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[Review]

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Molecular Markers and Their Application in Mulberry Breeding

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Mulberry (*Morus* spp.) is an economically important tree crop being cultivated in India, China and other sericulturally important countries for its foliage to feed the silk producing insect *Bombyx mori* L. Genetic improvements of mulberry lag behind to the same in many other economically less important crops due to the complexity of its genetics, the breeding behavior, and the lack of basic information on factors governing important agronomic traits. In this review, the general usage and advantages of different molecular markers including isoenzymes, RFLPs, RAPDs, ISSRs, SSRs, AFLPs and SNPs are described to enlighten their applicability in mulberry genetic improvement programs. Application of DNA markers in germplasm characterization, construction of genetic linkage maps, QTL identification and in marker-assisted selection was also described along with its present status and future prospects.

Key words: Molecular markers, mulberry breeding, marker assisted selection, QTL.

Introduction

Mulberry (*Morus* L.), of the family *Moraceae*, is believed to have originated at the foothills of the Himalaya and has been distributed in the warm and moist climatic zones between 50N Lat. and 10S Lat. (Koidzumi, 1917). Sanjappa (1989) reported that the genus *Morus* comprised of ca. 68 species and majority of these species occur in Asia, especially in China (24 species) and Japan (19). In India, *Morus* is represented by four species *M. indica* L., *M. alba* L., *M. laevigata* Wall., and *M. serrata* Roxb. (Brandis, 1874; Hooker, 1885). In sericulturally important countries like China, India, Japan, the major economic product of mulberry is the leaf, which is being used for rearing the silk producing insect *Bombyx mori* L. However, in most European countries including Turkey and Greece, mulberry is grown for fruits rather than foliage (Gerasopoulos and Stavroulakis, 1997; Ercisli, 2004). In India the mulberry acreage is ca. 282 244 hectors (Datta, 2000) while China has ca. 733,000 hectares (Yongkang, 2000). A rough estimation on the economicreturn of sericulture points to the fact that mulberry cultivation cost alone covers more than 60% of the total expenditure of the cocoon production (Das and Krishnaswami, 1965). This in turn necessitates to have mulberry plantations with high outputs. Keeping this in view, research in sericulture has given much emphasis on the genetic improvement of mulberry. Although conventional methods of breeding and selection coupled with mutation breeding have helped in developing a few new verities like V1, RFS-175, S1635, $BC_2$59 in India and Heybai, Lujiaoyo, Bei Ou No.1, Lun Jio 109, Sha No. 2 in China with high yielding potential, greater adaptability and higher response to agronomic practices, the full potential of this crop has yet to be harnessed. The major bottle necks in achieving the goal of developing varieties with high yield potential and wider adaptability are lacks of information on inheritance of important agronomic traits such as leaf yield, resistance to abiotic stresses (water, alkalinity, frost, etc.), biotic stresses (root knot nematode, root rot, tukra, etc.), and efficient nutrient uptake and leaf quality. Most of these traits are under polygenic control with considerable environmental influence and genotype-by-environment interaction on trait expression. Thus, these traits are the most difficult to breed for, typically requiring large-scale multi-environment testing in order to make progress from selection. Furthermore, it is also known that developing a new mulberry variety by conventional methods of breeding and selection may take a minimum of 15-20 years and at the end of all these long process release of a new variety cannot even be guaranteed. Thus, in order to curtail the breed-

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Table 1. Merits and demerits of different molecular markers commonly used in plant breeding

Features	RFLP (µg)	RAPD	ISSR	AFLP	SSR	SNP
Amount of DNA	> 10	0.02	0.02	< 1.0	< 1.0	< 1.0
Purity of DNA	high	high	moderate	moderate	moderate	high
Technique used	hybridization	PCR	PCR	PCR	PCR	PCR
Prior genomic information	needed	no	no	no	needed	needed
Reproducibility	high	low	high	high	high	high
Developmental cost	low	low	low	moderate	high	high
Amenability to automation	low	moderate	moderate	moderate	high	high
Nature of markers	Co-dominant	dominant	dominant	dominant	Co-dominant	Co-dominant
Cost per analysis	high	low	low	moderate	low	low

ing period, expenditure and also to enhance the efficiency of the selection process, dependable marker systems are essential. In this context, molecular markers like DNA markers appear to be quite promising as they help selection for quantitative traits. Therefore, together with conventional breeding methods, DNA marker techniques should be utilized to accelerate genetic improvements in mulberry.

