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Marker Assisted Selection: A Novel Approach for Crop Improvement

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The process of marker-assisted selection, or marker-aided selection (MAS), selects a trait of interest indirectly by considering a marker linked to the trait (e.g., quality, productivity, disease resistance, and biotic stress tolerance) rather than the trait itself. This integration of marker data with traditional selection approaches has become a widely studied and recommended method for advancing breeding programs. This technique has been extensively researched and recommended for animal and plant breeding. Here, we combine marker data with conventional selection to choose the best candidate for a future breeding program. Nowadays, the majority of MAS investigations employ DNA-based markers such as microsatellites, single nucleotide polymorphisms (SNPs), random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and restriction fragment length polymorphism (RFLP). In this case, we discussed several kinds, techniques, and other features of marker Assisted Selection.

Keywords: Marker; selection; linkage; polymorphism.

1. INTRODUCTION

1.1 Marker Assisted Selection (MAS)

Marker-assisted selection, or MAS, indirectly selects a particular plant phenotype based on the banding pattern of linked molecular (DNA) markers. The underlying premise of MAS is that the existence of a marker that is strongly linked to the gene of interest can be used to infer the presence of a gene. This approach of MAS in current breeding technologies permits the selection by genotype using DNA markers being closely linked with the desired gene (Khlestkina, 2014). If the marker and gene are located far apart, double crossover recombination events will reduce the chance that they will be passed on to the offspring.

Marker-assisted selection or MAS, is the process of indirectly selecting a trait of interest by employing a marker, whether it be morphological, biochemical, or based on DNA/RNA variation. Through an indirect selection process, a marker linked to a trait of interest is used to pick it. For instance, when MAS is used to identify people who have a disease, the existence of the disease is determined by a marker allele linked to the disease, not the severity of the condition (Ahamad et al., 2021). A linked allele is thought to be connected to the target gene or quantitative trait locus (QTL). Characters that are late in development, have poor heredity and are challenging to assess are good candidates for MAS. Sax (1923) first showed the relationship between a purely inherited genetic marker and quantitative characteristics in plants, although he had also found that in beans (*Phaseolus vulgaris* L.), there was a segregation of seed size linked to a seed coat colour marker. Rasmusson (1935) showed that a simple inherited gene for flower colour is linked to the quantitative feature of flowering time in peas.

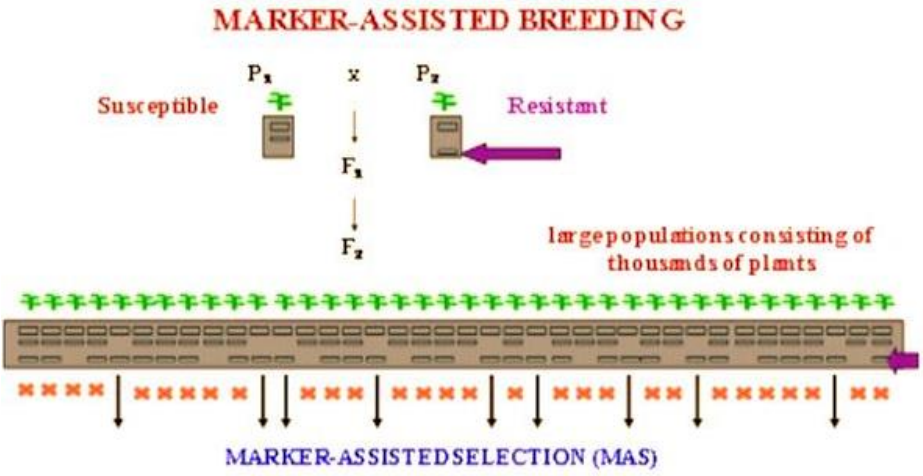

Fig. 1. Method whereby phenotypic selection is based on DNA marker

2. FEATURES OF MARKER ASSISTED SELECTION (MAS)

The primary characteristics of MAS are outlined in brief below

i. **Terms Used Besides:** Marker-aided breeding (MAB) and marker-assisted selection (MAS) are other names for the same concept. It is not the same as gene-assisted selection (GAS), which is selection based on QTLs (quantitative trait loci) (Wilcox, 2007, Luby et al., 2009).

ii. **Prerequisites:** Marker-assisted selection has two prerequisites. These are: (i) a close relationship between the target gene and the molecular marker; and (ii) a high degree of heritability in the target gene.

iii. **Application:** MAS can be used to improve an animal's or plant's genetic makeup. It applies to both self-pollinated and cross-pollinated plant species equally.

iv. **Markers Employed:** MAS use a variety of molecular markers. Among the most commonly used molecular markers are single nucleotide polymorphisms (SNP), amplified fragment length polymorphisms (AFLP), restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), and simple sequence repeats (SSR) or microsatellites. Furthermore, different species use molecular markers in different ways (LR Schaeffer , 2006).

