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Application and Advantage of Marker Assisted Selection in Plant Breeding: Review

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ABSTRACT

Conventional and modern plant breeding methods play a significant role in crops genetic improvement for desirable traits. Conventional breeding relied entirely on morphological markers, and selecting plants with desirable traits required several generations and many years. Selecting economically important traits such as yield, quality, and stress tolerance is challenging due to their polygenic nature and the strong influence of environmental factors. This challenge has driven plant breeders to explore second- and third-generation breeding techniques, such as marker-assisted selection (MAS). The present article was reviewed with the aim of understanding the principles and applications of marker-assisted selection in plant breeding. Various types of markers and their functions were articulated effectively. Single nucleotide polymorphism markers are among the most recent and advanced markers in plant breeding. They are highly effective for identifying genomic regions linked to desirable traits, thus enabling breeding accuracy and efficiency. MAS is an essential tool in plant breeding activities, including crop improvement. The applications of MAS in selecting breeding lines/populations, recurrent selection, marker-assisted backcrossing, and gene pyramiding were briefly discussed in the article. MAS plays a key role in shortening the breeding cycle and facilitating rapid, cost-effective selection. It significantly contributes to increasing genetic gain in desired traits compared to conventional breeding methods.

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Introduction

Since the late 19th century, plant breeders have relied on phenotypic-based selection to improve crops for desirable traits and develop new varieties. This selection involves assessing external and internal traits such as plant habits, disease resistance, yield, quality, and yield-related characteristics. Improved varieties were developed solely by selecting plants with desirable morphological traits. Over the years, plant breeding techniques have become increasingly sophisticated and time-consuming. Developing a new, improved crop variety through phenotypic selection can easily take more than 10 years [1].

Many agriculturally important traits, such as yield, quality, and disease resistance, are controlled by multiple genes and are referred to as quantitative traits (polygenic). The regions within genomes that contain genes associated with a particular quantitative trait are known as quantitative trait loci (QTLs). The identification of QTLs based solely on conventional phenotypic evaluation is not possible [2]. A major breakthrough in the characterization of quantitative traits, which created opportunities to select for QTLs, occurred with the development of DNA (or molecular) markers in the 1980s. In alignment with this, Reported that DNA markers in plant and animal breeding have introduced a new realm in agriculture known as 'molecular breeding' [3,4]. These markers are tightly linked to agronomically important genes (a process called gene 'tagging') and can be used as molecular tools for marker-assisted selection (MAS) in plant breeding. Consequently, some studies suggest that DNA markers will play a vital role in enhancing global food production by improving the efficiency of conventional plant breeding programs [5,6].

Marker-assisted selection (MAS) involves the use of genetic markers to track regions of the genome that encode specific characteristics of a plant [7]. Similarly, defined MAS as the selection for a trait based on genotype using associated markers, rather than the phenotype of the trait [8]. Additionally, confirmed that MAS involves using a marker as a substitute for, or to assist in, phenotypic selection, making it more efficient, effective, reliable, and cost-effective compared to conventional plant breeding methodologies [2].

In recent years, molecular marker technology, particularly DNA-based markers, has become increasingly popular among plant breeders. Molecular markers are widely used to characterize genetic diversity in germplasm collections, map and identify genes associated with important traits, mine superior alleles, and facilitate marker-assisted selection (MAS) of desirable genes/traits in breeding programs. DNA markers are more advantageous compared to conventional or morphological markers, as they meet the characteristics of an ideal genetic marker system: unlimited in number, insensitive to environmental conditions, highly reliable, and easily automated for convenience and cost-effectiveness [9].

Selecting plants in a segregating progeny that contain appropriate combinations of genes is a critical component of plant breeding [10,11]. Moreover, plant breeders typically work with hundreds or even thousands of populations, which often consist of large numbers of individuals and conduct selection within a specific time frame [10,12]. Marker-assisted selection can greatly increase the efficiency and effectiveness of addressing such challenges in plant breeding compared to conventional breeding methods. Once markers tightly linked to genes or QTLs of interest are identified, breeders can use specific DNA marker alleles as a diagnostic tool to identify plants carrying the desirable genes before conducting field evaluations of large numbers of plants. MAS provides an excellent opportunity for plant breeders to improve the efficiency and genetic gains of their breeding programs in various ways.

