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IN VITRO PROPAGATION OF NEEM FROM SEEDLING AND MATURE TREE

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Azadirachta indica A. Juss. commonly known as Neem is medicinally and pesticidally important tree. Its seed viability lost for few months which falls off rapidly after two weeks. Thus conventional multiplication through seeds is limited. Present paper described *in vitro* plantlet formation from nodal explant of mature tree and seedling. Nodal explant from mature tree when cultured on Murashige and Skoogs medium (with half concentration of NH_4NO_3 and KNO_3) supplemented with 0.5–5.0 mg/l benzyladenine produced 3-4 shoots in 4 weeks. The number of shoots increased along with increase in the shoot length when second subculture was done on MS + 2.5 mg/l BA. Similar results were obtained with nodes excised from young seedling. However, addition of 50 mg/l Adenine sulphate in the medium was found to be better for shoot growth. These shoots when transferred to MS + 0.5 mg/l BA further elongated. In vitro developed shoots were rooted on MS medium supplemented with 1.0 – 3.0 mg/l NAA.

Key words : Neem tree, *Azadirachta indica*, *in-vitro* propagation

Clonal selection and propagation of tree species using tissue and organ culture technique has a considerable potential in the improvement of economically important trees that have been under cultivation for many generations. The reviews of Bonga (1977) and Durzan and Campbell (1974) amply discussed the significance of tree tissue culture work. Although tissue culture of herbaceous angiosperms has been extensively studied, trees have not received as much attention (Zimmerman, 1985). A majority of the woody plants are difficult to propagate vegetatively by traditional methods like cutting, grafting, layering etc. Such methods, although successful in some instances, still have limitations and cannot be applied for all economically valuable species. Regeneration of plantlets is an integral part of any tree modification/improvement programme. it is also important in the cloning of superior or elite trees, as the potential benefits of using clonal planting stock in afforestation or reforestation have been recognized for a long time (Thorpe and Biondi, 1984).

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Azadirachta indica A. Juss. popularly known as **Neem** is one of the most valuable tree species. The plant is a source of animal feed, fuel, shade, medicine and insectivide. Neem is potentially one of the most suitable and valuable tree species for arid zones. It can grow on a wide range of soil up into pH 10 (Chaturvedi *et al.* 1985). It also occurs in Tropical dry deciduous and thorn forests and in tropical dry evergreen forests. It grows in arid and semi-arid tropical and subtropical zones in the plains and lower foot-hills.

Neem seeds do not retain viability for more than a few months and viability falls off rapidly after two weeks (Dent, 1948). Thus it faces problems in its rapid multiplication. A very little efforts have been made for its in vitro micropropagation and improvement. Rangaswamy and Promila (1972) reported regeneration of shoots from embryos and decapitated seedlings. Naina *et al.* (1989) obtained transgenic plants where as Gautam *et al.* 1993 reported development of shoot and roots from anther derived callus of *Azadirachta indica*. In the present report, young axillary buds as well as axillary buds from mature selected elite trees were successfully used for micropropagation of neem.

MATERIALS AND METHODS

Fresh young and juvenile shoots were collected in spring season from selected well grown mature trees. Nodal segments with single axillary buds were used as explants for the induction of multiple shoots. The explants were sterilized with 0.1 per cent mercuric chloride solution for 10-15 minutes and washed three to four times thoroughly with sterile distilled water and cultured on the sterile medium.

Surfaced sterilizing of nodal segments obtained from field-grown trees was difficult and have certain limitation due to phenolics exudate. Therefore, seedlings were raised aseptically to get explants for direct inoculation. For this, seeds were collected from plus trees as selected by state forest departments. After removal of outer fleshy covering the seeds were thoroughly washed with running tap water first, and then with 1.0 per cent cetavelon solution for 5 minutes. After rinsing with tap water, the seeds were surface-sterilized with 0.1 per cent mercuric chloride solution for 5 minutes followed by 3-4 times washing with sterile distilled water. The seeds were then cultured on agarified basal MS medium and kept for germination. After 5-8 weeks of germination, the nodal segments were excised from these aseptically raised seedlings and cultured on medium containing different growth regulators singly or in combinations. Three different basal medium tested were Murashige and Skoog (MS 1962) (half concentration of NH_4NO_3 & KNO_3), Gamborg (B_5 1968), and Lloyd and McCown (WPM 1981). Cultures were maintained at 25 $\pm$ 1 C with 16 h illumination photon flux density of 30 μ mol $m^{-2}s^{-1}$, from white fluorescent tubes.

RESULTS AND DISCUSSION

Germination of seeds

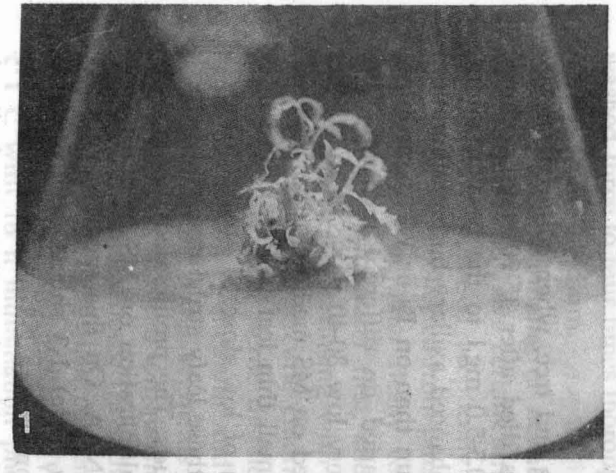
Seed germination and seedling growth was better in MS medium as compared to WPM and B_5 medium. Among the freshly collected seeds, 70-80 per cent of them germinated within 8-12 days. However, the seeds stored for 3 months showed only 40-50 per cent germination and the germination rate declined further with increase in seed storage time. The in vitro seedlings displayed considerable variation in growth rate. For in vitro propagation explants (shoots) were derived from seedling with 5-10 cm or longer shoots. On a hormone free semi-solid or liquid medium, generally a single (very rarely two) unbranched shoot developed from a seed.

