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Molecular Markers in Plant Breeding-II. Some Pre-requisites for Use

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Abstract: This paper describes some of the pre-requisites for applying molecular markers in plant breeding or crop improvement programmes. This includes, possible answers to some of the very pertinent questions regarding marker-assisted plant breeding. For example, I) how an effective marker system is to be selected, ii) how, when and where these expensive technologies can be used efficiently and iii) what particular problem would be solved just by using a particular marker system. The most commonly used marker systems including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), micro-satellite or simple sequence repeat (SSR) and random amplified polymorphic DNAs (RAPDs) have been described in detail. The advantages and disadvantages (if any) of these marker systems and their practical utilization in different areas of crop improvement programmes have been discussed. Different methods of integrating molecular markers in conventional plant breeding programmes have also been described in greater detail with special reference to varietal identification and germplasm characterization, marker-assisted selection for qualitative and quantitative traits and abiotic stress tolerance. It is suggested that in all these cases, cost-effective application of markers can best be achieved through collaboration with those who excel in this technology. This collaboration is also necessary in order to integrate the systems and technologies to deliver the product in minimum possible time and with minimum extra input/investment in terms of operational expenses.

Key words: AFLP, background selections, DNA markers, foreground selections, RAPDs, RFLP, SSR

Introduction

In a previous paper we have described in detail different types of markers that can contribute significantly in accelerating the characterization/development of crop varieties (Farooq and Azam 2002). However, before embarking on such an endeavor particularly in this country and in any other country like Pakistan, it is imperative to know where, when, and how such markers are to be employed in plant breeding systems. The most important step in marker-assisted breeding is the selection of character(s) to be analyzed and a suitable marker system to be utilized. This paper provides answers to some of these questions in order to I) make utilization of DNA markers an efficient yet simple, relevant, and cost effective technique and ii) make marker-assisted breeding (plant molecular breeding) as an essential component of conventional plant breeding programmes.

Possible objectives to be pursued with DNA markers: Before initiating marker-assisted breeding, it is imperative to know I) what specific purpose is to be achieved through the use of markers, ii) which specific problem should we concentrate upon, and iii) how these expensive technologies should be used, especially in the developmental phase. Answer to these questions will depend upon I) the location of laboratory where the research work is to be conducted, ii) availability of infrastructure and resources, knowledge and skills to handle swiftly, conveniently and thoroughly all aspects of plant molecular breeding.

Molecular markers can conveniently be used in plant breeding programmes for the characterization of germplasm, assessment of genetic diversity in the collections used for crop improvement, and identification of crop varieties via DNA fingerprinting. Laboratories that are fully capable to handle marker-assisted breeding can focus on I) development of a high throughput, co-dominant marker system (not available elsewhere) for economically important crops established in the region, ii) marker-assisted selection with the help of quantitative traits loci (QTL) identified elsewhere in such crops, iii) tagging important agronomic gene(s) with appropriate marker system, iv) assessment of introgression from wild species to cultivated varieties and v) development of multiple trait mapping population based on parents established already in the region.

The most crucial amongst all these objectives is management of the genetic resources, systematic reduction of which has resulted into narrow genetic base of major agricultural crops. Awareness of the genetic diversity and of genetic resources will therefore, remain a vital component of all breeding programmes. The most important task for germplasm managers is to know the degree of overlapping in the accessions that originated from morphological variation and eco-geographical distribution. Markers-mediated characterization of such germplasm can reveal variation in accessions that not only helps germplasm managers but also provides information, which can be used to enhance the efficiency of a breeding programme (Laurent *et al.*, 1994). Similarly, for crop improvement programme where wild germplasm is to be utilized, characterization of wild species is of prime importance in order to mark duplicate and genotypically different accessions showing grossly identical morphology. DNA marker can provide a very effective way to identify and characterize such accessions thereby enhancing the efficiency of any crop improvement programme (Farooq *et al.*, 1995).