In this paper, I review the progress in addressing the issues pertaining to identification and utilization of molecular markers in mulberry breeding and related questions surrounding the genetic improvement of mulberry. I outline some of the routinely used molecular markers, their application and comparative advantages. Finally, I discuss the progress towards identifying the genetic markers directly associated with leaf yield and its contributing characters in mulberry.

Molecular markers

Ideal markers should be polymorphic, multiallelic, codominant, non-episatic, neutral and insensitive to environment (Table 1). Morphological markers do not adequately meet these criteria. They are insufficiently polymorphic and are mostly dominant. Further, they are influenced by the environment and sometimes other associated traits. However, most of the biochemical and molecular markers have all the above characteristics. The following are some of the most important molecular markers being used widely in plant genome analysis.

Isozymes markers

Isozymes are multiple forms of enzymes that share a common substrate but differ in electric mobility (Markert and Moller, 1959). Isozymes are revealed when tissue extracts are subjected to electrophoresis in various types of gels and subsequently submersed in solutions containing enzyme-specific stains. Isozymes generally exhibit Mendelian inheritance, co-dominant expression, complete penetrance and are free of pleiotropic and epistatic interactions (Weeden, 1989). The isozyme technique is fast, cheap and simple. However, isozyme markers are not as plentiful as DNA markers and sometimes interpretation of zymograms become difficult due to complex banding profiles arising from polyploidy or duplicate genes. In addition, proteins with identical electrophoretic mobility (comigration) may not be homologous (Morell *et al.*, 1995). Isozyme studies in plants have demonstrated that pattern and band intensities differ depending on tissue types and developmental stages (Montarroyos *et al.*, 2003). Although Isozymes are the first molecular markers used in plant breeding (Tanksley and Orton, 1983), due to their low polymorphism its utilization has been mostly restricted to a few sporadic attempts of genetic diversity analysis in mulberry (Hirano and Naganuma 1979; Hirano, 1982; Venkateswarlu *et al.*, 1994). Hence, no further discussion will be made on isozyme in this review.

DNA markers

Recent advances in the field of plant molecular genetics have contributed a number of DNA markers with varying properties and usage. Depending on the techniques, theses DNA markers can be broadly classified as hybridization based markers and polymerase chain reaction (PCR) based markers. In hybridization based markers the DNA profiles are visualized by hybridizing the restriction enzyme-digested DNA blotted onto a solid membrane with radio labeled probe. Important among them is restriction fragment length polymorphism (RFLP). In PCR based marker system, *in vitro* amplification of a particular DNA sequences is carried out with the help of specifically or arbitrarily chosen primers and a thermostable enzyme, called *Taq* polymerase. The amplified fragments are separated electrophorectically on polyacrylamide gels or agarose gels and the banding patterns are detected by either staining or by autoradiography. Some of the important PCR based marker systems are random amplified poly-

morphic DNA (RAPD), inter simple sequence repeats (ISSR), amplified fragment polymorphism (AFLP), simple sequence repeats (SSR), expressed sequence tag (EST) and single nucleotide polymorphism (SNP) (Table 1).

Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism was the first developed DNA-based marker technique (Fazuoli *et al.*, 2000). The technique of RFLP analysis includes isolation of DNA, digestion of DNA with a restriction enzyme, separation of DNA fragments by agarose gel electrophoresis, transfer of the separated restriction fragments to a filter, detection of individual restriction fragments by nucleic acid hybridisation with a radioactively labelled probe and scoring of RFLPs by direct observation of autoradiograms. Polymorphism observed in RFLP analyses is based on the length of fragments generated by digestion with restriction enzymes. RFLP variability in plants could be due to (1) base sequence changes, which add or eliminate restriction sites, (2) rearrangements in the genome such as insertion or deletions, or (3) unequal crossing over or replication slippage. It appears that most RFLP variability in plants is caused by genome rearrangements rather than nucleotide sequence change. RFLP markers are simply inherited Mendelian characters and proven to be abundant in most organisms (Kochert, 1994). Further, it is noted that RFLPs are co-dominant markers, thus has the capability to distinguish both homozygotes and heterozygotes of a locus. Consequently, it is more informative than dominant markers. However, this technique requires relatively large amounts of highly purified and high molecular weight DNA, isolation of such a large quantity of DNA is often difficult due to non availability of plant materials. Further the process of RFLP is time consuming and laborious besides the need of utilizing hazardous radioisotopes. Therefore, it is not a very promising techniques for large-scale analyses involving evaluation of segregating populations for genetic mapping or extensive screening of germplasm collections (Halward *et al.*, 1992).

