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20. Marker-Assisted Breeding Program

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Abstract:

Considerable developments have been made in biotechnology due to the challenges faced by plant breeders which led to more effective and efficient selection systems and accelerated breeding process. The marker-assisted breeding programme has replaced the traditional phenotypic- pedigree-based selection system. It is an indirect selection process where a trait of interest is selected not based on the trait but on a marker linked to it. Molecular marker-assisted breeding (MAB) is the application of molecular biotechnology (DNA markers) to practical breeding and selection, and is a novel strategy and powerful method for crop improvement. It provides significant advantages as compared to conventional breeding methods. Presently this procedure has been extensively utilised in different crops. This chapter will explain briefly about marker-assisted selection, general procedures, Markers and applications of marker-assisted breeding (MAB). Integration of MAB into conventional breeding programs represents an optimistic strategy for future crop improvement.

Keywords

DNA Marker, Breeding Population, Crop Productivity, Polymorphism, QTL

20.1 Introduction:

20.1.1 Marker-Assisted Selection:

Marker-assisted selection or marker-aided selection (MAS) is an indirect selection process for a trait of interest based on markers (biochemical. Morphological or DNA/RNA variation) linked to a trait of interest (e.g., productivity, disease resistance, tolerance, and quality), rather than on the trait itself. It is one tool utilized in breeding companies and research Institutes for the fast development of improved varieties, giving a possibility to select desirable traits more directly using DNA markers.

A. Markers:

- A marker is a gene or a DNA sequence with a known location on a chromosome that can be used to identify individuals or a species.

- The gene of interest and the marker tend to move together during segregation of gametes due to their proximity on the same chromosome and concomitant reduction in recombination between the markers and gene of interest.
- If the gene is not known, markers linked to the gene of interest can still be used to select for an individual with desirable alleles of the gene of interest.

B. Markers Types:

The majority of MAS work in the present day uses DNA-based markers. However, the first markers that allowed indirection selection were morphological markers.

i. Morphological - markers that are often detectable by eye, by visual inspection. Examples of these type markers include the presence or absence of an awns, plant height, grain color, seed shape etc.

ii. Biochemical- a protein can be extracted and observed; for example, isozymes and storage proteins.

iii. Cytological- the chromosome banding produced by different strains; G-banding, Q-banding, R-banding etc.

iv. Molecular markers or DNA based- a unique (DNA sequence), occurring in proximity to the gene or locus of interest, can be identified by a range of molecular techniques such as;

- Restriction Fragment Length Polymorphism (RFLP)
- Amplified Fragment Length Polymorphism (AFLP)
- Random Amplified Polymorphic DNA (RAPD)
- Cleaved Amplified Polymorphic Sequences (CAPS)
- Simple Sequence Repeat (SSR) Length Polymorphism
- Single Strand Conformational Polymorphism (SSCP)
- Single Nucleotide Polymorphism (SNP)
- Expressed Sequence Tags (EST)
- Sequence Tagged Sites (STS)

C. Properties of DNA Marker for MAS:

- Markers should exhibit high level of polymorphism. In other words, there should be variability in the markers. It should demonstrate measurable differences in expression between trait types and/or gene of interest.
- Evenly distributed across the whole genome (not clustered in particular regions)
- Marker should be co-dominant. It means, there should be absence of intra-locus interaction. It helps in identification of heterozygotes from homozygotes.
- The marker should be multi-allelic. It useful in getting more variability/ polymorphism for a character.
- There should be absence of epistasis. It makes identification of all phenotypes (homo- and heterozygotes) easy.

- Markers should be insensitive to environment. This property is also found in almost all the DNA markers.
- Low or null interaction among the markers allowing the use of many at the same time in a segregating population.
- Abundant in number.

20.2 Steps in Marker Assisted Selection (MAS):

In marker-assisted selection, RFLP markers are widely used for the genetic improvement of crop plants for various economic characters.

The Marker Aided Selection Consists of Five Important Steps:

20.2.1 Selection of Parents:

The selection of suitable parents is an important step in marker-aided selection. The parents should be such so that we can get usable level of polymorphism (variation) in the RFLP markers. In other words, parents with contrasting characters or divergent origin should be chosen. This will help in identification of DNA of both the parents and also their segments in F_2 generation in various recombination. For selection of parents, we have to screen germplasm and select parents with distinct DNA. 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.

