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Application of Plant Tissue Culture Techniques for the Genetic Improvement and Yield Maximization in Moringa (Moringa oleifera)

Moringa is a popular vegetable for curry, gravy and side dish preparations, both leaves and fresh pods are used in culinary purposes. The important breeding objectives in moringa are development of short statured drought and saline tolerant annual types with higher number of pods and higher levels of iron and zinc. Besides these, off season production is also one of the important breeding objectives. Applications of conventional breeding approaches in Moringa are very difficult due to its highly cross pollinated nature and heterozygosity expressions. Hence, biotechnological approaches are the best option for further improvement of Moringa. Micro-propagation and Doubled haploid techniques will pave the way for genetic improvement in Moringa for genetic uniformity.

INTRODUCTION

India is the major producer of Moringa with a production of 2.2 to 2.4 crore quintals of young pods from an area of 0.95 lakh acres per year. The approximate yield per acre is 250 quintals per acre. Andhra Pradesh ranks number one in area and production Tamil Nadu is one of the leading state in Moringa production, because of prevailing

genetic and climatic diversity. Recent years, states like Maharashtra, Karnataka, Andhra Pradesh and Telengana started growing annual moringa as fodder crop using annual moringa variety PKM-1. The cheapest source of iron is the main reason for leaves as fresh fodder which has many roles in animal health.

As popular vegetable for curry, gravy and side dish preparations, both leaves and fresh pods are used in culinary purposes. Leaves are richest sources of iron containing (120 ppm) of digestible and available iron and one of the richest sources of iron containing plant products. Apart from iron, leaves and pods also contain zinc, calcium, phosphorous, soluble proteins, anti-oxidants, vitamin C and D to appreciable amounts. Trees can be grown in kitchen backyard as component of nutritional garden and supply of the greens and pods can be met throughout the year.

BREEDING OBJECTIVES OF MORINGA

1. Development of short statured annual plant types with sturdy stems with higher number of pods
2. Development of Drought and Saline tolerant annual types suitable for saline and dryland areas
3. Development of higher iron and zinc accumulating lines for bio fortification purpose
4. Genetic induction of off season flower induction and pod formation

DIFFICULTIES ASSOCIATED WITH BREEDING OF MORINGA

Moringa is highly cross pollinated crop due to protoandry nature. It is mediated by wind, bees and other insects. Due to its heterozygosity, genetic populations are always heterogeneous and not uniform. It is a tedious, laborious and time consuming process to self the selected populations to develop homozygous lines to be used in hybridization for breeding methods *viz.*, population improvement, and hybrid derivative. Though inbreeding depression is very low in moringa, obtaining true homozygosity is still a long way to achieve using selfing methods. Due to heterozygosity nature and propagation through open pollinated seeds, the population is always heterogenous and performance of populations cannot be predicted through progeny testing. So, larger scale planting of genetically uniform populations propagated through seeds are not possible. Seeds are always not true to type. Annual Moringa, PKM-1 is an open pollinated population improved from the local type ***Eppodhum vendran*** upon continuous inbreeding. PKM 1 is the most popular annual moringa variety of TNAU across southern states, released during 1989. Demands for seeds are increasing every year due to its multi purposes *viz.*, nutritive diet and fodder value.

There is only one released variety from TNAU viz., PKM-1 popular among farmers. Most of the locally grown types are open pollinated progenies grown in their natural habitats.

NECESSITY FOR BREEDING OF MORINGA CULTIVARS

1. High yielding varieties should be developed combined with improved nutritive value and tolerance to abiotic stresses like drought and salinity.
2. Dwarf statured plants with sturdy stems will be highly useful for high intensity planting and high input responses cultivations for medium period (3-5 years).

UTILIZATION OF TISSUE CULTURE TECHNIQUES FOR GENETIC IMPROVEMENT AND YIELD MAXIMIZATION

I. MICRO-PROPAGATION TECHNIQUES

Organogenesis through nodal and shoot tip cultures will generate true to type plants of selected lines with increased yield combined with nutritional qualities in a shorter period.

STEPS

1. Selection of Superior mother plants in Open Pollinated populations/ Natural Habitat

Superior mother plants should be selected from segregating populations/ open pollinated populations/ perennials in natural habitats. Selection should be based on yield, quality parameters and plant type aimed from the current breeding objectives. Thorough biochemical analysis should be carried out for metabolites, minerals and proteins and superior lines should be tagged.

2. Surface sterilisation of nodes and shoot tips

Suitable and simple protocol should be developed for elimination of surface and endophytic microbes which cause contamination *viz.*, 70% alcohol treatment followed by treatment with chlorox and finally with fungicides treatment. The tip of the shoots

and nodes should be surface sterilized by using above chemicals.