Mature axillary bud culture

In starting aseptic culture of the axillary buds from mature aged tree, the main hazard faced was that of exudation of phenolic substances into the medium. A biweekly change of medium and incorporation of 100mg/l activated charcoal, 50mg/l citric acid and ascorbic acid at the initial stage into the medium under different sets of experiments overcame this problem. Also the use of juvenile buds of fresh sprouts of monsoon season collected from trees showed very less phenolic exudation.

Multiplication of shoots

The excised axillary buds from seedling and tree, when cultured on MS basal medium, devoid of growth regulators died after 15-20 days without showing any growth. Incorporation of BA 0.5-5.0 mg.l to the basal medium resulted in multiple shoot formation. The enhanced axillary bud proliferation and shoot growth were better on MS medium than on B_5 or WPM medium. Proliferation of shoot from axillary bud varied on different medium. The frequency of axillary bud break remained very low 30-40 per cent in all the experiments tried. Axillary bud when cultured on MS medium supplemented with 5.0 mg/l BA, 3-4 shoots proliferated. Small thin leaf like structures also proliferated via axillary branching. Addition of low dose of NAA (0.1mg/l) in the medium upto some extent prevented these leafy structures, and helped to developed distinct shoots from axillary buds. The proliferated shoots were excised and were subcultured for shoot multiplication on MS medium supplemented with 5.0 mg/l BA + 0.1 mg/l NAA. On this medium cultured shoots grew and from every node (axillary node) 1-3 shoots originated. A small callus also developed at the base, from where 2-3 shoots formed. At increased concentration of NAA (1.0 mg/l) the shoot formation rate declined with formation of callus at the cut end. The in vitro shoots were subsequently



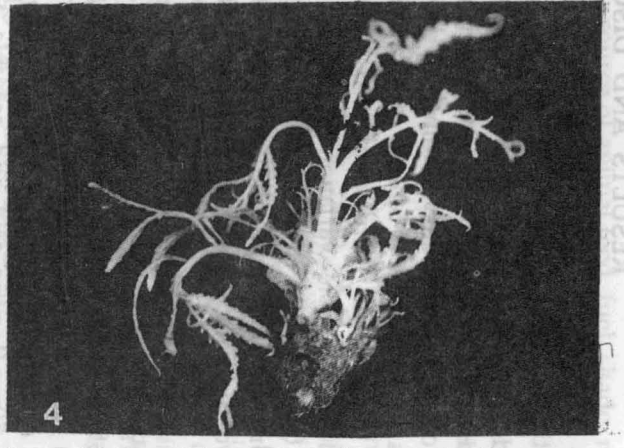


Fig. 1. Excised axillary bud of seedling cultured on MS + 5.0 mg/l BA + 50 mg/l Adenine sulphate, note proliferation of shoots
Fig. 2. Shoot multiplication on MS + 5.0 mg/l BA + 0.1 mg/l NAA supplemented medium
Fig. 3. Initial shoot proliferation from axillary bud of nature tree
Fig. 4. Stage, when proliferated shoots were excised from the axillary buds for shoot multiplications.

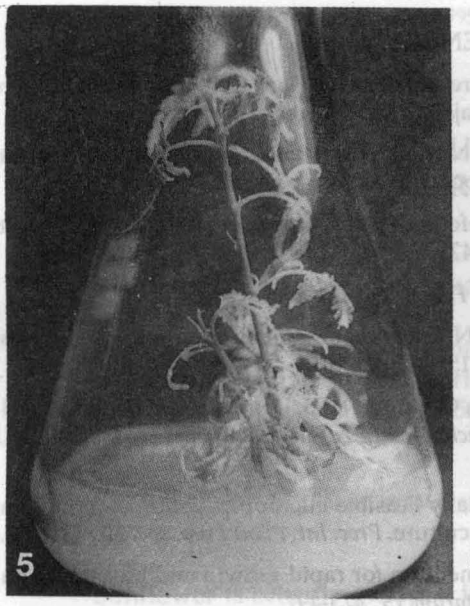


Fig. 5. *In vitro* shoot elongation on MS + 2.5 mg/l BA

Fig. 6. *In vitro* root formation on MS + 1.0 mg/l NAA supplemented media (Plantlet formation)

excised/isolated and sub-cultured on MS + 2.5 mg/l BA for further growth and shoot multiplication. The rate of shoot multiplication was quite low in case of axillary buds of seedlings. An addition of 50 mg/l Adenine sulphate was found to be better for shoot growth and multiplication in this case.

Rooting of shoots

The in vitro raised shoots failed to root on a hormone free basal medium, thus various auxins were attempted for rooting trials. All rooting experiments were carried out with modified MS basal medium as it gave best results in subsequent experiments. Of the various auxins treatments, best results were obtained with 1.0 - 2.0 mg/l NAA supplemented MS medium. Generally 2-3 roots emerged from the cultured shoots. In all the experiments small callusing was observed at the base of the shoots.

The present results of micropropagation of neem through tissue culture technique is encouraging as it overcome the plant regeneration problem arising due to rapid loss of the germinability of the seeds. The insecticidal plant constituents (Pradhan et al. 1962) including the terpenoids azadirachtina greatly varied in neem trees. A high valued neem tree can now be multiplied true to its type from above technique as well.

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