Genetic enhancement for agronomically important traits is challenging because most of these traits are genetically complex, controlled by multiple genes, and influenced by environmental factors. Classical breeding methods and tools are insufficient to address such complexities. However, molecular markers provide a means to precisely identify the number of genes controlling complex traits, locate them on linkage maps, and quantify their effects. This enables breeders to selectively manipulate complex traits in breeding programs [9]. Therefore, the objective of this review was to understand the principles and applications of marker-assisted selection in plant breeding.

Literature Review

Genetic Markers

Genetic markers are biological features, i.e., traits, enzymes/proteins, and fragments of DNA that are inherited from parent to progeny following Mendelian segregation. These features are used to keep track of an individual or a gene, hence called 'markers'. Genetic markers encompass four major markers, viz., morphological markers, cytological markers, protein/isozymes, and DNA markers [9].

Morphological Markers

Historically, plant breeders used easily observable morphological traits such as leaf shape, flower color, pubescence, pod/seed color, seed shape, and others to distinguish individuals as well as to use as a proxy in selection, when they are linked with other agronomic traits. Morphological markers have been reported in several crops, viz. rice, coffee, wheat, soybean, tomato and corn. For example, has listed more than 300 morphological traits that can be used for genetic analysis in rice [13]. A few associations/linkages between morphological and agronomic traits have been successfully used for selection in breeding populations. Early 1923, Karl Sax found an association between seed color and seed size in Phaseolus vulgaris that helped in selecting plants with large seeds.

Cytological Markers

The variations in banding patterns of the chromosome are called 'cytological markers'. They are used for chromosome characterization, detection of mutations and studying taxonomical relationships. The distribution of euchromatin and heterochromatin is the basis for variations in the banding pattern. Generally, the variations are visualized in terms of color, width, order and position of the bands created by staining the chromosomes. In recent times, fluorescent in situ hybridization (FISH) is used to detect and localize the presence or absence of specific DNA sequences on chromosomes using fluorescence-labeled DNA or RNA as probes. Cytological markers were widely used in physical mapping and linkage group identification. In line with this, have mapped the genes controlling seed storage proteins to chromosome 1R of rye using cytological markers [14]. Additionally, reported a translocation-based cytological marker that is associated with winter hardiness in oat [15].

Protein Markers

Some of the enzymes are present in multiple forms in an individual and still carry out the same function. Such variants of enzymes are called 'isozymes' and used as genetic markers. The isozymes are enzyme variants that are the products of different genes and thus represent different loci, whereas, enzyme variants produced by different alleles of the same gene are called allozymes. Isozymes are the first set of molecular markers. Enzyme variants differ in the amino acid sequences causing differences in size and charge, which can be visualized by gel electrophoresis. This class of markers was widely used in population genetic analysis during 1980s. However, protein-based markers are not stable across tissues, organs and developmental stages, and the number of informative marker loci is too small to use in gene mapping studies [9].

DNA Markers

Technological advances have enabled us to visualize the differences in DNA sequences itself across genotypes, which have led to the advantages of DNA-based markers. DNA markers are the variations observed in a particular portion of the DNA among the individuals of a species. These variations may be due to different mutations such as insertions, deletions and substitution or errors in replication of randomly repeated DNA. Since 1980, several DNA marker technologies have been developed which differ on the basis of polymorphism and the methodology to detect it [16-18]. These DNA markers are differentiated according to their function. Major of them are: Restriction fragment length polymorphism (RFLP) markers, Randomly-amplified polymorphic DNA (RAPD) markers, Amplified fragment length polymorphism (AFLP) markers, Microsatellite markers and Single nucleotide polymorphism (SNP) markers

Application of Markers Assisted Selection in Plant Breeding

The two major applications of DNA markers in plant sciences are detailed chromosome mapping and selection and introgression of both simple and quantitative traits. In addition, DNA markers are also used for germplasm evaluation, genetic diagnostics, phylogenetic analysis, study of genome organization and screening of transformants [19]. MAS' application has become an important tool in some areas of plant breeding and crop improvement.

