

*Review*

# Effect of molecular markers in horticultural crop breeding program

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Mapping and tagging of agriculturally important genes have been greatly facilitated by an array of molecular markers in crop plants. Marker-assisted selection (MAS) is gaining considerable importance as it would improve the efficiency of plant breeding through precise transfer of genomic regions of interest (foreground selection) and accelerate the recovery of the recurrent parent genome (background selection). MAS have been widely used for simple inherited traits than for polygenic traits, although there are few success stories in improving quantitative traits through MAS. They are been used to monitor DNA sequence variation in and among the species and create new sources of genetic variation by introducing new and favourable traits from landraces, wild relatives and related species and to fasten the time taken in conventional breeding, germplasm characterization, genetic mapping, gene tagging and gene introgression from exotic and wild species. The success of MAS depend on many critical factors such as the number of target genes to be transferred, the distance between the target gene and the flanking markers, number of genotypes selected in each breeding generation, the nature of germplasm and the technical options available at the marker level. The power and efficiency of genotyping are expected to improve with the advent of markers like single nucleotide polymorphisms (SNP). Although genetic maps have been developed for most important fruit and vegetables species and a number of horticulturally important gene loci have been tagged, only a few are reported. New, easy to perform allele testing methods are needed to bridge this large gap between marker development and application. This review discusses the basic requirements and the potential applications of MAS and the significance of integrating MAS into conventional plant breeding programmes.

**Key words:** DNA sequence, gene introgression, genetic maps, germplasm characterization, polygenic traits.

## INTRODUCTION

Conventional plant breeding is primarily based on phenotypic selection of superior individuals among segregating progenies resulting from hybridization. It is often time consuming as breeding a new variety takes between

eight and twelve years and even then, the release of improved variety is not guaranteed. Hence, breeders are extremely interested in new technologies that could make this procedure more efficient. Molecular marker-assisted selection, often simply referred to as marker-assisted selection (MAS) offers such a possibility by adopting a wide range of novel approaches to improving the selection strategies in horticultural crop breeding. Molecular markers are powerful research tools that make it possible to determine the genetic makeup of plants; they also serve as reference points to compare differences in DNA sequence and consequently, the allele composition between plants.

In particular, markers have provided a rapid method to screen parental germplasm for genetic variation, develop genetic linkage maps and tag genes controlling important

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**Abbreviations:** MAS, Marker-assisted selection; LD, linkage disequilibrium; LE, linkage equilibrium; MAB, marker-assisted backcrossing; BC, backcross; QPM, quality protein maize; QTL, quantitative trait loci; SNP, single nucleotide polymorphism; RAPD, random amplified polymorphic DNA; AFLP, amplified fragment length polymorphism; SSR, simple sequence repeat; STS, sequence tagged site; EST, expressed sequence tag; PCR, polymerase chain reaction.

traits. Both high density maps and markers linked to traits can assist in selecting breeding progeny carrying desirable alleles. Thus, molecular markers bring a systematic basis to traditional breeding, enhancing its precision and expediting the process (Kumar, 1999; Collard et al., 2005). In addition, a better understanding of the genetic and genomic control of horticultural traits achieved through molecular markers can help design more efficient breeding strategies and map – based isolation of genes aided by DNA markers can provide clones of specific genes for genetic engineering of horticultural crop species. This article discusses the role of molecular markers in horticultural crop breeding programme in increasing the efficiency of conventional breeding.

### **SALIENT REQUIREMENTS FOR MAS**

**The success of a marker-based breeding depend mainly on three important factors:**

- (1) A genetic map with an adequate number of uniformly-spaced polymorphic markers to accurately locate desired quantitative trait loci (QTLs) or major genes.
- (2) Close linkage between the QTL or a major gene of interest and adjacent markers.
- (3) Adequate recombination between the markers and the rest of the genome.

**Relationship between markers with respect to genes of interest also play an important role in the success of MAS. Three kind of relationship exist:**

- (1) The molecular marker is located within the gene of interest, which is most favourable and preferred situation for MAS but it is difficult to find. It is referred to as gene-assisted selection.
- (2) The marker is in linkage disequilibrium (LD) with the gene of interest throughout the population. LD is the tendency of certain combination of alleles to be inherited together. Selection using these markers can be called LD-MAS.
- (3) The marker is in linkage equilibrium (LE) with the gene of interest throughout the population, which is a most difficult and challenging situation for applying MAS.

