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MARKER-ASSISTED AND GENOMIC SELECTION: A PARADIGM SHIFT IN HORTICULTURAL CROP BREEDING

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ABSTRACT

Plant breeding has undergone a significant paradigm change as a result of the development of molecular marker technology, which holds that better breeding results from identifying and characterizing the genetic loci governing a trait. Through marker-assisted selection (MAS), DNA markers have the potential to significantly increase the accuracy and efficiency of traditional plant breeding. Numerous mapping investigations of quantitative trait loci (QTLs) for various crop species have shown a wealth of relationships between DNA markers and traits. For agricultural experts, achieving a significant impact on crop development with MAS is the biggest challenge of the coming decades. The discovery of underlying key genes in gene pools and their transfer to desirable traits of major plant breeding programs have been accomplished through the use of marker assisted selection and molecular breeding. Molecular markers give traditional breeding a methodical foundation, increasing its accuracy and speeding up the procedure. Furthermore, map-based gene isolation facilitated by DNA markers can yield clones of particular genes for genetic engineering of horticultural crop species, and a deeper comprehension of the genetic and genomic control of horticultural traits attained through molecular markers can aid in the design of more effective breeding strategies. The function of molecular markers in horticultural crop breeding programs in boosting the effectiveness of traditional breeding is covered in this article.
Keywords **:** gene, marker-assisted breeding (MAB), genomic selection, horticulture.

Introduction

Scientific productivity gains in key food, feed, and industrial crops have been and will continue to be primarily driven by plant breeding. Plant breeding is accomplished using two methods: conventional and marker-assisted breeding (MAB) (Breseghello and Coelho, 2013). In order to create a better crop variety, conventional breeding entails hybridization between

heterogeneous parents followed by selection over several generations. Since the 1990s, marker-assisted selection (MAS) has been a significant plant breeding strategy due to encouraging analysis results for identifying QTL or tagging genes (Coors, 2008). MAB was developed to address the shortcomings of traditional breeding and uses molecular markers for indirect selection on desired traits in crop species. It requires little phenotypic data during the training phase (Collard and Mackill, 2008).

There are two types of MAB: genomic selection (GS) and marker-assisted selection (MAS). To choose plants with a desirable allele influencing the target attribute, MAS uses molecular markers that are known to be connected to phenotypes or traits of interest. It is only effective for traits that are primarily influenced by a small number of quantitative trait loci (QTLs); for complex quantitative traits that are influenced by a large number of minor QTLs, the method is even less effective than traditional phenotypic selection (Zhao *et al*., 2014). The primary reason is that linkage mapping and genome-wide association mapping (GWAS) frequently estimate QTL effects for small QTLs in a biased manner. As a result, research communities spent decades trying to figure out how to handle these complicated features, which led to the development of GS. Unlike MAS, which is based on a small number of markers, GS uses a broad set of marker information spread over the entire genome to evaluate an individual's genetic merit.

Genomic Selection

Genomic selection (GS) is a promising method that uses molecular genetic markers to create new breeding plans and models based on markers for genetic assessment. It offers chances to boost the genetic gain of complex features per unit of effort and expense in plant breeding. GS offers fresh chances to boost plant breeding programs' effectiveness (Bernardo and Yu, 2007; Heffner *et al.,* 2009; Crossa *et al*., 2010; Lorenz *et al.,* 2011). All genetic variance may be fixed by the GS, which can also precisely choose people with greater breeding value without the need to gather phenotypes specific to these individuals. As a result, the breeding cycle has been shortened, and early generation breeding material can now be selected and intercrossed more quickly. Numerous writers have determined that the expected annual genetic gain from applying GS is several times more than that of conventional breeding, and recent studies have demonstrated that GS has the potential to transform crop breeding (Bassi *et al.,* 2016). Due to labour and land-use costs, the cost of phenotyping is rising, but the cost of genotyping has drastically decreased in the era of next generation sequencing or NGS (Davey *et al.,* 2011). This has enhanced the utility of GS in crop improvement. Attempts to solve the issue of growing global hunger would directly benefit from this since it will increase the genetic evaluation of germplasm in crop improvement programs and speed up the supply of crop varieties with enhanced yield, quality, and biotic and abiotic stress tolerance.