v. **Efficacy:** The relative efficacy of MAS is highest for traits with low heritability if the marker loci account for a sizable amount of the additive genetic variance. In other words, when a trait has a low heritability, MAS can be useful. Furthermore, MAS works better than phenotypic selection alone in populations that are comparatively big. According to some experts, MAS may eventually lose its effectiveness in favour of phenotypic selection. This is because undesirable alleles are more likely to be fixed at QTLs with small effects under MAS as opposed to phenotypic selection (Testolin, 2002). It could be the consequence of the intensive selection of early-generation QTLs, which had notable effects under MAS. But this problem comes after a long time (Francia et al., 2005).Genetic mapping of major genes and quantitative traits loci (QTLs) for many important agricultural traits is increasing.

vi. **Accuracy:** The accuracy of molecular markers is very great. The state of the environment has no effect on them. A new breeding technique called MAS is available to help choose more valuable and accurate individuals from breeding populations. Heritable qualities can be connected to the DNA molecule that regulates them thanks to MAS.

vii. **Rate of Advancement:** MAS is a quick technique for improving crops. For instance, in traditional breeding, in order to identify a recessive trait transferred through backcrossing, one selfing is necessary following each backcross. A faster pace of crop improvement work is achieved through the detection of recessive alleles even in heterozygous conditions thanks to MAS.

viii. **Improved Traits:** Both polygenic and oligogenic traits can be improved by MAS. Little progress has been made with polygenic qualities through the application of MAS, which was previously only used for the genetic improvement of oligogenic features.

ix. **Content Created:** Non-transgenic genotypes or cultivars are developed as a result of MAS. To put it another way, MAS is employed in the creation of non-transgenic cultivars. The general population is against the transgenic cultivars. Conversely, customers approve of the cultivars created by MAS.

x. **Price:** When compared to phenotypic selection, MAS is far more expensive. Equipment, consumables, infrastructure, labour, and the DNA extraction procedure are among the expensive components of MAS. A modern and well-stocked laboratory is necessary for MAS (Wannemuehler 2018).

3. THE PURPOSE OF MARKER-ASSIS-TED SELECTION

The most practical use of MAS for plant breeders is the utilisation of DNA-based markers for essentially three purposes:

- Tracking down advantageous alleles (dominant or recessive) over generations; to build up advantageous alleles,
- Finding the best candidates among segregating offspring by analysing the allelic makeup of a portion or of the complete genome and

- Severing the potential connection between undesired loci and advantageous alleles.

Transferring a single genomic area from a donor to a recipient line can result in a notable improvement in a trait when the expression of the target trait is controlled by a single gene or by a gene that accounts for a large portion of the trait's phenotypic variance. In backcross (BC) programs, MAS is being used more and more to hasten the recurrent parent's recuperation. By using molecular markers instead of traditional backcrossing, BC breeding can be more efficient in at least three ways:

Selection for a marker allele from the donor parent at a locus close to the target gene can improve the efficiency and accuracy of selection for traits that are challenging to phenotype; markers can also be used to select rare progenies from recombination and BC progeny with less donor parent germplasm in the genome outside the target region. Close to the target gene, reducing the consequences of linkage drag and further selfing generations are needed following each backcross in the transfer of recessive genes through conventional breeding which results in a technique that is prohibitively low for the majority of breeding needs (Singh et al., 2015).

1. When heritability is low to moderate, there is little chance of choosing better genotypes. Plant breeders address this issue in classical breeding by creating and evaluating offspring from several crossings, applying minimal selection pressure, conducting repeat tests, and evaluating later generations. Breeders that choose to focus on features with low to moderate heritability face the following challenges (Kashi, Y., E.M. Hallerman and M. Soller. 1990):

- To guarantee the presence of one or more superior genotypes in the chosen sample, a large number of progeny must be chosen (low selection intensities must be used); even with low selection intensities,
- The most exceptional genotypes generated by a cross may not be present in the chosen sample when heritability is low and samples are small. These findings suggest that when small samples of children are tested and the heritabilities of the qualities to be selected are low or moderate, the likelihood of choosing an exceptional genotype is extremely low.

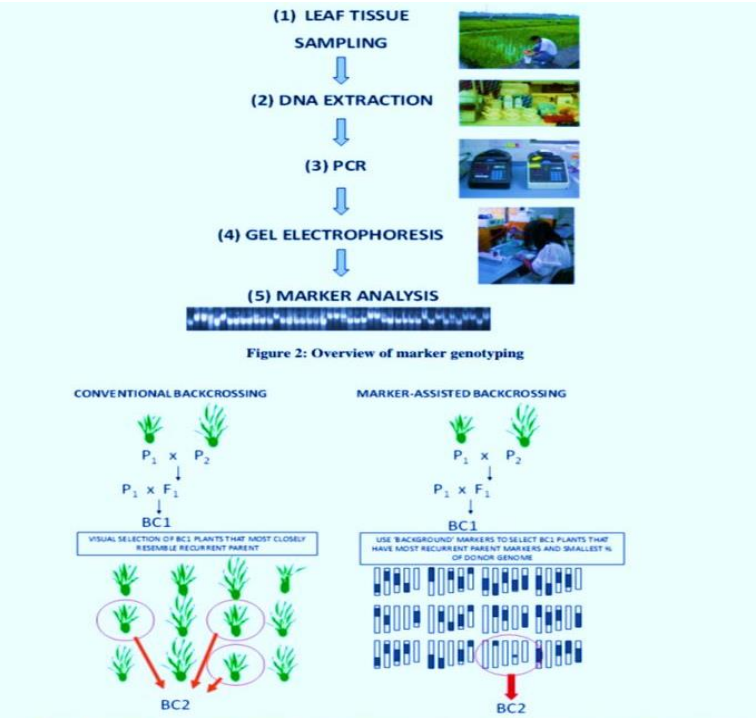

Fig. 2. Comparison between conventional backcrossing and background selection during marker assisted backcrossing

