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Organogenesis from Callusing Cotyledon Explants of *Leucaena leucocephala* (Lam.) de Wit

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ABSTRACT

The cotyledon explants excised from *in vitro* grown seedling of *Leucaena leucocephala* showed organogenesis when cultured on B_5 medium containing auxins and cytokinins individually or in various combinations. The various types of morphogenetic responses were callusing, rhizogenesis and caulogenesis. This is another report where micropropagation of leguminous trees is experimented through *in vitro* culture techniques, otherwise most of the leguminous trees are very much recalcitrant to such studies that has hindered the improvement of these plants through genetic transformation.

Keywords: *Leucaena leucocephala*, Caulogenesis, Rhizogenesis, Callusing, Recalcitrant

Leucaena leucocephala (Lam.) de Wit is a very fast growing leguminous tree and suitable for various purposes such as fire wood, fodder, paper pulp,cattle feed,etc. Due to the high content of mimosine in seed, its use as food is not recommended. However, some methods have been developed to remove mimosine content from leaves, pods and seeds (Soedarjo and Borthakur, 1996).

Most of the leguminous trees are recalcitrant for *in vitro* culture studies due to presence of several metabolites and inhibitory compounds and high levels of lignin in the wood. In some cases success has been achieved by culturing axillary buds obtained from *in vitro* as well as *in vivo* grown plants (Goyal *et al*. 1985). In our earlier report, the juvenile axillary bud and cotyledon explants formed callus as well as rooting (Gautam *et al*. 1985).

MATERIALS AND METHODS

Seeds of *Leucaena* being a legume has a very hard seed coat. To improve early germination, seeds were rubbed on sand paper from their chalazal end, a process called scarification was employed. Within a week, it was possible to get 90% seed germination. After rubbing, seeds were surface sterilized with saturated Chlorine water for 30 minutes. After that seeds are washed thoroughly with sterilized distilled water and inoculated aseptically on agar medium. Within 12-15 days 4-6 cm long seedlings were formed. The cotyledon explants were excised from these seedlings and cultured on media augmented with various growth hormones. For culturing cotyledon explants from *in vitro* grown seedling, B_5 medium (Gamborg *et al*. 1968) was used. In some experiments, yet another hormone, Zeatin (Zn) has been used along with B_5 medium. All cultures were maintained in the culture room at 25 -27^{o}C temperature and 50-60 % relative humidity under a continuous cool white light (approx.500 lux) produced by Philips Fluorescent tubes of 40 watts. The following criteria were taken into account for recording the observations:

(i) Total number of explants inoculated and total number of explants survived (excluding the infected ones),

(ii) Percentage of explants callusing, rooting and producing shoots,

(iii) Number of roots and shoots per explant.

RESULTS AND DISCUSSION

Cotyledonary explants formed callus, shoots and roots in 22 days after culture on B_5 + 1,2 and 4

mg/l BA. The morphogenic response varied in relation to the nature and concentration of auxins and cytokinins (Table 1). During differentiation, the responding explants remained green for almost two weeks. Subsequently, they started callusing either at the cut ends or all along the margin (Fig.1B). They produced callus in almost all the combinations tried (Table 1). Even the B_5 basal medium supported callusing (36%) and rooting (23%). However, addition of hormones appreciably increased the percentage of callusing explants up to 100 % in several combinations. Various concentrations of BA were quite effective in producing sufficient callusing and rooting (Fig. 1C). In addition, it also induced shoot formation though at a low frequency (2-5%). After three weeks of inoculation, shoots differentiated on 1,2 and 4 mg/ l BA supplemented media (Fig.2 A). Multiple shoots were organised on B_5+ BA (2 mg/l ; Fig. 2B). Ten weeks later, rarely small roots initiated from the basal ends of shoots (Fig. 2 C; Arrow). As compared to BA, Kn was less effective and it completely inhibited rhizogenesis at a low concentration (0.02 mg/l). However, its higher concentration (2.15 mg/l) supported good rhizogenesis (50%). Adenine (0.13 and 1.35 mg/l) and Zeatin (0.02,0.21 and 2.19 mg/l) supported both callus as well as root production (Table 1). Auxin, like NAA at low concentrations (0.01 and 0.18 mg/l), suppressed callusing but enhanced root differentiation. However, at higher concentrations (1.86 mg/l) the response was reversed since callusing was enhanced but rooting was inhibited. IAA generally produced both callus as well as roots (Fig.1D). However, 2,4-D mainly supported callusing but inhibited organogenesis.