The goal of genomic selection is to build a training population of individuals for whom both genotype and phenotype data are available. Then, using this data, a statistical model is developed that links variation in the individuals' observed phenotypes to variation in their observed genotype marker loci. More generations of parents and offspring produced a more potent training population than a single-generation person, and more generations and markers of people produced a more potent training population (TP). A prediction population made up of people for whom genotypes are available but phenotypes are not is then subjected to the statistical model derived from genotype and phenotype. The foundation of GS is the similarity between the breeding population (BP) and training population (TP) in the LD between marker and trait loci. This resemblance could be due to the breeding population being chosen from the training population or derived from it, or it could be because of the high marker density, which puts every characteristic locus out of equilibrium with at least one marker locus throughout the target species' whole population. The genomic selection (GS) prediction model is trained by genotyping and phenotyping the training population. Phenotyping's primary function in genomic selection is to compute the impact of markers and cross-validation. The program then uses the genotypic data from the breeding material to determine these lines' genome estimated breeding values (GEBV).

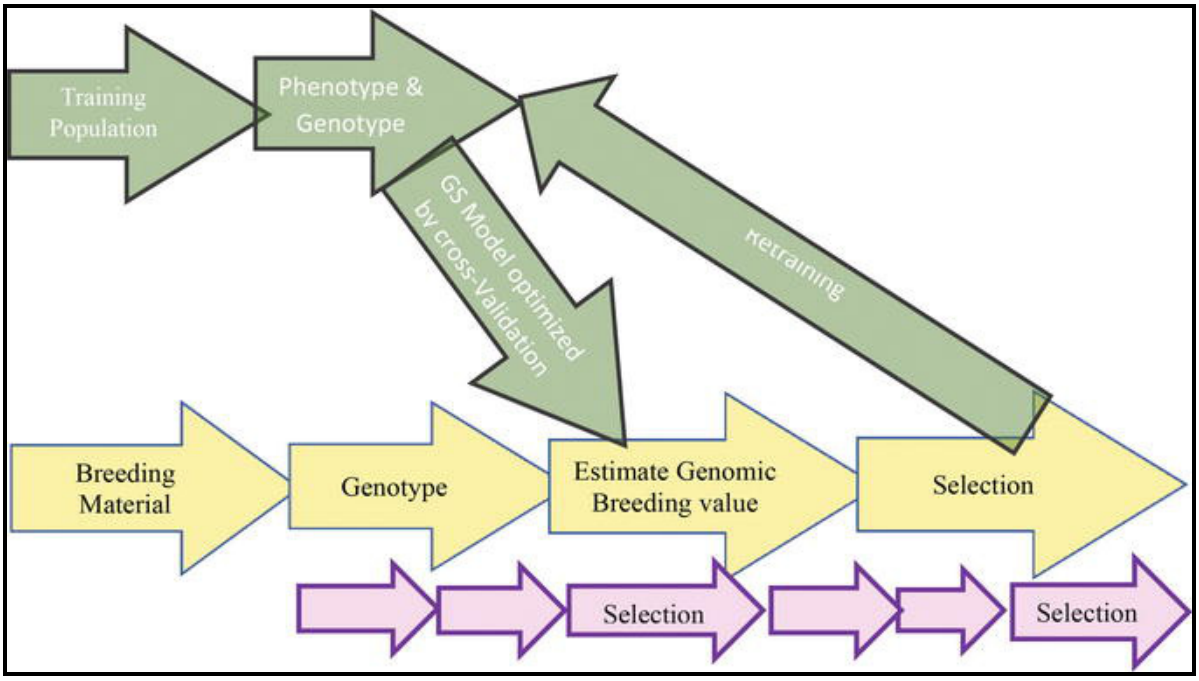

Fig. 1: Genomic selection scheme. Information on phenotype and genotype for a training population allows estimating parameters for the model (Perez-de-Castro *et al.,* 2012).

