLP, SSR and RAPD markers in the areas of DNA fingerprinting, measurement of genetic distance and heterosis, marker-assisted selections and abiotic stress tolerance are being described. Various difficulties that a user can encounter during the ontogeny of marker's application have also been discussed. Marker mediated varietal fingerprinting and germplasm characterization appeared most common and most pervasive application with AFLP and SSR markers. Being cost effective, easy to handle and devoid of any radioisotope requirement, SSR markers are considered as the most suitable and reliable system for DNA fingerprinting. Capturing heterosis appeared most difficult with very little success due to lack of a facile marker system that could unconditionally identify the heterotic groups, population and progenies. Marker-assisted selections for qualitative traits appeared most successful after DNA fingerprinting while for quantitative characters, major disease resistance genes and genes controlling QTL for abiotic stress tolerance, the success is limited. It is anticipated that application of markers will remain restricted in these areas till the allele-specific markers are available and the cost of marker analysis is reduced significantly.

Key words: DNA finger printing, heterosis, marker-assisted breeding, QTL, stress tolerance

Introduction

Although utility of DNA markers has been recognized and established in many plant-breeding programmes, the primary obstacles to their widespread use are lack of facile marker system and availability of resources to support their use. Several factors are to be considered before the markers are integrated into a particular breeding system. Some of the important factors include nature of crop and traits undergoing selection, availability of resources for sustainable use of different marker system, use of radioactive compounds, size of population to be analyzed/characterized, level of polymorphism exhibited by the parents and requirement of inter-population and inter-specific transfer of marker data. Cross-pollinated species and complex polygenic traits are difficult to handle. For large sample sizes, DNA extraction and purification would be expensive and time consuming. Timely availability of radioisotopes would be difficult especially in the developing countries. Marker systems requiring acrylamide gel would be challenging. Management of data generated by AFLP and SSR marker systems would require careful planning and execution especially when it is to be shared between two laboratories and personals working on different population and/or crop species under altogether different environments.

With all these limitations, successful application of molecular markers in plants are reported in the areas of I) diagnostics and DNA fingerprinting, ii) measurement of genetic distance and heterosis, iii) marker-assisted selections and vi) tolerance to abiotic stresses. In this paper, successful application of molecular markers to resolve some of the issues pertinent to these areas are being described along with some of the limitations and problems that a user can encounter while integrating markers into practical breeding programmes.

Diagnostics and fingerprinting: One of the simplest and most pervasive application of DNA markers in plants is DNA fingerprinting, a technique that was forecasted by Soller and Beckmann (1983). Fingerprinting can be used to identify and monitor germplasm after its release for commercial cultivation. Seed companies and national registration agencies have an interest in DNA fingerprinting because the technology can be used to protect intellectual property, establish identity and assess purity. For these reasons, DNA fingerprinting has to be an integral part of every breeding programme. The most powerful and extensive applications of DNA fingerprinting has been considered in germplasm management programmes to get the insurance that an accession used in a breeding programme is genetically uniform and that the diversity in the available germplasm is sufficient to fulfill the requirement of broadening the genetic base of crops on sustainable basis. Numerous studies have been made previously to achieve this objective via estimating genetic diversity and/or measuring genetic distance between different accessions. Most of these studies made through the use of RFLP markers (Siedler *et al.*, 1994; Graner *et al.*, 1994) indicated that accurate information on genetic diversity in the available germplasm could only be collected through the use of molecular markers. This is particularly important in cases where germplasm to be utilized is closely related, its ancestry obscured or its pedigree either inaccurate and/or lost. Despite this, molecular marker technology has not yet been able to make a significant impact on characterization, management and utilization of germplasm. One of the several reasons is the inconsistency of results obtained with different marker systems. As mentioned earlier, RFLPs are the most reliable markers to be used for this purpose as exemplified in wheat (Sasanuma *et al.*, 1996), sugarcane (Oropeza and Eva, 1996), rice (Zheng *et al.*, 1994), alfalfa (Pupilli *et al.*, 2000) and several other crop species. However, they were not found suitable for inter-specific comparison (Castagna *et al.*, 1997).

