# Accelerating Crop Improvement Using Marker-Assisted Selection Supriya Ambawat<sup>1\*</sup> and Subaran Singh<sup>2</sup>

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#### Abstract

Conventional methods of plant breeding are time consuming and affected greatly by changing environment and climatic conditions. But, DNA markers have great potential to develop the efficiency and precision of conventional plant breeding via marker-assisted selection (MAS). The large number of mapping studies and quantitative trait loci (QTLs) for diverse crops species have provided an abundance of DNA marker-trait associations. During past many years, MAS has been found to be a widely used application in plant breeding as it offers great potential for increasing the genetic gains in crops by stacking favorable alleles at target loci and reducing the number of selection cycles. Different approaches of MAS are available which can facilitate us from complex genomic research to genic and finally at proteomic level for useful breeding purposes in agricultural field.

#### Introduction

The development and use of molecular markers during the last few decades have played a significant role in the crop improvement. The efficiency of plant breeding has accelerated upto a large extent through marker assisted selection (MAS). Selection for the desirable alleles of a gene or quantitative trait locus (QTL) on the basis of molecular markers linked to it instead of phenotype generated by this allele is known as marker-assisted selection (MAS). This term was first used by Beckmann and Soller in 1983. In comparison to the time consuming and tedious methods of conventional plant breeding, molecular markers are more useful in identifying the economically important traits in the breeding material for further improvement in lesser time. MAS can help to meet the demands of increasing population by increasing crop yields and mitigating the effects of different biotic and abiotic stresses which are the big challenges for agriculture in the present scenario. Owing to genetic linkage, DNA markers can be used to detect the presence of allelic variation in the genes underlying these traits. Thus, DNA marker technology derived from research in molecular genetics and genomics offers great potential for plant breeding. By using DNA markers, efficiency and precision of plant breeding has greatly increased in several crops like rice, maize, pearl millet, barley etc. (Table 1). It is useful in selecting traits with low heritability like yield, selection for various traits at seedling stage e.g., fruit and seed characteristics. It is also helpful for indirect selection for recessive alleles, pyramiding of resistance genes as well as effectively utilizes green houses and off season nurseries in rapid generation advancement. MAS can be conducted at F2 or F<sub>3</sub> stage and plants with desirable genes or QTLs are selected and alleles can be fixed in the homozygous state. Plants with undesirable gene combinations can be discarded and thus save the resources which can be used to focus on fewer lines for later stages of breeding program.

## Marker assisted selection and its need

The use of DNA markers in plant breeding is called marker-assisted selection (MAS) and is a component of the new discipline of 'molecular breeding'. Integration of recent advances in genome research, biotechnology and applications of molecular markers in combination with conventional practices of plant breeding has created new directions for plant breeding as interdisciplinary science for crop improvement. Molecular markers have widely been used as new era of plant molecular research. It involves DNA fingerprinting to detect polymorphism among different individuals. It has become a basic tool in crop improvement via plant breeding methods to enhance crop yield and combating the different adverse conditions and ultimately enhance genetic gains in different crops. It is a tool that can help plant breeders to select desirable traits more efficiently, screen many traits which may be either extremely difficult or time consuming. With MAS, individual plants can be selected based on their genotype. For most traits, homozygous and heterozygous plants cannot be distinguished by conventional phenotypic screening but MAS can be useful to resolve this issue.



