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MICROPROPAGATION OF *ACACIA SENEGAL* (L.) WILLD VIA COTYLEDONARY NODES

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Cotyledonary node explants of *Acacia senegal* (L.) Willd, differentiated multiple shoots on MS-medium supplemented with various cytokinins, auxins and other additives. Maximum shoot proliferation from single explant was obtained on MS-medium supplemented with BAP (1.5 mg/l), Kn (1.5 mg/l), NAA (0.5 mg/l), adenine sulphate (25.0 mg/l), ascorbic acid (10.0 mg/l) and glutamine (146.0 mg/l) Individual shoots when subcultured on the rooting medium - 1/4 MS-medium fortified with IAA (3.0 mg/l), produced healthy roots in 80 % cultures. Attempts are being made to transfer *in vitro* regenerated plantlets of *Acacia senegal* to pots.

Key Words : *Acacia senegal,* micropropagation, cotyledonary node.

Tissue and organ culture techniques are being increasingly developed for the propagation of many forest tree species, for improving afforestation, woody biomass, and conservation of elite and rare germplasm (Bonga and Durzan, 1982; Bajaj, 1986). *Acacia senegal* (L.) Willd is an economically and ecologically important species. This woody tree legume is a multi-purpose vegetation element in arid and semi-arid regions. Callus formation and induction of cell suspension culture in *A. senegal* has been reported by Hustache *et al.* (1986), plantlet regeneration has been reported by Badji *et al.* (1993) and multiple shoot formation from mature nodal explants of *A. senegal* was reported by Gupta *et al.* (1994).

The present investigations were undertaken to define optimal culture conditions for high frequency plant regeneration of *A. senegal* using cotyledonary node explant.

MATERIALS AND METHODS

Seeds of *Acacia senegal* were procured from Central Arid Zone Research Institute (CAZRI), Jodhpur, India. They were washed thoroughly with 2% extran detergent and then with sterile distilled water. Subsequently they were surface sterilized with 0.2% mercuric chloride for 5 min and rinsed thoroughly with sterile distilled water and were germinated on plain sugar-agar medium fortified with BAP (1.0 mg/l). The pH of all media was adjusted to 5.8 before autoclaving at 15 psi for 20 min.

One centimeter long segments of cotyledonary nodes were excised from 15 days-old seedlings and cultured on MS (Murashige and Skoog, 1962) medium supplemented with BAP/Kn (1.0-6.0 mg/l) and NAA (0.1-0.5 mg/l) for raising multiple shoots. Various other additives viz. adenine sulphate, ascorbic acid and glutamine were also added in the media. The media contained 3% sucrose and 0.8% agar. All the cultures were maintained at 28±2°C under 16 hours photoperiod and 3000 lux light intensity.

3-4 cm long shoots were excised for rooting. They were recultured on MS-medium (1/4, 1/2 and full strength) fortified with various auxins viz. IAA, IBA and NAA in different concentration (1.0-6.0 mg/l). Instead of agar, phytagel (0.2%) was used in rooting medium. Six replicates for each treatment were taken and all experiments were repeated twice.

The seedlings obtained on BAP augumented medium were short and sturdy unlike the weak and elongated seedlings obtained on plain sugar-agar medium.

RESULTS AND DISCUSSION

The cotyledonary node was found to be a very good explant for high frequency regeneration of *A. senegal.* Within 5-7 days following inoculation, shoot buds differentiated in cotyledonary node explants when grown on MS-medium supplemented with BAP, Kn and NAA (Fig. 1). All the explants implanted on BAP or Kn alone and BAP and Kn in combination and in concentration of 1.0-6.0 mg/l showed varied response with respect to number of shoot

