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# Crop Improvement in Self Pollinated Crops Through MAS

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## Introduction

Full form of MAS is Marker Assisted Selection. It is the method of selecting the desirable characteristics or traits in a breeding scheme for improving the characteristics by targeting the DNA sequence.

Through marker-assisted selection, DNA markers have immense potential to increase the efficiency and precision of traditional plant breeding. A plethora of DNA marker–trait relationships have been discovered as a result of a huge number of Quantitative Trait Loci (QTLs) mapping investigations for various crop species.

Markers used in MAS programme are:

**a.** RFLP

**b.** SSR

**c.** RAPD

**d.** SNP

A marker is a DNA sequence that can be easily identified and whose inheritance can be tracked. It is a gene of known function and location that allow the studying of inheritance of the gene.

## Boons of MAS

- Ease of use
- Small amount of DNA is required
- Repeatability of results
- High rate of polymorphism

Knowledge of markers linked with qualities vital to breeding programmes is the most important part of MAS.

Steps included in conducting MAS programme

Tissue sampling

⇓

DNA extraction

⇓

PCR

⇓

Gel electrophoresis

⇓

Marker analysis

## A. Tissue Sampling

**a.** It is the first and foremost part prerequisite for this programme and keen hygenic conditions are maintained since from the stage of tissue sampling to gel documentation process.

**b.** Different equipments, such as petri dishes and glass wares, must be sterilised in an autoclave at 121 degrees Celsius and 15 pounds of pressure for 15 minutes.

## B. Procedure of DNA Extraction

**a.** Raising of seedlings in sterilized petri dishes.

**b.** Maceration of plant tissue by using liquid nitrogen.

**Materials Required are**

- Buffer
- Microfuge tubes
- Mortar and pestle
- Liquid nitrogen
- Microfuge
- Absolute ethanol ( ice cold)
- 70% Ethanol ( ice cold)
- Water bath
- Chloroform : Iso Amyl alcohol ( 24:1)
- Sterile distilled water
- Agarose
- Agarose gel electrophoresis system
- Ethidium bromide
- Gel documentation software.

**c.** Place the plant extract and buffer in a microfuge tube and incubate it in a recirculating water bath at 55 degrees for 15 minutes.

**d.** After that, spin it for 5 minutes at 12,000 rpm in the centrifuge to mix the cell debris, and then collect the supernatant using a micropipette and transfer it to a clean microfuge tube.

**e.** To each tube add 250 microliter of chloroform: Iso amyl alcohol (24:1) and mix

**Centrifuge usage for mixing cell debris**

**Transfer of the supernatant to microfuge tube**

the solution by inversion. After mixing rotate the tubes manually or centrifuge for better mixing of the cell contents. Invert the tubes slowly several times to precipitate the DNA, by adding 50 microliter of ammonium acetate followed by ice cold absolute ethanol. Generally after adding these it will be precipitated out of solution.

**f.** After this place the inverted microfuge tubes in the ice box at freezer for 1 hr to precipitate to thick mass for best outcome.

**g.** After precipitation transfer it the microtube containing 500 microliter of 70% ethanol and spin it for about a minute at 13,000 rpm.

**h.** By this repeated process the DNA will be pelletized and remove all the supernatant and dry the pellet under hygenic condition for about 15 min until it becomes dry.

**i.** Do not allow it to be over dry because it will be hard to re-dissolve.

**j.** Re suspend the DNA in sterile DNase free water approximately 50-400 microliter of water and RNase (10 micro litre) prior to dissolve the DNA to remove any RNA in the isolation process.

**k**. After resuspension, DNA is incubated

for 20 minutes at 65 degrees. DNA integrity will be determined by agarose gel electrophoresis, while concentration and purity will be determined by spectrophotometry.

### C. PCR (Polymerase Chain Reaction)

For DNA amplification, the polymerase chain reaction is a powerful and sensitive technology. Taq DNA polymerase is a widely used PCR enzyme.

**Steps undergoes in PCR are**

**a.** Denaturation

**b.** Annealing

**c.** Extension

**Process undergoing in PCR is described in brief**

**a. Denaturation:** For pure DNA templates, a 35-second denaturation at 95 degrees is sufficient. For difficult templates such as GC rich sequences denaturation time should be done for about 2-4 minutes at 95 degree to denature the template.

