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In-vitro rooting and Acclimatization in certain Mulberry varieties of M₅, V1, S36 and Anantha

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ABSTRACT:

Abnormal changes (protuberant, opened stomata), caused by the high humid atmosphere within culture vessel can be minimized by transferring the plantlets into the culture vessels with ventilation and acclimatized *in vitro* for 15 days and transferred to pots and maintained in outdoor conditions for better survival rate. A different percentage of root inductions were observed with different concentrations of hormones such as NAA, IAA and IBA. Root induction was observed in explants cultured on half-strength MS medium fortified with 2% sucrose and any of the three auxins.

KEYWORDS: In-vitro rooting, Mulberry, Vitamins, auxins, NAA, IAA and IBA

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INTRODUCTION

Adventitious root for measure is a key step in micro propagation which are induced by an auxin. Direct shoot and root organogenesis are special ways of morphogenesis in plants¹. The most commonly used auxin for root formation is IBA. Rooting remains one of the critical steps of initial multiplication of fruit tree species especially the replication of high rooting percentage and optimal root quality. Consistent high frequency rooting of mulberry has been more difficult to achieve that shoot multiplication. An efficient rooting treatment yields a high percentage of rooted shoots and high quality root system in tissue culture raised plants which is necessary for acclimatization also. The influence of endogenous growth factors, their transport and decomposition, in experiments using intact plants is unknown, and it is likely that these are important in any response to an applied hormone, which could alter the relative amounts of the growth factors within the tissue² The greater efficiency of IBA versus IAA in root formation is probably due to its progressive conversion (β -oxidation)³ The present study showed the effects of NAA and IBA on root induction of Mulberry explants. The presence of activated charcoal in the rooting medium, improved the rooting quality but reducing the rooting percentage in apple root stalks. Reduction of MS salts sucrose in root elongation medium showed decreased rooting⁴. Rooted plantlets were hardened in plastic cups containing sterile sand and soil mix (1:1; w/w) for 21 days and subsequently transferred to clay pots (l x b x h: 12 x 12 x 12 cm) in shade with a survival of 65 and 52% for diploid and triploid cytotypes, respectively. The time required for field establishment of micro-propagated triploid was about 60 days⁵. The aim of the acclimatization study was to obtain information on their suitability to natural Climatic conditions, Not only does acclimatization prepare them with a margin of safety but some microorganisms, insects, and plants tolerate experimental exposure at temperatures far colder or warmer than ever occur in nature. It seems strange that adaptability enables these organisms to be prepared to encounter conditions beyond their natural experience. Another surprising characteristic of acclimatization is its anticipatory nature it can develop before the change occurs.

MATERIALS AND METHODS

Actively growing plant tissues requires a continuous supply of inorganic chemicals, which constitute the macronutrients and micronutrients. Potassium nitrate was used in combination with ammonium nitrate in MS medium whereas potassium nitrate was used as a single nitrogen source in B₅. Phosphate was supplied as sodium dihydrogen phosphate in B₅ and potassium dihydrogen phosphate in

MS. Calcium chloride was added for the calcium requirement in both media. Concentrated stock solutions of micronutrients listed in the tables were prepared. Iron stock was prepared separately to avoid problem with iron solubility, and it was prepared in a chelated form as the sodium salt of ferric ethylene di-amine tetra acetate. Different concentrations of 2, 4-D, IAA, NAA and Kinetin were used to study the callus initiating abilities and regenerating abilities of explants of mulberry varieties. These growth regulators were dissolved in suitable solvent before preparing stock solution. Thiamine- HCl, Nicotinic acid, pyridoxine HCl were added in both the media. The amount of thiamine was relatively more in B₅ medium than in MS medium. Glycine was added to MS medium.

0.1% activated charcoal was supplemented to the nutrient media as it adsorbs secondary products secreted by cultured tissues. 20,000 mg/l sucrose was added for both MS and B₅ media. Sterilized double distilled water was employed in all tissue culture media, including the water used during the culture procedure. For semi solid media, add agar at a final concentration of 6-10 g/litre prior to autoclaving. It is important to use a good quality, bacteriological grade agar for plant cell culture work. The formulation for MS and B₅ media were given in table 1 and 2. Approximately 50 ml of double distilled water was taken in a 100 ml beaker. Salts were weighed according to the first column of the table I. weighed salts were dissolved separately. Solution was transferred to the 100 ml of volumetric flask and made up to the mark. This micronutrient stock was stored under refrigeration. To 50 ml of double distilled water, weighed Na₂EDTA (according to column 1 in Table I) was added and boiled to dissolve. Weighed FeSO₄.7H₂O was added to the boiling solution. After 5 minutes the solution was transferred to the volumetric flask of 100 ml capacity. DDH₂O was added to make the solution to final volume. Iron stock was stored at room temperature. Vitamins are weighed according to the column 1 of table I and dissolved in 50 ml of DDH₂O. This vitamin mixture was transferred to the 100 ml volumetric flask and double distilled water was added to the final volume. Vitamin stock was stored under refrigeration. 10 mg of 2, 4-D was dissolved by adding 2-3 drops of ethanol. Few ml of DDH₂O was added and then transferred to the volumetric flask. This was made up to 100 ml by adding double distilled water. 10 mg kinetin was dissolved in few drops of 1 N HCl. About 10 ml of DDH₂O was added and transferred to the volumetric flask (100 ml). Kinetin was made up to the final volume by adding double distilled water. Indole auxins can be dissolved in 1 N Na OH IAA was dissolved in few drops of 1 N Na OH and this was transferred to a volumetric flask of 100 ml after adding 10 ml of distilled water. DDH₂O was added in order to make up the solution to the final volume.