Random amplified polymorphic DNA (RAPD)

Random amplified polymorphic DNA was developed independently by Welsh and McClelland (1990) and Williams *et al.* (1990), which uses arbitrary short oligomers (usually 10-mer) which anneal to random homologous target sites within the genome. Variation among individuals for RAPD profiles mostly arises from base pair substitutions that modify or eliminate the primer binding sites. Insertions in the genomic sequence that separate the primer binding sites to a distance which prohibit amplification or cause length changes of the amplified product results in RAPD profile variation among individuals (Moeller and Schaal 1999). RAPD has many advantages over RFLP such as it requires only a small amount of template DNA, does not require sequence information for primer construction, is easy and quick to assay, has low cost, generates large number of DNA fragments per reactions. However, it suffers from less reproducibility and dominant nature of the markers (Rafalski, 1997). Relative to other methods, RAPD markers tend to underestimate genetic distances between more distantly related individuals, for example in inter-specific comparisons (Powell *et al.*, 1996).

Intersimple sequence repeats (ISSR)

Inter simple sequence repeat (ISSR) technique was first reported by Ztetkiewicz et aI. (1994), which involves amplification of DNA segments between two identical microsatellite repeat regions oriented in opposite direction using primers designed from microsatellite core regions. The technique uses microsatellite primers, usually 16-25 bp long, of di-nucleotide, tri-nucleotide, tetra-nucleotide or penta-nucleotide repeats to target multiple genomic loci. The primers can be either unanchored (Meyer *et al.*, 1993; Gupta *et al.*, 1994) or more usually anchored at 3' or 5' end with 1 to 4 degenerate bases extended into the flanking sequences (Zetkiewicz *et al.*, 1994). ISSR primers generate polymorphism whenever one genome misses the sequence repeat or has a deletion or insertion or translocation that modifies the distance between the repeats. Usually di-nucleotide repeats anchored either at 3' or 5' end reveal high polymorphism (Blair *et al.*, 1999; Joshi *et al.*, 2000; Nagaoka and Ogihara, 1997). The primers anchored at 3' end give clearer banding pattern as compared to those anchored at 5' end (Tsumura *et al.*, 1996; Blair *et al.*, 1999; *Nagaoka and Ogihara, 1997*). In general, primer; with (AG) (GA), (CT), (TC), (AC), (CA) repeats show higher polymorphism than those with (AT) repeats as the primers with (AT) repeats tend to be self-annealed. ISSR markers are generally considered as dominant markers following Mendelian inheritance (Tsumura *et al.*, 1996; Ratnaparkhe *et al.*, 1998), However, there are incidence where they segregated as co-dominant markers and helped to distinguish homozygotes from heterozygotes (Wu *et al.*, 1994; Akagi *et al.*, 1996; Sankar and Moore, 2001). Though, ISSR technique is simple, quick and less costly like the RAPD technique, ISSR markers have high reproducibility than RAPD primers. The studies on reproducibility showed that about 92-95% of the scored fragments could be repeated across DNA samples of the same cultivars and across separate PCR runs (Fang and Roose, 1997; Moreno *et al.*, 1998). Since the development of ISSR

markers does not need prior knowledge of the genome to be analysed, it has been used universally for plant genome analysis. ISSR markers have been used for characterization of germplasm (Bornet *et al.*, 2002), to estimate the extent of genetic diversity at inter- and intra-specific level in a wide range of crop species (Salimath *et al.*, 1995; Nagaoka and Ogihara, 1997; Joshi *et al.*, 2000), to identify DNA markers closely linked to important agronomic traits (Ratnaparkhe *et al.*, 1998; Levin *et al.*, 2000), to determine the distribution of microsatellites in the genome (Gupta *et al.*, 1994; Nagaoka and Ogihara, 1997; Blair *et al.*, 1999; Pasakinskiene *et al.*, 2000), to test the hypothesis of speciation and to study the history of colonization of plant communities.