4. THE NEED FOR MARKER-ASSISTED SELECTION

Productivity attributes differ between and between populations in all domestic animal species. There are genetic and environmental components to this variation. Issues in using genetic variation within or between populations for genetic enhancement of animals raised for agriculture exist because the most important agricultural qualities exhibit polygenic and quantitative genetic variation. Due to the polygenic nature of trait variation, segregating allelic variations at several loci dispersed throughout the genome affects the trait value. Environmental influences have a significant impact on trait expression due to the quantitative character of trait variation. Individual polygenes in heredity cannot be identified or tracked due to these properties (M Soller, 1994).

Polygenic loci that cause genetic variation in quantitative characteristics are commonly referred to as "Quantitative Trait Loci," or QTL, while traits that show polygenic quantitative genetic variation are sometimes referred to as "Quantitative Traits." Because of the relative "interchangeability" of the effect of QTL on trait value, the presence of a favourable allele at any QTL influencing a given trait will usually increase trait value. Because of this, individuals in populations with high trait values will generally have a higher-than-average proportion of favourable alleles occupying their QTL. The offspring generation is therefore more likely than the parent generation to have a higher average trait value and an overall greater frequency since selection chooses these individuals to be the progenitors of the next generation. Based on theoretical considerations, qualities that manifest early are easily evaluated in both sexes and are not significantly influenced by microenvironmental influences are the most likely to be selected for (Falconer, 1989).

In these circumstances, there is rapid genetic improvement due to intense and precise selection, short generation intervals, and so on. Regretfully, the only characteristic that nearly satisfies this requirement is juvenile growth rate! On the other hand, there is a vast and diverse range of characteristics and circumstances where phenotypic selection is more accurate and intense, but the generation interval is longer. Certain characteristics manifest later in life (e.g., limb issues), are unique to one sex (e.g., milk amount and composition), or are challenging to assess (e.g., illness resistance, feed efficiency, body composition). In certain situations, it is not possible to get phenotypic data on every member of the paternal population (Barendse et al., 1994, Dekkers JCM, 2007). As a result, there is less intense selection. Some features, like the production of milk and eggs, fertility, and udder infections, are highly influenced by microenvironmental conditions, resulting in low heritability (Lipkin et al., 1993). In these situations, there is a lack of close correlation between phenotypic and genetic values. This lowers the selection's precision.

There are biometric techniques that offer some genetic advancement even in the aforementioned circumstances. These techniques, referred to as "family" selection, entail choosing individuals based on the typical phenotypes of their offspring or siblings. But they also slow down genetic advancement by decreasing selection intensity (since room and resources for raising children are few), which only allows for the evaluation of a small number of families, and frequently by lengthening the generation gap (especially when progeny testing is involved).

1. Family selection techniques can sometimes be costly. For example, in dairy cattle progeny testing, a large number of young candidate bulls are kept for four or five years until the records of their daughters are accessible. However, when dealing with genetic variation that occurs between populations, the primary flaw in phenotypic selection and biometrical approaches becomes evident (Hayes et al., 2007). Therefore, even if crosses of lines or strains can occasionally boost output, biometrical methods are unable to predict which pair of parent lines will produce a good hybrid. Biometrical techniques can only direct additional cross-improvement once such a pair has been found (aside from through the time-consuming procedure of "reciprocal recurrent selection," a family-based selection program). Biometrical techniques are totally useless when it comes to helping animal breeders use the vast genetic resource represented by the hundreds of Landraces and regional breeds of livestock and poultry through selection in artificial populations or through introgression programs

(Georges et al., 1994). Separating linked negative alleles for production from beneficial alleles for adaptation (and vice versa) and coordinating effective selection for disease resistance, productivity, and adaptability simultaneously seem to be the main obstacles. Because native breeds and landraces have a remarkable ability to adapt to local macroenvironmental conditions, animal breeders are unable to adapt highly enhanced breeds currently in use for these particular settings. Furthermore, by employing the livestock breeds that have already been enhanced, breeders cannot precisely enhance the productive qualities of landraces (Dekkers JCM, 2007).

4.1 Steps in Marker Assisted Selection (MAS)

RFLP markers are frequently employed in marker-assisted selection to genetically modify crop plants for a range of economically important traits.

4.2 Five Crucial Processes Make Up Marker-Assisted Selection

i. Parent selection,
ii. Population growth for breeding,
iii. DNA isolation from every plant
iv. Rating RFLPs, as well as
v. Association with physical characteristics.