Consistency of RAPD markers (the most commonly used marker system due to its cost effectiveness and ease of handling) is also debatable. For example, when sorghum germplasm consisting of 42,000 accessions was analyzed with RAPD markers, the clusters developed were not even close to those obtained on the basis of morphological and agronomic data (Dahlberg *et al.*, 2002). It was thus, concluded that if core collections are to be developed and their validity checked, a single technique would not do the job. On the other hand, when 269 land races of common beans were analyzed with RAPD markers, the grouping corresponded in part to that formed earlier on the basis of morphological and agro-ecological criteria (Beebea *et al.*, 2000). Also, differences in soybean cultivars originated in USA and China and genetic diversity in HOPE maize population has been accurately determined through RAPD markers (Zenglu *et al.*, 2001; Popia *et al.*, 2000) respectively. Contrary to this, when RAPD marker obtained in tomato was converted into SCAR (sequence characterized amplified region), it was unable to differentiate two parental cultivars under a variety of polymerase chain reaction conditions (Yiping and Stommel, 2001). Farooq *et al.* (1998) successfully fingerprinted rice using RAPD markers. In this study, several radiation induced mutants of an aromatic, long grain rice cultivar Basmati were discriminated from parents and from each other: an observation in conformity with morphological variation detected in the field. The fingerprints thus produced were also used to detect adulteration of the finest quality Basmati varieties with inferior quality rice. DNA

fingerprinting has also been used successfully in characterization of morphologically identical accessions of wild rice species *Oryza punctata* (Farooq *et al.*, 1995). These findings helped establish that under certain conditions, DNA fingerprinting through RAPD markers can ensure the genetic uniformity of a particular accession in a breeding program. Inference from these studies is that fingerprinting with RAPD markers is conditional, while in most cases it would perhaps not be absolutely reliable.

Selected micro-satellite, DAF (DNA amplification fingerprinting) and AFLP markers may prove very suitable for fingerprinting because of their high level of polymorphism. For example Anderson *et al.*, (2001) successfully used DAF markers to separate 14 genetically putative Bermuda grass [*Cynodon dactylon* (L). Pers] strains produced in 1930 from those being currently cultivated in Oklahoma, USA. Similarly, when 105 bread wheat varieties released between 1932 and 1995 in Argentina, were characterized with SSR and AFLP markers (Manifesto *et al.*, 2001), a subset of 10 highly informative SSR markers allowed discrimination of all the 105 cultivars. The data thus obtained was complemented by the information derived earlier by utilizing AFLP markers to quantify genetic diversity in wheat breeding programme across Argentina. The objective of this study was to know if modern wheat cultivars released by the private sector have lower genetic diversity compared with those released earlier by the public sector. Although both SSR and AFLP marker systems were unable to find any significant difference of the genetic diversity between public and private sector cultivars, significant differences were found between the profiles generated for the samples taken from breeding programmes that have released large number of cultivars. The studies thus, indicated that both SSR and AFLP markers are highly reliable for varietal finger printing especially in cases where development of new varieties is based on narrow genetic base and they are to be released quickly for commercial cultivation.

Measuring genetic distance and prediction of heterosis: In order to predict mean performance of a hybrid, it is important to select parents that can ensure a sufficient genetic variance. However, due to inherent complexity of genetic variance, its error-free estimation is difficult. Plant breeders are looking for methods to enable them identify a progeny that can maintain performance standards for most of the agronomic characters and exceed the level of target variety in few of the characters. Such progenies usually have high heterotic values.

