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Shoot Regeneration and Somaclonal Variation from Leaf Callus Cultures of *Plumbago zeylanica* Linn.

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Abstract: The morphogenetic potential of leaf callus cultures of *Plumbago zeylanica* was investigated to develop reliable protocols for shoot regeneration and somaclonal variation. Maximum callus proliferation was obtained Murashige and Skoog (MS) medium supplemented with 2.0 mg L^{-1} BAP. The maximum shoot regeneration (16.3±0.51) was achieved in five weeks when callus cultured on MS medium containing 0.75 mg L^{-1} BAP, 1.0 mg L^{-1} IAA and NAA each. Regenerated shoots were rooted on half strength MS medium supplemented with 0.5 mg L^{-1} NAA. The rooted plantlets were successfully established in soil. Calli derived from leaf explants cultured on MS medium fortified with 2.0 mg L^{-1} BAP, when subcultured on MS medium fortified with 2.0 mg L^{-1} BAP, 1.5 mg L^{-1} Kin and 1.0 mg L^{-1} NAA induced somaclonal variation.

Key words: *Plumbago zeylanica*, medicinal plants, shoot regeneration, somaclonal variation

INTRODUCTION

Plumbago zeylanica Linn. is belongs to the family plumbaginaceae. Their roots are the main source of plumbagin, an alkaloid used as anticancer drug (Krishnasamy and Purushothaman, 1980; Jayaraman, 1987; Lin *et al*., 2003). It is used as an irritant of the skin, in the treatment of rheumatism, piles, diarrhea and anasarca (Anonymous, 1989). Recently, antioxidant activity (Tilak *et al*., 2004) and anti allergic activity (Dai *et al*., 2004) reported from its stem and roots. There are a number of reports elucidating the chemical and pharmacological properties of *P. zeylanica*. However, the limited work done on *in vitro* studies include direct regeneration from leaf explants (Das and Rout, 2002) regeneration from callus culture (Rout *et al*., 1999b) and clonal propagation (Rout *et al*., 1999a). In tissue culture, somaclonal variation is often achieved through indirect organogenesis via callus production. This study describes an efficient method of indirect plant regeneration and somaclonal variation from leaf callus cultures of *P. zeylanica*.

MATERIALS AND METHODS

Plants of *P. zeylanica* were collected from Botany Field Research Laboratory, University of Madras, Chennai, India. Actively growing shoots were used as the explants source. Leaves taken from the apical region were surface sterilized with 70% ethanol for 60 sec and 0.1% mercuric chloride for 10 min, washed 3-4 times with sterile distilled water. Leaf discs 0.5-1.0 cm in length, were prepared and inoculated onto Murashige and Skoog (1962) medium. Basal medium supplemented with BAP (0.5-3.0 mg L^{-1}) were used for callus culture from leaf explants. The primary calli were separated from initial explants after 4 weeks and subcultured every 4 weeks. The calli were transferred onto MS medium supplemented with different concentration and combinations of BAP, IAA and NAA to induce shoot regeneration. For somaclonal variation, leaf callus were subcultured on MS medium supplemented with different concentration and combinations of BAP, Kin and NAA for 5 weeks and subcultured every 5 weeks. Individual shoots, which were 3-4 cm long were excised from the shoot clump and transferred to half strength MS medium containing NAA (0.1-2.0 mg L^{-1}).

All the media contained 0.8% agar and 3% sucrose, the pH was adjusted to 5.8 using 1 N NaOH or 0.1 N HCl and media were dispensed into culture vessels and autoclaved for 15 min at 121°C and a 16 h photoperiod provided by cool fluorescent tubes (3000 Lux). After 30 days rooted plantlets were taken out of culture tubes, washed thoroughly with sterile distilled water to remove agar and transplanted into plastic pots containing sterilized soil, sand and vermiculite (1:1:1). The potted plants were irrigated with MS basal salt solution (1/4 strength) devoid of sucrose and myo-inositol every 4 days for 3 weeks. After 3 weeks the plants were kept under shade for 2 weeks and then transferred into the field (Botany Field Research Laboratory, University of Madras, Chennai).

RESULTS AND DISCUSSION

A variable amount of callus produced from leaf explants when they were cultured in the presence of BAP. Callus initiation appeared, for leaves after 7-10 days and callus was light brown and friable. Callusing was best (89%) on medium supplemented with BAP 2.0 mg L^{-1} (Table 1). This was opposed to the reports by Rout *et al.* (1999a, b) who showed that there was no sign of callus formation when explants were cultured on media without auxin. Callus proliferation declined at lower concentrations of BAP and only small amounts of callus were obtained at higher concentrations of BAP. The primary calli were subcultured twice successively. Calli obtained in each subculture were tested further for shoot regeneration. In both the subcultures, the shoot regenerative ability of the callus was not changed significantly. The shoot primordial started appearing from the light brown friable callus obtained on medium containing BAP on passage to MS medium containing BAP, IAA and NAA. The maximum number of shoots (16.3±0.5) were regenerated from callus cultured on MS medium containing 0.75 mg L^{-1} BAP, 1.0 mg L^{-1} IAA and NAA and the percentage of shoot induction was 95±1.0 (Table 2 and Fig. 1). These results are better than recent report (Rout *et al.*, 1999b). Low frequency of regeneration of shoot buds was marked in the medium having higher concentration of BAP. Similar findings had been observed