20.2.2 Development of Breeding Populations:

This is the second important step for the application of marker assisted selection. The selected parents are crossed to obtain F_1 plants. F_1 plants between two purelines or inbred lines are homogeneous (alike phenotypically) but are heterozygous for all the RFLPs of two parents involved in the F_1. The F_2 progeny is required for the study of segregation pattern of RFLPs. Generally, 50-100 F_2 plants are sufficient for the study of segregation of RFLP markers.

20.2.3 Isolation of DNA:

The third important step is the isolation of DNA from the breeding population. The main advantage of MAS is that DNA can be isolated even from the seedlings and we need not wait for the flowering or seed development stage. The DNA is isolated from each plant of F_2 population. Standard procedures are available for DNA isolation. The isolated DNA is digested with a specific restriction enzyme to obtain fragments of DNA. The DNA fragments of different sizes are separated by subjecting the digested DNA to agarose gel electrophoresis. The gel is stained with ethidium bromide and the variation in DNA fragments can be viewed in the ultraviolet light. The DNA of chloroplasts, when digested with a specific enzyme, produces about 40 fragments of different sizes. The nuclear DNA of higher plants, when digested with specific restriction enzymes, produces millions of fragments in a continuous range of sizes. It is a tedious job to identify individual DNA fragments in such cases.

20.2.4 Scoring RFLPs:

The polymorphism in RFLPs between the parents and their involvement in the recombinants in F_2 population is determined by using DNA probes. The labelled probes are used to find out if the fragments have similarities.

The probe will hybridize only with those segments which are complementary in nature. Generally, 32P is used for radioactive labelling of DNA probes.

Now non-radioactive probe labelling techniques are also available. In this way, RFLPs are determined.

20.2.5 Correlation with Morphological Traits:

The DNA marker (say RFLPs) is correlated with morphological markers and the indirect selection through molecular markers is confirmed.

Once the correlation of molecular markers is established with morphological markers, MAS can be effectively used for the genetic improvement of various economic traits.

20.3 Applications of Marker Assisted Selection (MAS):

In crop improvement programmes MAS can be used in various ways. In other words, MAS has several useful applications in plant breeding.

Important applications of MAS in plant breeding are briefly presented below:

- MAS is very effective, efficient and rapid method of transferring resistance to biotic and abiotic stresses in crop plants.
- It is useful in gene pyramiding for disease and insect resistance.

- It is being used for transfer of male sterility and photo period insensitivity into cultivated genotypes from different sources.
- MAS is being used for improvement of quality characters in different crops such as for protein quality in maize, fatty acid (linolenic acid) content in soybean and storage quality in vegetables and fruit crops.
- MAS can be successfully used for transferring desirable transgene (such as Bt gene) from one cultivar to another.
- MAS is very effective in introgression of desirable genes from wild into cultivated genotypes.
- MAS is equally effective in genetic improvement of plants and animals.
- MAS is useful in genetic improvement of tree species where fruiting takes very long time (say 20 years) because for application of phenotypic selection we have to wait for such a long time.
- MAS has wide application for genetic improvement of oligogenic traits as compared to polygenic traits.

20.3.1 Advantages of Marker Assisted Selection (MAS):

- It can be performed on seedling stage thus reducing the time required before a plant's genotype is known. For example, characters such as grain or fruit quality, flower color, male sterility, photoperiod sensitivity that express late in the life of a plant can be screened in the seedling stage.
- The accuracy of MAS, is very high because molecular markers are not affected by environmental conditions.
- MAS is a rapid method of crop improvement. It takes 3-5 years to develop a new cultivar against 10-15 years taken by the conventional method of breeding.
- MAS leads to development of non-transgenic cultivars which are acceptable to everybody. In other words, it does not involve transgene. Hence there is no question of gene silencing.
- When recessive allele determines the traits of interest, they cannot be detected through phenotyping evaluation of heterozygous plants. MAS permits identification of recessive alleles even in heterozygous condition and thus speeds up the progress of crop improvement programmes.
- MAS permits screening traits that are extremely difficult to express and time consuming to score phenotypically. For example, screening for traits such as root morphology and resistance to biotic (insects and diseases) and abiotic stresses (drought, salinity, heat, frost etc.) is very easy through MAS.
- MAS is a very effective method in accumulating multiple genes for resistance to specific pathogens and pests within the same cultivar. This process is called gene pyramiding.
- MAS requires only a small amount of plant tissue for DNA testing.
- MAS permits mapping or tagging of quantitative trait loci (QTL) which is not possible by conventional methods.
- maintenance of recessive alleles during backcrossing or for speeding up backcross breeding in genera
- pyramiding multiple monogenic traits or several QTL for a single disease resistance with complex inheritance.
- A consideration that may affect cost-effectiveness of MAS is that multiple markers can be evaluated using the same DNA sample.