In the real context of MAS, DNA- based markers can be effectively utilized for two basic purposes: (i) Tracing favourable allele(s) (dominant or recessive) across generations and (ii) identifying the most suitable individual(s) among the segregating progeny, based on allelic composition across a part or the entire genome.

### **FOREGROUND SELECTION AND BACKGROUND SELECTION**

The use of molecular markers to trace the presence of target genes is referred to as foreground selection while

their use for accelerating the recovery of the recurrent parent genome is referred to as background selection. Marker-assisted backcrossing (MAB) improves the efficiency of backcross breeding in three ways: (i) If the phenotype of the desired gene cannot be easily assayed, backcross (BC) progeny possessing a marker allele from the donor parent at a locus near/within the target gene can be selected with a good probability of carrying the gene, (ii) markers can be used to select BC progeny with least amounts of donor parent germplasm in the genome outside the target region and (iii) markers can be used to select rare progeny that are the result of recombination near the target gene, thus minimizing the effects of linkage drag.

Transfer of recessive genes through conventional breeding requires additional selfing generations after every backcross, a procedure that is prohibitively slow for most commercial breeding purposes. Melchinger (1990) presented an approach for calculating the minimum number of individuals and family size required in recurrent backcrossing but due to lack of allele-specific markers practical examples of this approach in plant breeding is limited. One successful example of foreground selection is the conversion of normal maize lines into quality protein maize (QPM) through marker -assisted transfer of a recessive mutant allele, *opaque 2*, using allele- specific molecular markers (Babu et al., 2004). Plastow (1999) reported that in animal breeding, the availability of an array of allele-specific markers has been facilitating applications of this approach on a commercial scale to eliminate disease and stress-susceptibility genes.

Marker-assisted background selection was proposed by Young and Tanksley (1989) and experimented by many scientists (Hospital, 1992; Frisch, 1999; Visscher, 1996) . This strategy has been used extensively in commercial maize breeding programmes, particularly for selection of lines carrying transgenes conferring herbicide tolerance or insect resistance (Yu, 1996). Several parameters need to be optimized in the background selection programs; flanking markers for the target allele are necessary to remove linkage drag.

### **APPLICATIONS OF MOLECULAR MARKERS IN CROP BREEDING**

**Trait tagging and marker- assisted-selection of horticulturally important genes**

One of the most practical applications of DNA-based markers in breeding programme is the ability to select phenotypic traits using markers tightly linked to genes controlling the trait. The ability to select plant based on the genotype rather than the phenotype is extremely attractive to plant breeders because many associated problem with phenotypic selection will be avoided using DNA-markers. The likelihood of identifying a gene by a

marker is inversely proportional to the distance between the gene and the marker. Interactions with other genetic and environmental factors limit the effectiveness of phenotypic evaluations. In addition, most fruit trees have a high level of heterozygosity that makes visual selection difficult but selection based on allele composition will avoid this problem. Ability to select breeding progeny early at the seedling stage is another advantage of using molecular markers. The number of trees that needed to be maintained in a fruit tree breeding programme can be reduced by eliminating progeny that do not carry the desirable allele at the seedling stage, saving space, time, labour and other resources. One common goal of most fruit, vegetable and ornamental breeding programme is to improve genetic resistance to major diseases, fruit size and number which collectively determine the yield potential (Monforte et al., 2001; Alpert and Tanksley, 1996), fruit tree shape, bud dormancy, cold hardiness and fertility factors such as male sterility, self incompatibility and reduced fruit set (Gökce et al., 2002; Pomper et al. 1998). For flowering ornamental species, traits as flower colour, size and petal number are being studied for tagging, for example, genes controlling double corolla and pink flower colour have been tagged (Debener and Mattiesch, 1999). Genes controlling height and compactness of ornamental plants are of high interest to green house crop breeders. Many of the fruit related traits are controlled by relatively large number of loci termed quantitative trait loci (QTLs) each making positive or negative contribution to the phenotype. DNA markers are especially useful in selecting for such quantitative traits that prove difficult to select due to phenotypic assessment alone. QTL regions controlling such traits have been identified in a few horticultural crops such as tomato (Grandillo et al., 1999), apple (Conner et al., 1998), peach (Dirlewanger et al., 1999).