Selection from Breeding Lines/Populations

Markers are used for selecting qualitative as well as quantitative traits. MAS can aid selecting for all target alleles that are difficult to assay phenotypically. Especially in early generations, where breeders usually restrict their selection activities to highly heritable traits because a visual selection for complex traits like yield is not possible with only few plants per plot being available. MAS is said to be effective, cost- and time-saving. To improve early generation selection, markers should decrease the number of plants retained due to their early generation performance, and at the same time they should ensure a high probability of retaining superior lines (Figure 1) [20]. An important prerequisite for successful early-generation selection with MAS are large populations and low heritability of the selected traits, as under individual selection, the relative efficiency of MAS is greatest for characters with low heritability [21].

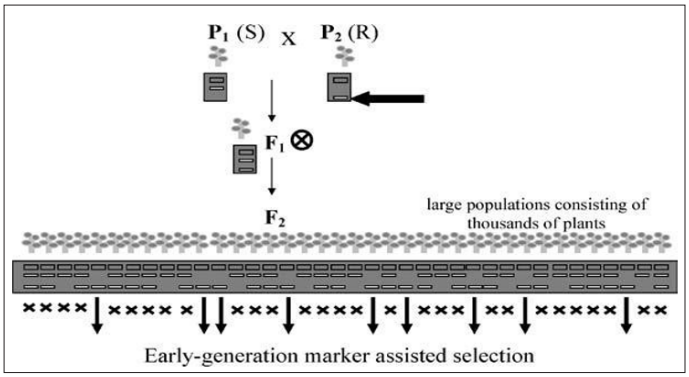

Figure 1: Early Germination Marker Assisted Selection
Source: Ribaut and Hoisington,1998 [4].

A susceptible (S) parent is crossed with a resistant (R) parent and the F1 plant is self-pollinated to produce a F2 population. In this diagram, a robust marker has been developed for a major QTL controlling disease resistance (indicated by the arrow). By using a marker to assist selection, plant breeders may substitute large field trials and eliminate many unwanted genotypes (indicated by crosses) and retain only those plants possessing the desirable genotypes (indicated by arrows). Note that 75% of plants may be eliminated after one cycle of MAS. This is important because plant breeders typically use very large populations (e.g. 2000 F2 plants) derived from a single cross and may use populations derived from hundreds or even thousands of crosses in a single year.

Marker-Assisted BackCrossing (MABC)

Backcrossing is used in plant breeding to transfer (introgress) favorable traits from a donor plant into an elite genotype (recurrent parent). In repeated crossings the original cross is backcrossed with the recurrent parent until most of the genes stemming from the donor are eliminated. However, the donor segments attached to the target allele can remain relatively large, even after many backcrossing generations. In order to minimize this linkage drag, marker assays can be of advantage. Markers can be used in the context of MABC to either control the target gene (foreground selection) or to accelerate the reconstruction of the recurrent parent genotype (background selection). According to in traditional backcross breeding the reconstruction of the recurrent parent genotype requires more than six generations, while this may be reduced to only three generations in MABC [22].

Traditional backcrossing is especially efficient if a single allele is to be transferred into a different genetic background, for example, in order to improve an existing variety for a specific trait. However, if the performance of a plant is determined by a complex genotype it is unlikely that this ideal genotype will be attained through MABC only [23].

Marker-Assisted Recurrent Selection (MARS)

The improvement of complex traits via phenotypic recurrent selection is generally possible, but the long selection cycles impose restrictions on the practicability of this breeding method. With the use of markers, recurrent selection can be accelerated considerably. In continuous nursery programs preflowering genotypic information is used for marker assisted selection and controlled pollination. Thus, several selection-cycles are possible within one year, accumulating favorable QTL alleles in the breeding population [24].

Markers are also frequently used to select parents with desirable genes and gene combinations, and MARS schemes involve several successive generations of crossing individuals based on their genotypes. The achievable genetic gain through MARS is probably higher than that achievable through MABC [23].

Pyramiding

Using MAS, several genes can be combined into a single genotype. Pyramiding is also possible through conventional breeding but phenotypically testing individual plants for all traits can be time-consuming and sometimes very difficult. The most frequent strategy of pyramiding is combining multiple resistance genes. Different resistance genes can be combined in order to develop broad-spectrum resistance to, e.g., diseases and insects. Either qualitative resistance genes can be combined or quantitative resistances controlled by QTLs. An example for the combination of resistance QTLs is the pyramiding of a major stripe rust resistance gene and two QTLs in the same genotype [25]. In order to pyramid disease or pest resistance genes that have similar phenotypic effects, and for which the matching races are often not available, MAS might even be the only practical method – especially where one gene mask the presence of other genes [26,27].

