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MARKER ASSISTED SELECTION: A MODERN TOOL TO ACCELERATE CROP IMPROVEMENT

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ABSTRACT

Producing sufficient food to meet the demand of vastly growing population and eradication of rural poverty is one of the critically important issues that the world is facing. At the current pace, the world population is expected to cross the mark of nine billion people by 2050 adding further pressure to already exhausted food production systems. Considering the increasingly volatile climate, it will be difficult to maintain the crop production in conjugation with the demand, resulting in increased food prices affecting people who already spend the highest percentage of their disposable income on food. In addition to climate change, limited water resource availability and poor soil health have the potential to restrict food crop production (Varshney *et al.*, 2017). In last few years there is a great change in population in rural &urban area. A large population in developing world is shifting from rural to urban area. Within the next decade more than half of the world population, an estimated 3.9 billion will be living in urban area. The metro cities of future are taking shape in developing countries and will affect social& environmental aspect of concern countries and ultimately an alarm for food security. The Urban populations are not able to feed themselves by subsistence food production, and their eating patterns differ from those of rural folk (Singh, 2023). In the event of these challenges, there is a need to look for new ways of breeding for food crops and other plant species by using modern technologies. Marker assisted selection (MAS) is a novel plant breeding technique that uses molecular markers to select the plant carrying genomic regions, which are involved in expression of trait of interest. MAS is used on a broad level for improving the cultivars for various biotic and abiotic stresses.

Key words : Marker Assisted Selection, Crop improvement, Hunger, Food security, Sustainable development.

Introduction

Over active nature of human being is affecting the environment of earth in adverse manner. This is leading a threat to global sustainable agriculture development and ultimately food security. The Food and Agriculture Organization of the United Nations (FAO) defines "food security" as a state of affairs where all people at all times have access to safe and nutritious food to maintain a healthy and active life . This means that in order to enjoy food security, there must be on the one hand a provision of safe, nutritious, and quantitatively and qualitatively adequate food and, on the other, rich and poor, male and female, old and young must have access to it. Furthermore, with increases in the world population, the availability of agricultural land is decreasing. Under these constraints, to meet the rising demand for food, agricultural production must increase by an estimated 50% without greatly increasing water usage or expanding the total land area dedicated to agriculture. Smallholder farmers, especially from underdeveloped and developing countries with limited access to agricultural inputs or agricultural markets, are likely to be affected by rising production costs and climate volatility. As per the United Nations' estimates, more than 790 million people globally do not have access to sufficient nutritious food (https://sustainabledevelopment.un.org/sdg2), posing a threat to achieving the Sustainable Development Goal (SDG) target of zero hunger (universal access to safe, nutritious and

sufficient food at all times of the year).

In the event of these challenges, there is a need to look for new ways of breeding for food crops and other plant species by using modern technologies. Modern breeding approaches that have the capability to reduce breeding cycle time provide more precision in selection and more efficient use of genetic variation can be exploited to increase the rate of genetic gains in breeding programs. The rapid decline in the cost of sequencing and genotyping has led to the development of new tools and strategies that can transform the way, we breed plant species. In the past, the cost of genotyping restricted the regular use of markers in breeding. In most cases a limited number of markers for the target regions were used for selecting the lines based on presence or absence of agriculturally important alleles. Development of crop varieties using conventional breeding approaches has been effective but time-consuming and labor-intensive. Recent advances in the next-generation sequencing (NGS) technologies have been able to reduce the cost of genotyping and sequencing. This has enabled the use of the high-throughput and cost-effective high-density genotyping. These low-cost genotyping platforms have accelerated the use of markers in the breeding programs using genome-wide approaches (Varshney *et al*., 2014).