### **Gene introgression from wild germplasm**

Markers can be employed for crop improvement in introgressing beneficial traits wild germplasm into crop cultivars. Markers linked to the genes from the wild parent (donor) parent as well as marker distributed throughout the genome of the improved cultivar (recurrent) parent in the form of genetic map, are used in selection of breeding progeny. Markers will be used in tracking desirable alleles from the donor parent and also, it will help reduce the genetic background of the donor parent in the progeny. Ribaut and Hoisington (1998) reported that marker-assisted selection achieved complete conversion to recurrent parent genome in three backcrosses compared with minimum of six backcrosses needed in conventional selection in maize. Successful introgression of fruit size and other quantitative fruit traits from exotic tomato species have shown that it is possible to apply molecular markers in the improvement of such complex traits (Fulton et al., 2000). Advanced backcross

QTLs have been performed on a number of crosses between wild tomato species and elite tomato lines (Tanksley and Nelson, 1996). Many QTL controlling a wide range of fruit traits have been found and mapped (Grandillo et al., 1999). Studies have shown that one cannot predict the genetic make up of exotic background based on phenotype alone and so, markers should be employed to fully exploit the potential of exotic and wild germplasm (Albert and Tanksley, 1996).

### **Germplasm characterization**

Molecular markers are used to evaluate variation in existing germplasm. Multiloci markers like random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers that can scan the entire genome quickly are efficient for this purpose. Molecular markers can help identify the genetic diversity or lack of it in the material available to breeders because understanding the genetic relationships among the germplasm helps to select appropriate parental plants for crossing and make informed decisions on breeding strategies too. Many horticultural crops might have very narrow genetic base which need to infuse genetic donors in the breeding programs (Sosinski et al., 2000). Wild relatives of crop plants are source of beneficial traits for crop improvement. The use of molecular markers to study genetic relatedness between wild and cultivated species provides information on selecting closest wild relatives to use in breeding programmes especially when crossing between the wild species and the cultivated species is difficult to perform (Huang and Sun, 2000; Jarret and Austin, 1994) (Tables 1 and 2).

Molecular markers can be used to identify core collections at germplasm repositories of collection centers in order to eliminate duplicate and unidentified materials but only represent the diversity available in all accessions present in the entire collection. This narrowing of the genetic materials allow breeders to use them more efficiently. This type of collection has been obtained for vegetable (Staub et al., 2002). Molecular markers also allow for parental verification of breeding progeny. Gaiotto et al. (1997) reported that nuclear DNA derived markers could be employed to identify the pollen parent in poly-crosses and open crosses and to estimate the level of outcrossing. Molecular markers verify hybrid origin of progeny (Pooler et al., 2002) and resolve uncertainty in parentage (Rajapaske et al., 2001). Codominant and multiallelic markers such as simple sequence repeat (SSR), sequence tagged site (STS) and expressed sequence tag (EST) markers are efficient in parental analysis.

### **Construction of genetic linkage maps**

Prior to the invention of molecular markers, map construction was based on phenotypic mutations which

**Table 1.** List of frequently used molecular markers.

<b>Abbreviations</b>	<b>Molecular makers</b>
AFLP	Amplified Fragment Length Polymorphism
CAPS	Cleaved Amplified Polymorphic Sequences
EST	Expressed Sequence Tag
IRAP	Inter-Retrotransposon Amplified Polymorphism
REMAP	Retrotransposon-Microsatellite Amplified Polymorphism
RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
RAPD	Random Amplified Polymorphic DNA
STS	Sequence Tagged Site
SCAR	Sequence Characterized Amplified Region
SSR	Simple Sequence Repeat
PCR	Polymerase Chain Reaction
ISSR	Inter- Simple Sequence Repeat amplification