Heterosis, the most controversial affair in the genetic terminology, is a phenomenon in which an F1 hybrid of two genetically dissimilar (diverse) parents shows increased vigor at least over the mid-parent value or over better parent or commercial variety. Only certain crosses (F1 hybrids) express heterosis which, is caused by a combination of partial or complete dominance or epistasis (Moll and Stuber, 1974) at loci controlling the trait, different desirable alleles or allele frequencies in a population to be crossed. Heterosis is different and can be measured for any character. However, capturing heterosis is a difficult task because identification of lines exhibiting superior hybrid performance in extensive field tests is still the most costly and time consuming effort. For achieving real heterotic yield gains, it is essential to identify heterotic groups, genotypes or populations (preferably divergent) that produce high yielding progenies after crossing. Parameters used for such an identification include I) per se performance, ii) mitochondrial complementation, iii) combining ability and iv) genetic diversity determined by geographical origin or through the use of neutral markers (Burmmer, 1999). The genetic distance estimated through such markers distributed throughout the genome should (in principle) provide a mean to predicting hybrid performance prior to making and evaluating the actual cross (Vienne *et al.*, 1992). Molecular markers (RFLP, RAPD and AFLP) have been used to directly assess, the genetic diversity and/or genetic distance between parental genotypes of wheat (Sasanuma *et al.*, 1996: Mayburg *et al.*, 1997; Sun *et al.*, 1998), *Lens* species (Ahmad, 1996), *Allium cepa* (Le Thierry *et al.*, 1997) and other crops. Using RFLP, RAPD and AFLP markers, the association of genetic distances with F1 performance and heterosis has been tested in maize (Lee *et al.*, 1989), soybean (Cerna *et al.*, 1997), rice (Maroof *et al.*, 1997), forage crop (Brummer, 1999) and alfalfa (Brummer *et al.*, 2000). In all these cases, the predictive value of RFLP data for hybrid performance has been found I) promising for crosses between lines belonging to the same heterotic group, ii) practically zero for crosses between unrelated lines from genetically divergent heterotic groups and iii) medium for mixtures of both (Boppenmaier *et al.*, 1992). RAPD markers have also been used to determine the extent of diversity among inbred lines and to aid in the choice of superior crosses to be made (Lanza and Souza, 1997). In most of these cases, correlation between RAPD-based genetic distance and hybrid performance were very low to predict heterosis.

Many reasons are reported for the poor correlation between genetic distance and hybrid performance, which need careful consideration. For example, it is absolutely essential for a selected marker system to identify specific marker loci with tight linkage to those chromosomal segments, which determine the expression of the traits of interest (Bernards, 1992). This is imperative because some of the chromosomal regions are more important than others in their contribution to F1 yield performance and heterosis and can only be identified through selecting a marker system with adequate genome coverage. The markers that do not have adequate genome coverage will not assess the genetic distance between the two species accurately. This is perhaps the case with RAPD markers because they are dominant (Rafalski *et al.*, 1990) and cannot detect heterozygots from the homozygots and they are not reproducible (Jones *et al.*, 1997). This has been established during mapping of quantitative trait loci affecting grain yield and related traits. In these studies, magnitude of genetic effects assessed with different marker systems for a particular QTL contributing to yield and yield components varied from 5-25% of the phenotypic variance (Stuber *et al.*, 1992) and indicated the need of selecting an appropriate markers system. The marker loci selected without any tight linkage with such QTL would largely result in low correlation. The small sample size of the parental lines and of the progenies that are assessed especially, when RFLP markers are to be utilized (due to their inherent complexity) might be another reason as to why genetic distance failed to have a predictive value for heterosis.

Compare to RFLP and RAPD markers, a significant improvement in the correlation between genetic distance and hybrid performance has been noted in maize by using AFLP markers (Ajmone-Marsan *et al.*, 1998). The reasons for improvement included I) analysis of large number of samples in relatively short time, ii) selection of an AFLP marker strongly linked to loci that affect a quantitative trait and iii) high resolution (required to resolve clearly, the light and dark bands coming out of a heterozygous individuals) of poly-acrylamide gels used for AFLP analysis.

The parameters used to determine the genetic distance differ also in their ability to predict heterosis because different parameters do not have same genetic basis of measurement. For example they are either measured on the basis of coefficient of parentage, variation of traits among the parents and progenies and performance variation in the segregating progeny (Souza and Sorrells, 1991). The power of molecular markers explored by the breeders to predict heterosis in this range of genetic distances seems rather low. The only relevant loci in terms of phenotypic expression of heterosis are those affecting the QTL. Since the number of QTL with significant effect for any given characters is limited, the prediction could be improved by selecting markers in linkage disequilibrium with QTL which will provide an effective means for predicting heterosis and would thus expedite field screening. However, it is imperative to see whether such linkage can be identified with reasonable experimental expenditure and whether the associations identified between markers and a QTL

would be valid across a wide range of germplasm. The studies so far reported clearly indicate that for predicting heterosis and hybrid performance, it is imperative that I) parents and progenies exhibiting significantly better agronomic performance over commercial varieties are identified, ii) characterized with a selected co-dominant molecular marker system capable of a) producing high level of reproducible polymorphism, b) covering the entire genome and c) be easily utilized with large population size to determine genetic distances and iii) producing meaningful correlation between genetic distance and F1 performance.

