Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

In-vitro rooting and Acclimatization in certain Mulberry verities of M_5, V1, S36 and Anantha

Varaprasad P. [1*] and and A. Vijaya Bhaskara rao[2]

[1]Department of Sericulture, Sri Krishnadeveraya University, Anantapur-515003, A.P. India
[2]Department of Ecology and Environmental Sciences, Pondicherry Central University, Pondicherry

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

*** Corresponding author**

P. Varaprasad

[1]Department of Sericulture, Sri Krishnadeveraya University,

Anantapur-515003, A.P. India

E mail: pustela9@gmail.com

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 plants[1]. 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 tissue[2] 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 rooting[4]. 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 days[5].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 B_5. Phosphate was supplied as sodium dihydrogen phosphate in B_5 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- HCI, Nicotinic acid, pyridoxine HCI were added in both the media. The amount of thiamine was relatively more in B_5 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 B_5 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 B_5 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 Na_2EDTA (according to column 1 in Table I) was added and boiled to dissolve. Weighed $FeSO_4.7H_2O$ was added to the boiling solution. After 5 minutes the solution was transferred to the volumetric flask of 100 ml capacity. DDH_2O 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 DDH_2O. 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 DDH_2O 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 HCI. About 10 ml of DDH_2O 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. DDH_2O 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.

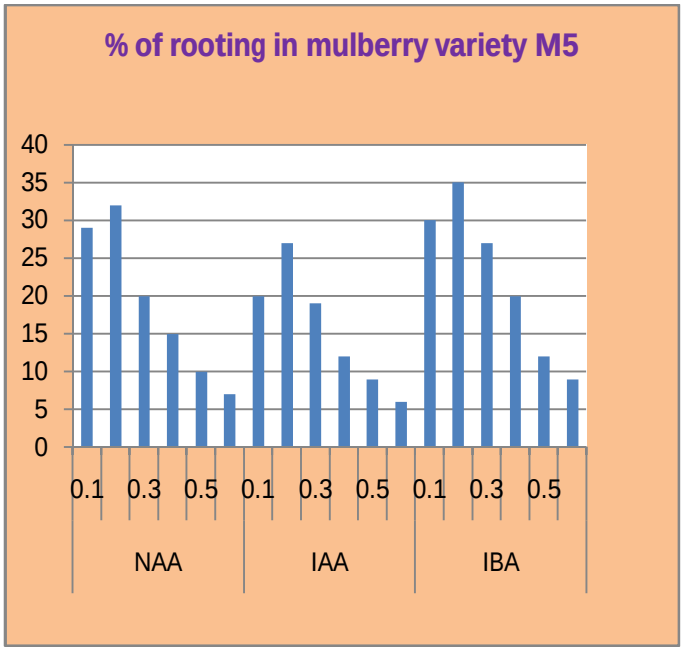

Figure 1: Effect of different concentrations of auxins invitro rooting ability after 20 days of aseptic shoots of mulberry variety M5

A similar trend was also found in the mulberry variety V_1 (Fig 2), S_{36} (Fig 3), and Anantha (Fig. 4).

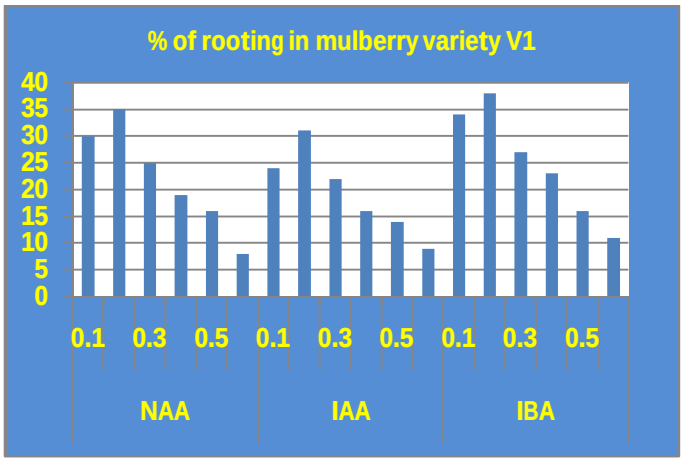

Figure 2: Effect of different concentrations of auxins invitro rooting ability after 20 days of aseptic shoots of mulberry variety VI

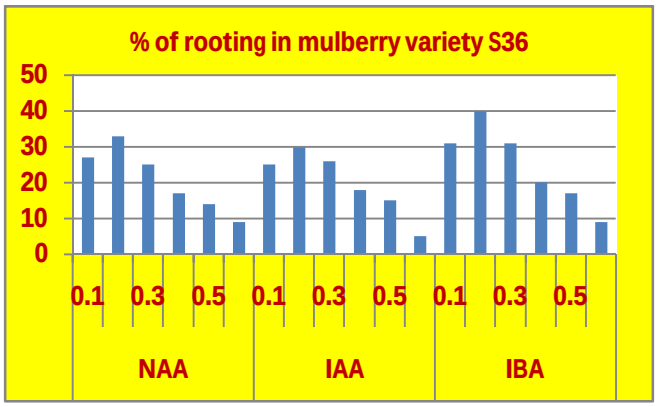

Figure 3: Effects of different concentrations of Auxins *In vitro* rooting ability after 20 days of aseptic shoots of mulberry variety S36

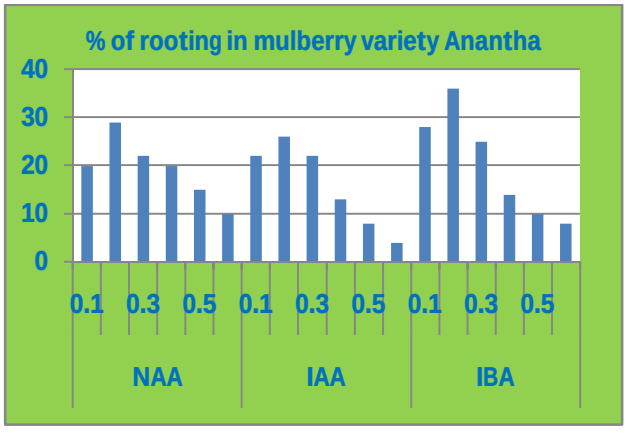

Figure 4: Effect of different concentrations of auxins invitro rooting ability after 20 days of aseptic shoots of mulberry variety Anantha

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^{-l}) 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 liwinia*[6], *Hedoema multiflorum*[7].

