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PROPAGATION OF *LEUCAENA LEUCOCEPHALA THROUGH* TISSUE CULTURE

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Abstract

Clonal propagation of woody species through tissue culture technique has received much importance these days because the multiplication process is quite speedy and the ramets so produced are devoid of pathogens. Plantlets of Ipil Ipil (*Leucaena leucocephala*) were obtained through multiplication of ex-plants produced from aseptically raised germinates of the species. In the basal media (MS + 5 micro molar BAP), ex-plants started differentiating shoot buds at high frequency. These aseptically developed plantlets were shifted to earthen pots for rooting without applying any root-promoting hormone. These plantlets showed a good growth response in earthen pots and attained one-meter height within a period of two months.

Introduction

Ipil Ipil (*Leucaena leucocophala*) is a multipurpose leguminous tree, which provides nutritious fodder. Sheep and goats relish its leaves.

Investigation on *in-vitro* morphogenesis of juvenile tissues and *in-vitro* shoot multiplication has been achieved by apical as well as axillary buds obtained from seedlings. The present experiment was conducted with the objective to investigate *in-vitro* growth response for its multiplication so that the technique may effectively be used for propagation superior ortets.

Materials and Methods

Experiment was conducted at Plant Tissue Culture Laboratory, Punjab Forestry Research Institute (PFRI), Faisalabad. Seeds of *Leucaena leucocephala* were collected from plus trees growing at PFRI Arboretum. Seeds were surface sterilized with 70% ethyl alcohol for 5 minutes and with 1% bleach solution for 25 minutes. Seeds wee then washed with sterile water to remove

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possile traces of sodium hypochlorite. The surface sterilized seeds were inoculated on MS basal media supplemented with different concentrations of BAP (Benzylaminopurine). Apical and axillary segments were taken from 10 days old germinates.

MS basal media contained 3% sucrose with 0.9% bacteriological agar. MS basal media was augmented with different concentrations of BAP. pH was adjusted at 5.7 before autoclaving. For rooting, the elongated shoots were transferred to soil without application of any rooting hormone.

Culture was maintained at 25–27°C with 16 hours light and 8 hours dark period. Observations were recorded on percent culture responding to multiple shoot formation, average number of shoots, percent of shoots rooted, number of days taken for rooting, average length of shoots after establishing in soil, etc.

Results and Discussion

After initiation the multiplication of shoots (apical segments of hypocotyle) started on MS media supplemented with different concentrations of BAP. Shoot proliferation occurred within 25–30 days of incubation. No difference in regeneration response was noticed due to different concentrations of BAP, but maximum proliferation was observed in shoots subjected to 5 μM of BAP (Table 1).

In general BAP has been frequently reported to induce better shoot multiplication than other cytokinins particularly in tree species (Ahmad, 1989). Rao and Lee (1982) have reported its effectiveness in juvenile as well as mature tissue of *Callophyllum inophyllun*, *Eugenia* spp. and *Fragraea fragrans*. Tricoli *et al.* (1985) in *Prunus serotina* and Gupta *et al.* (1981) in *Eucalyptus* spp. have reported its effectiveness.

Table 1. Effect of BAP on multiple shoot formation

Concentration of BAP in MS media (μM)	Culture response (%)	Average number of shoots (after 4 weeks)	Correlation between 1&2	Correlation between 1&3	Correlation between 2&3
0	10	2			
5	70	9			
10	62	7	-0.04	0.11	0.90
15	40	4			
20	38	5			
25	30	6			

Maximum 70% culture responded to the multiple shoot formation among the media combinations tested. Maximum 9 shoots were developed after 4 weeks of inoculation on the media with 5 micromolar BAP.

Rooting and acclimatization of rooted shoots

When shoots attained the height of 5–6 cm, they were transferred to earthen pots for rooting without applying any rooting hormone. These contained soil taken from tree growing area. These earthenpots were covered with polythene sheet to retain moisture. The root initiation was detected after two weeks of transplanting. Eighty-five percent rooting was assessed within 20–25 days from the surviving plantlets. Polythene sheet was gradually removed to acclimatize the plantlets. On the emergence of first pair of leaves, the polythene sheat was completely removed. At the age of 2 months, plants attained height of 1 m. The survival percentage in pots was 80%.

Conclusion

It is concluded that Ipil Ipil (*Leucaena leucocephala*) can be successfully propagated through tissue culture technique. As far as the concentration of BAP in MS media is concerned, 5 micro molar proved to be optimum. No significant correlation was found between the concentration of BAP and culture response or number of shoots developed.

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