Salient requirements for mas

Three key elements are necessary for marker-based breeding to be successful:

(1) A genomic map that has enough evenly spaced polymorphic markers to precisely identify key genes or targeted quantitative trait loci (QTLs).

(2) A strong association between nearby markers and the QTL or a key gene of interest.

(3) A sufficient amount of recombination between the markers and the genome.

Main types of DNA markers used in MAS

When using DNA markers in MAS, there are five primary factors to take into account: cost, level of polymorphism, technical process for the marker test, quantity and quality of DNA needed, and reliability (Mackill & Ni 2000; Mohler & Singrun 2004).

Reliability: Target loci and markers should be closely connected, ideally within a genomic distance of less than 5 cM. The reliability of the markers to predict phenotype will be significantly increased by the use of intragenic or flanking markers.

DNA quantity and quality: Large quantities of high-quality DNA are needed for several marker approaches, which can occasionally be challenging to get in practice.

Technical procedure: Two important factors to take into account are the technique's degree of simplicity and time commitment. Simple, fast, and high-throughput techniques are ideal.

Level of polymorphism: In breeding material, the marker should ideally be highly polymorphic (i.e., differentiate between several genotypes), particularly in core breeding material.

Cost: The feasibility of MAS depends on the cost-effectiveness of the marker assay.

Relationship between markers with respect to genes of interest also plays an important role in the success of MAS.

There are three different types of relationships: (1) the molecular creator is found within the gene of interest, which is the most advantageous and desired scenario for MAS, but it is challenging to detect. (2) Throughout the population, the marker and the gene of interest are in linkage disequilibrium (LD). The tendency of a certain allele combination to be inherited jointly is known as LD. LD-MAS is the term for selection that makes use of these markers. (3) One of the hardest and most complex circumstances for implementing MAS is when the marker is in linkage equilibrium (LE) with the gene of interest across the population.

DNA-based markers can be used successfully in the actual context of MAS for two main purposes:

(i) Tracking a favorable allele (either recessive or dominant) across gene-rations,

(ii) Determining which individual or individuals among the segregating progeny are the most suitable based on allelic composition across a portion or the entire genome.

Applications of Molecular Markers in Crop breeding

Marker assisted selection of horticulturally important genes

Using markers that are closely connected to the genes governing phenotypic features is one of the most useful uses of DNA-based markers in breeding programs. Plant breeders find it very appealing to be able to choose plants based on their genotype rather than their phenotype since DNA markers will help them avoid many of the issues that come with phenotypic selection. When it comes to selecting for quantitative qualities that are hard to find via phenotypic evaluation alone, DNA markers are particularly helpful. A few horticultural crops have been found to have QTL regions governing these properties such as tomato (Grandillo *et al.,* 1999), apple (Conner et *al.,* 1998), peach (Dirlewanger *et al.*, 1999).

Another benefit of employing molecular markers is the ability to choose breeding offspring early in the seedling stage. By removing offspring that lack the desired allele at the seedling stage, a fruit tree breeding program can save space, time, labour, and other resources by lowering the number of plants that must be maintained. The majority of fruit, vegetable, and ornamental breeding programs share the objective of increasing genetic resistance to major diseases, fruit size and quantity, which together determine the potential yield (Monforte *et al.,* 2001; Alpert and Tanksley, 1996), fruit tree shape, bud dormancy, cold hardiness, and fertility factors like male sterility, self incompatibility, and decreased fruit set (Gökce *et al.,* 2002; Pomper *et al.,* 1998). Features including flower colour, size, and petal count are being researched for tagging in blooming ornamental species; for instance, genes governing the double corolla and pink flower colour have been tagged (Debener and Mattiesch, 1999).

