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Cytokinin induced multiple shoot induction from node explants of *Daemia extensa* (Jacq.) R.Br – A potentially important medicinal plant

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In vitro shoot and multiple shoot induction was achieved in one of the important medicinal plants of Asclepiadaceae family, *Daemia extensa* (Jacq.) R.Br., which has been historically been used to treat a wide assortment of diseases. Murashige and Skoog (1962) medium supplemented with 1.0 mg/L BAP was found to be optimum to induce shoots (100 %) directly from the node explants. Significant increase in the number of shoots per explant was found in MS medium supplemented with 1.0 mg/L BAP and 15 mg/L adenine sulphate. All the tested combinations have little effect on increasing the number of shoots. The present study established reliable and reproducible protocol for rapid multiple shoot induction from node explants of *Daemia extensa* using different concentrations and combinations of cytokinins.

Key words: Veliparutthy, Micropropagation, Shoots, Benzyl amino purine, Adenine sulphate, *Daemia extensa*.

INTRODUCTION

Daemia extensa (Jacq.) R.Br, commonly known as veliparutthy is a slender foul smelling perennial milky twining herb belonging to the family Asclepiadaceae. The plant is distributed in warm regions with temperate climates. The plant is astringent, thermogenic, ementic, expectorant, emmenagogue, antihelminthic and laxative. The whole plant is useful in urothrorrhoea, inflammations, asthma, amenorrhoea and leucoderma. The plant extract is useful in uterine and menstrual disorders and in facilitating parturition. Tissue culture techniques are now becoming popular as alternative means of vegetative propagation. Micropropagation involves multiplication of genetically identical individuals by asexual reproduction within a short span of time with tremendous potential for the production of high quality plant based medicines (Murch *et al*., 2000). The advantage with micropropagation is most of the *in vitro* propagated plants of many important medicinal species were found to be uniform, showing less variation in the secondary metabolite content than their wild counterparts (Yamada *et al*., 1991).

Several workers in past have micropropagated some of the important Asclepiadaceae members such as *Ceropegia bulbosa* (Patil, 1998; Britto *et al.*, 2003)), *Hemidesmus indicus* (Misra *et al*., 2003; Patnaik and Kishore, 1996) and *Holostemma ada-kodien* (Martin, 2002, 2003). Since very scarce information is available about micropropagation about this important medicinal plant, an attempt was made to develop a reproducible protocol for shoot and multiple shoot induction from nodal explants of one of the tissue culture recalcitrant medicinal plants of Asclepiadacea family,*Daemia extensa* (Jacq.) R.Br. using various concentrations of benzyl amino purine and adenine sulphate.

MATERIALS AND METHODS

Collection of explants:

Node explants were collected from healthy shoot materials of *Daemia extensa* from different places in Alagappa University, Karaikudi. Random survey was conducted to choose healthy plants to collect suitable explants for culture initiation.

Culture medium:

MS (Murashige and Skoog, 1962) medium supplemented with different concentrations of benzyl amino purine (BAP) (0.1 – 1.2mg / L were used for shoot induction. For multiple shoot induction MS medium supplemented with 1.0mg /L BAP and 5 – 20 mg /L adenine sulphate were used. The pH of all media was adjusted to 5.75 before adding 0.8% agar. About 40 ml of moltened media was dispensed in to Magenta boxes (Sigma, St.Louis, USA) and autoclaved at 15Ib and 121° C for 18 min. All the media were kept at 26 ± 2° C for 3 days before use.

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In vitro shoot induction:
The shoot segments after removing the leaves were cut into 2 cm pieces, each containing a single node region and washed under running tap water for 15 min, followed by brief washing with sterile distilled water. Node explants (1.25 cm) were surface sterilized in 70 % (v/v) ethanol for 60 sec followed by 0.1%(w/v) mercuric chloride for 6 min. Explants were thoroughly washed in sterile distilled water and blot dried on sterile Whatmann 1 mm filter paper.