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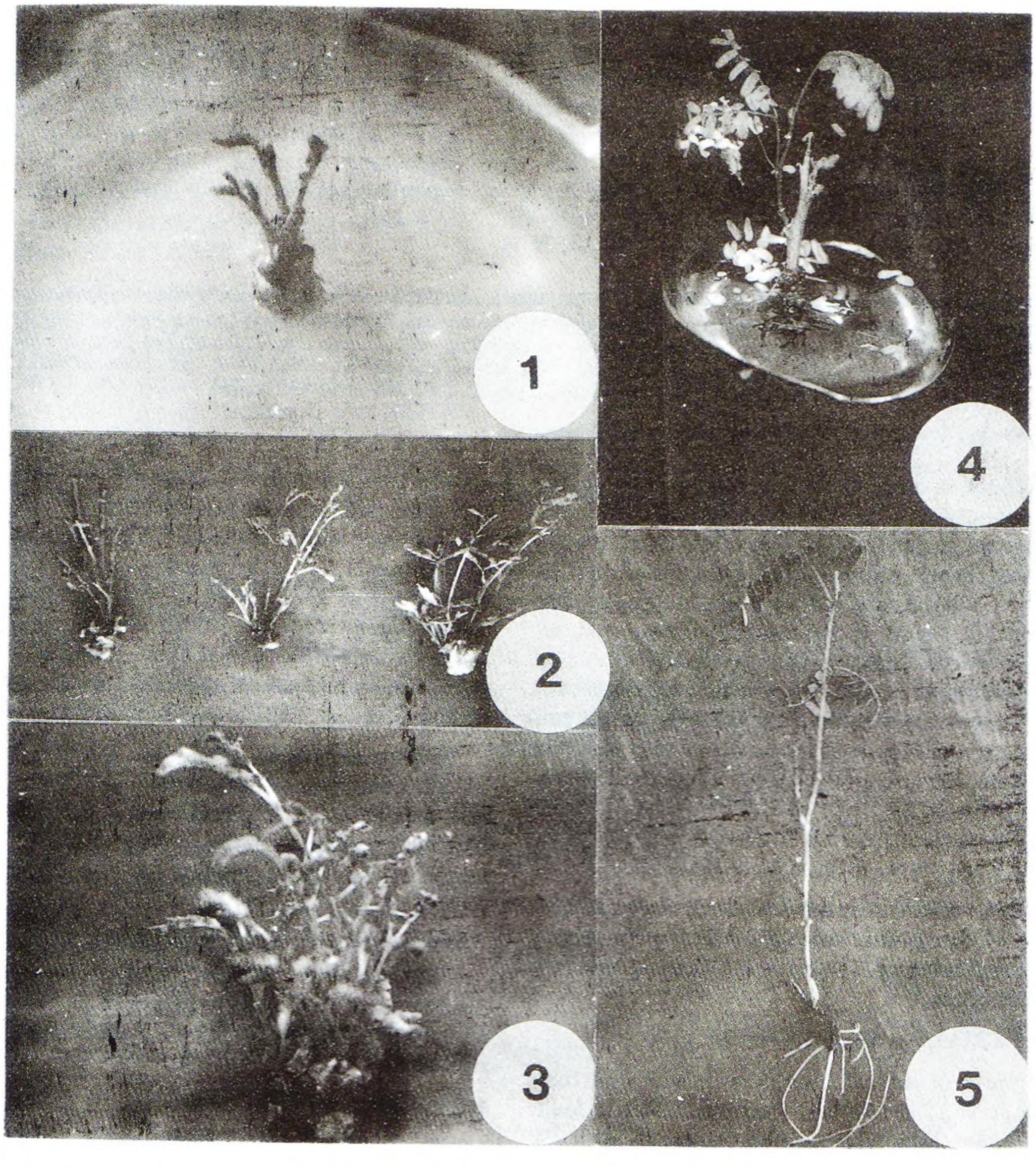

Figures 1-5 Micropropagation of *Acacia senegal* (L.) Willd via cotyledonary node.
Fig. 1. Shoot bud initiation on MS-medium supplemented with BAP (1.5 mg/l), Kn (1.5 mg/l), NAA (0.5 mg/l), ascorbic acid (10.0 mg/l), adenine sulphate (25.0 mg/l) and glutamine (146.0 mg/l) after 5-7 days of inoculation. Fig. 2. Increase in the number shoots after 10, 15 and 20 days following inoculation of explant, on the same medium. Fig. 3. Multiple shoot proliferation (20-22) from the single explant after 30 days of inoculation. Fig. 4. Root induction in regenerated shoots on 1/4 strength MS-medium fortified with IAA (3.0 mg/l). Fig. 5. A complete *in vitro* regenerated plantlet of *Acacia senegal*.

buds obtained per explant (Table 1). However a combination of BAP (1.5 mg/l) and Kn (1.5 mg/l) along with NAA (0.5 mg/l), ascorbic acid (10.0 mg/l), adenine sulphate (25.0 mg/l) and glutamine (146.0 mg/l) was found to be the optimal media for maximum shoot proliferation from cotyledonary node explant. An increase in the number of shoots is observed after every 5th day (Fig. 2).

Table 1. Effect of Cytokinin/s on shoot proliferation from cotyldeonary node.