Marker-assisted selections: Although, marker-assisted selection (MAS) offer promise for I) indirect selection of desirable plant free of environmental, pleiotrophic or epistatic effects, ii) the ability to discriminate between homo and heterozygote, iii) pyramiding of genes, iv) monitoring the introgression and v) identification of recombinants possessing least amount of linkage drag and donor DNA flanking the gene of interest. However, reports on actual application of MAS are still limited. Among them, successful application includes identification of homozygous or heterozygous resistant plants against barley mild mosaic virus (BaMMV) and barley yellow mosaic virus BaYMV (Tuvesson *et al.*, 1998). There are numerous resistance sources against these viruses, but resistance to the cultivars of European winter barley to BaYMV and BaMMV depends entirely on a single recessive gene (ym4), which confers complete immunity. This gene is located on chromosome 3 and is mapped there with RFLP markers. Two RFLP markers (MWG10 and MWG 838) flanking this gene are 1.6 cM apart and are thus, highly attractive for the breeding programmes. One of the markers (MWG 838) was converted into a PCR based STS marker. Based on this marker, plants possessing homozygous or heterozygous resistance against BaMMV/BaYMB were selected and are being used actively in commercial breeding programmes. Barley stripe rust caused by *Puccinia striiformis* f. sp. *hordei* is another disease that cause severe quality and yield losses. Two RFLPs and one AFLP markers were used to map on chromosome 3 and 4, the QTL conferring resistance to barley stripe rust. These QTL were later introgressed through marker-assisted back crossing into elite breeding material lacking resistance. The selected backcross lines were converted into doubled haploids. These doubled haploid lines after phenotyping for stripe rust resistance were characterized with different markers to confirm the target gene and to identify lines with maximum percentage of recurrent parent genome. The procedure allowed rapid development of resistant germplasm of barley on commercial scale (Toojinda *et al.*, 1998).

Two populations of common beans (*Phaseolus vulgaris* L.) derived from recombinant inbred lines (RILs) were grown at 8 locations for 5 consecutive years (1990-1994) under stressed and non stressed conditions. The objective was to identify RAPD markers linked with drought resistance. Four RAPD markers were identified in one population and 5 in other that were significantly correlated with yield under drought stress, yield under non-drought stress and/or mean yield across a broad range of environment. Marker-assisted selection improved the performance of the first population by 11% under stress condition and 8% under non-stress condition while, conventional selections made on the basis of yield failed to improve performance. In the second population, on the other hand, no improvement in the performance was observed because heritability of yield in this population was three times less than that in the first population under both stressed and non-stressed conditions (Schneider *et al.*, 1997).

Rice blast is one of the most devastating diseases, which can be effectively controlled by host plant resistance approach. In genes pyramiding programme, using three near isogenic lines (NILs), resistance genes Pi-1, Pi-z5 and Pi-ta were characterized. The three genes were mapped with RFLPs to obtain closely linked DNA markers for markers assisted selection. Pair wise crosses were made between carrier isogenic lines with recurrent parent. The F1 plants were selfed to obtain approximately 150 plants for each cross. Southern analysis was used to select plants with two genes in homozygous condition. The carrier of two homozygous genes was further crossed with the plant carrying 3rd resistance gene in order to complete pyramiding of 3 genes to achieve durable resistance against rice blast (Hittalmani *et al.*, 1995).

Marker-assisted breeding has also been successfully used to develop a super broccoli variety that contains 100 times the concentration of cancer-fighting sulphoraphanes as normal broccoli do. This was achieved through a number of biochemical and DNA fingerprinting techniques to identify *Brassica* species that could be effectively used in breeding programmes with cultivated broccoli. The species belonging to Sicilian *B. villosa-rupestris* complex were bred with cultivated broccoli, which greatly increased sulphoraphane levels without significantly increasing the levels of more volatile unpalatable compounds (Faulkner *et al.*, 1998). The development of super broccoli took only 4 years to complete with markers assisted breeding compared with 10-15 years that may take through conventional breeding. This variety is already being tested for human consumption.