Table 1: Morphogenic responses of cotyledonary explants on B_5 medium scored after 71 days of inoculation

Hormones (mg/l)	Explants			
	Survived	Callusing %	Rooting %	Shooting %
Control (B_5)	88	36	23	0
0.1 BA	59	90	29	0
0.2 BA	18	67	06	0
0.4 BA	37	95	16	0
0.8 BA	26	92	15	0
1.0BA	31	100	23	03
2.0 BA	44	93	16	02
4.0 BA	41	100	10	05
0.02 Kn	12	67	0	0
2.15 Kn	16	100	50	0
0.01NAA	12	0	08	0
0.18 NAA	14	0	14	0
1.86 NAA	12	17	0	0
0.01 IAA	24	25	29	0
0.17 IAA	28	50	25	0
1.75 IAA	28	75	46	0
0.22 2,4-D	16	38	0	0
2.21 2, 4-D	16	50	0	0
0.13 Ad*	24	50	29	0
1.35 Ad	22	55	05	0
0.02 Zn**	12	100	50	0
0.21 Zn	12	92	67	0
2.19 Zn	20	95	55	0
2.25 BA + 1.75 IAA	12	58	08	0
2.25 BA + 0.17 IAA	34	50	03	0
2.25 BA + 0.01 IAA	32	69	03	0
1 BA + 0.5 IAA	22	100	14	0
1 BA + 1 IAA	12	75	25	0
0.5 BA + 1.5 IAA	12	92	0	0
0.22 BA + 1.75 IAA	24	58	33	0
0.22 BA + 0.01 IAA	36	56	5	0
0.02 BA + 1.75 IAA	24	50	0	0
0.02 BA + 0.17 IAA	28	32	0	0
2.25 BA + 0.18 NAA	14	93	21	0
2.25 BA + 0.01 NAA	14	100	0	0
1 BA + 0.5 NAA	12	100	50	0
1 BA + 1 NAA	12	100	67	0
0.5 BA + 2 NAA	12	100	0	0
1 Kn + 0.5 NAA	15	100	0	0
0.21 Kn + 0.01 NAA	16	0	0	0

* *Adenine;* ** *Zeatin.*

The regeneration through tissue culture is difficult in tree taxa (Bonga, 1977), plantlets have been developed successfully in *L. leucocephala* from juvenile tissue in this study. According to Saafi and Borthakur (2002) BA was effective in producing shoots from cotyledon explants in *Leucaena* similar to our study, however they used MS medium as