Identification and description of cultivars and varieties is essential particularly when breeders need to register their newly produced varieties with the seed companies. This is a routine activity of all the breeding programmes in both the developing and developed countries. With the advent of DNA markers, detailed description of a particular variety with reference to its taxonomy, genetics and phylogeny can be easily obtained. Thus, two of the most commonly encountered problems i.e. characterization of germplasm and varietal identification, can be resolved easily in a plant-breeding laboratory equipped only with a PCR machine, gel electrophoresis equipment and a gel photographic system. Achievement of the remaining objectives requires a very well-established laboratory and comprehensive knowledge on marker-assisted breeding. In both the cases, cost-effective application of markers can best be achieved through collaboration with those who excel in this technology. This collaboration is also necessary in order to integrate systems and technologies to deliver the product in minimum possible time and with minimum extra input/investment in terms of operational expenses. In countries where agriculture is an industry, applied plant breeders usually develops partnership with companies involved in marker technologies for contracting out small jobs to get better value for investment. For swift and meaningful application of markers in plant breeding, laboratories in developing countries must develop collaboration/partnership with such service laboratories. The laboratories that are fully equipped to handle all aspects of marker-assisted breeding, usually work in a systematic way to integrate molecular markers in gene tagging, and transfer of characters controlled by one (dominant/recessive) or more genes. In all these cases, choice of marker(s) is of prime importance to solve a particular problem and requires comparative understanding of the marker systems used frequently in practical plant breeding programmes.

Table 1: Comparison of different marker systems

	RFLP	RAPD	AFLP	M. satellites
Principle	Restriction and S. Blotting/ Hybridization	Amplification with random primers	Restriction and selective PCR amplification	PCR of simple sequence repeats
Type of polymorphism	Single base change, Insertion, Deletion	Single base change, Insertion, Deletion	Single base change, Insertion, Deletion	Change in repeat length
Genomic abundance	High	Very high	Very high	Medium
Level of polymorphism	Medium	Medium	Very high	High
Dominance	Co-dominant	Dominant	Dominant/Co-dominant	Co-dominant
DNA required	2-10 μg	10-25 ng	500 ng	50-100 ng
Sequence information	No	No	No	Yes
Radioactive requirement	Yes/No	No	Yes	No/Yes
Development cost	Medium	Low	Medium/high	High
Start up cost	Medium/high	Low	Medium/high	High
Portability of Tech. Lab./crop	High/High	Medium/Nil	High/Nil	High/low
Suitable application	Comparative mapping	Varietal/hybrid identification, marker-assisted selection	Fingerprinting, fast mapping, region specific marker saturation	Frame./Region-specific Mapping, Fingerprinting MAS

For more details see Anonymous (1998)

How to choose a suitable marker system?: In a previous paper, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNAs (RAPD), amplified fragment length polymorphism (AFLP) and single sequence repeats (SSRs) or micro-satellites were suggested to be the most commonly used DNA markers (Farooq and Azam, 2002). The choice of any of these marker systems depends upon I) the use of radioactive compound, ii) the number and amount of samples to be processed, iii) the anticipated level of polymorphism between the parents, iv) the size of the population developed on the basis of these parents and v) whether or not, the generated data would be used in different populations/laboratories. A comparison of the most commonly used marker systems with special reference to these variables is presented in Table 1.

Restriction fragment length polymorphism (RFLPs) was the first to develop (Soller and Beckmann, 1983) and are still widely used marker system especially in practical plant breeding. Most of the studies in systematic and evolution (Witkus *et al.*, 1994) and molecular maps of different crop species were initially made through the use of RFLP markers. A comprehensive study on these maps is documented by Phillips and Vasil (1994). In addition to that, voluminous literature is available on utilization of RFLPs in other aspects especially in DNA fingerprinting (Tanksley, 1983), phylogenetic analysis (Zheng *et al.*, 1994), detection of genetic variability in rice (McCouch and Tanksley, 1991), genetic dissection of maturity (Phillips *et al.*, 1992), identification of chromosomal regions responsible for regeneration of RFLPs (Armstrong *et al.*, 1992), comparative mapping of chromosomes in wheat, rye and barley (Devos *et al.*, 1993), analysis of wide compatibility in rice varieties (Zheng *et al.*, 1994), QTL mapping (Byrne *et al.*, 1996), gene tagging (Zhang *et al.*, 1995), and identification of ecotypes (Pupilli *et al.*, 2000). Thus, RFLP markers have the potential to address with power, precision and accuracy, all issues of plant breeding in which markers can be applied.