NAA can also be dissolved in 1 N Na OH. The same procedure given to the IAA stock was followed to prepare NAA stock. The Hormone stock solutions were stored in refrigerator. All the stock solutions were labeled including the concentration and date of preparation. All the stock solutions were used within 30 days and discarded after 30 days.

RESULTS AND DISCUSSION

Effect of different concentrations of auxins for *invitro* rooting ability after 20 days of aseptic shoots of mulberry variety M5 were presented in table figure (1). A different percentage of root inductions were observed with different concentrations of hormones such as NAA, IAA and IBA. Root induction was observed in explants cultured on half-strength MS medium fortified with 2% sucrose and any of the three auxins. Among all the three auxins IBA showed best rooting response compared to IAA or NAA.

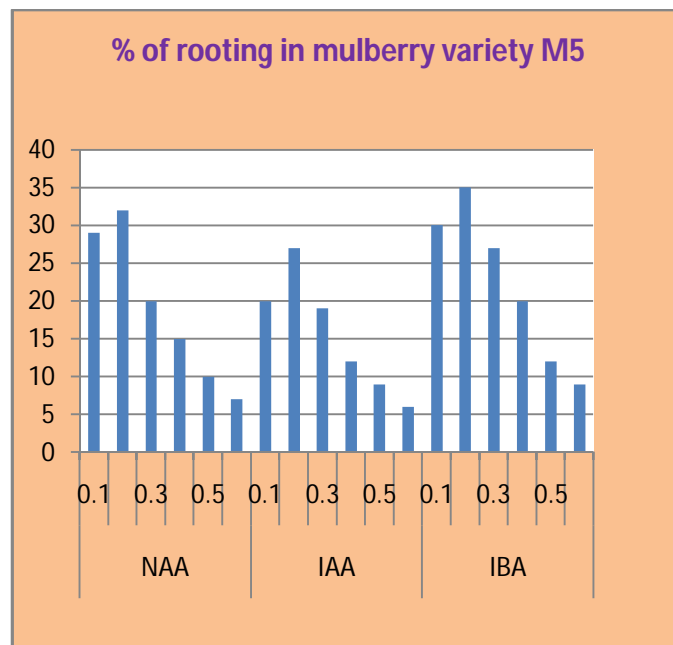


Figure 1: Effect of different concentrations of auxins invitro rooting ability after 20 days of aseptic shoots of mulberry variety M5

A similar trend was also found in the mulberry variety V₁ (Fig 2), S₃₆ (Fig 3), and Anantha (Fig. 4).

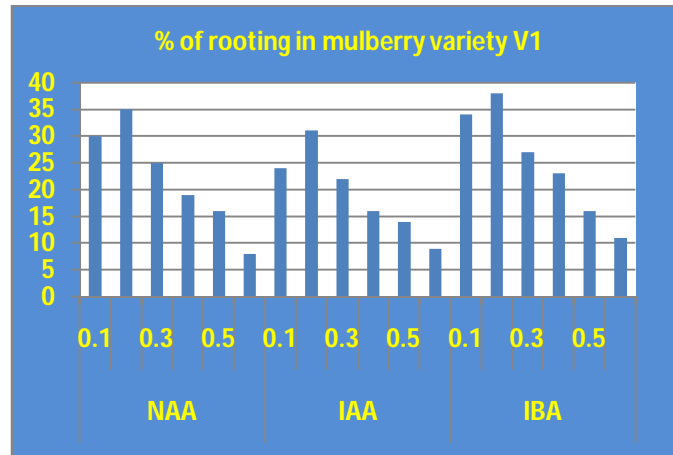


Figure 2: Effect of different concentrations of auxins invitro rooting ability after 20 days of aseptic shoots of mulberry variety VI

