COST EFFECTIVE MICROPROPAGATION OF POLYSCIASFRUTICOSA (L.) HARM.

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ABSTRACT
Polysciasfruiticosa(L.) Harm is plant which belonging to Araliaceae family, also known as Ming Aralia. Micropropagation is a technique to develop whole plant from the part of the plant in In-vitro or controlled environmental conditions. The research work focused on the micropropagation of Polysciasfruiticosa(L.) Harm. from the shoot apex of the plant. Here only the cytokinin was used for the production of whole plant and M. S. Media. Generally, in Micropropagation auxin is used for the rooting phase but in this research work only kinetin which was used for the better growth for rooting also. Mostly the plant producing rare flowers and propagated through cuttings. Here M. S. Media with one Kinetin hormone standardized for the successful production of the plant lets of Polysciasfruiticosa (L.) Harm. with this technique within 60 days we can produce no. of plantlets of Polysciasfruiticosa (L.) Harm.

Keywords: Micropropagation, Polysciasfruiticosa (L.) Harm., M. S. Media, Kinetin

INTRODUCTION
Polysciasfruiticosa(L.) Harm is plant which belonging to Araliaceae family, also known as Ming Aralia. It is dicot shrub native to India. This is shade loving and planted for its foliage purposes. It has compound leaves with seven or more than seven leaflets. Generally, the leaves are deeply lobed and opposite arrangement is observed. The growth of the plant is seen highest from 19-29ºC temperature. Its sensitive plant for any type of stress specially it cannot survive at high temperature. It bears rare flowers and mostly used as an ornamental foliage plant. It is not directly edible by any animals or humans. The leaves have so many important phytochemical constituents and that can be utilized for drug designing.

MATERIALS AND METHODS
The shoot apexes (tips) of Polysciasfruiticosa (L.) Harm were selected for the propagation of plants through In-vitro and In-vivo approaches. The Experimental work was completed at Plant Biotechnology Laboratory and Botanical Garden of Gujarat University.

In-vitro production of Plantlets:

Sources of Explant:

Shoot tips were collected with sterilized scalpel from Botanical Garden of Gujarat University. So, shoot tips were used as an explant for the production of plantlets. All the shoot tips were sterilized with the help of 0.1%HgCl2 solution and 70% methanol and rewashed with Grade-1 Distil water.

Aseptic Conditions for Production:

Culture room and the laboratory or transfer room were sterilized through Fumigation technique (Potassium iodide and Formaldehyde were used for it with 2:4 ratio). All the glassware and miscellaneous agents were washed with soap solution and rapped with papers and then sterilized through Autoclave (121ºC for 20 min). Laminar Air flow hood, weighing scale and all the other small equipment like micropipette were sterilized with 0.1% mercuric
chloride solution and 70% methanol.

Preparation of M. S. Media for the production of plantlets:

Here for the practical work most widely used media Morashige and Skoog’s media (1962) was used. For the preparation first all the Major, Minor, Iron and Vitamin stalk solutions were prepared as per the Table-1. Only Cytokinin was used as PGRS (plant Growth Regulator). Here all the chemicals used for the preparation of stalk solution were Hi Media and SRL company.

Different stalk solutions were prepared in the amount of 500ml (Major, Minor and Iron) and 100ml (Vitamin) and then for the preparation of 1 litter M. S. Media 50ml from Major, 50ml from Minor, 50ml from Iron and 10ml from Vitamin stalk were taken and sequentially dissolved and other chemicals which were separately weighed like Myo Inositol, Agar-Agar, Glycine and Sucrose were added for the preparation of media. (Here Grade-1 Purified water was used for the preparation of media with the help of Genie Direct Pure (Rephile) Instrument was used for the preparation of Purified water). After the preparation of media, it was sterilized with the help of autoclave at 121ºC temperature for 20 minutes. After Autoclave sterilization the kinetin 0.5mg was added in the media and then under the Laminar Air Flow Hood in all the sterilized culture flasks and Glass jars media was poured about 50ml in each vessel. All the vessels with media were transferred in Culture room where 25±1ºC temperature and sterilized conditions were maintained. After 24 hrs media was ready for the Inoculation process.

