Rooting of *Eucalyptus citriodora* explants on non-sterile sand as a cost-effective means of mass propagation

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Nodal segments of a fifteen year old tree of *Eucalyptus citridora* when cultured on Murashige and Skoog medium (MS medium) supplemented with Benzylaminopurine (BAP) 1.0 mg-1 and 10% coconut milk (CM) initiated shoots. The latter produced multiple shoots after 5-6 subcultures on MS medium supplemented with low concentration of BAP (0.25 mg-1) and 10% CM. The induction of roots wes observed 8 to 10 days after transplanting of microshoots on non-sterile sand which reduced the costs incurred during rooting in the laboratory.

Keywords: *Eucalyptus citriodora*, Micropropagation, MS medium, Benzylaminopurine, sand rooting, afforestation.

Eucalyptus citriodora Hook. (eucalypt tree) is locally called as masala. The tree is commercially valued for soft wood. Essential oil (citrionella) from its leaves is also used in perfumery and as an insect repellent. The plant produces pole better than that of few other eucalyptus such as *E. camaldulensis*. In Nepal large scale plantation of different species of eucalyptus including *E. citriodora* has been done. As sexual breeding may not produce efficient clones due to outcrossing, the desirable clones can be produced in large scale, through tissue culture technique. Micropropagation of *E. citriodora* from seedling had been done by Laxmi Sita (1981) and from mature tree by Gupta *et al.* (1981). Their reports showed that *in vitro* rooting was very costly for commercial production. The present study therefore, attempts to reduce the production cost, through rooting of tissue cultured shoots on non-sterile sand.

Materials and methods

Small twigs of a fifteen year old *E. citriodora* tree growing at the Royal Botanical Garden, Godawary were collected. These twigs were dipped in tap water for 24 to 48 hours to leach out any phenolic compounds. Nodal segments were excised and kept in running tap water for 3-4 hours, and surface washed with sterilized distilled water. Finally the nodal segments were surface sterilized with 0.01% Mecuric chloride solution for 2-3 minutes and then washed with sterilized distilled water to remove any traces of the sterilant. The Nodal segments (4-5 mm) were cultured on MS medium (solidified with 0.7% agar and pH adjusted to 5.8 before autoclaving at 25 lb pressure for 15 min) and supplemented with growth hormones. Cultures were incubated at 25 ± 2^{0} C.

Microshoots (4-5cm long) were excised, washed in water and then allowed to root in a propagator containing only sun dried sand. Water was regularly sprayed to keep the sand moist.

Results and discussions

Small green shoots were observed on MS medium (with BAP 1.0 mg-1 and 10% CM) within four to five weeks. These shoots multiplied when subcultured on MS media with low BAP concentration (0.25mg-1) and 10% CM. After 5-6 subcultures in this

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concentration, a maximum rate of shoot proliferation was observed and this composition was used for routine shoot multiplication. After 6-8 weeks of culture, the number of shoots varied from 50-60 in a 250 ml flask (fig. 1). The high concentration of BAP (1-5 mg-1) resulted stunted growth. The growth of microshoots was not good in MS medium supplimented with 0.5-2.0 mg-1of Kinetin. Callusing and browning was not observed in the medium containing low BAP concentration.

Two months old shoots rooted in a propagator containing only sand. The root induction was observed 8-10 days after they were transferred to sand. Fifteen days after rooting, the seedlings were transferred to polybags for field establishment (fig. 2 and 3).

Gupta *et al.* (1981) reported a proliferation of *E. citriodora* in MS medium supplimented with calcium panthothenate, biotin, and BAP. The present study indicated that a higher shoot proliferation from a single nodal segment could be achieved from MS medium supplimented with BAP alone. This considerably reduces the use of extra chemicals for tissue culture raised plants. Stimulation of axillary buds directly through the micropropagation is genetically more stable than shoot regeneration from callus culture. In the latter, there is a great chance of genetic erosion, mutation, variation in ploidy level at each periodic subculture (Burley,1989). The present study had become successful to avoid such genetic erosion. Similar observation was found in *E. camaldulensis* cultured from mature tree where no phenotypic variation was noticed eight years after platation in Sagarnath forest (personal communication).

Rooting of microshoots of *E. citriodora* in non-sterile sand reduces the cost of seedling production. The commercial application of this technique could therefore be recommended.

References

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Fig. 1

Fig. 1: S hoots sprouting 6-8 weeks after culture

Fig. 2: Roots developing 8-10 days after being transferred to sand

Fing. 3: Seedling transferred on polypots 15 days after rooting

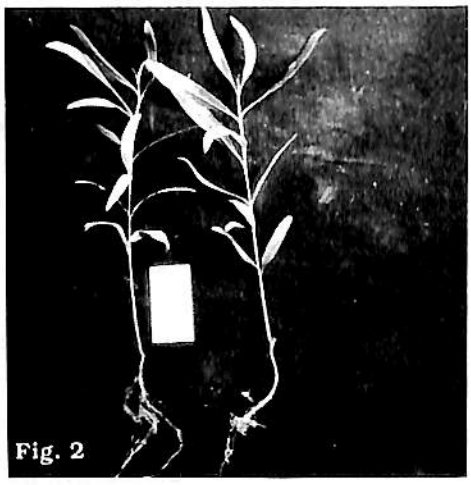
Fig. 2


Fig. 3