Most of the agriculturally important characters are multigenic, strongly influenced by the environment and expensive to evaluate directly. Quantitatively inherited traits have strong genetic components but, under normal conditions of measurements, cannot be measured by individually recognizable loci. Such quantitative traits can best be selected phenotypically provided they have high heritability (Falconer and Mackay, 1996). However, with the help of DNA markers, such complex traits have been resolved into discrete QTL, which can then be modified through marker-assisted selections. The individual effect of QTL, their interaction and usefulness in a breeding programme can all be evaluated once, they are well-defined in terms of DNA markers. Complex QTL like yield and nutritional value can also be manipulated through marker-assisted selection provided a reference population is available. This is imperative because genetic heterogeneity of trait(s), epistatic gene action and unknown genetic constitution among the potential parents at the QTL regions may become a limiting factor. Thus, many factors are to be considered seriously during the process of detecting and locating the QTL. These include I) type of population and progeny (Jansen and Stam, 1994), ii) population sampling strategies (Wang and Paterson, 1994), iii) determination of the genetic location of a QTL in the selected population, iv) threshold level for detecting QTL (Rebai *et al.*, 1994) and v) estimation of genetic effects of the selected QTL (Hayashi and Ukai, 1994). Lee (1995) has explained all these factors in grater detail. According to him, prospects for marker-assisted selection and transfer of polygenic traits are less certain, while benefits may be limited to specific situations. Nevertheless, encouraging results have generally been obtained through marker-assisted breeding aimed at transferring quantitative traits from non-adapted resources to elite breeding line e.g., drought tolerance and corn borer resistance to tropical maize.

Tanksley and Nelson (1996) suggested to combine QTL analysis and backcross breeding and applied it to the improvement of NILs (Near Isogenic Lines) to analyze introgression of genome segment transferred from wild tomato (Zamir and Fridman, 1999). Studies in rice (Xiao *et al.*, 1998) also confirmed marker-assisted selection of QTL transferred from a wild rice species *Oryza ruffipogon*. Marker-assisted early testing (in F2) of combining ability in maize (Eathington *et al.*, 1997), for QTL in doubled haploid line of barley (Romagosa *et al.*, 1999) and in sorghum (Tuinstra *et al.*, 1998) have also been known. In addition to this, a successful and very practical example of marker-assisted transfer of QTL was witnessed when soybean insect resistant (SIR) QTL were identified in soybean germplasm with RFLP markers (James *et al.*, 2001). Introgression of these QTL into registered cultivars, germplasm released and/or breeding lines was detected with SSR markers. This analysis was made on the plants in which SIR QTL were tracked already through pedigrees system. Amongst 15 such SIR genotypes, 13 exhibited introgression of SIR-M QTL with some

possessing minimum linkage drag as a consequence of phenotypic selection. The study stressed the need of markers assisted introgression and selection of SIR QTL, into elite breeding lines.

Integration of markers in back crossing for selection of quantitative traits will significantly increase the efficiency of a breeding program. It may also be useful for selection of mapping population among progenies of an advanced generation. However, several factors are to be considered before they are practically utilized. For example, only verified effects of important QTL can be included in such studies as over estimation or environment specific QTL may reduce the efficiency of selection index. Application of MAS for the improvement of recurrent population or experimental hybrids would require strong and established association between a QTL and a marker for the entire population for which, at the moment, cost effective and realistic solution does not exist. If marker-assisted selection for QTL is to be applied in the long term breeding programs, it would be imperative to understand that MAS combined with phenotypic selection will be superior initially but will become inferior when QTL approaches fixation (Hospital *et al.*, 1997). To fix resources for marker-assisted breeding, efforts required for obtaining reliable QTL estimates, comparative cost of phenotypic and marker data assessment, the genetic and non-genetic population parameters including population size, number of trials, replications per trial and the operational possibilities of the breeder should be thoroughly considered. Since different mapping populations generally share only small sets of common QTL (Lynch and Walsh, 1998), in very few cases association between a marker and a QTL would appear valid in genetically broad breeding population. Such situations can lead to highly conserved association between a marker and QTL, which needs to be considered before embarking on such an endeavour because it can significantly affect the efficiency of marker-assisted breeding. To cope with such situations, it is imperative to observe marker allele frequency changes in long-term experiments (Vuylsteke, 1999) or to identify marker(s) that can explain significant portions of the combing ability in diallel or factorial crosses. It is anticipated that such an exercise might reveal universally applicable markers.