Below is a quick discussion of these:

2. **Selection of Parents:** One crucial stage in marker-assisted selection is choosing appropriate parents. In order to obtain a useful degree of polymorphism (variation) in the RFLP markers, the parents must be such. Put another way, children should be raised by parents who have different personalities or backgrounds. This will facilitate the identification of the parents' DNA as well as the segments belonging to them in the F_2 generation through different recombinations (Knott, S.A. and C.S. Haley. 1992).

   We must filter the germplasm in order to choose parents whose DNA is different. For MAS, pure (homozygous) parents should be employed. Plants in self-pollinating species are typically homozygous. Inbred lineages serve as parents in species that are cross-pollinated.

i. Development of Breeding Populations: This is the second crucial stage in using marker-assisted selection. To create F_1 plants, the chosen parents are crossed. Although F_1 plants between two pure-lines or inbred lines are homogenous (phenotypically similar), they are heterozygous for all two parents' RFLPs.

The analysis of the RFLP segregation pattern necessitates the F_2 progeny. Typically, 50–100 F_2 plants are enough to explore RFLP marker segregation (Beckmann et al., 1988).

ii. Isolation of DNA: Isolating DNA from the breeding population is a crucial third step. The primary benefit of MAS is that DNA can be extracted from seedlings without waiting for blooming or the stage of seed development. All of the F_2 population's plants have had their DNA separated. There are established protocols for isolating DNA.

To obtain DNA fragments, the extracted DNA is digested using a particular restriction enzyme. By using agarose gel electrophoresis to separate the digested DNA, DNA fragments of varying sizes are separated. Under ultraviolet light, the ethidium bromide-stained gel allows the variation in DNA fragments to be seen.

When a certain enzyme is used to degrade chloroplast DNA, around 40 fragments of varying sizes are produced. When some restriction enzymes are used to digest the nuclear DNA of higher plants, millions of pieces in a continuous range of sizes are produced. In these situations, identifying each particular DNA fragment is a laborious task (N Bumstead, J Palyga 1992).

iii. Scoring RFLPs: DNA probes are used to identify the polymorphism in RFLPs between the parents and their participation in the recombinants in the F_2 population. The labeled probes are employed to identify the comparable pieces.

The probe will only form hybrids with segments that are complementary to one another. 32P is typically used to

radioactively label DNA probes. There are also non-radioactive probe labeling methods accessible as well. RFLPs are calculated in this manner.

iv. **Correlation with Morphological Traits:** It is established that indirect selection occurs through molecular markers when DNA markers, such as RFLPs, are associated with morphological markers. When the molecular markers and morphological markers are shown to be correlated, MAS can be utilized to improve many economic features genetically (Beckmann et al., 1983).

By increasing selection intensity, decreasing generation intervals, and improving prediction accuracy, MAS is expected to enhance rather than replace traditional breeding systems, increasing the rate of genetic improvement. Additionally, selection for sex-limited features based on markers may occur early in life or in individuals of both sexes (Lande et al.,1990, Smith, 1967). Since MAS is a type of indirect selection, there is a chance of a decreased genetic response if the marker association data is erroneous (Ribaut et al., 1997). The distance between the markers and target traits, the kind of linkage phase, and the degree of linkage disequilibrium all influence the connection between the markers and the QTL. Consequently, a high-density gene map with closer linkage is necessary for the effective application of MAS55. It is estimated, that an average (Andreescu et al., 2007).

A 10 cm (5–20 cm) marker density with 200–250 makers should be adequate to detect the marker–QTL association23. Gene maps with an average marker interval greater than 5 cm have been available up until recently (Walling et al., 1998). But as of right now, high-resolution maps have been produced with 2.5 cm or even lower marker density (Ihara et al., 2004). Therefore, MAS entails two steps: finding marker loci associated with the QTL and using this knowledge by appropriately incorporating it into currently running breeding programs.

4.3 Identification of Marker QTL Linkage

Molecular markers can be found in either non-coding or coding sequences, and they have the ability to reveal genetic differences at QTL. According to Ron and Weller (2007), there are essentially four design options for marker QTL linkage analysis in cattle.

- Making a backcross between the F_1 and one of the original populations, or using F_2 populations crossing two comparable F_1 populations.
- Using a half-sib sire design, in which a random sample of females is mated to heterozygous sires for the markers, and every offspring is genotyped.
- Alternatively, utilizing a granddaughter design that has been genotyped using progeny testing to assess a sire and their sons.
- Making crosses between people with extreme phenotypes for a single trait or set of traits.
- We also utilize animals from populations with significant variation in critical traits, or from lines that have undergone divergent selection. There are two methods for determining which molecular markers are connected to the QTL.

Changes in the coding sequence: Gene expression can be directly impacted by DNA polymorphisms that arise in and around structural and/or regulatory sequences of physiologically significant genes (e.g., hormone, milk protein, and MHC genes), which can lead to individual differences in productivity and health (disease susceptibility/resistance). As such DNA polymorphisms can exist in genes that are predisposed to being connected or associated with a performance variable of interest, they can be chosen as markers (Beckmann and Soller, 1990).