For shoot induction, nodal explants were again trimmed into 1.0 cm and transferred to MS medium supplemented with 0.1 – 1.0 mg /L BAP. Cultures were incubated at 26 ± 2° under a 16/8 h photoperiod for 26 – 28 days at a relative humidity of 65%.

Multiple shoot induction:
Node explants (1.0 cm long) were used as explants for multiple shoot induction on MS medium fortified with 1.0 mg/L benzyl amino purine and 5 – 20 mg/L adenine sulphate. After two weeks of culturing at 26 ± 2° under a 16/8 h photoperiod shoots were subcultured onto fresh medium for proliferation.

All the experiments were repeated thrice (each with 15 explants) and the response was scored after 26 – 28 days of culture initiation. The data pertaining to mean percentage of culture showing response, number of shoots per explants and shoot length were scored and presented in mean ± SE.

RESULTS AND DISCUSSION

The results scored on the above-mentioned aspects (shoot and multiple shoot induction) are summarized in the following order.

In vitro shoot induction :
In order to assess the effect of different concentrations of benzyl amino purine (0.1 - 1.2 mg/L) on shoot induction from nodal explants, explants were surface sterilized and inoculated onto MS media supplemented with various concentrations of benzyl amino purine. Shoot induction was monitored after 24-28 days of inoculation by counting the number of shoots induced from each explant. Shoot induction was observed in all the concentrations of benzyl amino purine tested with variation in per cent response of shoot induction. The highest per cent of shoot induction (99.4) was observed in MS with 1.0 mg/L benzyl amino purine followed by 80.4 and 80.2 in the medium containing 0.8 and 0.7 mg/L benzyl amino purine respectively (Table 1). The number of shoots produced from nodal explants on medium with 1.0 mg/L BAP was 3.8 with an average height of 2.5cm (Figure 1.A). We found an increase in the per cent response of shoot induction and number of shoots with an increase in the concentration of benzyl amino purine from 0.1 mg /L to 1.2. The percentage of explants exhibiting shoot induction was found to be between 40 – 80 in most of the concentrations of benzyl amino purine tested except MS medium supplemented with 1.0 mg /L benzyl amino purine. After 26 – 28 days of culture, nodal explants derived shoot cultures were subcultured to MS medium fortified with same concentration of hormone for shoot elongation. Significant elongation has been achieved in medium with 0.8 and 1.0 mg/L benzyl amino purine. There was no significant variation in shoot length between the different concentrations of benzyl amino purine except in the case of medium with 0.2 mg/L producing average shoot length of 2.74 cm (Table 1). The shoots subcultured to fresh medium with same concentration of benzyl amino purine proliferated additional 3 – 4 shoots after 26 days of culture.

In general, the nodal explants cultured on medium with benzyl amino purine developed pale yellow intermediate callus at the basal portions due to the accumulation of auxins at the basal cut ends (Figure 1.A). The effect of benzyl amino purine in inducing shoot induction was already reported in some of the important medicinal plants of Asclepiadaceae family members such as *Ceropegia bulbosa* (Patil, 1998; Britto *et al.*, 2003)), *Gymneme elegans* (Komalavalli and Rao, 2000) and in *Holostemma ada-kodien* (Martin, 2002). The promotive effect of benzyl amino purine on shoot induction and multiplication was well understood in various plants like *Phytolocca decanta* (Demeke and Huges, 1990), *Saussuriea lappa* (Arora and Bhojwani, 1989), *Clerodendran colebrookianum* (Mao *et al.*, 1995), *Trichopus zeylanicus* (Krishnan *et al*., 1995) and in *Woodfordia fruticosa* (Krishnan and Seeni, 1994).