Cytokinin/s level		No. of shoot buds per explant *Mean±0.05 SE (X)
Kn	BAP	
1.0	-	0.83 ± 0.79
2.0	-	2.33 ± 0.54
4.0	-	3.17 ± 1.23
6.0	-	3.83 ± 1.54
-	1.0	1.67 ± 0.55
-	2.0	3.17 ± 0.79
-	4.0	8.0 ± 2.20
-	6.0	9.17 ± 2.60
1.0	1.0	12.33 ± 2.46
1.5	1.5	19.67 ± 1.58
2.0	2.0	17.33 ± 0.86
3.0	3.0	18.5 ± 1.60

*Values are 95% confidence limits for mean.

Incorporation of additives like ascorbic acid in culture media checked leaching and browning of cultures and also showed auxilliary effects on shoot growth. 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.

Mathur and Chandra (1983) reported development of plantlets in mature nodal explants of *Acacia nilotica* on auxin containing media. According to them, auxin was essential for shoot differentiation. On the other hand some workers have reported that cytokinin is obligatory for shoot differentiation in other plant species (Arya and Shekhawat, 1987; Dewan *et al.*, 1992). In another species of the same genus, viz. *A. albida* Duhoux and Davies (1985), regenerated plantlets in cotyledonary buds on MS-medium containing Nitsch's vitamins and BA (3.0 mg/l) along with NAA (0.5 mg/l).

From the single explant 20-22 shoots were obtained on the above mentioned media after 30 days of inoculation (Fig. 3). A harvest of sturdy shoots was made after each subculture and the number of shoots produced thus increased.

The shoots rooted on 1/4 MS-medium fortified with IAA (3.0-5.0 mg/l). Sturdy and profuse rooting was obtained on IAA - 3.0 mg/l and it was upto 80% (Fig. 4), whereas thin, long and less roots were obtained on IAA - 5.0 mg/l augumented medium in upto 60% of cultures. Use of IAA in higher concentrations (> 5.0 mg/l) produced callus. Induction of roots with IAA is also reported earlier by Mittal *et al.* (1989) in *A. auriculiformis*, and also in *Acacia nilotica* by Dewan *et al.* (1992).

The complete *in vitro* regenerated plantlets of *A. senegal* are thus ready (Fig. 5) and attempts are being made to gradually transfer them to pots.

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REFERENCES

Arya H C & N S Shekhawat 1987 Micropropagation of certain fruit and timber trees through tissue culture regeneration and establishment. In *Proc Symp Plant Cell and Tissue Culture of Economically Important Plants* (G M Reddy ed) Hyderabad India p 159-163.

Badji S, Y Mairone, I Ndiaya, I Merlin, P P Danthu, P Neville & J P Colonna 1993 *In vitro* propagation of gum arabic tree (*Acacia senegal* (L.) Willd) I Developing a rapid method for producing plants. *Plant Cell Reports* **12** 629-233.

Bajaj Y P S 1986 (ed) *"Biotechnology in Agriculture and Forestry" 1. Trees* Springer - Verlag Berlin Heidelberg New York.

Bonga J M & D J Durzan 1982 Vegetative propagation in relation to juvenility maturity and rejuvenation. In *Tissue Culture in Relation to Forestry* (eds) Bonga J M and D J Durzan) Publ Martinus-Nijhoff/Dr W Junk p 387-412.

Dewan A, N Kanan & S C Gupta 1992 *In vitro* micropropagation of *Acacia nilotica* sub sp *indica* Brenan via cotyledonary node. *Plant Cell Reports* **12** 18-21.

Duhoux E & D Davies 1985 Shoot production from cotyledonary buds of *Acacia albida* and the effect of saccharose on root production. *J Pl Physiol* **121** 175-180.

Gupta P, V Patni, U Kant & H C Arya 1994 *In vitro* multiple shoot formation from mature trees of *Acacia senegal* (L) Willd. *J Ind bot Soc* **73** 331-332.

Hustache G, F Branoud & J Joseleau 1986 Callus formation and induction of a cell suspension culture from *Acacia senegal*. *Plant Cell Reports* **5** 365-367.

Mathur I & N Chandra 1983 Induced regeneration in plantlets of *Acacia nilotica. Curr Sci* **52** 882-883.

Mittal A, R Agrawal & S C Gupta 1989 *In vitro* development of plantlets from axillary buds of *Acacia auriculiformis* - a leguminous tree. *Plant Cell Tissue and Organ Cultures* **19** 65-70.

Murashige T & F Skoog 1962 A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol Plant* **15** 473-497.