Polymorphism in non-coding sequences: This method uses non-coding sequence variants, such as flanking or intergenic areas, as surrogate markers for linkage analysis (Smith and Simpson, 1986). Nowadays, highly polymorphic microsatellite markers are being used to find QTLs that are economically significant.

5. MAS INTEGRATION WITH SELECTION PROCESSES

Breed selection and the integration of outstanding traits from several breeds can both benefit from the application of molecular information (Collard and Mackill, 2008). The tactics are covered in the section below.

5.1 Between Breed Selection

It is possible to use linked and direct markers when making breed selections. Marker-assisted introgression may be employed if there is a slight

genetic variation between the two breeds regarding the characteristic of interest. When a hybrid breed repeatedly crosses with the recipient breed, the target gene from the donor breed is transferred into the recipient breed's gene pool (Michelmore, I, 1991). This process is known as introgression. There will be one or two inter crossings after several back crossings. The recipient's QTL will be fixed by these interconnections. Examples include the bare neck gene found in broiler chickens with low body weight (Cahaner et al., 1993).

5.2 Within Breed Selection

The lowest response to selection is predicted unless all the genes affecting the trait are included in the QTL EBV. Selection based only on QTL or marker information disregards information that is available on the additional genes (polygenes) that affect the trait. However, this approach can be appealing when the phenotype is expensive or difficult to record (e.g., disease features meat quality, etc.). It only requires the phenotypes necessary to assess marker effects. In the short run, selection based on the sum of the QTL and polygenic EBV is expected to produce the highest response; but, because of losses in polygenic response, the long-term response may not be ideal. According to Deckers and van Arendonk (1998), it is possible to generate QTL and polygenic EBV indexes that optimize long-term response or a mix of short- and long-term responses (Dekkers and Chakraborty, 2001). Selection on the sum of QTL and polygenic EBV, however, is anticipated to be almost ideal if many QTLs are used and the focus is on increasing shorter-term response. Achieving optimal selection on multiple EBVs, indices, and genotypes while taking into account the inbreeding rate and other pragmatic factors is a challenging issue. Mate selection techniques may be employed to address such issues, and it is anticipated that when genotypic data for a greater number of regions is used more widely, specialized knowledge about individual QTLs will simply become less relevant and contribute to the prediction of whole EBV or whole genotype (JCM Dekkers, 2004).

6. APPLICATIONS OF MARKER ASSISTED SELECTION (MAS)

MAS can be employed in crop improvement programs in a number of ways. Stated differently, there are a number of beneficial uses for MAS in plant breeding.

Below is a brief overview of several significant plant breeding applications of MAS:

i. MAS is a very quick, easy, and successful way to give crop plants tolerance to both biotic and abiotic stressors.
ii. It helps with gene pyramiding for resistance to insects and diseases.
iii. It is employed to introduce photoperiod insensitivity and male sterility into genotypes that have been cultured from various sources.
iv. MAS is being utilized to improve quality characteristics in a variety of crops, including the storage quality of vegetables and fruit crops, the fatty acid (linolenic acid) content in soybeans, and the protein quality in maize.
v. MAS is capable of becoming used for transferring desirable transgene (such as Bt gene) from one cultivar to another.
vi. MAS is highly good at introducing desired wild-type genes into genotypes that have been cultivated.
vii. MAS is equally useful for enhancing an animal's or plant's genetic makeup.
viii. Because phenotypic selection cannot be used until a very long time in the future, MAS is helpful in genetic development of tree species whose fruiting takes a very long time (Strauss et al., 1992).
ix. Compared to polygenic qualities, oligogenic traits can be genetically improved more widely through the use of MAS.

6.1 Achievements of Marker Assisted Selection (MAS)

Higher levels of resistance to the bacterial blight pathogen were displayed by the pyramided lines. Through MAS, two rice cultivars that are resistant to bacterial blight-Angke and Conde-have were made available in Indonesia. Three genes-Pil, Piz5, and Pita-have been pyramided for blast resistance in the vulnerable rice variety Co 39 using PCR and RFLP-based markers.

MAS has been used to genetically modify a range of field crops for various economic features, such as maize, barley, rice, wheat, sorghum, soybean, chickpea, pea, sunflower, tomato, potato and some fruit crops (Zhuchenko et al., 1979, Foolad et al., 2012). The creation of disease-resistant cultivars for a variety of crops has been the main use of MAS.

6.2 Here are a Few Noteworthy Instances of MAS Usage

i. **Rice:** MAS has been effectively applied to rice in order to create cultivars that are resistant to blast and bacterial blight. Four genes (Xa4, Xa5, Xa13, and Xa21) for resistance to bacterial blight have been pyramided using STS (sequence tagged site) markers.

ii. **Maize:** MAS has been used to transform normal lines of maize into quality protein maize (QPM) lines by utilizing the opaque 2 recessive gene. The International Center for Wheat and Maize Improvement (CIMMYT) in Mexico is the location of this effort.
For this, three SSR markers found in the opaque 2 gene (Umc 1066, Phi 057, and Phi 112) have been employed. The MAS is easy to use, quick to process, and precise for converting regular maize lines into QPM.

iii. **Soybeans:** Nematodes, or cyst nematodes, are a major concern in soybeans and affect most types. There is a resistance gene (rhg 1) accessible. Using the SSR marker, MAS has been used to generate nematode-resistant lines in soybeans (Sat 309).