Utilization of RFLP markers requires a lot of skill in using short-lived radioactive material: the availability of which often becomes a limiting factor especially in the developing countries. Another limiting factor could be the large sample size to be analyzed like that of F_2 segregating population. In this case, utilization of RFLP markers may not be feasible, as it requires extraction of DNA in ultra pure form, restriction digestion, and lengthy and expensive process of isotopic labeling. Such difficulties have opened up a debate on the feasibility of routine application of RFLPs in large scale crop improvement programme. Another difficulty frequently encountered by RFLP users is the detection of low (1-3 loci) or non-existent polymorphism in closely related species/varieties (Waugh and Powell, 1992). It was essentially this difficulty that led to the search for a new marker system.

The well-developed Polymerase Chain Reaction (Mullis *et al.*, 1986) generated a new class of molecular markers termed as Randomly Amplified Polymorphic DNAs (RAPDs) (Williams *et al.*, 1990). Contrary to RFLPs, procedure for RAPD markers is technically simple, quick to perform without using radioisotopes and requires small amount of DNA. Cost effectiveness (Francisco-Ortega *et al.*, 1993) of RAPD markers and the ease of handling makes this system well suited to the programmes using large population. Thus, RAPD markers were preferred over RFLPs because of I) simplicity of the technique, ii) the high level of polymorphism, and iii) the low cost compared with RFLPs and iv) the simplicity of probes used for detection of polymorphism (Waugh and Powell 1992). RFLPs require either species specific and low copy genomic or cDNA clones while RAPDs require a random 10-mer oligonucleotide. It is because of these advantages that RAPDs have extensively been used for constructing linkage maps to manipulate swiftly and with precision the monogenic and polygenic characters. However, since RAPD markers segregate in simple Mendelian manner (Rafalski *et al.*, 1991), linkage analysis with the gene of interest could be time consuming and laborious. Thus, for quick identification of markers linked with the trait of interest, it is advisable to use RAPD markers in conjunction with Near Isogenic Lines (NILs). With this procedure and using NILs from tomato, Martin *et al.* (1991) found three markers tightly linked with *pto* gene; the analysis completed in 4 weeks compared with > 8 weeks required with RFLP markers.

Several other attempts have also been made to use RAPD markers, which also include identification of the two fusion parents in a somatic hybrid that was not possible even with RFLP markers. In addition, RAPD markers have been used extensively for accelerating the construction of genetic linkage maps (Martin *et al.*, 1991; Michelmore *et al.*, 1991), measuring variation in a population (Crochemore *et al.*, 1996), identification of cultivars and wild species (Farooq *et al.*, 1994,1995), gene tagging (Wang *et al.*, 1995), identification of hybrids (Farooq *et al.*, 1996), genetic analysis of disease resistance (Geunhwa *et al.*, 1997), linkage studies (Sun *et al.*, 1997), determination of heterogeneity in plants (Huff, 1997), mapping plant genome (Malyshev and Kartel, 1997), identification of alien chromatin and introgression in lines (Peil *et al.*, 1997), identification of phenotypically identical accessions (Farooq, 2001), measuring genetic distance and level of genetic variability (Gherardi *et al.*, 1998), and sex determination in plants (Rapaport *et al.*, 1998). Use of RAPDs on such a wide scale necessitates that this technique is applicable simultaneously in different laboratories involved in coordinated research in order to allow a comparison of data generated under different working conditions. Such an exercise can be fruitful only if the selected technique(s) can yield reproducible results. Unfortunately, however, RAPDs profiles have not been found thoroughly reproducible (Jones *et al.*, 1997). Another difficulty is that because of being dominant, RAPDs cannot detect heterozygots in a segregating population.