Molecular breeding is approach in which we use tightly linked molecular markers for identification of our desired traits in breeding population (Singh and Upadhyay, 2016). Marker assisted selection (MAS) MAS is used on a broad level for improving the cultivars for various biotic and abiotic stresses. It is extremely helpful for screening of those traits that expresses late in plant development. This technique is simple in application and has high level of accuracy for introgression of genes or QTLs from wild sources known as marker assisted back crossing. MAS approach known as marker assisted gene pyramiding used to transfer multiple genes or QTLs from multiple sources at the same time into a single genotype with high level of precision. Similarly other approaches such as marker assisted recurrent selection and genomic selection is helpful for the improvement polygenic traits in plants. The basic need of any plant breeding programme is the presence of genetic variation. Variation should be present at morphological level or DNA level in any plant population and utilization of this genetic variation for the selection of superior crop genotypes is the ultimate aim of a plant breeder. With the development of molecular marker technology in 1980 and continuous advancement in this technology helps the breeder in detection of variation in early phase of growth in plants and use this variation in selection of superior genotypes. Marker

assisted selection is a new smart breeding technique where selection of a phenotype is made on the basis of genotype of a marker. In MAS, molecular marker linked to the target gene or gene of interest is used to select the plants. Nowadays, MAS has gaining much importance as it is the simple and rapid technique of transfer genes from wild relatives to the cultivated varieties for disease resistance, insect resistance, salt tolerance, drought tolerance, flood tolerance, fruit quality etc. With the help of MAS technology, multiple genes from different sources can be transfer simultaneously into a single cultivar with high precision. MAS have high accuracy and applicable to all crop species whether it is self pollinated, cross pollinated or asexually reproducing species (Soni *et al*., 2023).
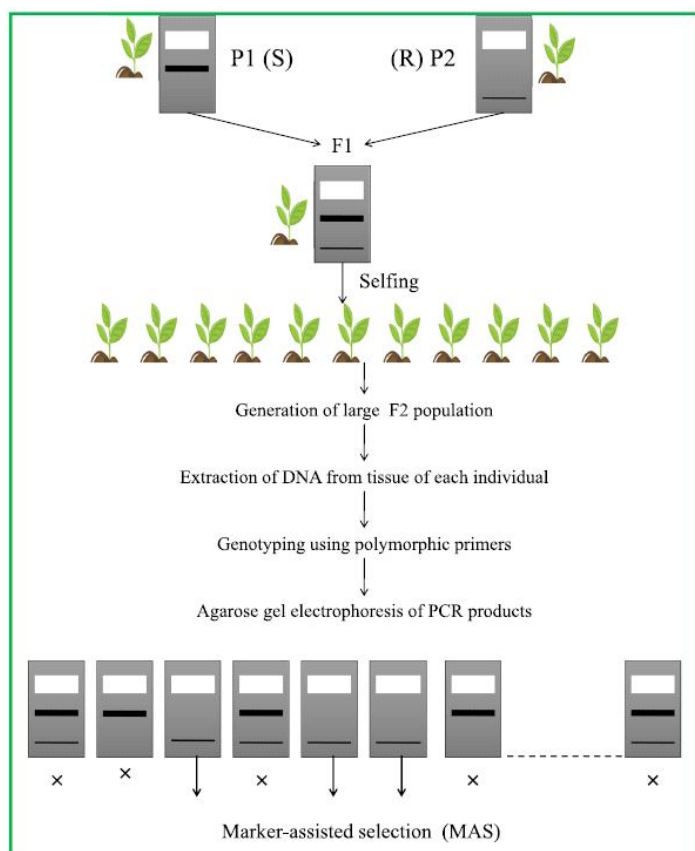
Marker Assisted Selection

- It is an **indirect selection process**, where expression of a trait or gene of interest is selected not based on the expression of itself but due the marker (Morphological, Cytological, Biochemical or Molecular) linked to it.

- MAS is useful for traits those are
  1. Difficult to measure.
  2. Exhibit low heritability.
  3. Expressed late in development.

- MAS is a rapid method of breeding where phenotypic selection is based on DNA markers or molecular markers.