To genetically enhance a variety of qualities, MAS has been applied to a variety of crops. Salt resistance, insect resistance, disease resistance, and shattering resistance are significant qualities that MAS has improved in a number of crops. It has also been utilised to transfer a number of features to various crop plants, such as higher protein content, earliness, male sterility, and insensitivity to photoperiod. In MAS, a variety of crop plants have been marked with several types of DNA markers. Numerous molecular markers have been widely used in MAS in a range of crops, including microsatellites, random amplified polymorphic DNA (FAPD), restriction fragment length polymorphisms (RFLP), and simple sequence repeats (SSR) (Begna, T. 2020).

Additional markers that have been applied to certain crops include SCAR markers, expressed sequence tags (EST), sequence tagged site (STS), and amplified fragment length polymorphism (AFLP). SNPs, or single nucleotide polymorphisms, are also employed. SNPs for every major grain crop have been found. Fruit and fodder crops are also being genetically improved through the application of MAS. MAS has been widely employed in pomegranate, apple (Longhi et al*.,* 2013), and pear crops among fruit crops (Ru et al*.,* 2015). Based on RFLP, RAPD, SSR, and AFLP markers, MAS is used in fruit crops. MAS is used in various fruit crops to increase fruit yield, improve fruit quality for storage, and increase resistance to disease (Ibitoye *& Akin-Idowu* 2010, Ibitoye *& Akin-Idowu (*2011). Based on RFLP, RAPD, and AFLP markers, MAS is utilized in vegetable crops, primarily in tomato and potato, for disease resistance. It has been discovered that MAS is helpful for genetically enhancing tree crops like coconut and rubber (Drew, R, 2016, K Choudhary, OP Choudhary 2008, Dirlewanger, *et al,* 2004).

The majority of field crops contain associated markers and genes for a number of significant features that have been found; they are used for MAS.

6.3 Advantages of Marker Assisted Selection (MAS)

Compared to conventional breeding methods and phenotypic selection, MAS offers a number of benefits.

A few significant benefits of MAS are covered in brief below:

1. **Precision:** Because molecular markers are unaffected by environmental factors, MAS has extremely high accuracy. Even if the characters have a low heredity, it is still incredibly effective.
2. **Quick Approach:** MAS is a quick technique for improving crops. The process of creating a new cultivar takes three to five years, whereas the traditional breeding procedure takes ten to fifteen years.
3. **Non-transgenic Product:** MAS promotes the creation of universally palatable non-transgenic cultivars. To put it another way, transgenes are not involved. Therefore, gene silencing is not an issue.
4. **Recessive Allele Identification:** Crop development initiatives move more quickly because of MAS, which makes it possible to identify recessive alleles even in heterozygous conditions. Stated differently, it works just as well for improving recessive traits genetically.

5. **Early Characteristic Identification:** Traits that manifest late in a plant's life can be detected early thanks to MAS. For instance, traits that manifest late in a plant's life, such as flower colour, male sterility, grain or fruit quality, and photoperiod sensitivity, can be checked for in the seedling stage. To put it another way, DNA testing at the seedling stage can reveal information about traits that will manifest later.
6. **Screening of Difficult Traits:** MAS has been applied to diverse crops in order to genetically improve a range of traits. Important traits that have been enhanced in several crops by MAS include salt resistance, insect resistance, disease resistance, and shattering resistance.
7. **Gene pyramiding:** Multiple gene accumulation for resistance to particular diseases and pests within a single cultivar can be achieved extremely successfully with the help of MAS. Gene pyramiding is the term for this procedure. Gene introgression breeding programs frequently use maker assisted backcrossing. A useful and efficient breeding method for finding, following, keeping, combining, and pyramiding disease-resistant genes is MAS.
8. **Tiny Sample Required for Testing**: For DNA testing, MAS just needs a tiny sample of plant tissue. Put differently, tiny breeding populations can be used for MAS. Additionally, MAS can be used at any phase of the plant's growth.
9. **Acknowledges QTL Mapping**
   Quantitative trait loci (QTL) can be mapped or tagged using MAS, which is not achievable using traditional methods.
10. **Extremely Repeatable**:
    The DNA fingerprinting method, on which the MAS is based, yields extremely dependable and repeatable findings.

These benefits could translate into either improved line development or increased efficiency in breeding programs. For instance, using DNA marker tests instead of labor-intensive or challenging field testing may result in time and labor savings. Furthermore, because environmental influences have an impact on field trials, selection based on DNA markers might be more reliable. One other advantage of MAS is that it may result in a reduction in the overall number of lines that require testing. Because so many lines can be dropped at an early stage using MAS, breeding designs can be more successfully implemented (Luby, DV, 2001). Increased target character selection efficiency, which would enable some features to be "fast-tracked" because some genotypes are simple to identify and choose. Additionally, "background" markers could be employed to hasten the recovery of recurrent parents during marker-assisted backcrossing (Hospital et al., 1997).