Amplified Fragment Length Polymorphism (AFLP) technique (Vos *et al.*, 1995) was thus introduced that combines the power of PCR and precision of RFLP making it fairly complicated (Karp *et al.*, 1997). The technique is composed of reliable and informative

probes to describe complex genetic structures (Powell *et al.*, 1996). Like all newly invented markers, AFLP has also been used extensively in phylogenetic studies of wheat (Heun *et al.*, 1997), *Solanum* (Kardolus *et al.*, 1998) and rice (Agarwal *et al.*, 1999), analyses of diversity and genetic variation (Riesberg *et al.*, 1999) and in paternity analyses (Krauss and Peakall, 1998). In few cases, complex taxonomic groups have also been described (Muluvi *et al.*, 1999).

One of the most important advantages of AFLP technique is that compared with RAPD, RFLP and even SSR markers, it generates higher polymorphism which is also largely reproducible (Jones *et al.*, 1997). It is because of this reproducibility that AFLP has been considered essential for gene flow studies, plant variety registration (Law *et al.*, 1998) and for paternity analysis (Krauss, 1999). Just like RAPDs, AFLP technique does not require sequence information. Being based on PCR, it can provide high throughput (Krauss and Peakall, 1998), which makes the technique ideal for large-scale population studies. It has also been used on dried samples and is thus suitable for material difficult to sample *ex-situ* (Harris and Robinson, 1994). Because of the high multiplex ratio (number of loci to be analyzed per experiment; Rafalski *et al.*, 1996), probability of analyzing the whole genome (rather than a segment of genome) is higher with AFLP compared with any other marker system (Harris, 1999). The steps that may limit the use of AFLPs include I) proficiency in molecular biological techniques, ii) availability of resources compared with suitability of the technique i.e., if the problem is solved with RAPDs then available resources should be invested in establishing the RAPD technique, while for obtaining quality data, investment made in RAPDs would be considered a wastage of money, and iii) choice of restriction enzyme (Ridout and Donini, 1999) and primer (Hartl and Seefelder, 1998; Kardolus *et al.*, 1998; Lerceteau and Szmidt, 1999). Since different combinations of primer and enzyme can produce variation in quality and quantity of the data, target material should first be screened with different primer combinations, which may not be feasible due to high cost of AFLP technique. In addition, scoring AFLP data can become a limiting factor due to the difference in methods used (Escarvage *et al.*, 1998; Angiolillo *et al.*, 1999; Agarwal *et al.*, 1999) and interpretation of the physical state of the bands (Gift and Stevens, 1997). Thus, computer software (Krauss and Peakall, 1998) and fluorescent detection methods are recommended to detect the AFLP fragment that may further raise the cost.

Simultaneous occurrence of dominant, and co-dominant banding pattern on the gels is another factor that limits the application of AFLPs in population genetics studies (Lynch and Milligan, 1994). However, increasing the sample size in combination with large number of polymorphism can overcome this limitation (Krauss and Pekall, 1998). The problem of whether or not, co-migrating bands are homologous, can lead to serious misinterpretation of AFLP data collected particularly for phylogenetic analysis: a difficulty that can be overcome by comparatively less expensive RFLP markers.

Micro-satellites also known as short tendem repeats (STRs) or simple sequence repeats (SSRs) are sequence units generally less than 5 base pair long (Bruford and Wayne, 1993). They result from errors in DNA replication (Moxon and Willis, 1999) during copying of repeat regions that can change the number of repeats (Jarne and Lagoda, 1996). The variability at micro-satellite loci is due to the differences in the number of repeat units. These are easily detectable as variation in length of a DNA fragment obtained through PCR amplification of the whole genome. Micro-satellites detect variation at individual loci and have thus been used in gene flow and mating systems (Chase *et al.*, 1996), paternity (Streiff *et al.*, 1999) and diversity analyses (Rosetto *et al.*, 1999; Seefelder *et al.*, 2000). Due to high variability, micro-satellites have also been used to re-study species in which other marker systems were unable to detect variation (Echt *et al.*, 1998). The most significant application of micro-satellites is in cross species analysis (White and Powell, 1997a,b), which stimulated some phylogenetic studies especially in Avocado (Mhameed *et al.*, 1997) and *Hordeum* (Proven *et al.*, 1999).