Germplasm characterization

To assess variance in the current gemplasm, molecular markers are employed. For this, multi-loci markers that can swiftly scan the entire genome, such as amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD), are effective. Because knowing the genetic relationships among the germplasm aids in choosing the right parental plants for crossing and informing breeding strategy decisions, molecular markers can assist in identifying the genetic diversity or lack thereof in the material available to breeders. Numerous horticultural crops may have a relatively limited genetic background, necessitating the use of genetic donors in breeding initiatives (Sosinki *et al.,* 2000). Beneficial features for agricultural enhancement can be found in the wild cousins of crop plants. Finding the closest wild relatives to utilize in breeding programs is made easier by using molecular markers to examine the genetic relatedness of wild and cultivated species, particularly in cases where crossover between the two species is challenging (Huang and Sun, 2000; Jarret and Austin, 1994).

Construction of genetic linkage maps

Before the development of molecular markers, phenotypic mutations which are uncommon, originate from diverse genetic backgrounds, and are challenging to combine into a single population were used to generate maps. Genetic mapping of horticulturally significant species has been substantially enhanced by the development of techniques to produce DNA markers, and this discovery made it possible to make maps using just one set of progenies. Molecular markers in genetic mapping are brief DNA segments that serve as waypoints along the chromosomes, creating a scaffold of the complete genome. The tomato (Bernatzky and Tanksley, 1986) and rose (Rajapaske *et al.,* 2001) linkage maps were among the first to be created. They were created by combining several crosses of the principal Solanaceae (Tanksley *et al.,* 1992) and Brassicaceae (Quiros, 2001) crops. Different levels of genomic coverage and marker saturation are provided by genetic linkage maps that are produced through the use of molecular markers.

Gene introgression from wild germplasm

In order to introduce advantageous features from wild germplasm into crops cultivars, markers can be used for crop improvement. When choosing breeding offspring, markers connected to the genes of the wild parent (donor) and those dispersed across the genome of the enhanced cultivar (recurrent) parent in the form of a genetic map are employed. In addition to helping to lessen the donor parent's genetic influence on the offspring, markers will be used to track beneficial alleles from the donor parent. In contrast to the minimum of six backcrosses required for traditional selection in maize, Ribaut and Hoisingnton (1998) found that marker-assisted selection achieved complete conversion to recurrent parent genome in three backcrosses.

The application of molecular markers in the enhancement of complex traits has been demonstrated by the successful introgression of fruit size and other quantitative fruit features from exotic tomato species (Fulton *et al.,* 2000). Numerous crosses between elite tomato lines and wild tomato species have undergone advanced backcross QTLs (Tanksley and Nelson, 1996). Numerous QTLs that regulate a variety of fruit characteristics have been identified and mapped (Grandillo *et al.,* 1999). Studies have demonstrated that one cannot predict the genetic makeup of exotic background based on phenotype alone and hence, markers should be applied to fully exploit the potential of exotic and wild germplasm (Alpert and Tanksley, 1996).

Potential of marker-assisted selection for crop improvement

The biggest issue facing molecular breeding in the early 21st century is making a noticeable difference in crop development, even with the vast potential of MAS in plant breeding. To have a bigger impact, solutions for the MAS challenges listed above must be created. The following are the most crucial elements that should allow MAS influence to be realized in the near future:

a. A higher degree of integration between MAS, QTL mapping/validation, and traditional breeding;
b. Meticulous preparation and implementation of QTL mapping studies, particularly for complex quantitative characteristics, with a focus on validating results before MAS;
c. Optimization of MAS techniques such marker genotyping and DNA extraction, particularly with regard to efficiency and cost reduction, and
d. Effective data storage solutions, ranging from publically accessible databases to internal laboratory information management systems (LIMS).