- MAS accelerating the breeding program where plant having gene of interest selecting in early phase of growth irrespective of expression of gene of interest in later phase of growth of plant.

- MAS is used to determine if plant having gene of interest in minimum time interval.

- MAS decreases the costs incurred in screening of target traits.

Key components for Marker Assisted Selection

The success of marker assisted selection depends on following factors:

1. A genetic map with sufficient number of uniformly spaced polymorphic markers to accurately locate desired QTLs or gene of interest.

2. A tight linkage between QTLs or gene of interest and molecular marker.

3. An Adequate recombination between molecular markers linked to gene of interest and rest of genome.

4. A good analysis method of large number of individuals

Fig. 1 : Basic strategy for Marker Aided Selection (MAS) (Hasan *et al.*, 2021).

in a time and in cost effective manner.

General steps in Marker Assisted Selection

- Selection of parents
- Development of Breeding Populations
- Isolation of DNA
- Genotyping using Polymorphic Primers
- Agarose gel electrophoresis of PCR products

1. Selection of Parents: Parents with contrasting characters or divergent origin should be chosen.

- The parents that are used for MAS should be pure (Homozygous).
- In self-pollinated species, plants are usually homozygous.
- In cross-pollinated species, inbred lines are used as parents.

2. Development of Breeding Populations : The selected parents are crossed to obtain F_1 plants. F_1 plants between two pure lines or inbred lines are homogeneous (alike phenotypically) but are heterozygous for the gene of interest. The F_2 progeny is required for study of segregation pattern of gene of interest. Generally, 50-100 F_2 plants are sufficient

for study of segregation of gene of interest linked to molecular marker.

1. Isolation of DNA : The main advantage of MAS is that DNA can be isolated even from seedlings and we need not to wait for flowering or seed development stage. The DNA is isolated from each plant of F_2 population.

2. Genotyping using Polymorphic Primers : The polymorphism between the parents and their involvement in the recombinants in F_2 population is determined by using polymorphic primers. The primer will hybridize only with those segments, which are complementary in nature.

3. Agarose gel electrophoresis of PCR products : PCR amplified products were subjected to agarose gel electrophoresis and separated bands were visualized under UV transilluminator and after analysis of molecular data, plants carrying gene of interest were selected.

Molecular marker technology : Molecular marker technology is an integral part of Agriculture Biotechnology. PCR based molecular markers are considered as boon for development of agriculture (Singh, 2010 a). Due to ample applications of Agriculture Biotechnology, it is considered as a weapon for scientist to fight hunger, malnutrition and poverty (Singh, 2009 b). Actually the science of plant genetics traces back to Mendel's classical studies on garden peas. Since, then researchers have been identifying, sorting and mapping single gene markers in many species of higher plants. In early part of twentieth century, scientist discovered that Mendelian factors controlling inheritance which we now call genes were organized in linear fashion on chromosomes. Actually the first genetic map was produced by Sturtevant with segregation data derived from studies on drosophila. The markers on first genetic map were phenotypic traits scored by visual observation of morphological characteristics of the flies. Generally a marker must be polymorphic that is it must exist in different forms so that the chromosome carrying the mutant gene can be distinguished from the chromosome with normal gene by the form of marker it also carries. This polymorphism in the marker can be detected at three levels *i.e* phenotypic level (by Morphological markers), difference in proteins (by Biochemical markers) or difference in the nucleotide sequence of DNA (by Molecular markers).

Morphological markers : Morphological markers

generally match to the qualitative traits that can be scored visually. They have been found in nature or as the result of mutagenesis experiments. Chang (1976) and Takahashi (1984) had recognized three eco-species in *Oryza sativa* i.e., *Japonica, Javanica* and *Indica*. Matsuo (1952) and Oka (1953) did the genetic differentiation among these three eco species by morphological and phenological analysis. These markers are highly influenced by environmental factors.