3. Inoculation into multiple shoots induction media through direct regeneration
The above sterilized explants are to be inoculated onto the Murashige and Skoog medium by adding various quantities of Benzyl Amino Purine (BAP) and exposed to night conditions for Seven days. After one week, cultures should be transferred to light conditions for induction of shoots from nodes and shoot tips. Shoots formation will be observed after 21 days of cultures. Sub culturing will be done if there is necessary for induction of shoots onto the same medium combination.

4. Multiple shoots induction
Shoots will be transferred to MS medium added with mixtures of BA and NAA for multiple shoots induction and incubated under light conditions. Multiple shoots will be observed after 21 days of culture under light.

5. Rooting induction
Multiple shoots will be transferred to ½ MS medium added with various quantities of Indole Butryic Acid (IBA) and incubated in day environments. Ancillary roots will be formed after 21 days of culture in the rooting medium.

6. Acclimatisation in green house
Rooted plantlets should be transferred to sterile pot mixtures and kept it in culture room for 48 hours and later in the evening hours will be transferred to green house for hardening. Hardening processes will take place for one week.

7. Establishment in field and analysing the yield, quality and other performances
Tissue culture plants with intact pot mixtures will be transplanted in the main field. Required cultural practices will be followed along with IPM and INM practices. Yield and quality parameters will be estimated for fresh pods and greens along with suitable controls. Yield advantages will be recorded.

8. Analysis of genetic fidelity through DNA finger printing
In commercial micro propagation techniques, appearance of soma-clonal variants should be avoided and tissue culture progenies should be true to type without any compromise. Hence, DNA samples from tissue culture plants established in main field should extracted and Finger printing analysis should be performed for confirming genetic fidelity and stability. If there is any deviation, tissue culture protocol should be revised with less numbers of subcultures to avoid appearance of soma-clonal variations.

9. Establishment of axenic plants *in vitro*
Superior plants with genetic fidelity and stability should be selected and inoculated into shooting media. Shoots will be transferred to rooting media and subsequently nodes and shoot tips are being multiplied and maintainer as axenic plants for continuous supply of aseptic explants.

Advantages of micro propagation in moringa

1. True to type plants production
2. Faster multiplication rate
3. Seasonal independence and year around production of plantlets
4. Free from diseases and pests
5. Media enrichment effects on yield and quality traits
6. Uniform growth, flowering and maturity

II. DOUBLED HAPLOID TECHNIQUE FOR PRODUCTION OF INBRED LINES

Scope of inbred line development in moringa

1. Inbred lines can be used as potential donors for biotic and abiotic stress tolerances
2. Can be directly used as parents in heterosis breeding and population improvement
3. Basic plant physiological and pathogens inoculation studies can be effectively performed with inbreds

Moringa is being a highly cross pollinated crop which has heterozygous and heterogeneous nature. Development of inbred through inbreeding takes longer periods. There is no available scientific reports on development of true inbred so far nationwide and globally. Hence, anther and microspore cultures followed by induction of haploid plantlets to develop doubled haploidy will be highly reliable and relevant technology for moringa improvement.

STEPS

1. Optimization of pollen mother cell (PMC) stages through cytological techniques viz., age,

morphology of buds should be correlated with appearance of tetrads and microspores.

2. Surface sterilization of unopened buds.
3. Inoculation into anthers in callus induction medium added with various levels of 2, 4-Dicholoro Phenoxy Acetic Acid and Benzyl Adenine.
4. Intact buds will be made a small hole using syringe needles and inoculated into suspension media.
5. Callus induction frequency will be observed from anthers and microspores cultures.
6. They will be transferred to somatic embryos induction medium followed by regeneration.
7. Regeneration percentage will be calculated for anthers and microspore cultures.
8. Haploid plantlets with roots transferred to secondary rooting medium for roots proliferation.
9. Rooted haploids plantlets will be hardened in green house and haploidy will be confirmed.
10. Spontaneous generation of doubled haploidy will be studied and the proportion will be derived.
11. If the proportion is very low, artificial induction will be done by treating the shoots with colchicine during flowering initiation.
12. Doubled haploid lines will be confirmed by seed set and cytological techniques.
13. Doubled haploid lines will be studied for performance under field conditions and evaluated with genetical studies.
14. Inbred will be grouped based on genetical analysis.
15. Characterized inbred will be utilised in heterosis and varietal development programmes.

CONCLUSION

Bio-technological approaches *viz.,* micro-propagation and Doubled haploid techniques are very much useful for genetic improvement of Moringa for genetic uniformity.