**Table 2.** Different marker systems and their comparison.

<b>Marker</b>	<b>Advantages</b>	<b>Disadvantages</b>
RFLP	<ul style="list-style-type: none"> <li>-unlimited number of loci</li> <li>-co-dominant</li> <li>-many detection systems</li> <li>-can be converted to SCARs</li> <li>-robust in usage</li> <li>-good use of probes from other species</li> <li>-detects in related genomes</li> <li>-no sequence information required</li> </ul>	<ul style="list-style-type: none"> <li>-labour intensive</li> <li>-fairly expensive</li> <li>-large quantity of DNA needed</li> <li>-often very low level of polymorphism</li> <li>-can be slow (often long exposure times)</li> <li>-needs considerable degree of skills</li> </ul>
RAPD	<ul style="list-style-type: none"> <li>-results obtained quickly</li> <li>-fairly cheap</li> <li>-no sequence information required</li> <li>-relatively small DNA required</li> <li>-high genomic abundance</li> <li>-good polymorphism</li> <li>-can be automated</li> </ul>	<ul style="list-style-type: none"> <li>-highly sensitive to laboratory changes</li> <li>-low reproducibility within and between laboratories</li> <li>-cannot be used across populations nor across species</li> <li>-often see multiple loci</li> </ul>
SSR	<ul style="list-style-type: none"> <li>-highly polymorphic</li> <li>-fast</li> <li>-robust</li> <li>-can be automated</li> <li>-small quantity of DNA</li> <li>-multi-allelic</li> <li>-does not require radioactive labelling</li> <li>-co-dominant</li> </ul>	<ul style="list-style-type: none"> <li>-high developmental and startup cost</li> <li>-usually single loci even in polyploids</li> <li>-species-specific</li> <li>-difficult interpretation because of stuttering</li> </ul>
ISSR	<ul style="list-style-type: none"> <li>-robust in usage</li> <li>-can be automated</li> <li>-highly polymorphic</li> </ul>	<ul style="list-style-type: none"> <li>-usually dominant</li> <li>-species specific</li> </ul>

**Table 2.** Contd.

AFLP	<ul style="list-style-type: none"> <li>-can be automated</li> <li>-no sequence information required</li> <li>-small DNA quantities</li> <li>-can be adapted for different uses e.g. cDNA-AFLP</li> </ul>	<ul style="list-style-type: none"> <li>-marker clustering</li> <li>-dominant</li> <li>-technique is patented</li> <li>-evaluation of up to 100 loci</li> </ul>
IRAP/REMAP	<ul style="list-style-type: none"> <li>-highly polymorphic depends on the transposon</li> <li>-robust in usage</li> <li>-can be automated</li> <li>-species-specific</li> </ul>	<ul style="list-style-type: none"> <li>-alleles cannot be detected</li> <li>-can be technically challenging</li> </ul>
SNP	<ul style="list-style-type: none"> <li>-robust</li> <li>-suitable for high throughput</li> <li>-different detection methods available</li> <li>-can be automated</li> <li>-polymorphism are identifiable</li> </ul>	<ul style="list-style-type: none"> <li>-very high development costs</li> <li>-require sequence information</li> <li>-can be technically challenging</li> </ul>
Morphological	<ul style="list-style-type: none"> <li>-usually fast</li> <li>-usually cheap</li> </ul>	<ul style="list-style-type: none"> <li>-few in number</li> <li>-often not compatible with breeding aims</li> <li>-need to know the genetics</li> </ul>
Protein and Isozyme	<ul style="list-style-type: none"> <li>-fairly cheap</li> <li>-protocol for any species</li> <li>-fairly fast</li> <li>-co-dominant</li> <li>-require no sequence information</li> </ul>	<ul style="list-style-type: none"> <li>-often rare</li> <li>-often different protocol for each locus</li> <li>-labour intensive</li> <li>-sometimes difficult to interpret.</li> </ul>
<b>Marker</b>	<b>Advantages</b>	<b>Disadvantages</b>
Microarray	<ul style="list-style-type: none"> <li>-highly abundant</li> <li>-co-dominant</li> <li>-single base changes</li> <li>-no gel system</li> <li>-suitable for high throughput</li> <li>-highly polymorphic</li> <li>-highly reliable</li> <li>-small DNA quantity required</li> </ul>	<ul style="list-style-type: none"> <li>-very high development and start-up costs</li> <li>-portability unknown</li> </ul>
SCARS/CAPS	<ul style="list-style-type: none"> <li>-highly reliable</li> <li>-small DNA required</li> <li>-co-dominant</li> <li>-usually single locus</li> <li>-species-specific</li> </ul>	<ul style="list-style-type: none"> <li>-very labour intensive</li> </ul>
STS/EST	<ul style="list-style-type: none"> <li>-fast</li> <li>-cDNA sequences</li> <li>-non-radioactive</li> </ul>	<ul style="list-style-type: none"> <li>-substantially decreased levels of polymorphism</li> <li>-sequence information required.</li> </ul>