The Barley Yellow Mosaic Virus (BaYMV) complex as an example is a major threat to winter barley cultivation in Europe. As the disease is caused by various strains of BaYMV and Barley Mild Mosaic Virus (BaMMV), pyramiding resistance genes seems an intelligent strategy. However, phenotypic selection cannot be carried out due to the lack of differentiating virus strains. Thus, MAS offers promising opportunities. Suitable strategies have been developed for pyramiding genes against the BaYMV complex [1].

Application in Germplasm Storage, Evaluation and Use

Marker-assisted germplasm evaluation is an important tool in the acquisition, storage and use of plant genetic resources (PGR) and the evaluation of germplasm can be considerably improved with the assistance of markers [28]. Markers can be used prior to crossing to evaluate the breeding material. Also, mixing of seed samples can be discovered using markers instead of growing plants to maturity and assessing morphological characteristics [29]. In order to broaden the genetic base of core breeding material, germplasm of diverse genetic background for crossings with elite cultivars can be identified with the assistance of markers and markers are on the whole a valuable tool for characterizing genetic resources, delivering detailed information usable in selecting parents [30]. According to molecular markers can be used for (i) differentiating cultivars and creating, maintaining, and improving heterotic groups; (ii) assessing collections and identifying germplasm redundancy, underrepresented alleles, and genetic gaps; (iii) Monitoring genetic shifts that can occur during medium- or long-term storage, regeneration, domestication, and breeding; (iv) identifying unique germplasm; and (v) constructing core collections [31].

Choice of Marker

In general, there is no perfect marker. Markers vary in their attributes such as abundance, genomic distribution, level of polymorphism, basis of polymorphism, technical requirements, cost, etc., and the choice depends mainly on the application. Other factors influencing the selection of markers include accessibility, technical expertise, turnaround time and level of polymorphism in the study material, DNA quantity and quality requirement, transferability between laboratories and populations and cost [9].

Table 1: Comparison of Most Commonly used Marker Systems (Adopted from Korzun, 2003)

Feature	RFLPs	RAPDs	AFLPs	SSRs	SNPs
DNA required (g)	10	0.02	0.5-1.0	0.05	0.05
DNA quality	High	High	Moderate	Moderate	High
PCR-based	No	Yes	Yes	Yes	Yes
Number of polymorph loci analyzed	1.0-3.0	1.5-50	20-100	1.0-3.0	1.0
Ease of use	Not easy	Easy	Easy	Easy	Easy
Amenable to automation	Low	Moderate	Moderate	High	High
Reproducibility	High	Unreliable	High	High	High
Development cost	Low	Low	Moderate	High	High
Cost per analysis	High	Low	Moderate	Low	Low

Advantages of Marker Assisted Selection

Shortening the Breeding Cycle

The MAS helps breeders to select plants carrying desirable genes at heterozygous state itself; therefore, selfing can be avoided and a season can be saved (Figure 2). This is especially important for traits controlled by recessive genes as well as crops that are highly season bound. Conventional backcrossing will not give any clue about the genome status of the backcross progenies. Based on a simulation study, reported that ~1 % of the backcross progenies may have the maximum of recurrent parent genome. When MAS is applied, those progenies (with ~97 % of recurrent parent genome) can be identified with one or two generations [32].

Some studies involving markers for disease resistance have shown that once markers have been developed for MAS, it is cheaper than conventional methods [33]. In other situations, phenotypic evaluation may be time-consuming and difficult. Therefore; using markers is preferable to save time of evaluation [33-35].

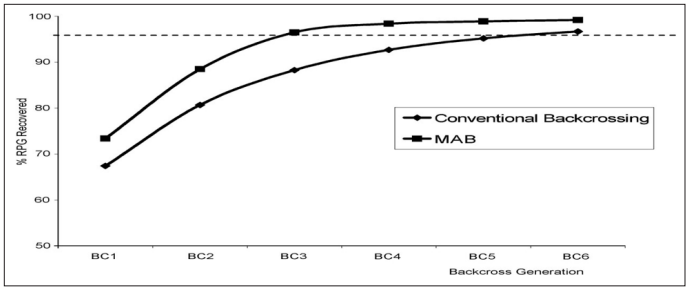

Figure 2: The Graph of Recurrent Parent Genome (RPG) Recovery using Marker Assisted Backcrossing (MAB) and conventional backcrossing.