Another approach to achieve maximize the effect of MAS could be through direct use of markers specific for QTL-alleles (Sorrel and Wilson, 1997) such as STS (sequence tagged site). Studies conducted so far revealed, that marker-assisted selections are highly suitable for I) introgression of exotic germplasm and ii) improving material derived from mapping population. However, in most of the practical situations, pure MAS is generally considered as equivalent to phenotypic selections and QTL need to be verified before being applied to MAS.

Marker-assisted foreground selection (Melchinger, 1990) are not being used frequently due to lack of allele specific markers which is a must. However, when three PCR primers derived from an RFLP marker linked with a major drought tolerance QTL, were used to select drought tolerant plants (Ribaut *et al.*, 1997), they were successfully selected in BC_2F_1 generation after tagging only 300 plants instead of screening a population of 2300 in conventional back-cross method up to BC6 generation. Thus, through markers assisted back crossing, not only the time for transferring a gene but also the size of the test population was reduced significantly. Marker-assisted foreground selection will certainly become more popular as allele-specific markers become available through genomic programme. Ragot *et al.*, (1995) transferred *Bt* gene from a maize variety Lancaster to Stiff Stalk maize inbred line. In these experiments, *Bt* gene was fused with herbicide tolerance gene *pat*. Therefore, foreground selections for *Bt* were not followed by markers rather selections were followed by spraying each BC generation with herbicide while RFLP markers were used for background selection that saved two BC generations.

For established breeding programme with some skill in handling markers, transfer of single important QTL alleles from an inferior donor parent through foreground selection will attract more attention. Marker-assisted background selections, on the other hand, will become a standard breeding technique when transgenic crop cultivars become more popular in future. For laboratories with reasonable resources to handle large marker-assisted breeding program, marker-assisted pyramiding of major disease resistance genes would become a prominent task. However, for resource deficient laboratories, this application will remain limited as long as the costs of markers analysis are high.

Molecular markers for abiotic stress tolerance: Drought, salinity and cold are the three major abiotic stresses based on depletion of water (Bartel and Nelson, 1994). Tolerance to these stresses is a complex polygenic character that exhibits continuous variation due to simultaneous segregation of several genes that are also influenced by the environment. To dissect such complex traits into discrete QTL and their further mapping required strong linkage with molecular markers. Such markers can be obtained from a saturated genetic linkage map of the parents, which, can only be constructed through crossing population possessing highly diverse response towards a particular stress.

Since, tolerance to the above-mentioned, abiotic stresses is a relative measurement due to their strong interaction with the environment and with each other, therefore, availability of suitable parents for crossing is difficult. Hence, marker-assisted breeding for abiotic stress is not a common practice. Nevertheless, QTL for responses to drought stress have been reported in rice (Champoux *et al.*, 1995; Quarrie *et al.*, 1997), maize (Leberton *et al.*, 1995), soybean (Mian *et al.*, 1996), wheat (Quarrie *et al.*, 1994), barley (Teulat *et al.*, 1998) and sorghum (Tunistra *et al.*, 1996). QTL for salt tolerance have also been noted in tomato (Foolad *et al.*, 1998; Foolad and Chen 1998; Foolad and Jones, 1993). Most of such QTL have been mapped using RFLP (Champoux *et al.*, 1995; Foolad *et al.*, 1998; Leberton *et al.*, 1995; Mian *et al.*, 1996), RAPD (Foolad and Chen, 1998), combination of RFLP and isozymes markers (Quarrie *et al.*, 1994), combination of RFLP and AFLP (Quarrie *et al.*, 1997) and, a combination of RFLP, RAPD and morphological markers (Sutka *et al.*, 1997). The characters evaluated through these QTL include root characteristics of rice (Champoux *et al.*, 1995), osmotic adjustment and other characteristics related with water relation in soybean, barley and maize (Mian *et al.*, 1996; Teulat *et al.*, 1998; Leberton *et al.*, 1995), leaf abscisic acid content in rice and wheat (Quarrie *et al.*, 1997; Quarrie *et al.*, 1994), leaf ash content in soybean (Mian *et al.*, 1996) and seed yield, yield component and plant architecture in common bean (Tar'en *et al.*, 2002). In addition to this, cold tolerance related QTL such as winter hardiness in barley (Hayes *et al.*, 1993), frost resistance in wheat (Sutka *et al.*, 1997), *in-vitro* freezing tolerance of acclimated and non-acclimated oilseed *Brassica* (Teutonico *et al.*, 1995) and drought avoidance or tolerance in sorghum (Tuinstra *et al.*, 1996; 1997a,b and 1998) have also been reported. Among all these characteristics, drought tolerance is the one for which QTL analysis holds great promise because genetics and physiological mechanisms involved in the expression of drought tolerance are poorly understood. They are controlled by many genes and are dependent on the timing and severity of moisture stress that makes it one of the most difficult traits for characterization.