#### Table 1. Marker-assisted selection in different crops

S. No.	Crop	Genotypes/genes used	Variety improved by MAS	Traits and resistant genes
1.	Rice	Pusa basmati I	Improved Pusa Basmati I	Bacterial blight (Xa13 + Xa21)
2.	Rice	Tapaswini	Improved Tapaswini	Bacterial blight (Xa4 + Xa5 +
				Xa13 + Xa21)
3.	Rice	Wuyujing3	K01, K04	Low-amylose content
				gene(Wx-mq)
4.	Maize	Shrunken2 (sh2)-based	Sweet corn hybrids (ASKH-1	Introgression of crtRB1 and o2
		sweet corn	& ASKH-2) were introgressed	genes into shrunken2 (sh2)-
			with crtRB1 and o2 genes	based sweet corn
5.	Maize	Vivek QPM9	Pusa Vivek QPM9 Improved	QPM + provitamin A
6.	Pearl	Restorer parent H	HHB 67 Improved	Downy mildew resistance
	millet	77/833-2-202		
7.	Barley	rym1, rym5, rym4,	F <sub>1</sub> derived doubled haploids	Barley yellow mosaic virus
		rym9, rym11 genes		
8.	Barley	Rspx (QTLs 4, 7 QTL 5)	F1derived doubled haploids	Stripe rust



# Fig 1. MAS approach for crop improvement Essential requirements for MAS

- Linked marker: Coinheritance or cosegregation of a gene of interest and a molecular marker is very useful. Marker should be <5 cM from a gene or QTL; ideally, the marker could be allele specific or at least gene-based. Whenever the markers are more than 5 cM away from the gene or QTL, using a pair of flanking markers can greatly improve reliability but increases time and cost.
- Marker should be polymorphic and codominant markers provide more information than dominant markers.

- Availability of efficient screening techniques to handle large populations will be very helpful.
- Marker loci should be evenly distributed throughout all the loci in the chromosome.
- The technique should be cost effective and user friendly with high reproducibility.



# Fig 2. Inheritance and co-segregation of a gene of interest in MAS

#### **MAS Breeding Schemes**

There are different approaches for markerassisted selection as described below:

- Marker-Assisted Backcrossing (MABC)
- Marker-Assisted Gene Pyramiding
- Early Generation MAS
- Combined Approaches

## Marker Assisted Backcrossing (MABC)

A backcross program based on markers is known as marker-assisted backcrossing. Backcrossing



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is a plant breeding method most commonly used to incorporate one or a few genes into an adapted or elite variety. The use of DNA markers in backcrossing greatly increases the efficiency of selection. Backcrossing aims to achieve the different criteria like effective selection of target loci, minimize linkage drag and accelerate recovery of recurrent parent. It is based on foreground selection which includes indirect selection for the target gene or QTL on the basis of linked marker genotype. It is highly preferable when phenotypic evaluation for the target trait is problematic due to any reason. The effectiveness of foreground selection will depend primarily on the genetic distance between the marker and the target gene or QTL. The closer is the marker to the gene or QTL, the greater will be the efficiency of foreground selection. The recovery of recurrent parent genome is made possible by background selection. The markers should be sufficiently dense and almost evenly distributed throughout the genome to facilitate effective selection for the recurrent parent genome. With conventional backcrossing, it takes a minimum of six BC generations to recover the recurrent parent genome but there may still be several donor chromosome fragments unlinked to the target gene. Using markers, it can be achieved by two to four backcrosses. Thus, the use of background selection during MABC, accelerate the development of recurrent parent genome. The recombinant selection is a special type of background selection that aims to reduce the donor parent genome flanking the target gene/QTL. Using conventional breeding methods, the donor segment can remain very large even with many BC generations. By using a pair of flanking marker (e.g. less than 5cM, preferably ~1 cM, on either side), linkage drag can be minimized. Recombinant selection ensures the transfer of the target gene/QTL with a minimum of the donor parent genome to minimize linkage drag. Since double recombination events occurring on both sides in one generation for the target locus are extremely rare, recombinant selection is usually performed using at least two BC generations.

#### Marker-Assisted Gene Pyramiding

Pyramiding is the process of simultaneously combining multiple genes or QTLs together into a single genotype. Pyramiding is extremely difficult to achieve using conventional methods but it is possible through MAS. Phenotyping for multiple forms of resistance in a single plant is almost impossible by

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conventional approaches but screening at early growth stage and at early generations for several genes is practically useful and made possible by MAS. Thus, it is widely used to combining multiple disease resistance genes for specific races of a pathogen. It also useful in minimizing linkage drag and reduces breeding cycle thus less time consuming and makes crop improvement faster.



