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IN VITRO DIFFERENTIATION FROM CULTURED EXPLANTS OF *LEUCAENA LEUCOCEPHALA*

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ABSTRACT

The epicotyl and hypocotyl explants from *in vitro* grown seedlings were cultured on various media to exploit their morphogenetic potential using various plant growth regulators in various concentrations and combinations. In general leguminous trees are very much recalcitrant to morphogenetic studies. However, in this study both explants produced profuse calli as well as roots in most of the combinations tried. Very small shoots developed at very low frequency only in some combinations. Both explants produced a large amount of callus that can be used for regenerating plants in large numbers, i.e. indirect mode of organogenesis.

.Key words: Hypocotyl, Epicotyl, *in vitro,* organogenesis

INTRODUCTION

The success of improving leguminous forest trees mainly depends on evolving an efficient protocol for *in vitro* regeneration. During the last decades, marginal success has been achieved in developing an *in vitro* regeneration system for leguminous plants. The juvenile tissues have responded better than those excised from field grown old trees. *Leucaena leucocephala* belongs to the family Fabaceae and is an important leguminous tree species having several applications. It is a very fast growing tree that can grow on any soil type without proper irrigation and much care. It is most suitable for the paper pulp industry and its seeds are used to produce decorative items. Its leaves are rich in protein content and are used as cattle feed. In previous studies, its morphogenetic potential has been studied using cotyledon and hypocotyl pieces, but no report is known of using epicotyl explants. In some studies successful regeneration of leguminous tree species from various cultured explants has been reported (Tomar & Gupta, 1988). In *Leucaena* also successful in vitro regeneration has been reported (Gautam *et. al*., 1985; Shirish, *et.al*., 2008).

MATERIAL AND METHODS

The seeds of *Leucaena* were obtained from Pratap Nursery, Dehradun and washed thoroughly with tap water to remove dust and debris. These seeds were then germinated in a simple agar medium containing 0.8% agar. As its seeds have a very hard seed coat so these were rubbed from the chalazal end on sandpaper. This increased seed germination efficiency up to 80%. The seeds were surface sterilized using saturated Chlorine water for 30 minutes and then thoroughly washed with sterilized distilled water at least three times. Finally seeds were inoculated on agar medium using Laminar Flow under aseptic conditions. Within two weeks 4-6 cm long seedlings were developed with prominent regions of epicotyls and hypocotyls. One cm long pieces of epicotyls and hypocotyls were excised from seedlings and cultured on culture media having auxins and cytokinins. B_5 medium developed by Gamborg *et at*.1968 was used in this study. Cultures were maintained in the culture room having 25^0 C temperature and continuous white light from Philips Fluorescent tubes.

RESULTS AND DISCUSSION

Response of Epicotyl Explants: The responding epicotyls started callusing within 2-3 weeks on basal medium. The calli were of different colours, such as whitish- green, yellowish- green, whitish

-yellow and greenish-brown and were either compact or fragile in nature. Even the amount of callus produced per explant after 60 days of inoculation varied considerably. After 60 days of inoculation mainly callus was produced (77%) with very little rooting in 3% of the cultures. Callusing and rooting responses were enhanced by supplementing the media with various hormones (Table1; Fig A-C).

Table1: In Vitro morphogenic response of epicotyl explants of *Leucaena leucocephala* on B5 medium supplemented with hormones after 71 days of culture.

Hormones (mg/L)	Explants		
	Survived	Callusing (%)	Rooting(%)
Control	125	77	3
0.1 BA	17	88	18
2.25 BA	15	100	7
0.02 Kn	14	93	15
2.15 Kn	31	94	6
0.02 Zn	16	100	13
0.21 Zn	16	100	69
2.19 Zn	17	100	59
0.17 IAA	25	100	36
1.75 IAA	26	96	72
0.01 NAA	22	100	18
0.18 NAA	21	100	24
1.86 NAA	15	100	7
0.13 Ad	28	71	14
1.35 Ad	25	64	24
0.5 BA +1.5 IAA	15	100	0
1 BA + 0.5 IAA	30	100	0
1 BA + 1 IAA	12	100	0
0.5 BA + 2 NAA	18	94	0
1 BA + 0.5 NAA	12	92	0
1 BA + 1 NAA	12	100	0
2 BA + 1 IBA	15	93	0
1 Kn + 1 IAA	20	100	10

Fig.1.A-C. Morphogenic response of epicotyl explants. A. Segments of epicotyl calli on B_5 medium.

B.Differentiation of roots from epicotyl explants on B_5+ 1.75mg/L IAA after 28 days of inoculation.

C.Production of callus as well as roots on B_5 + 0.1 mg/L BA.