Sorghum is one of the most drought tolerant grain crops and is therefore, an excellent model plant to study mechanism involved in drought tolerance. Tuinstra *et al.* (1996) developed recombinant inbred (IR) lines for sorghum and evaluated for their response to drought in a series of pre- and post-flowering stress environment for grain yield, stability of yield, rate and duration of grain filling, seed weight and stay green and associated traits. Several regions of the genome were found associated with the expression of yield or yield components under pre- and post-flowering drought and under fully irrigated conditions. In most of these cases, the marker allele associated with higher yield under fully irrigated conditions was also associated with improved tolerance or agronomic performance under drought. On the other hand, two separate linkage groups were found strongly associated with agronomic

performance under pre-flowering drought but not under full irrigation. These finding suggested the mediation of these loci in the expression of pre-flowering (Tuinstra *et al.*, 1996) and post flowering (Tuinstra *et al.*, 1997a) tolerance independent of mechanisms that control yield. Several QTL for stay green were identified on five linkage groups of which, QTL on three linkage groups were also found positively associated with grain yield under non-drought conditions. This indicates that there may be a physiological link between the expression of stay green under post flowering drought and grain yield under non-drought conditions. Although, these studies identified regions of the sorghum genome that conditions the expression of drought tolerance, it provided little information concerning the expression of individual QTL. This information has been obtained through analyses of near isogenic lines (NILs) differing in QTL with molecular markers (Tunistra *et al.*, 1997b). NILs contrasting at three loci were evaluated by testing markers flanking each target QTL for the differences in the size of the genomic region differentiating each set of the NILs. Agronomic evaluation of these NILs indicated large differences in yield and seed weight associated with each QTL marker. In most of the cases, NILs contrasting for a specific locus differed also in phenotype as was predicted by QTL analysis (Tuinstra *et al.*, 1998). Detailed analysis of such differences in agronomic performances indicated their possible association with heat tolerance, water status and expression of stay-green and suggested that these loci mediate the expression of drought tolerance via different biological mechanisms.

Another success story in which molecular marker have been used for abiotic stress tolerance is recorded for Pearl millet (*Pennisetum glaucum* L. R.Br.). Pearl millet is staple food and fodder crop of millions of poor rural families in the dry land agricultural environment of Asia and Africa. The crop is not commercially important (Anonymous, 1996) but yield losses due to biotic and abiotic stresses are economically important. Department for International Development (DFID), UK, initiated the development and application of molecular markers for the improvement of yield and yield component of pearl millet hybrid cultivars. For five consecutive years (1994-1999), efforts remained targeted on identification of markers that flanked QTL associated with superior grain yield of pearl millet under terminal drought conditions. Mapping population for this study was developed by Yadav *et al.* (1999) by crossing thermo-tolerant, drought sensitive inbred pollinator line H77/833-2 from India with thermo-sensitive, drought tolerant breeding line PRLT 2/89-33 from ICRISAT (Hash and Witcombe, 1994). The parental lines, skeleton maps and skeleton mapped progenies from this mapping population were used in a series of markers assisted back crossing programme. In addition to this, Jump-started marker-assisted back-crossing was used, which resulted into transfer of drought tolerance QTL identified on linkage group 2 of PRLT 2/89-33 (thermo-sensitive, drought tolerant) to H 77/833-2 (thermo-tolerant, drought sensitive). The progenies were advanced to BC4 F1 where they segregated for the target QTL and its flanking markers based on marker genotypes of the non-recurrent parents used. Based on these markers, non-recurrent parent plants phenotypically identical to recurrent parent (H77/833-2) and possessing both temperature and drought tolerance were selected which are being used as an elite, drought tolerant male parent of several hybrid cultivars of pearl millet in India.

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