Unlike AFLP, micro-satellites generate large number of polymorphism at one particular locus. For example, one locus in soybean exhibited 26 alleles with SSR markers. Compared with single locus polymorphism generated by RFLP markers in rice, polymorphism generated by micro-satellite markers is reported to be six times high (McCouch *et al.*, 1997). Being strictly co-dominant, micro-satellite markers can detect heterozygotes which, compared with AFLP and RAPD markers, increases the efficiency and accuracy of population genetics measurements. It is because of this ability that micro-satellites are ideal markers for gene flow, hybridization and paternity analysis (Schlotterer and Pemberton, 1994). Like RAPD markers, only a PCR machine, gel electrophoresis apparatus and gel documentation/ photographic systems are required thus, making the system fairly cost effective.

Running SSR markers requires basic knowledge of molecular biology, handling of PCR and electrophoresis machines. Isolation and characterization of microsatellite regions, sequencing and the testing of micro-satellite primers can however, be time consuming and expensive. Hence, economically it is not advantageous to try developing new markers unless the required technology and expertise are already in hand. It would be most cost effective and efficient to have markers developed by a research laboratory with experience in this field. Analysis of SSR data requires a background in statistics. Even with an appropriate software package, it is imperative to know what software means and how it should be used to interpret data obtained from different loci.

The problems with micro-satellite markers are of varying nature. These include availability of a specific primer to analyze a particular organism and complex and expensive screening method with a success rate of as little as approximately 0.12% (Kelly and Willis, 1998). Due to co-dominant nature of these markers, less intense band in a profile of heterozygote usually escapes counting during screening which, may lead to misinterpretation of the data. Also, difficulty in differentiating less intense and small-size bands of up to 1-5 repeats that result from heterozygosity or error during amplification process (Ciofi *et al.*, 1998), difference (maximum up to 2.17 nucleotides) in the exact size of the allele and the one that appears on the automatic sequencing gels (Haberl and Tautz, 1999; Chavarriaga *et al.*, 1998) may also create problems. It is obligatory, therefore, to determine the real size of the allele first by sequencing before it is used as an internal standard. Micro-satellite markers contain variation within the SSR flanking and repeat regions that are composed of both point mutations and indels. Point mutations do not change the length of a micro-satellite product but the indels will. This may not only cause instability (Butler *et al.*, 1999) but will also lead to misinterpretation of the repeats (Blanquer-Maumont and Crouau-Roy, 1995). For example, an allele originally with 20 base pairs but having 6 base pairs in its flanking region, will co-migrate with an allele of 23 base pairs which do not contain insertion in its flanking region and will cause misidentification of the allele (Grimaldi and Crouau-Roy, 1997). This problem could have serious implications for phylogenetic studies. Presence of Null alleles resulting from mutation in binding site of one or both the SSR primers can also complicate the interpretation of a heterozygote (Morgante *et al.*, 1998) and cause mismatch between parents and offsprings (Pemberton *et al.*, 1998). It is because of these difficulties, that SSR markers are viewed as a very poor marker system for phylogenetic analysis (Jarne and Lagoda, 1996).

The comparison of advantages and disadvantages of different marker systems used frequently in crop improvement programmes suggests that it is the nature of the problem that determines the method of analysis. Thus, the most suitable system may not necessarily be the most modern or expensive. Therefore, it is imperative for the laboratories planning to employ DNA markers in breeding programmes to know which maker system is to be used for solving a particular problem?

