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ORGANOGENETIC DIFFERENTIATION IN CALLUS TISSUES OF *ACACIA SENEGAL* (L.) WILLD.

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Callus from hypocotyl and cotyledon explants of *Acacia senegal* was raised on MS-medium supplemented with 2,4-D (2.5 mg/l) in combination with BAP (0.5 mg/l). When transferred, differentiated into shoot buds on MS + BAP (2.5 mg/l) and Kn (2.5 mg/l) medium. Other additives viz, adenine sulphate (25.0 mg/l) ascorbic acid (10.0 mg/l) and glutamine (146.0 mg/l) were also added to the medium. The shoot buds elongated and developed on MS-medium with lower concentration of BAP and Kinetin (0.5 mg/l each). Rooting of regenerated shoots was achieved on 1/4 strength MS-medium fortified with IAA (3.0 mg/l).

Key Words : *Acacia senegal*, callus, differentiation, plantlets.

In vitro techniques have been employed successfully in the propagation of mature trees during the last decade. *Acacia senegal* (L.) Willd belongs to family Mimosoideae is an economically and ecologically important tree species of arid lands. It is used in the manufacture of pharmaceutical preparations, produces gum arabic, provides fodder for animals and plays an important role in struggling against desertification. In *A. senegal* callus formation and induction of cell suspension culture has been reported by Hustache *et al.* (1986), plantlet regeneration from cotyledonary node explant has been obtained by Badji *et al.* (1993) and multiple shoot formation from mature nodal explant was reported by Gupta *et al.* (1994). The present investigation was aimed at developing a suitable methodology for rapid propagation of *A. senegal* through callus tissue.

Seeds of *Acacia senegal* were procured from Central Arid Zone Research Institute (CAZRI), Jodhpur, India. They were washed thoroughly with 2% extran solution (mild commercial detergent) and then with sterile distilled water. Subsequently they were surface sterilized with 2% mercuric chloride. The sterilized seeds were germinated on sugar-agar medium fortified with BAP (1.0 mg/l).

Cotyledons and hypocotyl segments were excised from 15 days old seedlings for raising callus. For callus induction and organogenesis the basic medium used was modified MS (Murashige and Skoog's 1962) medium. The pH of the media was adjusted to 5.8 before autoclaving at 15 psi for 20 minutes. All the cultures were incubated at 28±2°C and exposed to 16 hrs photoperiod at a light intensity of 3000 lux and 50-60% relative humidity.

To induce shoot bud differentiation, callus tissue was transferred to shoot bud induction medium (MS+BAP/Kn (1.0-6.0 mg/l)]. For rooting, 3-4 cm long shoots were excised and were recultured on MS-medium (1/4,1/2 and full strength) enriched with various auxins viz., IAA, IBA and NAA separately at different concentrations (1.0-6.0 mg/l). In place of agar, phytagel (0.2%) was used in rooting medium. Six replicates for each treatment were taken and all experiments were repeated twice.

Seeds germinated on BAP augmented medium gave short and sturdy seedlings compared to weak and long seedlings on plain sugar agar medium. Callus induction was observed in both cotyledon and hypocotyl explants with IAA, NAA and 2,4-D.

The amount of callus produced with NAA (2.0-3.0 mg/l) and IAA (3.0-5.0 mg/l) separately was less compared to 2,4-D and was nonregenerative. Fast growing regenerative callus was produced on 2,4-D (2.5 mg/l) and BAP (0.5 mg/l) supplemented MS-medium (Fig. -1). The callus so produced was compact, green and possessed high regeneration potential. Similar results have also been reported by Kumar *et al.* (1991) in *Dalbergia sissoo* and Das *et al.* (1993) in *Acacia auriculiformis.*

The sign of shoot bud formation was noticeable within 20-25 days of callus transferred to shoot bud induction medium (MS + BAP/Kn (1.0 - 6.0 mg/l)]. Neither BAP nor Kinetin alone proved beneficial in