6.4 The advantages of MAS include

- Using molecular tests instead of time-consuming, technically complex field trials that must be conducted at specific times of the year or at specific locations can save time.
- Additionally, field trials' unreliable phenotypic evaluation caused by environmental effects can be eliminated.
- Finally, genotypes can be selected at the seedling stage.
- "Pyramiding" genes, or merging several genes at once;
- Prevent the transfer of unwanted or harmful genes (also known as "linkage drag"; this is especially important when genes from wild species are being introduced).
- When phenotypic evaluation is impractical due to quarantine constraints, low heritability features should be chosen for testing (e.g., exotic infections to be utilized for screening).

6.5 Limitations of Marker-Assisted Selection (MAS)

MAS has several advantages as discussed.

However, it has some limitations or drawbacks which are briefly below:

i. The MAS approach is expensive. It needs a well-stocked lab with pricey tools, glassware, and chemicals.
ii. The management of complex machinery, the isolation of DNA molecules, and the research of DNA markers all demand highly skilled labor for MAS.
iii. Finding different related DNA markers (AFLP, RFLP, RAPD, SSR, SNP, etc.) takes a lot of effort and time (Neves et al., 2012).

iv. Radioactive isotopes are occasionally used in MAS to identify DNA, which carries a significant risk to human health. This is a significant drawback of markers based on RFLP. In this sense, the PCR markers are safe.
v. It has been suggested that over time, phenotypic selection may prove to be more effective than MAS.
vi. Because QTL have small cumulative effects and are heavily impacted by genetic background and environmental factors, using MAS is more challenging for them (Bhat et al., 2010).

6.6 Future Outlook of Marker-Assisted Selection (MAS)

Plant breeding approaches should use MAS since it is seen to be a useful tool for improving crops. According to reports, wealthy nations have been the primary users of MAS due to the technology's high cost of infrastructure, equipment, chemicals, and glassware.

Many developing nations cannot afford the high cost of MAS technology. In order to facilitate the widespread adoption of this technology by the developing and underdeveloped globe, the following strategies and tactics could prove beneficial:

- The Consultative Group on International Agricultural Research (CGIAR), which encourages cooperative research and training on a global scale, need to fund expensive technologies like MAS. It will support the fast adoption of technology (MAS) in underdeveloped nations.
- The FAO and Rockefeller Foundation might be crucial in helping resource-poor nations have access to MAS technology.
- For the good of humanity as a whole and the private sector, MAS technology should be supported.
- Research institutes involved in MAS programs must collaborate internationally in order to fully utilize this technology.
- In order to give poor nations access to MAS technology, public and private groups must work together.
- Regional training programs for MAS technology capacity building should be organized by international agencies like the FAO and CGIAR.
- One tool that can be employed in the crop development program is marker-aided selection. It can expedite the advancement of breeding initiatives. It can reduce the amount of time needed to develop new types. This method cannot, however, be utilized in place of traditional breeding practices. Marker-assisted selection is not without its drawbacks.

6.7 The Relationship of Gene Mapping to Mas

DNA-level selection is intended to take the place of phenotypic selection in marker-assisted selection (MAS). MAS should ideally be based on a DNA-level screen for the specific sequence variant at each QTL associated with a favourable impact on trait value. To determine the coupling linkage relationships between certain favourable (or unfavourable) alleles at the linked QTL and specific alleles or haplotypes at the marker locus, it is actually sufficient to identify a marker or group of markers linked to the QTL of interest. Thanks to MAS, selection will be feasible at an early age, for both sexes equally, and without the requirement for costly trait evaluation (Soller et al., 1976).

Consequently, selection intensity will increase and the generation interval will shorten. MAS is not significantly impacted by changes in the microenvironment. This will increase the precision of the selection process. MAS will direct the usage of complimentary and additive dominant loci to improve cross-performance. Through the rapid identification and introduction of targeted, favourable alleles from resource populations to destination populations, MAS will improve resistance in the production features of enhanced breeds and landraces. Operationally, we pinpoint a number of methodologically distinct steps that culminate in the identification of a QTL in the DNA molecule that correlates to a certain functional position (A Darvasi, M Soller -1994, Darvasi et al., 1993).

Every phase allows for a more precise and tighter correlation between functional variation at the QTL and DNA level marker allele variation, and each step has its own suitable approach. Hence, each step can contribute to MAS in a rising amount. These actions are as follows:-

1. Finding the QTL of interest on the chromosomal areas (10–20 cm).

2. The precise location of the QTL (5 cm) within these areas.
3. Markers in close proximity to the QTL (1-2 cm) are identified.
4. The "narrow" region's possible "candidate" genes are identified.
5. Determination of the particular gene linked to variation in traits.
6. Determination of the gene's functional location.

The final outcome can be obtained in two ways. Examining the relationship between trait value variation and DNA level variation in genes known to be directly involved in the physiology and development of characteristics is the foundation of the first step, which jumps straight to the fourth step above. This is the "candidate gene" approach. The second is the whole approach outlined above, which is predicated on QTL mapping to progressively smaller regions of the chromosome. Once suitable candidate genes are identified, it is comparable to the candidate gene technique. Collins (1992) called this the "positional genetics" approach.