**b. Annealing:** It typically lasts for about 15- 60 seconds. Annealing temperature can be optimized by using temperature gradient PCR which starts at 5 degree below temperature.

**c. Extension:** Extension temperature is 72 degree Celsius. Extension times are generally 1 minute but extension of 5 min at the same temperature was found to be accurate.

### D. Gel Electrophoresis

DNA check run by gel electrophoresis: The PCR products generated using Taq DNA polymerase was checked by running on gel electrophoresis, to get the stable bands and to understand the molecular weight of DNA.

Components included under this are

**a.** Agarose concentration

**b.** Voltage

**c.** Electrophoresis buffer

**d.** Ethidium bromide.

**a. Agarose Concentration:** Varied sizes of DNA fragments can be resolved on gels with different agarose concentrations. Smaller DNA fragments can be separated more easily with higher agarose concentrations, while bigger DNA fragments can be resolved with lower agarose concentrations.

**b. Voltage:** Larger fragments travel proportionally faster than small fragments as the voltage applied to the gel is increased. Applying 5V is beneficial for achieving the best resolution of pieces larger than roughly 2kb.

**c.Electrophoresis buffer:** For DNA electrophoresis, several alternative buffers have been suggested. TAE (Tris-acetate-EDTA) and TBE (Tris-acetate-EDTA) are the most often used buffers (Tris-borate EDTA). Because of their ionic strength, these two buffers were shown to be more successful at moving DNA fragments. If we use water instead of buffer in the gel tank then there will be no movement of DNA fragments.

**d. Ethidium Bromide:** The reason for employing ethidium bromide is that it is a fluorescent dye that intercalates with the bases of nucleic acids, making it easy to spot DNA fragments in the gel.

**Check run procedure in gel electrophoresis**

### E. Gel Documentation and Marker Analysis

**a.** Casting gel is made by mixing agarose powder with electrophoresis buffer to the correct concentration, then heating it in the microwave until it melts completely.

**b.** After cooling the solution to around 60 degrees Celsius, it is poured onto a casting tray with a comb and allowed to solidify for nearly 45 minutes at room temperature.

**c.** The comb is removed once the gel has set, and the gel is put into the electrophoresis chamber and immersed in TBE buffer.

**d.** The lid and powder leads are placed on the equipment, and current is administered to DNA samples mixed with loading buffer pipetted into sample wells.

**e.** Bubbles emerging off the electrode indicate current flow in the gel electrophoresis tank, and DNA migrates towards the positive end.

**Procedure for Gel Documentation**

- There is a special soft ware designed to know about the gel documentation. Open the chamber door and load the gel into the chamber with the gloved hand. Centre the gel, using the monitor to assist in visualization.
- Close the door and switch on the UV light.
- On the menu bar, select 'Edit' and cursor down to 'Extract'. A new window will appear with final picture. We can see the bands clearly on the screen for the area of interest.
- Turn off the UV light once the work on soft ware is done.

**Advantages of MAS over conventional breeding method**

**a.** Marker assisted back crossing.

**b.** Pyramiding.

**c.** Early generation selection.

They have been described in brief:

**a. Marker Assisted Back Crossing**

- Effective selection of target loci.
- Minimize linkage drag

**b. Pyramiding**

- Widely used for combining disease resistance against specific race of pathogen.
- Also used to develop durable disease resistance.

**c. Early Generation Selection**

- MAS conducted at F2 or F3 stage.
- Plants with desirable genes are selected and alleles can be fixed in homozygous state.
- Plants with undesirable gene combinations can be discarded.

**Success Aspects by using MAS**

**a.**Disease resistance.

**b.** Insect resistance.

**c.** Drought resistance.

**d.** In pulses increment of essential amino acids like lysine, methionine and tryptophan.

**Conclusion**

It appears that contemporary breeding programmes are making success using widely used breeding techniques. Although the impact on variety development has been minor, MAS could substantially assist plant breeders in achieving this goal.

For the full potential of MAS to be realized, more integration with breeding programmes is required, as well as a thorough understanding of current barriers and the development of relevant solutions. Exploiting the benefits of MAS over traditional breeding could have a significant influence on agricultural improvement. In the near future, the high cost of MAS will continue to be a major impediment to its deployment in developing countries for some crop species and plant breeders.

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