What are the problems to be addressed through markers?: The utility of molecular markers in plant breeding is based on finding I) differences in the banding profiles (generally known as fingerprinting) obtained after gel electrophoresis (or Southern blotting) which allows inter- and/or intra-specific differentiation, and ii) tight linkages between markers and genes of interest which permits indirect selection for the presence of a desirable gene by assaying for the molecular marker. Such marker-assisted selections are unaffected by environmental factors, pleiotropy or epistasis. In addition, marker-mediated selection accelerates the pace of varietal improvement by at least two generations. For example, disease resistance is a qualitative character generally controlled by single dominant gene that can be transferred through backcross breeding. Backcross breeding (BC) allows transfer of one or more genes from agronomically inferior plant (donor) into an elite commercial cultivar while selection of plant carrying the target gene, like disease resistance, would be made through a phenotypic based assay. In a BC1 generation, the proportion of recurrent parent (RP) genome in the selected plants would be distributed within a mean of 75% and would be more skewed in the later generation (Frisch *et al.,* 1999). The same population would also contain plants that may contain 85% of the RP genome (Frisch, 1999). All these plants including those that possess the desired disease resistance gene can only be identified with the help of molecular markers flanking the target gene. This would not only save further back crossing (Tanksley *et al.,* 1989), but would also remove linkage drag which may otherwise take more than 10 BC generations (Murray *et al.,* 1980). Thus, in classical backcross breeding, the integration of markers would accelerate the conversion and removal of the linkage drag coming as baggage with the introgressed segment.

Most of the economically important traits of crops like high yield that show quantitative inheritance, are controlled by several genes and follow continuous variation caused by action and interaction of many genes and various environmental factor. Such quantitative traits (QTL) can best be selected phenotypically, provided they have high heritability (Falconer and Mackay, 1996). With the help of marker technology (Soller and Beckmenn, 1983) and the concept of QTL, a marker linked with a gene that influences a quantitative trait can be identified. Thus, with the help of molecular markers, it is possible to identify and map a QTL contributing to genetic variance of a trait. This would eventually help selecting a superior genotype without any effect of genotype and environment interaction.

Heterosis, the improvement of progeny over parents for yield and other traits when particular genotype are crossed, is one of the most common and important methods suggested to improve the genetic yield potential (Brummer, 1999). Only certain crosses express heterosis, which is caused by a combination of partial to complete dominance at loci controlling that trait and of different desirable alleles or allele frequencies in the populations being crossed. Molecular markers can help capture the heterosis through selection I) for agronomic traits, ii) of parents for inter-population cross, and iii) by improving the heterotic population for cross performance. If marker loci at or closely linked to genes for yield and other agronomic traits could be identified, divergent selections at these loci for contrasting alleles in each heterotic population would help improve the performance of inter-population hybrids without rigorous selections.

Tolerance to stresses like drought, salt and cold are complex polygenic quantitative traits, that display continuous phenotypic variation due to I) simultaneous segregation of several genes and ii) effects of environment, growth stage and tissue or cell size. Studying genetic loci controlling these traits individually is very difficult and cumbersome and so is the plant breeding for improvement of these traits. Using molecular markers, it has become possible to dissect and map these QTL on the genome, which can help assess/estimate their genetic effects.

How to apply markers?: Conventionally, marker-assisted breeding programmes begin only after the target gene or QTL mapping has identified the map position and closely linked flanking markers for donor parent gene blocks that contribute substantially to target trait's phenotypic variation in the mapping population. At this point, selection should be made for one or two genotyped progenies from the mapping population. This population must combine heterozygosity for the donor parent markers in the vicinity of the target QTL and homozygosity for the recurrent parent marker in the remaining mapped genome. There are two ways to use marker to achieve this objective. One way is to make extensive use of markers mapped in non-target regions of the genome. This is known as marker-assisted background selection and is needed to reduce the number of back crosses for the recovery of desirable segregant (Hospital et *al.,* 1992,1997). The alternative may be to use markers mapped only at points immediately flanking (and inside) the target region. This is known as marker-assisted foreground selection and used for rapid recovery of the recurrent parent genome. In this procedure (Melchniger, 1990), a donor parent (carrier of the target allele) is crossed with recurrent parent (RP) followed by back crossing the F_1 again with RP to generate sufficient number of BC1 plants. These BC1 plants are then genotyped with markers tightly linked with target allele followed by back crossing with recurrent parent. The presence of the target allele in these BC2 plants is then checked with the same linked marker. The selected plants (carrier of the target allele) will then be back crossed with the recurrent parent. This method is ideal for the programmes having allele specific markers in hand.