The benefits of MAS above traditional breeding must be fully utilised if MAS is to realise its full potential for crop improvement. Ex ante studies of different methods before experimenting may be necessary for this. The best breeding plans to optimise genetic gain and reduce expenses may be suggested by computer simulations (Kuchel *et al.,* 2005).

Future Prospective of Marker-Assisted Breeding

There are currently numerous commercially significant features that have been marked with DNA markers; however, there are very few examples of marker-assisted selection in horticultural crops. There seems to be a significant disconnect between using the established markers in breeding programs and actually labeling genes with them. There are several explanations for this deficiency in marker application. For marker-assisted selection to be successful, the majority of marker associations are insufficiently strong (Young, 1999). In certain cases, markers that identify a specific attribute are unique to a single crop line, while breeding is done with additional lines for which the created markers are not directly applicable. Tag manufacturers ought to be more broadly applicable to different crop offspring in order to solve this prevalent issue.

Breeding programs are likewise limited in their use by current marker technology. Effective markers for progeny selection should be technically straightforward procedures that may be used in a breeding environment rather than a lab. Simple allele-specific markers based on polymerase chain reaction (PCR) are best suited for screening a large number of offspring for marker-assisted selection. To effectively utilize the potential of molecular markers in breeding, more technological developments in marker analysis are required. For instance, creating techniques to conduct PCR direct from crushed leaf discs would eliminate the need for time-consuming DNA extraction and purification processes. Furthermore, a wider use of markers would be made possible by substituting non-gel-based, plus or minus tests for the currently employed gel electrophoresis techniques. Although these non-gel techniques are currently often employed in genetic diagnostic work on humans and animals, they have not yet been used on plants. Converting initial PCR-based allele-specific markers into single nucleotide polymorphism (SNP) markers and using colorimetric tests, such as the genetic bit analysis evaluated to identify alleles in a locus controlling male sterility in onions, is one technique to create a simplified diagnostic method (Alcala *et al.,* 1997). As an alternative, SNP producers could be used in conjunction with DNA chip technology to determine if a certain allele is present or absent. These developments in marker analysis technology must be easy to use and reasonably priced. Additionally, it should be simple to handle and manage marker data.

The transfer of genetic markers from the lab to the field is frequently hampered by a lack of adequate funds and resources, even when breeders and molecular technologists work together to ensure their successful usage. It can be difficult to apply methods like the sophisticated backcross QTL analysis, which has been effectively used in tomato, to other horticulture crops like fruit trees. Producing and maintaining sizable progeny sets of numerous advanced generations, screening in a variety of

settings, and conducting in-depth marker analysis will all demand enormous amounts of resources.

Conclusion

Although DNA markers are currently often employed in a variety of agronomic crop breeding programs, including those for maize, rice, and soybeans, their actual usage in the genetic enhancement of horticulture crops in general is still uncommon. But in the past ten years, there have been notable developments in the use of molecular marker technologies for crop enhancement in a wide range of horticulture crop species. Many horticultural crops are quite heterozygous, which makes genetic dissection and trait mapping challenging, in contrast to agronomic crops like maize, where recombinant inbred lines are readily available. Additionally, it is uncommon to find doubly haploid lines or lines with chromosomal additions and deletions for agricultural crop mapping. These elements have also played a role in the sluggish adoption of markers in horticulture crop breeding. Although molecular markers have been applied slowly, they have enormous potential for future horticulture crop genetic improvement. Simpler, more "breeder-friendly" markers for plants are on the future thanks to advancements in genetic testing techniques for both humans and animals, such as DNA chips and genetic bit analysis. Breeding programs will be closer to allele composition screening thanks to these technical advancements. Additionally, developments in the genomics of model species like rice and Arabidopsis should be combined with DNA marker technology in order to fully realise the potential of markers in genetic enhancement of horticulture crops.

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