Biochemical markers (e.g. Isozymes): Biochemical markers are proteins produced by gene expression. These proteins can be isolated and identified by electrophoresis and staining. Isozyme the different molecular form of same enzyme, that catalyzes the same reaction, are proteins. They are revealed on electrophoregram through a coloured reaction associated with enzymatic activity. They are the products of various alleles of one or several genes .The first molecular markers that initially were used extensively to study systematic of plants, animals and insects were isozyme (or isoenzyme). Glaszmann (1987) examined 1688 rice cultivars from different Asian countries for allelic frequencies at 15 Isozymes loci and analyzed the data by a multivariate analysis. Isozymes markers have been applied to assess the genetic variation but these markers represent only a minor portion of the genome and hence undesirable for practical breeding (Second, 1982). Because isozymes are post transcriptional markers, their expression is influenced by environmental changes leading to polymorphisms that might not reflect real differences at the molecular level.

D.N.A. based molecular markers : Molecular markers are also called DNA markers. It is a DNA sequence that is readily detected and whose inheritance can be easily monitored. The use of molecular markers is based on naturally occurring DNA polymorphism, which forms the basis for designing strategies to exploit applied purpose. A molecular marker has some desirable properties like

- It must be polymorphic
- Co-dominant inheritance
- Should be evenly and frequently distributed
- Should be reproducible
- Should be easy fast and cheap to detect.

No single marker meet all these requirements so there is need to develop a wide range of molecular markers. Molecular markers are also reported to explore genetic diversity in rice (Singh and Singh, 2012; Singh *et al*., 2013), which may also assist in management of Abiotic stress in rice.

Various types of molecular marker used in Marker Assisted Selection are as following:

- ➢ **Restriction Fragment length Polymorphism (RFLP) :** It is non PCR based marker. Botstein *et al.* used this for construction of genetic map first time. It was the first technology that enabled the detection of polymorphism at DNA sequence level. In this method DNA is digested with restriction enzymes, which cuts the DNA at specific sequences, electrophoresed, blotted on a membrane and probed with labeled clone.

- ➢ **Random Amplified Polymorphic DNA (RAPD) :** This marker system was developed by Welsh and McClelland in 1991. RAPD is performed in conditions resembling those of PCR using genomic DNA from the target fungal pathogen and a single short oligonucleotide (generally 10 mer). The DNA amplification product is generated from a region that is flanked by a part of 10 b.p priming site in the appropriate orientation. A particular fragment generated for one individual but not for other represents DNA polymorphism and can be used as a genetic marker. RAPD markers are used by various workers in different species of plants to find out genetic diversity (Quintela-Sabaris *et al*., 2005; Yogendra Singh, 2006) and diseases diagnosis in animals (Singh and Katoch, 2008).

- ➢ **DNA amplifying finger printing (DAF):** In this single arbitrary primer as short as 5 bases is used to amplify DNA using PCR machine. In this two temperature cycles are used as compared to three in RAPD. The step denaturation is exempted in this case. The amplified product of DAF are analysed on acrylamide gels and detected by silver staining.

- ➢ **Amplified Fragment length Polymorphism (AFLP) :** It is a combination of   RFLP & RAPD. This involves major steps as following:

- Cutting of DNA with Restriction Enzymes
- Double stranded oligonucleotides adapters are ligated to the ends of DNA fragments.
- Selective amplification of sets of restriction fragments is usually carried out with P [32] labeled primers designed according to sequence of adaptors plus 1-3 additional nucleotides.
- Gel electrophoresis and analysis of amplified fragments.

This is highly sensitive method for detecting

polymorphism throughout the genome and it is becoming increasingly popular. Aggarwal *et al.* (2002) analyzed the genetic diversity and interrelationships among 33 rice genotypes consisting of the traditional basmati, improved basmati-like genotypes developed in India and elsewhere, American long-grain rice and a few non-aromatic rice using a DNA marker-based approach fluorescent Amplified Fragment Length Polymorphism (f-AFLP).