The highest percentage of callusing (100%) was achieved in several combinations. While the highest percentage (72%) of rooting was attained on B_5 medium containing 1.75 mg/L IAA medium (Fig 1B).Roots developed either directly from the explants or through calli. During the present study it was observed that in addition to auxins, cytokinins could also support sufficient rooting when used alone. However, in general, simultaneous application of auxin and cytokinin was inhibitory. Only in one combination of 1 mg/L Kn + 1mg/L IAA, little rooting (10%) was observed. Amoo and Ayisire (2004) have reported callus production from cultured epicotyl explants using MS medium containing auxin, 2,4-D. In another study Jain and Babbar (2003) have studied the effect of various sugars on morphogenetic potential of epicotyl explants of *Syzygium cuminii*, a medicinally important fruit tree.

Response of Hypocotyl Explants: Hypocotyl explants were less responsive. Both basal as well as hormone supplemented media supported either callusing or rooting or both (Fig.2 A-C). However, the percentage of response was low on basal medium (Table 2). After nine days of inoculation, the responding segments started swelling. In 14 days, callus initiated either on the entire surface or at both cut ends of the explants (Fig 2 A). Pale- green or yellowish- green calli were more conspicuous at the cut ends. After nearly 22 days, rhizogenesis was observed in some combinations (Table 2; Fig. 2C). Out of three auxins tried, only IAA (0.17 and 1.75 mg/L) produced roots. The highest percentage of rooting (33%) was recorded on B_5 + IAA (0.17mg/L).

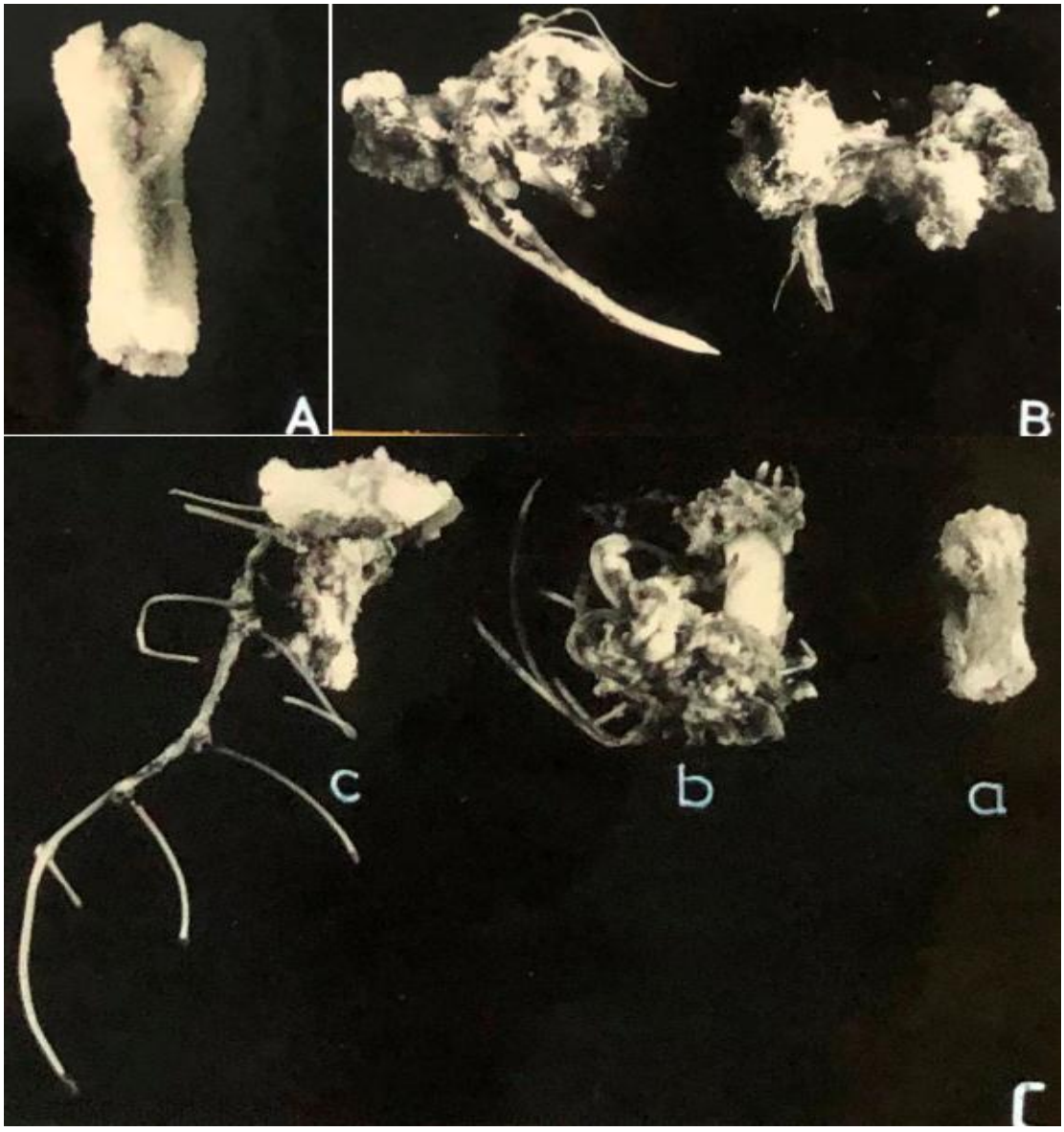

Fig.2.A Morphogenic response of hypocotyl explants.A.Swollen hypocotyl explant with calli at cut ends after 13 days of inoculation.B.Differentiation of roots on hypocotyl calli in B_5 + 0.17 mg/L IAA.C.Different concentrations of BA (0.1 and 0.4 mg/L) producing callus (a,b) as well as roots (c) from hypocotyl explants.