are rare and come from different genetic background and they are difficult to assemble into a single population. The advent of methods to generate DNA markers has greatly empowered genetic mapping of horticulturally important species and this discovery allowed map construction using only a single progeny set. In genetic mapping,

molecular markers consist of short segments of DNA that provide landmarks along the chromosomes which now provides a scaffold of the entire genome. One of the first linkage maps to be constructed is that of tomato (Bernatzky and Tanksley, 1986), roses for one diploid and tetraploid map (Rajapaske et al., 2001), map deve-

veloped from several crosses of major Solanaceae (Tanksley et al., 1992) and Brassicaceae (Quiros, 2001) crops. Genetic linkage map derived from the use of molecular markers provide various levels of genomic coverage and marker saturation.

## PROGRESS IN MARKER-ASSISTED-SELECTION

Though many economically important traits have now been tagged with DNA markers, instances of marker-assisted selection performed in horticultural crops are rare. A wide gap appears to exist between tagging genes with markers and actual application of the developed markers in breeding programs. This lack of marker application is due to a number of reasons. Most marker associations are not robust enough for successful marker-assisted selection (Young, 1999). In some instances, markers that tag a particular trait are specific to only one progeny line of the crop, whereas breeding is carried out with other lines for which the developed markers cannot be applied directly. To overcome this common problem, tagged markers should be more widely applicable to other progeny of the crop. Current marker technology also limits their application by breeding programs. Markers that can be effectively applied in selecting progeny should be technically simple methods that can be performed in breeders setting as opposed to a research laboratory. For screening a large numbers of progeny for marker-assisted selection, simple polymerase chain reaction (PCR) based allele-specific markers are the most appropriate. Further technological advancements are needed in marker analysis to fully realize the potential of molecular markers to breeding. For example, developing methods to perform PCR directly from crushed leaf disks would avoid lengthy DNA extraction and purification procedures. In addition, replacing currently used gel electrophoresis methods with nongel-based, plus or minus assays would facilitate more widespread marker application. These non-gel methods are now routinely being used in animal and human genetic diagnostic work, but are not yet applied to plants. One way to develop a simplified diagnostic method is to convert initial PCR-based allele specific markers into single nucleotide polymorphic (SNP) markers and use colorimetric assays, like the genetic bit analysis tested to select alleles in a locus controlling male sterility in onion (Alcala et al., 1997). Alternatively, SNP markers could be combined with DNA chip technology to test the presence or absence of specific alleles. These technological advances in marker analysis must be simple and cost-effective to perform. Handling and management of marker data should be made easy as well. Another reason for the large gap between marker development and application is the lack of close collaboration between the breeder and the molecular geneticist, as breeders trained in traditional ways alone are unable to apply these methods due to lack of skill and experience. When breeder and the molecular technologist cooperate for the effective use of molecular markers, often, lack of sufficient resources and primarily, funding, limits the

marker transition from the laboratory to the field. Applying techniques such as the advanced backcross QTL analysis that has been successfully carried out in tomato in other horticultural crops such as fruit trees is a challenge. This will require vast amounts of resources, to produce and maintain large progeny sets of several advanced generations and to screen under multiple environments as well as to carry out in-depth marker analysis.

## CONCLUSION

While DNA markers are now routinely used in a number of breeding programs of agronomic crops such as maize, rice and soybean, practical applications of DNA markers in genetic improvement of horticultural crops in general are still rare. However, the last decade has seen significant advancements towards application of molecular marker technology for crop improvement in a large number of horticultural crop species. Compared to agronomic crops like maize, in which recombinant inbred lines are available, many horticultural crops are highly heterozygous, making genetic dissection and mapping of traits difficult. In addition, doubled haploid lines and lines with chromosome deletions and additions are rarely available for map construction in horticultural crops. These factors have also contributed to the slow progress in the application of markers in breeding of horticultural crops. Despite the slow progress in application of molecular markers, they hold great promise for the genetic improvement of horticultural crops in the future. With advances in genetic testing methods in humans and animals, such as DNA chips and genetic bit analyses, simpler more 'breeder-friendly' markers are in the horizon for plants. These technological advances will bring screening for allele composition closer to breeding programs. In addition, to fully realize the potential of markers in genetic improvement of horticultural crops, advances in genomics of model species such as rice and Arabidopsis should be integrated with DNA marker technology.

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