IBA at different concentrations showed different response on rooting medium. The best response was found at 0.2 mg^{-l} 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.L[8], in Rotula aquatica Lour[9] in Strawberry[10] Cunilagalioides[11] and in Dalbergia latifolia Roxb[12]. Whereas at higher or lower concentrations of IBA induced delayed rooting. The results are coincided with the reports in Mungbean[13].

IAA was observed to be lesser responsive in root induction compared to IBA, these results agree with the findings of earlier report[14]. 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 method[19], i*n 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.

ACKNOWLEDGEMENTS

The first author, P.Varaprasad, gratefully acknowledged the University Grants commission,, New Delhi for the financial support through RGNF..

REFERENCES

1. Tang W, Newton RJ. Plant regeneration from callus cultures derived from mature zygotic embryos in white pine (*Pinus strobus* L.) Plant Cell Rep. 2005; 24:1–9.
2. Lindsay H, Northcote DH The influence of gibberellic acid and abscisic acid on cell and tissue differentiation of bean callus. J. cell. Sci. 1976; 20: 47-55.
3. Gaspar T, Hausman JF, Faivre-Rampant O, Kevers C, Dommes J Auxins in the Biology of Roots. In: Waisel Y., Eshel A.,Kafkafi L., (eds). Plant roots: The hidden half. Third edition. NY,Dekker, 2002; 21: 383-404.
4. Sharma S.K Bryan GJ. Millam Auxin pulse treatment holdthe potential to enhance efficiency and practicability of somatic embryogenesis in potato.Plant Cell Rep. 2007; 26: 945-950
5. Chattopadhyay, S. Gandhi Doss, S. Halder, A. K. Ali and A. K. Bajpai, Comparative micropropagation efficiency of diploid and triploid mulberry (*Morus alba* cv. S_1) from axillary bud explants. Afr. J. Biotech. 2011; 10 (79): 18153-18159.
6. Purohit SD, Kiran Tak *In vitro* propagation of an adult tree Feronia limonia L. through axillary branching. Ind. J. Exp. Biol. 1992; **30**:377-379.
7. Adolfina R Koroch, Hector R Juiani Jr,Victorio S Trippi.Micropropagation and acclimatization of Hedoma multiflorum. Plant. Cell. Tiss. Org. Cult.. 1997; 48:213-217.
8. Ignacimuthu S, Frankilin G, Melchias G Multiple shoot formation and in vitro fruting from cotyledonary nodes of Vigna mungo L. Hepper. Curr. Sci. 1997; **73**:733-735.
9. Martin K P Rapid in vitro multiplication and ex vitro rooting of Rotula aquatic lour a rare rhoeophytic woody medicinal plant. Plant cell Rep.2003;. **21**:415-420.

10. Indra D, Bhatt and Uppeandra Dhar. Micropropagation of Indian wild strawberry. Plant Cell, Tissue and Organ Culture. 2000; 60 (2), 83-88.
11. Pradhan, C., S. Kar, S. Pattnaik and P.K. Chand,. Propagation of *Dalbergia sissoo* Roxb. through *in vitro* shoot proliferation from cotyledonary nodes. Plant Cell Rep., 1998; 18: 122-126.
12. Fracaro F and Echeverrigaray S Micropropagation Cunila galioides, a popular medicinal plant of south Brazil. Plant Cell, Tiss. Org. Cult. 2001; 64: 1-4.
13. Suchita Tivarekar & Susan Eapen High frequency plant regeneration from immature cotyledons of mung bean. Plant cell Tiss. Org. cult.2001; **66**:227-230.
14. Thomas K, Agretius, KP, Marti, Molly,Hariharan In vitro clonal multiplication of Alpinia calcarasa Rose. Phytomorph. 1996; **42(2):**133-138.
15. Mohd Faisal, Naseem Ahmad, Mohammad Anis In vitro regeneration and mass propagation of Ruta graveolens L. multipurpose shrub. Hort. Sci. 2005; 40 (5): 1478-1480.
16. Afaque Quaraishi,Vijaya Koche, Mishra SKIn vitro micropropagation from nodal segments of Cleistanthus collinus. Plant. Cell. Tiss. Org. Cult.1996; 45:87-91.
17. Bhuyan AK, Patnaaik S,Chand PK Micropropagation of curry leaf tree (Murraya koenigii L.(Spreng) by axillary proliferation using intact seedlings. Plant Cell Rep. 1997; 16:779-782.
18. Kumar V, Radha A, Kumar Chitta SIn vitro plant regeneration of fig (Ficus carica L.cv.Gular) using apical buds from mature trees. Plant Cell Rep.1998; **17**:717-720.
19. Kapur A, Bhatnagar S, Khurana P Efficient regeneration from mature leaf explants of Indian mulberry via organogenesis. Sericologia. 2001; 41: 207-214.