Amplified fragment length polymorphism (AFLP)

The technique of amplified fragment length polymorphism is a combination of RFLP and polymerase chain reaction (PCR) techniques, hence, it combines the speed of PCR with the precision of RFLP (Vos *et al.*, 1995; Powell *et al.*, 1996). But like RAPD it requires only a small amount of DNA and it can also be readily automatable. Like RFLP, AFLP is a robust, reliable and reproducible technique that does not require sequence information of the target genome (Jones *et al.*, 1997). AFLP is carried out in four distinct steps (1) digestion of DNA using rare and frequent cutter restriction enzymes like *Eco* R1 and *Mse* I (2) ligation of double-stranded oligonucleotide adapters to the restricted sites (3) PCR amplification of restricted fragments with primers that bind to the adapter sequence, restriction site sequence and adjacent selective base(s) (4) acrylamide gel electrophoresis or capillary electrophoresis. The use of two restriction enzymes with different specificities enables the generation of a large number of DNA fragments suitable for PCR amplification. PCR amplification with specific primers reduces the number of DNA fragments to be amplified and ensures reliable and reproducible detection of restricted fragments. Acrylamide gel or capillary electrophoresis is sensitive and able to distinguish fragments differing in length by only one base pair (Miyashita *et al.*, 1999). AFLP can be used for DNA of any origin or complexity without any prior sequence information (Vos *et al.*, 1995) and a large number of markers that are randomly distributed throughout the genome can be visualized (Breyne *et al.*, 1997). AFLP markers are usually considered dominant markers since polymorphism is detected as presence and absence of fragments (Powell *et al.*, 1996).

Simple sequence repeats (SSR)

Simple sequence repeats (SSR) or microsatellite or short tandem repeat (STR) or simple sequence length polymorphism (SSLP) are tandem repeats of short (2-6 base pair) DNA fragments scattered throughout the genome that lie between conserved sequences (Litt and Luty, 1989). The three mechanisms that create a new allele at SSR loci are (a) replication slippage (b) unequal crossing-over and (c) genetic recombination. Replication slippage is considered to be a major factor affecting the repeat number for short tandem repeat sequences, whereas unequal crossing-over is thought to result in a very large number of alleles for long tandem repeat arrays (Huang *et al.*, 2002). In plant species, repeats containing (AT)n were found to be the most frequent dinucleotide repeat and the frequency of each class of SSR also appears to be different among plant species (Wang *et al.*, 1994). The SSR technique uses PCR to amplify DNA fragments by repeated cycles of DNA denaturation, annealing and extension using DNA polymerase enzyme and resulting DNA fragments are separated by banding on a gel. This method is rapid and reliable, markers are abundant and co-dominant. The major disadvantage of SSR is the need genomic information to develop primers, which is expensive and time consuming but. However, recent observations suggest that primers developed in one species may be useful to amplify microsatellite loci of closely related species (Hormaza, 2002).

Single nucleotide polymorphism (SNP)

The vast majority of polymorphisms exist in DNA sequence are single base pair differences. Recently much effort has been made to exploit the single nucleotide polymorphism (SNPs). SNPs are of interest because of their abundance and presence in single-copy DNA sequences. They may be found in both transcribed and non-transcribed regions and in some cases the direct cause for observed phenotypic variation. Because of the extremely high frequency of SNPs, meiotic recombination events are less likely to occur between two adjacent SNPs in comparison with other markers. Thus, several SNPs in a given DNA sequence may retain complete linkage disequilibrium for a considerable period of time. Thus, SNPs are going to play a major role in plant genome analysis, especially in QTL analysis. There are a number of ways by which SNPs can be investigated, a few among them are: Allele-specific amplification (D'Ovidio and Anderson 1994), Cleaved amplified polymorphic sequence (Konieczny and Ausubel 1993), Single stand conformation polymorphism (Orita *et al.*, 1989), denaturing gradient get electrophoresis (Fischer and Lerman 1979), Genetic bit analysis (Syvanen et al, 1990), Oligonucleotide chip-based hybridization (Chee *et al.*, 1996) and Matrix assisted desorption ionization, time of flight mass spectrometry (MALDI-TOF MS) (Ross *et al.* 1998). Further,

the continuing effort to improve the available techniques and the design of novel methods for SNP discovery and SNP genotyping will certainly expand the repertoire of this technology markedly.