Graph of PLABSIM computer simulations of recurrent parent genome (RPG) recovery using marker assisted backcrossing (MAB) and conventional backcrossing [36]. The use of markers can reduce the number of generations required to achieve the desired proportion of the recurrent parent genome (indicated by the dotted line).

Pyramiding of Multiple Genes

It has been possible to transfer only one or two genes into a single cultivar background through conventional breeding methods, especially for simply inherited traits as well as in some cases for pest and disease resistance owing to the availability of pathotypes or biotypes [37]. MAS also help the elimination of unreliable phenotypic evaluation associated with field trials due to environmental effects; selection of genotypes at seedling stage; gene 'pyramiding' or combining multiple genes simultaneously and avoid the transfer of undesirable or deleterious genes ('linkage drag'; this is of particular relevance when the introgression of genes from wild species is involved). MAS has great role in Selecting for traits with low heritability and testing for specific traits where phenotypic evaluation is not feasible (e.g. quarantine restrictions may prevent exotic pathogens to be used for screening) [2].

Breaking Undesirable Linkages

In backcrossing programme, it is possible that a large segment of wild chromosome is fixed in the plant selections. For instance, Ty1 and Ty3 genes conferring resistance to tomato yellow leaf curl disease are part of a large region (~32 cm) from Solanum chilense , and the lines homozygous for this region may show deleterious effects [38].

Based on a simulation study, reported that even after 20 rounds of backcrossing, the expected linkage drag around the target locus would be around 10 cm, when assumed that the target locus is at the centre of 100 cm chromosome [39]. Since MAS allows selection of plants which are heterozygous for target gene region, it is possible to have recombination in the region so that size of the linkage drag can be reduced further. A repulsion linkage refers to linkage of desirable allele of a target gene with undesirable allele of another target gene. For example, when resistance allele of a gene 'A' is closely linked with susceptible allele of a gene 'B', during the selection of resistance allele 'A', the susceptible allele 'B' also gets selected automatically [9].

Rapid and Cost-Effective Selection

Plant breeding is a number game. The probability of finding an ideal plant improves when a large number of segregating populations are generated and phenotyped rigorously. When 'genotypic information' of the parental lines or progenies is available to breeders beforehand, the undesirable progenies can be rejected timely, and only a small number of useful progenies can be followed up, which can save substantial amount of time and cost of a breeding programme. For instance, a detailed economic analysis by Kuchel on the use of MAS in an Australian wheat breeding programme showed that MAS enhanced the genetic gain over phenotypic selection as well as reduced the overall cost by 40 % [40].

Conclusion

Conventional plant breeding programs require a long period of time to complete one step or select desirable traits per cycle. Plant breeders depend on morphological markers to select desirable traits such as vigor, disease resistance, high yield, and quality traits. This has made plant breeding techniques increasingly sophisticated

and time-consuming over the years. Many agriculturally important traits such as yield, quality, and disease resistance are controlled by many genes, known as polygenic. These genes associated with a particular quantitative trait are known as quantitative trait loci (QTLs). The identification of QTLs based only on conventional phenotypic evaluation is not possible. Therefore, the characterization of quantitative traits to select for QTLs was initiated by the development of DNA markers in the 1980s. This unlocked a new realm in agriculture called 'molecular breeding,' which is tightly linked to molecular tools like marker-assisted selection (MAS). Marker-assisted selection involves the use of genetic markers to follow regions of the genome that encode specific characteristics of a plant in plant breeding. It plays a vital role in enhancing global food production by improving the efficiency of conventional plant breeding programs.

Marker-assisted selection has numerous applications in plant breeding activities, such as selection from breeding lines, recurrent selection, backcrossing, pyramiding, and germplasm storage. DNA markers are differentiated according to their function. Their choices depend on accessibility, technical expertise, turnaround time, and the level of polymorphism in the study material. Generally, MAS offers great advantages in shortening the breeding cycle, pyramiding multiple genes, breaking undesirable linkages, and providing rapid and cost-effective selection.

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