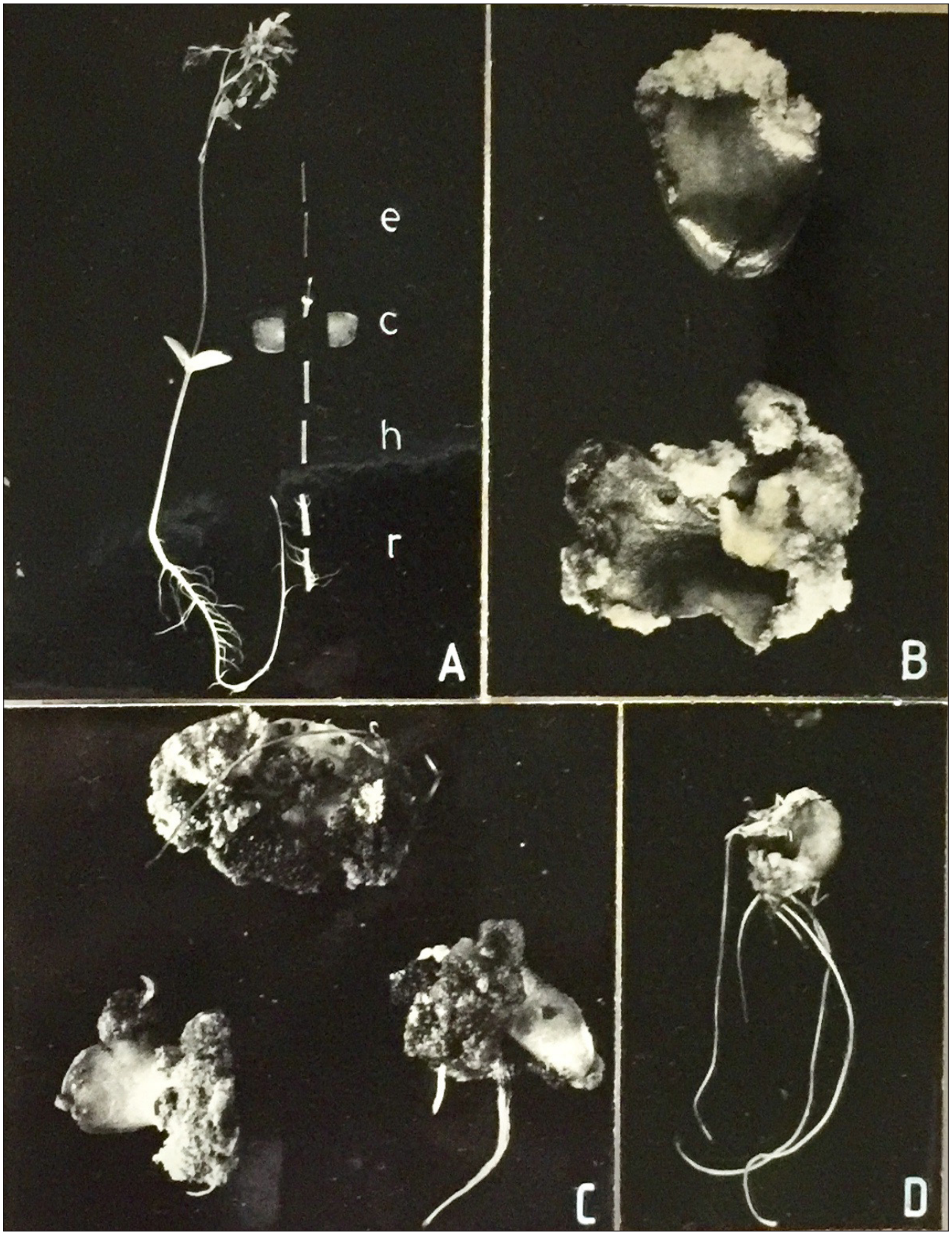

Fig. (1A) A young seedling showing root (r), hypocotyl (h), cotyledon (c) and epicotyl (e). (**1B)** Callus developed on cut surface and margins of cotyledon explant on B_5 + 1 mg/l BA. (**1C)** Differentiation of roots through callus developed on cotyledon explants on B_5 + 2 mg/l BA, (**1D**) Roots developed on cotyledon explants on B_5 + 1.75 mg/l IAA