Application of DNA markers in mulberry breeding

Challenges before mulberry breeders

Mulberry being an out crossing tree with high heterozygosity and long juvenile period, the breeders are faced with the following challenges. (1) understanding the basis of heterosis and prediction of hybrid performance, as it has already proved that developing homozygous plants through inbreeding or anther/pollen culture is not very easy (2) identification of useful genetic factors in divergent populations or lines, as phenotypic expression of mulberry varies with time and space, (3) introgression of desired genetic factors into elite breeding lines, selection of hybrids with desired traits and minimization of linkage drag (4) understanding and adjusting for genotype by environment interaction. These challenges can be met effectively and wisely, if we have proper understandings on (a) the number of genetic factors (loci) influencing expression of the traits, (b) the chromosomal location of these loci, (c) the relative size of the contribution of individual loci to trait expression, (d) pleitropic effects, (e) epistatic interactions among genetic factors, and (f) variation of expression of individual factors in different environments. Although a few attempts have been made in the past to understand the inheritance of certain quantitative traits like plant height, internodal length, leaf size, leaf weight and leaf yield using conventional methods of breeding and observation on morphological traits (Vijayan *et al.*, 1997a-b; Tikader and Dandin 2006) very little information could be generated from these investigations. Under this circumstance, for the manipulation and improvement of multigenic traits, maximum benefits from DNA-based markers need to be derived, which would enable to increase our understanding on the genetic bases underlying the quantitative trait variations.

Characterization and genetic relationships of parental materials

In India, the major germplasm of mulberry is maintained at Central Sericultural Germplasm Resources Center (CSGRC), Hosur, Tamil Nadu, though small germplasm collections could be seen at different Central Sericultural Research and Training Institutes situated in Mysore (Karnataka), Berhampore (West Bengal), and Pampore (Jammu and Kashmir). Presently, the germplasm of CSGRC, Hosur has more than 908 mulberry accessions (Indigenous-647 and Exotic-261), which were collected from 26 countries. In order to utilize these mulberry genotypes in different breeding programs, their proper identity, characteristics and genetic diversity among them are essential. Evaluation of such a large number of genotypes with morphological, biochemical and physiological traits is cumbersome, time consuming and labor intensive. Furthermore, afore said characters are influenced by developmental stages and physiological conditions of the plant, and are sensitive to environmental factors. In this context, molecular marker technique can play a vital role in identifying the genotypes and estimating the genetic diversity among them. The first advantage of molecular techniques is their capacity to detect genetic diversity at a higher level of resolution than other methods; furthermore, DNA-based assays are robust, speedy, information may be obtained from little amounts of plant material at any stage of development and it is not affected by environmental conditions.Owing to these adavantages, a number of investigations have been carried out with different molecular markers in mulberry. Molecular markers such as RAPD (Xiang *et al.*1995; Feng *et al.*,1996; Lou *et al.* 1998; Bhattacharya and Ranade 2001; Srivastava *et al.* 2004; Chatterjee et. al., 2004; Zhao *et al.* 2004) , AFLP (Sharma *et al.*,2000; Wang *et al.*,2001), SSR (Aggarwal and Udaykumar, 2004; Zhao *et al.*,2005) and ISSR (Vijayan and Chatterjee, 2003; Awasthi *et al.*2004; Vijayan, 2004; Vijayan *et al.*, 2004a-c Vijayan *et al.*, 2005; Vijayan *et al.*, 2006a-b; Zhao *et al.*, 2006, 2007) have been widely used to create molecular profile for genotypes, to estimate the genetic diversity among the germplasm accessions. These studies have identified many potential genotypes from the elite germplasm as well as wild relatives of the cultivated species, which can be used as parents in breeding programs with different goals. For instances, Vijayan and Chatterjee, (2003) Vijayan *et al.* (2005) worked out the genetic diversity among 34 mulberry accessions belonged to the cultivated species of mulberry, the genetically divergent parents can be used for heterosis breeding. Likewise, relationships and genetic diversities between cultivated and wild mulberry species have also been investigated (Vijayan *et al.*, 2004b) with a goal of identifying potential wild parents to incorporate desirable traits (stress resistance) into the elite and cultivating mulberry genotypes through introgressive breeding. In this context one should keep in mind that for accurate and unbiased estimates of genetic diversity adequate attention has to be devoted to: (i) sampling strategies, (ii) utilization of various data sets on the basis of the understanding of their strengths and constraints, (iii) choice of genetic similarity estimates or distance measures, clustering procedures and other multivariate methods in analyses of data; and (iv) objective determination of genetic relationships.