➢ **Allele Specific Associated primers (ASAP) :** In this specific allele either in homozygous or heterozygous state is sequenced and specific primers are designated for amplification of DNA template to generate a single fragment at stringent annealing temperature. In this marker system higher primer concentration is used and amplified product is analyzed on poly acrylamide gel followed by auto radiographic detection.

➢ **Simple Sequence Repeats (SSR) :** The term micro satellite was coined by Litt and Lutty in 1989. This is also known as Micro satellite marker system. These are present in genome of all eukaryotes. These are tandemely repeats of mono, di, tri, tetra and penta nucleotides with different length of motif (A, T, AT, AGG etc.). These are actually non-coding regions which remained conserved during the course of evolution and are ideal for DNA fingerprinting and varietal identification. They are valuable as genetic markers as they detect high level of allelic diversity based on the variability in the tandem repeats in the core unit and are co dominant. This SSR length polymorphism at individual loci is detected by PCR using locus specific flanking region primers where the sequence is known. SSR are ideal DNA markers for genetic mapping and population study of pathogens, because of their abundance.

➢ **Inter Simple Sequence Repeats (ISSR) :** This marker system is based on SSR marker system. In this marker system primers based on micro satellite are utilized to amplify inter SSR DNA sequences.

➢ **Expressed Sequence Tagged Marker (ESTM) :** Such markers are obtained by partial sequencing of random c-DNA clones. Such markers are obtained by partial sequencing of random c-DNA clones and are useful in cloning specific genes of interest and synteny mapping. This is also used in full genome sequencing and mapping Programmes and isolation of genes.

➢ **Single Nucleotide Polymorphism (SNP) :** This marker system belongs to third generation of marker systems. The frequency of occurrence of SNP in a genome is generally one SNP in every 100-3000 bp. The SNPs can be detected by two ways one is gel based assays and other is non gel based assays.

➢ **Cleaved Amplified polymorphic Sequences (CAPS) :** Konieczny and Ashubel (1993) first adapted the CAPS procedure for genetic mapping by developing a set of CAPS markers for use with *Arabidopsis.* In this marker system PCR amplified DNA fragments are digested with a restriction enzyme rendering restriction site polymorphism. The digested restriction fragments are subjected to gel electrophoresis (agarose) followed by ethidium bromide staining based visualization of bands.

➢ **Sequence Characterized amplified Regions (SCARs) :** A SCAR is a genomic DNA segment at a single genetically defined locus that is identified by PCR amplification using a pair of specific oligonucleotide primer. SCARs are inherited in a co dominant fashion in contrast to RAPDs, which are inherited in a dominant manner. SCAR marker system detects only one locus and the use of longer oligonucleotide primers allows a more reproducible assay than the one obtained with the short primers used for RAPD analysis.

Classification of Molecular markers (Table 1)

Comparison of important characteristics of different molecular markers used in MAS (Table 2)

Genetic and physical maps

Genetic or physical map assists to identify molecular markers associated with economically important traits, clone genes of interest and dissect agronomic traits. Researchers have constructed number of genetic maps with different populations using multiple types of molecular markers (Reinisch *et al.*, 1994).

Quantitative Trait Analysis : Major agronomically important traits, *i.e.* yield are controlled by polygenes or QTLs, interaction of QTLs $\times$ environment (E) and epistasis. Each gene of this polygene has a small influence on the phenotype, which is also effected by environmental conditions. Usually, the efficiency of MAS is reduced by QTL $\times$ E interaction and epistasis (interactions with other genes), which results as skewed QTL effect. Consequently, phenotyping and genotyping of quantitative

Table 1 : Classification of Molecular markers.