6.8 Potential Genes

The utilization of candidate gene technique has yielded noteworthy results in indicating the relationship between variations in milk protein output and cheese-making attributes and genetic polymorphisms at the lactalbumin and kappacasein loci in cattle (Bovenhuis et al., 1992). Although this is now thought to be due to linkage rather than a direct effect at the prolactin locus, exploration of RFLPs at the prolactin locus in dairy cattle also revealed an influence on milk production (Cowan et al*.,* 1990).

6.9 Genetics in Position

The quantitative trait was bean weight, and the markers were genes for the colour of the seed coat. As is well known, the first demonstration of a connection between QTL and genetic markers was made by Sax (1923). Following this experiment, Mather (Breese et al., 1957) and Thoday (1961) both carried out in-depth follow-up investigations on Drosophila. Comprehensive QTL mapping experiments had little impact on agricultural genetics because they require a set of genetic markers that span the entire genome at reasonable intervals (e.g., 20-40 cm) and segregate in the same population as the QTL, which was not even possible for the major agricultural plant and animal species (see, however, Zhuschenko et al*.,* 1979).

6.10 The Role of PCR in MAS

Following the identification, description, and sequencing of a direct or linked marker, a method called polymerase chain reaction (PCR) can be used to replicate a specific DNA region in order to produce enough DNA for a test. The PCR process is summarised in Fig. 2 on the following two pages. Kary Mullis created it in 1983, and it has since become one of the most widely used techniques in molecular biology. A small bit of DNA can be quickly and easily transformed into a relatively large amount of DNA using this method.

In natural systems, DNA replication needs access to the following nucleotides:

a. adenine (A), cytosine (C), thymine (T), and guanine (G);
b. the synthesis enzyme DNA polymerase;
c. a primer, which is a brief RNA molecule;
d. a strand of DNA that needs to be replicated;
e. and ideal reaction circumstances (temperature, pH).

Enzymatic unwinding of the DNA, synthesis of the RNA molecule, attachment of the DNA polymerase to the RNA, and synthesis of a complementary DNA strand follow. The elements and processes of the natural system are used in the laboratory when using PCR, yet there are three main variations:

1. Instead of using the RNA primer present in the natural system, DNA primers are used. Typically ranging from 18 to 25 nucleotides in length, DNA primers are engineered to bind to both ends of the target DNA region.
2. To the reaction mixture are added magnesium ions that are involved in DNA replication.
3. Taq, or another DNA polymerase enzyme that can tolerate high temperatures, is utilized.

In order to allow for the simultaneous synthesis of both strands of the DNA to be replicated, the DNA primers are complementary, or match up, to the opposing strands. C and G match, as do A and T. The reaction mixture includes primers complementary to both strands of DNA, thus using the opposite primer, the products of DNA synthesis can be replicated. The two primers' positions in relation to the targeted DNA region dictate how long the DNA needs to be replicated.

The length and position of the DNA copies on the original DNA are specified. The primer sequence is included in the new DNA strands since DNA replication begins with the primers.

This gives the new strands a sequence that primers can bind to in order to duplicate more DNA. Two significant advancements have made the PCR process simpler and the findings more consistent over time. The first was the discovery of Taq polymerase, a heat-stable DNA polymerase. Thermus aquaticus, the bacteria from which this enzyme was isolated, is the source of its name. It was found that this bacteria was present in the boiling hot spring water. The DNA polymerases that researchers had access to were destroyed at 65ºC until Taq polymerase was found. The high temperature needed to denature the DNA template (pattern) does not destroy the Taq enzyme.

As a result, employing this enzyme replaces the necessity of adding fresh enzyme to the tube for every copying cycle, which was frequently done prior to Taq's discovery. The three steps in the PCR process comprise the copying cycle. To maximize the reaction, the mixture's temperature is allowed to fluctuate at each stage. To get the required amount of DNA, the cycles are repeated as many times as needed.

STEP 1: Denaturation

To break the hydrogen bonds between the complementary bases, the double-stranded DNA that needs to be replicated is heated to about 95 degrees Celsius. Two single-stranded DNA fragments are produced as a result.

Step 2: Hybridisation or Annealing

To enable the DNA primers to generate hydrogen bonds between the bases of the template and the primers, the temperature is decreased to about 58ºC. This will allow the primers to attach to the corresponding sequence on the single-stranded DNA.

Step 3: Synthesis or Extension of DNA

The nucleotide bases A, C, T, and G are incorporated into the new DNA copy by the DNA polymerase during the replication step, which involves heating the reaction solution to about 72ºC. The new DNA strand is formed by connecting bases that are complementary to the template until it comes to the end of the region to be copied.

7. CONCLUSION

The majority of MAS investigations employ DNA-based markers such as microsatellites, single nucleotide polymorphisms (SNPs), random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and restriction fragment length polymorphism (RFLP). In this case, we discussed several kinds, techniques, and other features of marker Assisted Selection.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of this manuscript. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1. ChatGPT

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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