Inoculation of Explant:

All the sterilized seeds were inoculated separately in the jars or culture flasks under the sterilized conditions of Laminar Air flow hood. Different small equipments were used like forceps and scalpels for the inoculation process. After the inoculation of the seeds in the media all the jars and flasks were again transferred carefully at Culture room where 25±1ºC temperature and 16hrs light and 8hrs darkness was maintained. Incubation time was of 40 days.

| Stock   | Constituents                  | Quantity | Stock medium |
|---------|-------------------------------|----------|--------------|
|         |                               | 1 litter(gm) | 10 litter(gm) |
| A.      | Major Stock (gm)             |          |              |
|         | Ammonium Nitrate(NH4NO3)     | 1.65     | 16.5         |
|         | Potassium Nitrate (KNO3)     | 1.9      | 19           |
|         | Calcium Chloride(CaCl2.2H2O) | 0.44     | 4.4          |
|         | Magnesium Sulphate(MgSO4.7H2O)| 0.37     | 3.7          |
|         | Monobasic Potassium(KH2PO4)  | 0.17     | 1.7          |
| B.      | Minor Stock (mg)             | (mg)     | (mg)         |
|         | Potassium Iodide (KI)        | 0.83     | 8.3          |
|         | Boric Acid (H3BO3)           | 6.2      | 62           |
Manganese Sulphate (MnSO₄.4H₂O) 22.3 223
Cobalt Chloride (CoCl₂.6H₂O) 0.025 0.25 500 ml
Zinc Sulphate (ZnSO₄.7H₂O) 8.6 86
Sodium Molybdate (Na₂MoO₄.2H₂O) 0.25 2.5
Copper Sulphate (CuSO₄.5H₂O) 0.025 0.25

C. Iron Stock (mg) (mg)
Sodium EDTA (Na₂EDTA.2H₂O) 37.3 373 500 ml
Ferric Sulphate 27.8 278

(FeSO₄.7H₂O)

D. Vitamin Stock (mg) (mg)
Nicotinic Acid 0.5 5
Pyridoxine HCl 0.5 5 100 ml
Thymine HCl 0.1 1

E. Myo Inositol 100 mg
F. Glyine 2 mg
G. Agar-Agar 8 mg
H. Sucrose 30 gm

Table: 1 Showing the Composition and Components of M. S. Media (1962) preparation

RESULTS AND DISCUSSION

After 20 days of Inoculation of Explant the shoots development started and the plantlets which were transplanted in the different vessel with same media. After 20 days the plantlets were produced in the laboratory and the hardening process was completed. In the hardening process first the vessels with plantlets kept at 35-38º C at room temperature. After 24hrs all the plantlets were transplanted in the media and transferred in the Green house where 40-45% humidity was maintained. The media was used for the hardening which was composed of 60%cocopeat, 20% soil and 20% Organic Manure. After 10 days the media was changed (80% garden soil, 15% manure and 5% cocopeat) and all the plants were transferred in the Net house for 10 days. The mature plants were kept in the black nursery polythene bags. So, after 60days mature and healthy plants were obtained. Many scientists worked on the micropropagation. Asmaa Elsaayd Abdelhafez in 2012 worked on the tissue culture of Polysciasfruticosa (L.) Harm. He used different hormones here in this research work we have focused on one kinetin hormone.Vu Hoai Sam described the method of micropropagation of Polysciasfruticosa (L.) Harm and he used shoot tips and used different PGRs like BAP, IBA for the production of plantlets with MS.
Media. In this research work within short time duration many plantlets of *Polysciasfruticosa* (L.) were produced which were healthy and the technique is cost effective because here excess man power, soil, fertilizer used were avoided and the plantlets hardened within 20 days.

**CONCLUSION**

Micropropagation is the best technique for the production of healthy plantlets of *Polysciasfruticosa* (L.) Harm. The plant which is quite difficult to propagate through seeds canbe easily propagated through shoot tips only. The method also standardized with only kinetin application healthy plantlets with properly roots and shoots were obtained. Within 60 days we can obtained healthy plantlets of Polysciasfruticosa (L.) Harmwhich in any season. For In-vivo production the rooting phase takes time of 80days up to maturation phase so this method is fast and cost effective. In future Micropropagation of other plants can be done through single PGRs approach.

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Figure-A and B showing *In-vitro* production of aralia
Figure-C showing hardening of the plantlets. Figure-D showing ready plants for transplantation.

Figure: E and F showing hardened plants which were transplanted in the pots.