Genetic linkage map-a necessity for mulberry breeding

The speed and precision of breeding can be improved by the development of genetic linkage maps based on molecular markers to locate discrete chromosomal regions (QTLs), which control a number of complex polygenic traits. These maps indicate the order and relative genetic distance among the mapped loci. Mapping in outbreeding heterozygous perennial crops is not as advanced as in annual crops because they require more time and more space, given the long growing cycle and large crop size. Except for particular situations (Lanaud *et al.* 1995; Plomion *et al.* 1995) only progeny raised from cross between two heterozygous parents (F1 cross) are usually available. In this case, up to four alleles per locus may segregate, thus, the data are analyzed as a double pseudo-testcross (Grattapaglia and Sederoff 1994). In two-way pseudo-testcrosses dominant molecular markers segregate in 1 : 1 ratio because many alleles in highly heterozygous species are present in only one copy in one parent. These informative markers are used to build separate molecular maps for each parent (Grattapaglia and Sederoff 1994). New algorithms for recombination rate estimates also being developed, taking advantages of meioses occurring in both parents (Maliepaard *et al.* 1997; Ritter and Salamini 1996). However, in F1 crosses, marker phase (coupling or repulsion) can not always be deduced from parent and grandparent banding patterns (Carlson *et al.* 1991), which adds one source of complexity in the analysis. Nevertheless, several linkage maps, obtained by using molecular markers, are available today in out-breeding crops (Lespinasse et al 2000; Gao *et al.* 2005;). In mulberry, on the other hand, not much work has been done to develop linkage maps saturated with markers. As of todate, only a single molecular linkage map was reported from mulberry, which was made from RAPD, ISSR and SSR markers from 50 F1 full-sib progeny (Venkateswaralu *et al.* 2006). Two separate linkage maps for the female and male parents were also constructed using 94 testcross markers specific for female and male plants. The average map distances of 15.75 cM for the female and 18.78 cM of the male parents clearly indicated that this molecular map has less utility in identifying markers associated with quantitative trait loci for important agronomic traits. However, this initiative of developing linkage map with molecular markers may lead to the development of a molecular linkage maps saturated enough with markers. In this context, it is better to remember that a great potential for extremely fine genetic map is offered by the highly abundant single-nucleotide polymorphisms (SNPs), as they are the most abundant form of variation in any organism, which in turn offer the opportunity to uncover allelic variation directly within expressed sequences of candidate genes and to develop haplotypes based on gametic phase disequilibrium for analysis. However, in mulberry, as far as I know, no attempt has been made, as of to date, to develop SNPs that can be used for construction of genetic maps. Thus, serious attempts have to be made in this direction as a number of ESTs are now available from mulberry and also from closely related genra (GenBank No. EL772976 to EL812773), which can be assessed for identifying potential SNPs for mulberry.

Gene tagging/marker assisted selection

Marker-assisted selection (MAS) is emerging as a very promising strategy for increasing selection gains (Knapp, 1998). If sufficient mapping information is available, MAS can dramatically shorten the time required for genetic improvement of mulberry by allowing earlier selection of desired plants and reducing the plant population size to be assessed. Selection by molecular markers is particularly useful for tree crops with long juvenile period especially when the expression of the gene is recessive or the evaluation of the character is otherwise difficult as in the case of resistance to biotic or abiotic stresses (Luby and Shaw, 2001). Molecular marker-assisted selection involves scoring for the presence or absence of a desired plant phenotype indirectly based on DNA banding pattern of linked markers on a gel or on autoradiogram depending on the marker system. The rationale is that the banding pattern revealing parental origin of the bands in segregants at a given marker locus indicates presence or absence of a specific chromosomal segment which carries the desired alleles (Varshney *et al.*, 2004). MAS has many advantages over the conventional breeding program as it enable the breeder to select desirable hybrids at the seedling stages, eliminate extremely difficult field trails especially for diseases and pests, selection can be effected at a time for several traits, heterozygotes can easily be identified and distinguished from the homozygotes without resorting to progeny tests.