20.3.2 Limitations of Marker Assisted Selection (MAS):

- MAS is a costly method. It requires a well-equipped laboratory *viz.*, expensive equipment, glassware and chemicals.
- MAS requires well-trained manpower for handling of sophisticated equipments, isolation of DNA molecules and study of DNA markers.
- The detection of various linked DNA markers (AFLP, RFLP, RAPD, SSR, SNP etc.) is a difficult, laborious and time-consuming task.
- MAS sometimes involves the use of radioactive isotopes in labelling of DNA, which may lead to serious health hazards.
- It has been reported that MAS may become less efficient than phenotypic selection in the long term.

- The use of MAS is more difficult for QTL because they have minor cumulative effects and are greatly influenced by environmental conditions and genetic background.

20.3.3 Achievements of Marker Assisted Selection (MAS):

MAS has been used for genetic improvement of different field crops such as maize, barley, rice, wheat, sorghum, soybean, chickpea, pea, sunflower, tomato, potato and some fruit crops for various economic characters. MAS has been mainly used for developing disease-resistant cultivars in different crops.

i. Rice:

In rice MAS has been successfully used for developing cultivars resistant to bacterial blight and blast. For bacterial blight resistance four genes (Xa4, Xa5, Xa13 and Xa21) have been pyramided using STS (sequence tagged site) markers. One of the successful applications of MAS in breeding disease resistance was in Indonesia, and the release of two rice varieties 'Angke' and 'Conde', which are resistant to bacterial leaf blight infection.

The pyramided lines showed higher level of resistance to bacterial blight pathogen. For blast resistance, three genes (Pil, Piz5 and Pita) have been pyramided in a susceptible rice variety Co 39 using RFLP and PCR based markers.

iii. Maize:

In maize, normal lines have been converted into quality protein maize (QPM) lines through MAS using opaque 2 recessive alleles. This work has been done at CIMMYT (international centre for wheat and maize improvement, Mexico).

Three SSR markers (Umc 1066, Phi 057 and Phi 112) present within opaque 2 genes have been used for this purpose. The MAS used for the conversion of normal maize lines into QPM is simple, rapid and accurate.

ii. Soybean:

In soybean cyst nematodes pose serious problem and most of the varieties are susceptible to this parasite. The resistant gene (rhg 1) is available. In soybean, nematode resistant lines have been developed through MAS using SSR marker (Sat 309).

20.4 Reference:

1. Collard BC, Mackill DJ. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philosophical Transactions of the Royal Society B: Biological Sciences. 2008; 363(1491): 557-572.
2. Johnson GR. Marker assisted selection. Plant Breed. Rev. 2010; 24(1): 293-310.
3. Lande R, Thompson R. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics. 1990; 124(3): 743-756.

4. Singh BD, Singh AK, Singh BD, Singh AK. Marker-assisted selection. Marker-assisted plant breeding: principles and practices. 2015: 259-93.
5. Marker Assisted Selection (MAS): Meaning, Steps and Application. Marker-assisted selection – Wikipedia. Available from: biologydiscussion.com
6. Bustamam M, Tabien RE, Suwarno A, Abalos MC, Kadir TS, Ona I, Bernardo M, Veracruz CM, Leung H. Asian Rice Biotechnology Network: Improving Popular Cultivars Through Marker-Assisted Backcrossing by the NARES. Poster presented at the International Rice Congress. September 16-20, Beijing, China. 2002.
7. Xu Y, Crouch JH. Marker-assisted selection in plant breeding: from publication to practice. Crop Sci. 2008; 48: 391-407.