Table 2: Morphogenic response of *Leucaena leucocephala* hypocotyl explants cultured on B5 medium supplemented with various hormones for 71 days

Hormones (mg/L)	Explants Survived	Callusing (%)	Rooting (%)
Control	258	31	3
0.02 BA	28	0	0
0.1 BA	64	67	3
0.2 BA	56	9	2
0.4 BA	30	30	13
0.8 BA	43	14	0
1 BA	35	51	0
1.2 BA	14	14	0
2 BA	34	74	0
2.25 BA	40	60	0
4 BA	39	79	0
0,02 Kn	39	46	0
0.21 Kn	40	68	0
2.15 Kn	68	69	0
0.02 Zn	15	100	0
0.21 Zn	13	100	0
2.19 Zn	30	67	17
0.13 Ad	28	57	0
1.35 Ad	16	100	6
0.01 IAA	34	100	0
0.17 IAA	30	97	33
1.75 IAA	22	100	14
0.01 NAA	45	67	0
0.18 NAA	19	100	0
1.86 NAA	18	100	0
0.22 2,4-D	22	82	0
2.21 2,4-D	16	100	0
2.25 BA + 0.01 IAA	55	98	0
2.25 BA + 0.17 IAA	50	88	0
2.25 BA + 1.75 IAA	21	86	0
1 BA + 0.5 IAA	36	100	0
1 BA + 1 IAA	18	100	0
0.5 BA + 1.5 IAA	18	100	0
0.22 BA + 1.75 IAA	37	95	0
0.22 BA + 0.01 IAA	49	100	2
0.2 BA + 0.01 NAA	19	21	11
0.02 BA + 1.75 IAA	37	100	8
0,02 BA + 0.17 IAA	36	100	14
2.25 BA + 0.18 NAA	15	100	0
1 BA + 0.5 NAA	18	100	0
1 BA + 1 NAA	18	100	0
0.5 BA + 2 NAA	18	100	0
2 BA + 1 IBA	18	100	0
2.15 Kn + 0.01 NAA	20	45	0
1 Kn + 0.5 NAA	15	100	0
0.2 Kn + 0.2 NAA	18	100	0
0.21 Kn + 0.01 NAA	22	100	0
0.21 Kn + 0.01 IAA	21	100	0

On basal medium as well as those containing low concentrations of BA (0.1 - 0.4 mg/L), roots were induced (Fig.2C), while higher concentrations of BA (0.8 - 4 mg/L) were inhibitory. Adenine (1.35 mg/L) and Zeatin (2.19 mg/L) also produced rooting, though at much higher concentrations than that of BA. However, Kn (0.02 - 2.15 mg/L was ineffective in producing roots. BA in combination with IAA or NAA also produced roots, while other combinations of phytohormones were ineffective (Table 2), though profuse callusing did take place. The hypocotyl explants of *Parkia* sp. produced profuse callus on MS medium containing 2, 4-D

(Amoo and Ayisire, 2004). In this study the explant is same but medium and auxins are different for inducing callogenesis. The callus can be used for various purposes like differentiation of plants and even somaclonal variants.

REFERENCES

1. Amoo, S.O. and Ayisire, B.E. 2004. Callus induction from epicotyl and hypocotyl explants of *Parkia biglobosa* (Jacq.) Benth. Ife Journal of Science. 6: 95-100.
2. Gamborg, O.L.; Miller, R.A. and Ojima, K. 1968. Plant Cell Cultures-1. Nutrient requirements of suspension cultures of soybean root cells. Expl Cell Res. 50: 151-158.
3. Gautam, V.K.; Nanda, K. and Gupta, S.C. 1985. Morphogenic responses of a leguminous tree-*Leucaena leucocephala*. In : National Seminar on Reproduction Biology of Plants including Endangered Species. Institute of Advanced Studies, Meerut University, Meerut: 32-33.
4. Jain, N. and Babbar, S.B. 2003. Effect of carbon source on the shoot proliferation potential of epicotyl explants of *Syzygium cuminii*. Biologia Plantarum 47: 133-136.
5. Shirish, V. I. *et al*., 2008. Direct shoot organogenesis and plant regeneration from hypocotyl explants in selected genotypes of *Leucaena leucocephala*- a leguminous pulpwood tree. Indian J. of Biotechnology 7: 388-393.
6. Tomar, U.K. and Gupta, S.C. 1988. Somatic embryogenesis and organogenesis in callus cultures of the legume *Albizia richardiana* King. Plant Cell Rep. 7: 70-71.