Mapping quantitative trait Loci (QTL) is one of the most powerful tools being used these days in plant breeding. QTLs are generally recognized by comparing the degree of covariation for polymorphic molecular marker and phenotypic trait measurements. Different statistical methods can determine the linkages between marker loci and QTLs. Single marker approach, interval mapping, composite interval mapping (Zeng 1994) are a few among them. Single marker approach is based on ANOVA, or simple linear regression, and performs statistical tests based solely on single DNA marker information, and the calculations are based on phenotypic means and variances within each of the genotypic classes (Coffman *et al.*,

2003). No genetic map is required for single marker analysis. A serious limitation of this approach is confounding of the effect of one QTL by many others that influence the trait. Another serious limitation is that a QTL with major effect and loose linkage cannot be distinguished from a QTL with minor effect and tight linkage. Thus, single marker approach could not gain much popularity.The interval mapping, on the other hand, is the most popular approach for QTL mapping (Lander and Botstein 1989) as it allows asymptotically unbiased estimates of QTL location and effect, with the assumption that there is only one QTL on a chromosome is true (Lander and Botstein 1989). Composite interval mapping is a combination of simple interval mapping and multiple linear regression, which maps multiple QTLs more precisely (Zeng 1994), hence has the potential to increase the QTL resolution (Liu, 1998). A number of software packages have been developed for analyzing QTLs and many are freely available. Some of the important packages are MAPMAKER-QTL (Lander and Botstein 1989), QTLstat (Liu and Knapp 1997),QTL cartographer (Basten *et al.* 1998), Qgene and MAPQTL. In any pseudo-test cross experiment the only detectable QTLs are those for which one or both parents are heterozygous for alleles of strong alternative effect which are not masked either by dominance (Groover *et al.* 1994) or the environment in which the phenotyping is conducted (Bradshaw 1996). Lin and Ritland (1996) found that selective genotyping might decrease the power of mapping multiple linked QTLs. Some strategies (statistical analysis) for mapping QTLs in outbred populations are available (Hoeschele *et al.* 1997), but none is unbiased. Recent reports on the genetic architecture of QTLs in interspecific hybrids of trees support the existence of a few QTLs controlling large proportions of the total genetic variation (Grattapaglia *et al.* 1996).

In mulberry, though not much work has been done to detect QTLs, using multiple regression analyses a few markers associated with many important agronomical and biochemical traits have been identified. Vijayan and Chatterjee (2003) using ISSR markers identified 2 markers viz., 825_{1400} and 835_{750} associated with leaf yield. Later, Vijayan *et al.* (2006b) identified a number of ISSR markers associated with number of branches, total shoot length, leaf weight, internodal distance, leaf chlorophyll, protein, leaf moisture percentage (Table 2). Recently Kar *et al.* (2007) using the same techniques of multiple regression identified an additional set of ISSR markers, for leaf protein and sugar. However, the information on the inheritance pattern of these markers has not been worked out yet, which is essential for their utilization in marker assisted-selection processes. Therefore, before the induction of these markers in breeding programs their inheritance pattern and association with the respective traits in different genetic back grounds are to be assessed, as marker association with traits is known to be altered under different genetic back ground. Efforts in this direction are to be made urgently.

Conclusions

Mulberry, being a perennial tree crop with a long generation time along with difficulties in developing inbred lines and large segregating progeny populations has frustrated the development and testing of molecular based breeding strategies. However, this same limitation makes new strategies that improve breeding efficiency especially valuable. The vegetative propagating ability of mulberry offers a unique advantage over many other agronomic crops that a desirable and unique gene combinations, once achieved, can be captured and perpetuated for a long period. Thus, serious and concerted efforts must be made to harness the advantages of molecular marker technologies in germplasm assessment, parental selection, and evaluation of progenies.

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