# Fig 3. Scheme of marker-assisted backcrossing *Early Generation MAS*

In this MAS can be conducted at  $F_2$  or  $F_3$  stage. Plants with desirable genes or QTLs are selected and alleles can be fixed in the homozygous state. Plants with undesirable gene combinations can be discarded. Advantage of this approach is that resources are not wasted unnecessarily and they can that can be used later to focus on fewer lines during later stages of breeding program.

#### **Combined** Approaches

In some cases, a combination of phenotypic screening and MAS approach may be useful. This strategy is effective and useful in maximizing genetic gains when some QTLs have been unidentified by QTL mapping and the marker is not tightly linked to the target gene. It can be used to reduce population size for traits where marker genotyping is cheaper or easier than phenotypic screening. It is also useful when some highly desirable oligogenes generate undesirable pleiotropic effects, which limit their usefulness in crop improvement. For example, opaque2 (o2) mutant allele in maize is highly desirable, but it has the undesirable pleiotropic effect of making the kernels soft.

#### Advantages of marker-assisted selection

• MAS has high degree of accuracy and is a rapid method of crop improvement.



- It is free from the environmental effects and can be done even at the seedling stage.
- It permits selection for the target traits in off-season nurseries/greenhouses.
- Foreground selection greatly facilitates selection for such traits whose phenotypic evaluation is cumbersome, tedious or time-consuming.
- MAS can accelerate recovery of the recurrent parent genotype.
- It permits backcrosses to be made in succession during introgression of recessive genes.
- It enables stacking of oligogenic and polygenic resistance to obtain more durable and effective resistance to diseases.
- It can be combined with recurrent selections as MARS to effectively accumulate QTLs for the target traits.
- Genomic selection (GS) is the most ambitious specialization of MAS.

### Challenges in marker-assisted selection

The fundamental basis of plant breeding is the selection of specific plants with desirable traits. The goal of plant breeding is to assemble more desirable combinations of genes in new varieties. Using MAS, we usually go for selection at early stages to make the process faster. But, for breeding lines which become homozygous in F<sub>5</sub> or later, it is challenging to make the selection faster. Similarly, size and composition of a plant population is an important consideration for a breeding programme. The larger the number of genes segregating in a population, the larger the population size required in order to identify specific gene combinations. Typical breeding programmes usually grow hundreds or even thousands of populations and many thousands or millions of individual plants. Thus, the scale of breeding programmes also underlines the challenges of incorporating a relatively expensive technology such as MAS. It can greatly assist plant breeders in reaching the different goals but to realize the potential of MAS, there should be a greater integration with breeding programmes and the current barriers to be well understood and appropriate solutions should be developed. Identification and characterization of resistance genes is highly needed which can help to understand molecular mechanism of resistance and also facilitates gene introgression. Similarly, development of saturated linkage maps followed by association mapping/Genome Wide Association Mapping (GWAM), QTL analysis and comparative genomics are to be focused.

# Conclusion

MAS provides a systematic and efficient approach to select individuals with desired traits by utilizing genetic markers linked to those traits. This technology has revolutionized traditional breeding methods by accelerating the breeding process, increasing selection accuracy and enhancing the overall efficiency of breeding programs. Through MAS, breeders can identify and select individuals carrying desirable genetic traits early in the breeding process, saving time and resources compared to conventional breeding methods. This targeted selection approach allows breeders to focus on specific traits of interest, such as disease resistance, yield potential or quality characteristics leading to the development of improved varieties and breeds. Hands on training of researchers and research scholars on whole genome sequencing, bioinformatics tools used for genome sequence assembly and detection of *in silico* polymorphism is the need of the hour. A friendly collaboration between molecular biologist, biotechnologist and plant breeders is also required to achieve these goals.

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