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Micropropagation of *Crotalaria laburnifolia* L. – An ethnomedicinally important herbal species

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Abstract
A protocol for *in vitro* shoot multiplication in *Crotalaria labumifolia* L. through nodal explants was established. Excision and culture of the nodal segments from *in vitro* developed shoots on fresh MS medium with concentration of BA (1mg/1) facilitated development of multiple shoots. Subsequent cultures enhanced the rate of shoot proliferation. Shoots cultured on ½ MS medium containing NAA (0.5 mg/1) initiated roots well compared with IBA and this is the most suitable protocol studied in *C. labumifolia.* The present study is the first report on *in vitro* regeneration in this species

Keywords: Micropropagation, *Crotalaria,* Ethanomedicine, Herbal medicine.

INTRODUCTION

Crotalaria labumifolia L. is a potent ethnomedicinal herbal species due to its medicinal and several other properties. It is commonly known as Eswari and belongs to Fabaceae family. It is a rich source of amino acids, and alkaloids [1] and nearly 9 types of amino acids are so far reported. This species is very rich medicinally and various parts of this plant have been used in Ayurvedic medicine. The flower paste is rubbed over the snake bite for relief by local tribes [2]. The infusion of plants is used as a gargle [3] while the grinded leaves used for joint swellings [4]. It is a known fact that tissue culture techniques have been reported for conservation and multiplication of several medicinal plants [5-7].

In vitro regenerated medicinal plants could help in producing disease free healthy clones in large scale for extraction of various drugs. A micropropagation protocol was developed for this plant using nodal segments as explants with cytokinins like BA and Kn.

MATERIALS AND METHODS

Nodal explants of *Crotalaria laburnifolia* were collected from medicinal plant garden, Kakatiya University, Warangal, Andhra Pradesh, India. The explants were thoroughly washed with running tap water for 30 min, followed by removal of scales from the nodal segments, then washed with 5% v/v Tween 20 solution for 15 min and surface sterilized by passage through 70% ethanol for 1 min. These explants were sterilized with 0.1% $HgCl_2$ for 2 min, followed by three rinses with autoclaved sterile distilled water in aseptic conditions.

Nodal segments were surgically excised and inoculated into culture tubes containing MS medium [8] gelled with 0.8% of Agar and 3% sucrose was added into the medium as a carbon source and supplemented with various concentrations of 0.5, 1.0, 1.5, 2.0 and 3.0 mg/1 of cytokinins (BA and Kn) individually for multiple shoot formation. Then pH of the medium was adjusted to 5.7-5.8 with 1N NaOH / HCl prior to autoclaving for 121^0C, 15 lbs for 20min. All the cultures were incubated at 25 ± 2^0C under 3000 lux high intensity provided by white fluorescent lamp for 16 hours photoperiod. The data was tabulated and analyzed statistically. The sub culturing was carried out regularly at 4 week interval.

After four weeks of culture, the regenerated shoots were taken out and transferred to the rooting medium with various concentrations (0.25, 0.50, 1.0 and 2.0 mg/1) of auxins like NAA and IBA alone.

RESULTS AND DISCUSSION

Shoot multiplication was obtained from nodal explants cultured on MS medium containing different concentrations of BA and Kn (0.5, 1.0, 1.5, 2.0 and 3.0 mg/L). After lapse of 10 days multiple adventitious shoots formed directly from inoculated nodal explants in both hormonal treatments. However, early induction could be seen in BA than Kn. BA gave high percentage of response for shooting than Kn. Similarly multiple shoot induction was also more with BA than Kn (Table. 1).

The highest multiple shoot production (4.12) was observed on a medium containing BA l mg/L along with percentage of response (92%) which was also more. In case of multiple shoot production higher concentration of Kn (2.0 and 3.0 mg/L) produced less number of shoots per explant compared with BA. The similar trend of shoot length in both cytokinins was also observed as seen with the percentage of response and shoot production. Maximum shoot length (4.88) was noted in a medium containing BA l mg/L (Fig-1 c). Srinath Rao *et al*. [10] reported 2-3 shoots in *Crotalaria longipes* and *Vigna radiata* on MS medium supplemented with Kn. Direct adventitious multiple shoot induction was more in BA than Kn. Interestingly, with both hormones, number of shoots induction, percentage of shooting response and length of shoots were negatively correlated with increasing level of both hormones individually.