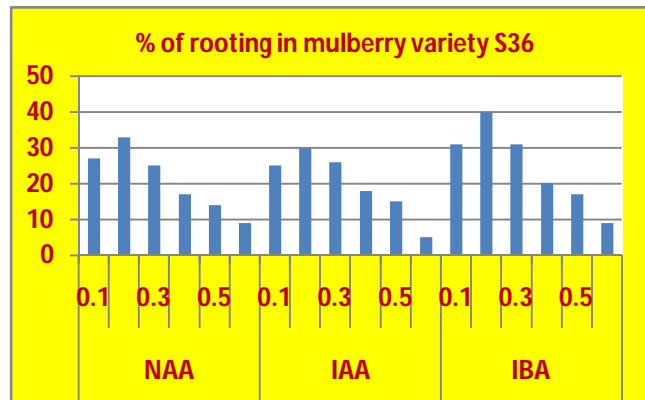


Figure 3: Effects of different concentrations of Auxins *In vitro* rooting ability after 20 days of aseptic shoots of mulberry variety S36

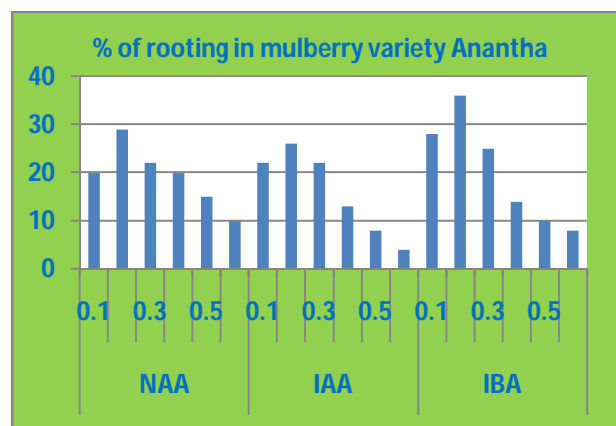


Figure 4: Effect of different concentrations of auxins invitro rooting ability after 20 days of aseptic shoots of mulberry variety Anantha

The regenerate shoots that contain at least 2-4 branches were subjected for rooting on MS full strength and half-strength media with 2% and 3% sucrose concentration supplemented along with different concentrations of auxins (IBA or IAA or NAA) (0.1 – 1.0 mg⁻¹) and MS basal medium was served as control. Results were recorded after 4 weeks. Root induction was observed in explants cultured on half-strength MS medium fortified with 2% sucrose and any of the three auxins. No roots were induced in explants inoculated onto basal medium. Among all the three auxins IBA showed best rooting response compared to IAA or NAA. The full strength MS medium supplemented with 3% sucrose and auxins resulted in callusing at the base of the shoots, whereas 2% sucrose concentration did not show any callusing. Similar observations were also reported in *Feronia livinia*⁶, *Hedoema multiflorum*⁷.

IBA at different concentrations showed different response on rooting medium. The best response was found at 0.2 mg⁻¹ with 3% sucrose in 1/2 strength MS medium. Nearly 6.1 roots each with about 4.2cm root length were induced. Similar reports were also found in *Morus alba*.L⁸, in *Rotula aquatica* Lour⁹ in Strawberry¹⁰ *Cunilagalioides*¹¹ and in *Dalbergia latifolia* Roxb¹². Whereas at higher or lower concentrations of IBA induced delayed rooting. The results are coincided with the reports in Mungbean¹³.

IAA was observed to be lesser responsive in root induction compared to IBA, these results agree with the findings of earlier report¹⁴. Similarly NAA also induced less frequent rooting in micro shoots than either IBA or IAA. Similar findings were also reported in *Ruta graveolens*¹⁵ and other plant species such as *Cleistanthus collinus*¹⁶, *Murraya koenigii*¹⁷ and in *Ficus carica*¹⁸ stating the superiority of IBA over either NAA or IAA in root induction in micro shoots in other plant species. Though there was a similar trend in the entire mulberry varieties in the above said parameters, there were significant percent variations among the mulberry varieties to different combinations of auxins and cytokinines. The regenerated complete plantlets were acclimatized to the natural environmental conditions as described in materials and methods. According to the method¹⁹, *in vitro* regenerated plantlets were transferred to earthen pots containing autoclaved soil + soilrite (1:1) and maintained in the culture room for about one month before transplanting them to the field. The survival rate of the transplanted plantlets was 80% for M5, 78% for V1, 75% S36 and 70 % for Anantha variety.

CONCLUSION: MS medium fortified with various auxins such as NAA, IAA and IBA in all four mulberry varieties. IBA was effective for *in vitro* rooting followed by NAA and IAA. *In vitro* plantlets with well developed roots were transferred to pots containing vermiculate and plantlets were subsequently acclimatized.

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