In marker-assisted background selections (Hospital and Charosset, 1997), marker alleles for recurrent parent genome are used for selection against donor background. In this procedure, strong linkage between marker loci and target locus is not required. However, the marker system that covers the entire genome is essential in order to select plants possessing maximum percentage of recurrent parent genome while still retaining target allele coming from the donor parent. Marker-assisted background selections procedure is ideal for the programmes working aggressively on transgenic crops or pyramiding major disease resistance genes. However, for laboratories with limited resources, this technique would have little application as long as the cost of marker analysis is high. Marker-assisted foreground and background selections are made simultaneously.

The advantage of marker-assisted back crossing is that once started, it cannot be abandoned because polymorphism characterized for donor and recurrent parent is usually based on phenotypic differences. The disadvantage is that since marker polymorphism is to be established first, it will take a long time to start actual marker-assisted back crossing. Also, since only the best marker genotype segregant present in the population is used in back crossing, it would remain a function of population size.

In Jump-started MAB programmes, back crossing begins during the development of mapping population before marker polymorphism of the two parents has been fully characterized. The F_1 plant selected from the cross of a donor and recurrent parent is back crossed with the parent that is the weakest for the target trait. This procedure uses the probability theory (Sedcole, 1977) and ensures that every possible QTL for the target trait is carried forward as rapidly as possible through the backcross generations. Upon availability of the suitable markers, these backcross generations can be used to identify (with minimum two markers per chromosomes) the segregants in which, individual chromosome arm from the donor parent may have been transferred into the recurrent parent genetic background. Through marker-assisted back crossing, such linkages can be used to rapidly detect one or more derivatives of the recurrent parent carrying a small homozygous segment of the donor consisting of target QTL. The major advantage of this procedure is early and rapid recovery of the recurrent parent genome in the non-target region due to early onset of back crossing programme. The disadvantage is that all of the efforts may go waste if the F_1 used, as non-

recurrent parent does not have a marker and QTL genotype identical to that mapped.

Factors affecting marker's application to QTL: Before applying markers to QTL, it is imperative to know that since, a quantitative trait is always controlled by quite a large number of genes (Falconer and Mackay, 1996) therefore, possible epistatic interactions of the QTL alleles with the genetic background of the material to be improved must be verified. In addition, power (probability of identifying a QTL), precision (agreement between repeated and independent estimates) and accuracy (agreement between estimates and true values) of a QTL must be understood. This depends upon type and size of the mapping population, number and distribution of true QTL, heritability, type and genome coverage of the selected marker system.

Marker-assisted selection may be based on marker information only (pure MAS) or on an index of marker plus phenotypic (combined MAS) data (Lande and Thompson, 1990). In both cases, application of markers can provide meaningful information only if they are tightly linked with target loci. Also, relative efficiency of MAS decreases as the number of QTL increases and their heritability decreases (Moreau *et al.,* 1998). This means that application of markers will be less effective for characters that are highly complex and governed by many genes and more effective if the QTL effects allow geometric distribution and only one or two of them explain the large portions of the genetic variance. In applying MAS to backcross breeding programmes, the population to be analyzed should contain at least one genotype having all favorable alleles for a particular QTL. Later, the number of QTL can be increased progressively but not beyond 6 QTL as transfer of all these would be prohibitively difficult in most practical situations. Also, greater the number of genes transferred, larger would be the portion of genome being fixed for unwanted genes. Cost effectiveness of MAS may also be considered before applying it to the breeding programmes. According to Xie and Xu (1998), marker-assisted selections are superior to phenotypic selections only if the cost of the later is not lower than the marker data.

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