For root induction *in vitro* regenerated shoots were gently

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removed from culture medium and inoculated on fresh half strength MS medium with IBA and NAA individually. Root induction could be seen within 10-14 days (and prolific roots were initiated with in a period of 3 weeks). Response was also compared with full strength MS medium but it showed less response than half strength MS medium with IBA and NAA (Table 2 & Figs-1 d, e). Induction percentage of rooting response and number of roots along with root length was more in half strength MS medium containing NAA at 0.5 mg/l in cape goose berry [11] and also in the present study (Table-2).

Compared with IBA, NAA was found to be best for inducing roots and percentage of rooting response was earlier reported in *Physails peruvriana* for induction of rooting with NAA followed by IBA [12].

The rooted plantlets were transferred to polythene cups containing autoclaved (2:1) soil mixture. Initially the pots were covered with perforated polythene bags for one week to prevent desiccation and to maintain humidity. 70% of healthy plantlets transferred to field, in field conditions survived and grew well. Morphological characteristics of the plantlets established in field conditions were identical to that of mother plants. This protocol could be useful for the induction of true type plants round the year irrespective of the season for the exploitation at industrial level for medicinal value at one hand and conservation of the species on the other hand. Further work is to be attempted to evaluate secondary metabolites in *in vivo* and *in vitro* levels

Table 1. Shoot Multiplication of *C. laburnifolia* on MS medium supplemented with different growth regulators

Growth regulator (mg/l)		% of response	Shoots/Explant Mean±S.E.	Shoot length (in cm) Mean±S.E.
BA	0.5	88	4.04 ± 0.21	4.23 ± 0.12
	1.0	92	4.12 ± 0.15	4.88 ± 0.12
	1.5	76	3.05 ± 0.17	3.31 ± 0.14
	2.0	68	3.11 ± 0.19	3.21 ± 0.09
	3.0	64	3.43 ± 0.17	3.16 ± 0.13
Kn	0.5	80	3.21 ± 0.18	2.33 ± 0.16
	1.0	82	3.12 ± 0.16	3.76 ± 0.22
	1.5	76	3.10 ± 0.22	3.40 ± 0.21
	2.0	72	2.88 ± 0.20	3.00 ± 0.17
	3.0	72	2.88 ± 0.19	3.09 ± 0.15

Data represents the Mean of 25 replicates. Mean followed by different cultures are significantly different at 5% level culture period 30 days.

Table 2. *In virto* rooting of *C. laburnifolia* on half strength M.S. medium fortified with IBA and NAA

Growth regulator (mg/l)	% of response	Roots Mean ± S.E.	Root length Mean±S.E.
½ MS + IBA			
0.25	60	2.01 ± 0.17	1.76 ± 0.04
0.50	72	3.34 ± 0.15	2.02 ± 0.06
1.0	68	2.83 ± 0.21	1.98 ± 0.03
2.0	65	0.73 ± 0.23	1.80 ± 0.03
½ MS + NAA			
0.25	60	3.36 ± 0.15	1.84 ± 0.08
0.50	92	4.31 ± 0.13	3.19 ± 0.18
1.0	80	4.00 ± 0.24	2.81 ± 0.13
2.0	72	3.44 ± 0.26	2.77 ± 0.11

Data represents the Mean of 25 replicates. Mean followed by different cultures are significantly different at 5% level culture period 30 days.

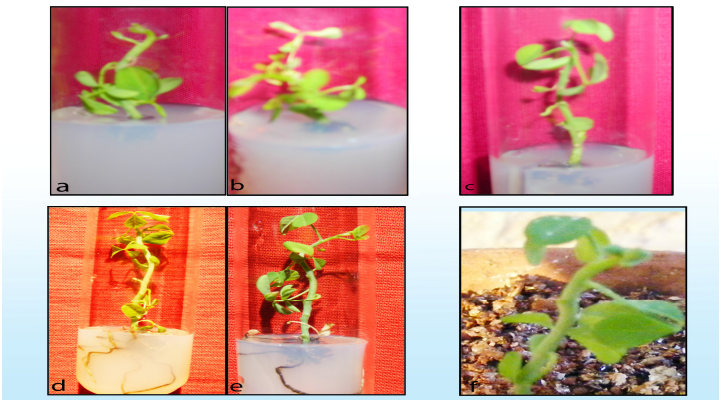

Plate1: *In vitro* propagation of *Crotalaria laburnifolia* L.

a&b: Shoot prolifiration, c: Elangation of shoot,
d&e : Root Initiation from excised shoot,
f: Hardened and acclimatized plant.

Fig 1. (a-f)

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