Class	Marker system	Abbreviation	Remarks
First Generation Molecular Markers	Restriction Fragment Length Polymorphism	RFLP	Based on restriction digestion and hybridization with probe
	Sequence Tagged Sites	STS	RFLP probes sequenced and converted in to PCR based STS markers
	Random Amplified Polymorphic DNA	RAPD	Random primers for PCR amplification
	Arbitrary Primed PCR	AP-PCR	RAPD primers of 10-15 bases in length for discrete amplification
	Sequence Characterized Amplified Regions	SCAR	RAPD marker termini sequenced for designing longer primer
	DNA Amplification Fingerprinting	DAF	Single random primer of 5 bases short length
Second Generation Molecular Markers	Simple Sequence Length Polymorphism	SSLP	Based on tandem repeat flanking sequence
	Variable Number of Tandem Repeats	VNTRs	Based on tandem repeat sequence hybridization by probe
	Random Amplified Micro satellite Polymorphism	RAMPO	Random primers used for amplification and then hybridized with micro satellite oligonucleotides probe
	Cleaved Amplified Polymorphic Products	CAPs	PCR amplified products digested by restriction enzymes
	Inter Simple Sequence Repeat	ISSR	Single primer based on SSR motif
	Amplified Fragment Length Polymorphism	AFLP	Detection of genomic restriction fragment by PCR amplification
	Allele Specific Associated Primers	ASAP	Specific allele sequenced and primers designed for amplification
Third Generation Molecular Markers	Expressed Sequence Tag markers	ESTs	Sequencing of random DNA clones
	Single Nucleotide Polymorphism	SNP	Non-gel based marker system and DNA sequence differs by single base
	Miniature Inverted Repeat Transposable Elements	MITE	Non autonomous transposable elements with strong target site preference

traits is complex undertaking, therefore repeated field test is needed for accurate characterization and stability test of the QTL effect. The analysis and mapping of QTLs are performed mainly for yield, yield associated components, biotic and abiotic stress resistance/tolerance.

QTLs Mapping : Bi-parental mapping uses two parents to develop a population for QTL identification. It is similar to major genes mapping, however it is more complicated due to existing following differences; usually larger population size, repeatedly evaluation of segregating populations in multiple environments (locations or years), genotyping of all progenies with all polymorphic markers

for constructing linkage map, analysis of the phenotypic data with appropriate statistics tool such as ASReml or SAS, an appropriate mapping method for identification of QTLs, such as composite interval mapping. Later step can be conducted with statistical softwares such as MapQTLs and QTL Cartographer (Van Ooijen, 2009).

Multiple-parent mapping uses more than two parents to develop a population for QTL identification. Obtaining of a high-resolution genetic map with a biparental population is very difficult or impossible due to low genetic diversity present within germplasms. Multiple-parent mapping ensures greater genetic diversity with increased

Table 2 : Comparison of important characteristics of different molecular markers used in MAS.

Characteristics	RFLP	RAPD	AFLP	SSR	SNP
Codominant/Dominant	Codominant	Dominant	Dominant	Codominant	Codominant
Number of detected loci	Single locus	Multilocus	Multilocus	Single locus	Single locus
Level of Polymorphism	Medium	Very high	High	High	High
Polymorphism type	Single base change, Indel	Single base change, Indel	Single base change, Indel	Change in length repeat	Single base change, Indel
Reproducibility	High	Low	Intermediate	High	High
Genomic abundance	High	High	High	Medium-high	Very High
PCR based	No	Yes	Yes	Yes	Yes
Required DNA Quantity (ng)	Large (10000)	Small (20)	Moderate (500-1000)	Small (50)	Small (>50)
Types of probes/primers	Low copy DNA	10 bp random primer	Specific sequence	Specific Sequence	Alleles-specific PCR primers
Cost	Moderate to high	Low	Moderate	Moderate to high	High
Genotyping throughput	Low	Low	High	High	High
Genomic coverage	Low copy coding region		Whole genome	Whole genome	Whole genome Whole genome

polymorphism frequency to improve the possibility of QTL identification.