Table 1: Effect of BAP on callus induction from leaf explants of *P. zeylanica*

Conc. (mg L^{-1}) BAP	Callus induction (%) Mean±SD
0.5	21.0±1.7d
1.0	52.1±1.8c
1.5	66.0±2.2b
2.0	89.7±1.7a
3.0	70.3±3.2b

Data followed by different letter(s) differ significantly within columns. The experiments were repeated twice, each experiment consisting of 25 replicates. Values represent the means±SD, Results were recorded after 30 days

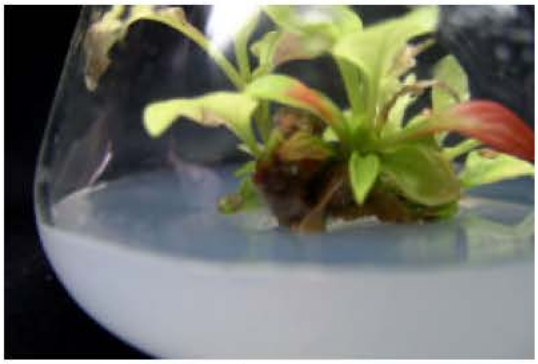
Fig. 1: Different stages of shoot regeneration from callus cultures of *P. zeylanica*

Table 2: Effect of PGRs on shoot regeneration from callus cultures of *P. zeylanica*

Conc. (mg L^{-1}) BAP +	IAA +	NAA	Shoot induction (%) Mean±SD	No. of shoots per calli Mean±SD
0.5	1.0	1.0	83.0±2.8b	7.6±0.8d
0.75	1.0	1.0	95.0±1.0a	16.3±0.5a
1.0	1.0	1.0	74.6±1.7c	12.3±1.6b
1.5	1.0	1.0	67.6±2.0d	9.0±1.0c
2.0	1.0	1.0	47.5±3.3e	4.1±1.3e
3.0	1.0	1.0	41.8±2.0e	3.0±1.0e

Data followed by different letters differ significantly within columns. The experiments were repeated twice, each experiment consisting of 25 replicates an average of 1.0 g callus. Values represent the means±SD, Results were recorded after 35 days

a



b

c

Fig. 2a-c: Different stages of somaclonal variation from callus cultures of *P. zeylanica*

Fig. 3: Rooting of *P. zeylanica*

Fig. 4: Hardening of *P. zeylanica*

Table 3: Effect of different concentrations and combinations of PGRs on somaclonal variation from callus cultures of *P. zeylanica*

Conc. (mg L^{-1}) BAP + Kin + NAA			Somaclonal variation (%) Mean±SD	No. of shoots per node Mean±SD
0.5	0.5	0.5	Callus	Callus
1.0	1.0	1.0	28.7±3.8c	1.9±0.1c
1.5	1.5	1.0	47.5±2.2b	3.3±0.5b
2.0	1.5	1.0	63.0±3.0a	5.0±1.0a

Data followed by different letter(s) differ significantly within columns. The experiments were repeated twice, each experiment consisting of 25 replicates an average of 1.0 g callus. Values represent the mean±SD, Results were recorded after 20 weeks

Table 4: Influence of half strength MS medium along with NAA on root induction

Conc. (mg L^{-1}) NAA	Root induction (%) Mean±SD	No. of roots per shoot Mean±SD
0.1	67.0±1.1c	7.1±0.8c
0.5	100a	12.5±1.9a
1.0	81.3±1.0b	9.5±1.2b
1.5	Callus + Root	Callus + Root
2.0	Callus	Callus

Data followed by different letter(s) differ significantly within columns. The experiments were repeated twice, each experiment consisting of 25 replicates. Results were recorded after 30 days

by Rout *et al.* (1999b). Somaclonal variation was induced from callus cultured on MS medium with BAP, Kin and NAA. The highest frequency of somaclonal variation was 63% on MS medium with 2.0 mg L^{-1} BAP, 1.5 mg L^{-1} Kin and 1.0 mg L^{-1} NAA. The variation may be due to plant growth regulators and number of subcultures. The frequency of somaclonal variation was below 63% and the mean number of somaclonal variant per gram of callus was below 5.0±1.0 on MS medium with low concentrations of PGRs (Table 3 and Fig. 2a- c). Somaclonal variation among regenerated plants from callus has been suggested as a useful source of potentially valuable germplasm for plant breeding and plant improvement. The diverse variation characteristic of somaclones highlights the fact that somaclonal variation may be an additional tool for crop improvement rather than interesting scientific phenomenon (Jain *et al.*, 1998 and Pareek, 2001). Regenerated shoots were transferred to half strength MS medium without growth regulator and did not show any root induction. Shoots (100%) rooted on half strength MS medium supplemented with 0.5 mg L^{-1} NAA with in 30 days (Table 4 and Fig. 3). At higher concentrations of NAA rooting was inhibited and callusing. Similar results observed by Rout *et al.* (1999b). Rooted plantlets were transferred to the field with 100% of survival rate (Fig. 4), which was an improvement over 90% survival obtained by Rout *et al.* (1999b).

An efficient protocol for plant regeneration and somaclonal variation from callus of *P. zeylanica* described in this report. However, the mechanism of somaclonal variation of regeneration plantlets of *P. zeylanica* deserves a through biological analysis going far beyond the present study.

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