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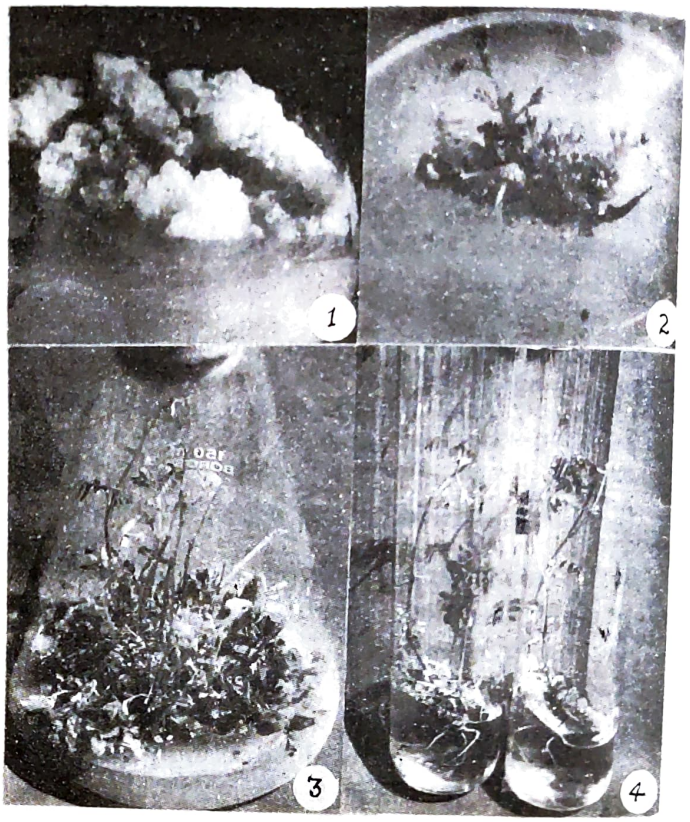
Table 1. Effect of Cytokinin/s on morphogenic response of *A. senegal* Calli.

S. No.	Treatment mg/l	No. of Shoot buds developed per unit callus mean*± 0.005 SE($\bar{x}$)
1.	BAP	
	1.0	-
	2.0	-
	3.0	-
	4.0	4.17 ± 0.79
	5.0	3.0 ± 0.93
	6.0	1.33 ± 0.54
2.	Kn	
	1.0	-
	2.0	-
	3.0	-
	4.0	-
	5.0	-
	6.0	-
3.	BAP + Kn	
	0.5+0.5	-
	1.0+1.0	1.33 ± 0.54
	1.5+1.5	6.17 ± 1.78
	2.0+2.0	6.0 ± 1.47
	2.5+2.5	9.33 ± 2.06
	3.0+3.0	8.5 ± 1.10

*Values are 95% confidence limits for mean.

differentiation of shoots from callus (Table -1). When BAP and Kinetin both were incorporated in combination in MS-medium, the frequency of shoot bud differentiation in callus rose considerably. The optimum level of shoot bud formation was observed in medium containing BAP (2.5 mg/l) and Kinetin (2.5 mg/l) along with adenine sulphate (25.0 mg/l), ascorbic acid (10.0 mg/l), and glutamine (146.0 mg/l). The entire piece of callus tissue turned into a mass of globular structures which later differentiated into shoot buds (Fig. 2). For shoot bud organogenesis, cytokinins (BAP and/or Kinetin) were found obligatory. Kumar *et al.* (1991) and Das *et al.* (1993) found that BAP gave better results while Kumar (1992) observed that use of Kinetin gave maximum shoot differentiation. But in case of *A. senegal* we found that BAP in combination with Kinetin gave maximum shoot bud differentiation.

The shoot buds so obtained failed to elongate on the same medium so they were subcultured on MS-medium containing lower concentration of BAP and Kn which was 0.5 mg/l each along with other additives (Fig. -3). Incorporation of additives like ascorbic acid in culture media checked leaching of phenolics and browning of cultures. Adenine sulphate was found to re-inforce the effect of other cytokinins and glutamine proved to be most effective to stop leaf fall in multiple shoots.



Figures1-4 Organogenetic differentiation in callus tissues of *Acacia senegal* (L.) Willd. 1. Regenerative callus induction on MS-medium supplemented with 2,4-D (2.5 mg/1) and BAP (0.5 mg/1). 2. Shoot bud regeneration on MS-medium augmented with BAP (2.5 mg/1), Kn (2.5 mg/1), adenine sulphate (25.0 mg/1), ascorbic acid (10.0 mg/1) and glutamine (146.0 mg/1). 3. Multiple shoot proliferation on MS-medium containing 0.5 mg/1 each of BAP and Kn along with other additives. 4. Root induction in regenerated shoots on 1/4 strength MS-medium fortified with IAA (3.0 mg/1).

The shoots so developed were rooted on 1/4 MS-medium fortified with IAA (3.0 -5.0 mg/l). Sturdy and profuse rooting was obtained on medium containing IAA - 3.0 mg/l (Fig. - 4). Induction of roots with IAA was also reported in *A. auriculiformis* by Mittal *et al.* (1989), in *Tamarindus indica* by Jaiwal and Gulati (1991) and also in *A. nilotica* by Dewan *et al.* (1992).

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