The utilization of the QTL mapping has been limited due to various constraints in plant breeding; lack of universally valid QTL-marker associations because new QTL mapping is required to identify QTL markers of a new Germplasm, strong QTL × E interaction, which varies phenotype between different locations or years, deficiencies of statistical analysis of QTLs, leading either underestimation or overestimation of the number of QTLs, lack of frequent QTLs with major effects on trait.

Association Mapping : Association mapping uses all alleles present in a data panel of phenotype and variety obtained through various experiments or variety tests on the basis of linkage disequilibrium. It has become a powerful tool for the identification of markers-associated with QTLs in plants (Hamblin *et al.*, 2011).

Allele Mining : Allele mining is an approach for identification of novel alleles or allelic variants of a candidate gene of interest on the basis of the information available about the genes, from a wide range of germplasm. Allele mining succes mainly depends on the diversity of genetic materials and availability of gene and genome sequence information of crop species. Usually local landraces and wild relatives are used for efficient allele mining, because they serve as reservoirs of useful hidden alleles. Sequence based allele mining and Eco Tilling are widely used approaches in the allele mining.

Sequence based allele mining strategy is simpler and less costly approach than Eco Tilling. Allele mining have wide range of applicability in crop improvement such as identiffication of alleles, characterization of allelic variation, identiffication of haplotypes, analysis of haplotypes diversity, evolutionary relationship and molecular markers development. Allele mining approaches have been used for identification of novel alleles of many blast resistance genes.

Merits of Marker assisted selection over Conventional breeding

1. In MAS, those traits which are expressed late in plant development such as grain quality, photoperiod sensitivity, male sterility etc. can be detected with the help of molecular marker linked with these traits, when plant is in seedling stage. Thus, saving time and efforts.

2. With the help of MAS, we can screen those traits that are extremely difficult, time consuming and expensive to score or measure such as tolerance to drought, salt, mineral deficiency and toxicity or resistance to specific races or biotypes of diseases or insects.

3. In MAS, a breeder can practice selection for different traits simultaneously, which is difficult or even impossible by conventional means.

4. With the incorporation of co-dominant markers, heterozygote can easily identified and

distinguished from the homozygote without resorting to progeny testing. This saves time and efforts.

5. In conventional methods of selection, single plants selected for many traits which is sometimes misleading due to environmental factors whereas in MAS, individual plants selected based on their genotypes.

6. Phenotyping is very easy in MAS as phenotype of an individual is selected on the basis of genotype of that individual.

7. MAS speedup the selection process and accelerate the progress of development of new variety.

8. In conventional breeding, selection is influenced by the environmental factors whereas in MAS, selection is done with the help of linked molecular marker, so there is no influence of environment.

Demerits of Marker Assisted Selection

1. MAS is a costly method, requires well equipped laboratory and well trained manpower for handling of costly equipments, isolation of DNA etc.

2. Detection of various linked molecular markers (RFLP, RAPD, AFLP, SSR, SNP etc.) is difficult, laborious and time consuming.

3. The utilization of MAS is very difficult in QTL study due to their cumulative effects, which are greatly affected by environmental factors and genetic background.

4. It is found that MAS may become less efficient than phenotypic selection in long term.

Conclusion

Marker assisted selection (MAS) is a novel plant breeding technique that uses molecular markers to select the plant carrying genomic regions, which are involved in expression of trait of interest. MAS are used on a broad level for improving the cultivars for various biotic and abiotic stresses. It is extremely helpful for screening of those traits that expresses late in plant development. Recently developed molecular breeding strategy such as allele mining and gene pyramiding holds greater potential for attaining long durable resistance against abiotic and biotic stresses in plants. Identiffication of superior and novel resistance alleles of the blast resistance genes is a vital task in the molecular breeding program. These novel alleles are extremely useful in crop breeding programs and can be used for development of superior and productive plants.

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