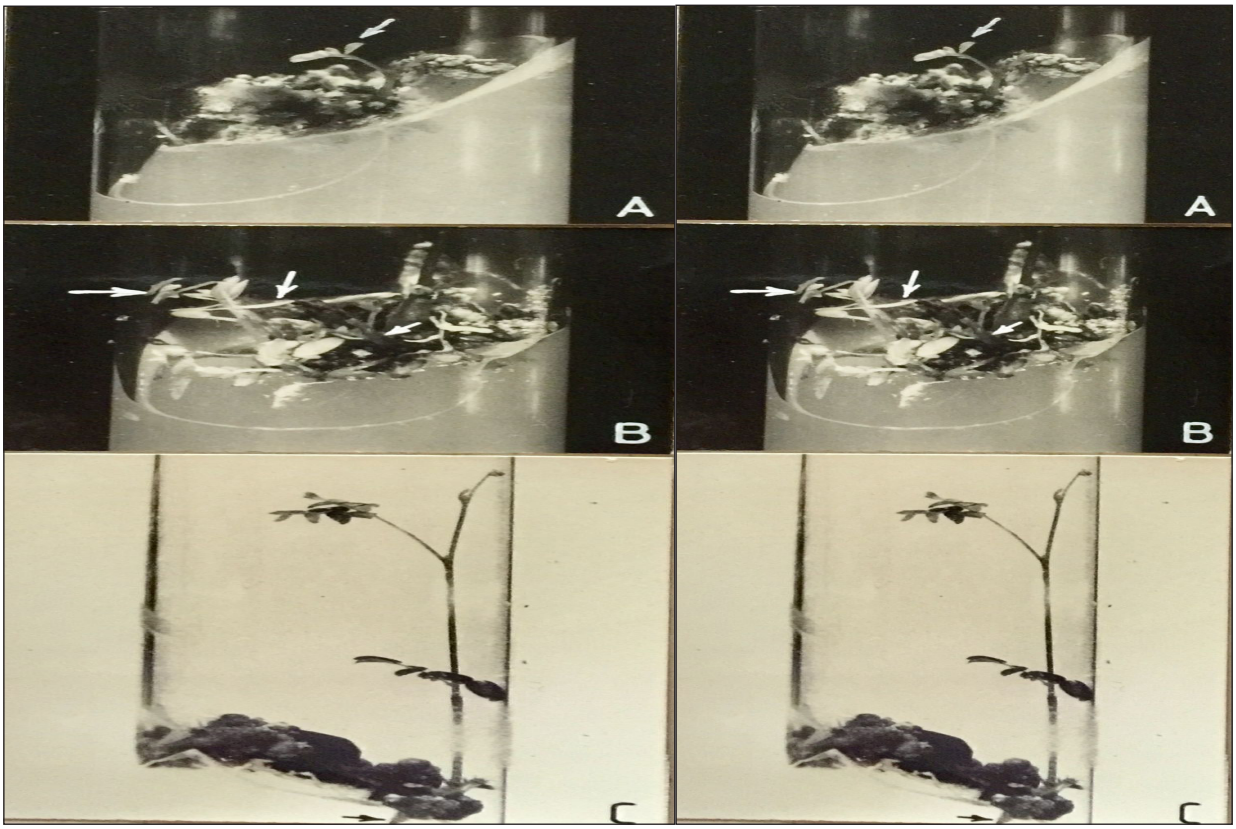

Fig. (2A) A young shoot (arrow) differentiated from callus, developed on cotyledon explant on B_5 + 1 mg/ l BA after four weeks. **(2B)** Production of multiple shoots (arrows) in 74 days old culture in B_5 + 2 mg/l BA. **(2C)** Proliferated shoot initiating root (arrow) on the same medium. Young shoots are also differentiating at the base of the old shoot

contrary to our report where B_5 medium was used. The production of shoots from cotyledons at a very low frequency has been reported in *Ceratonia* (Martins- Loucao and Rodriguez- Barrueco, 1981).

REFERENCES

Bonga, J.M. 1977. Applications of tissue culture in forestry. *In:* Reinert, J. and Bajaj, Y.P.S. (Eds), Applied and Fundamental Aspects of Plant Cell Tissue and Organ Culture. Springer-Verlag, Berlin, pp. 93-108.

Gamborg, O.L., Miller, R.A. and Ojima, M. 1968. Plant Cell Cultures-1. Nutrient requirements of suspension cultures of Soybean root Cells. *Expl. Cell Res*., **50**: 151-158.

Gautam, V.K., Nanda, K. and Gupta, S.C. 1985. Morphogenic responses of a leguminous tree- *Leucaena leucocephala. In:* National Seminar on Reproductive Biology of Plants including Endangered Species. Institute of Advanced Studies, Meerut University, Meerut, pp. 32-33.

Goyal, Y., Bingham, R.L. and Felker, P. 1985. Propagation of tropical tree, *Leucaena leucocephala* K67, by *in vitro* bud culture. *Pl. Cell Tiss. Org. Cult.,* **4**: 3-10.

Martins-Loucao, M.A. and Rodriguez-Barrueco, C. 1981. Establishment of proliferating callus from roots,cotyledons and hypocotyls of carob (*Ceratonia siliqua* L.) seedling. *Z. Pflazenphysiol.,* **103**: 297-303.

Saafi, H. and Borthakur, D. 2002. *In vitro* plantlet regeneration from cotyledons of the tree legume *Leucaena leucocephala*. *Plant Growth Regulation,* **38**: 279-285.

Soedarjo, M. and Borthakur, D. 1996. Simple procedures to remove mimosine from young leaves, pods and seeds of *Leucaena leucocephala* used as food. *Intl. J. Food Sci. Technol.,* **31**: 97-103.