Reculture:
To analyze the shoot induction ability of nodal explants from in vitro multiplied plants, nodal explants were used as an ideal source of explants for reculturing. Additional 2-3 shoots per node explants on MS medium fortified with 1.0 mg/L indicate the effectiveness of explants on multiple shoot induction without surface sterilization (Figure 1.B). A similar effect of the hormone in enhancing shoot induction has been reported in one of the Asclepiadaceae family members, *Ceropegia candelabrum* (Beena *et al.*, 2003). As expected, contamination rate has been drastically reduced in recultured nodal explants. The beneficial effect of stem nodal explants recultured from in vitro propagated shoots has been well documented in *Woodfordia fruticosa* (Krishnan and Seeni, 1994).

Table 1: Effect of benzyl amino purine on direct shoot induction from nodal explants of *Daemia extensa* (Jacq.) R.Br.

S.No	BAP concentration (mg / L)	% of explants for direct shoot induction (Mean ± SE)	No of shoots produced per explant (Mean ± SE)	Shoot length (cm) (Mean ± SE)
1	0.0	--	--	--
2	0.1	79.2 ± 0.41	1.22 ± 0.41	1.88 ± 0.41
3	0.2	40.6 ± 0.15	1.22 ± 0.41	2.74 ± 0.08
4	0.3	39.8 ± 0.41	1.44 ± 0.41	1.3 ± 0.41
5	0.4	60.4 ± 0.41	22 ± 0.41	1.76 ± 0.08
6	0.5	39.4 ± 0.35	1.88 ± 0.45	1.56 ± 0.07
7	0.6	59.2 ± 0.35	1.8 ± 0.45	1.86 ± 0.04
8	0.7	80.2 ± 0.45	2.2 ± 0.35	1.7 ± 0.04
9	0.8	80.4 ± 0.35	2.2 ± 0.35	2.02 ± 0.04
10	0.9	79.8 ± 0.35	1.8 ± 0.40	2.06 ± 0.04
11	1.0	99.4 ± 0.35	3.8 ± 0.25	2.5 ± 0.04
12	1.1	69.8 ± 0.25	1.8 ± 0.25	2.1 ± 0.04
13	1.2	60.0 ± 0.41	1.6 ± 0.25	2.09 ± 0.04

Table 2 : Effect of benzyl amino purine (1.0 mg /L) and adenine sulphate on multiple shoot induction from nodal explants of *Daemia extensa* (Jacq.) R.Br.

S.No	Adenine sulphate concentration (mg / L)	% of explants for direct shoot induction (Mean ± SE)	No of shoots produced per explant (Mean ± SE)	Shoot length (cm) (Mean ± SE)
1	0.0	--	--	--
2	5.0	98.4 ± 0.35	6.80 ± 0.35	2.56 ± 0.04
3	10.0	98.6 ± 0.45	6.80 ± 0.35	2.74 ± 0.08
4	15.0	98.2 ± 0.45	11.6 ± 0.35	2.10 ± 0.04
5	20.0	98.4 ± 0.45	9.8 ± 0.35	1.76 ± 0.08

In vitro multiple shoot induction:

In the present study, adenine sulphate when used in combination with benzyl amino purine induced multiple shoots. Among the combinations tested, benzyl amino purine (1.0) with 15mg/L adenine sulphate produced maximum number of shoots with intermittent callus at the basal cut end. (Figure 1.C). Of the various concentrations of adenine sulphate tested, 15 mg resulted in maximum number of shoots (11.6) followed by 20mg/ L (9.8), 10mg/L (6.5) and 5mg/L (6.8) (Table 2). Average number of shoots generated per explant on medium with 1.0mg/L benzyl amino purine and 15mg/L adenine sulphate is an improvement of almost 3 fold in the multiplication rate as compared with shoots induced on MS medium with 1.0 mg/L benzyl amino purine alone. Addition of 5 and 10mg/L adenine sulphate had no significant effect on number of shoots produced per explant. The presence of adenine sulphate initiated friable callus and suppress the formation of new shoots. A similar observation was reported in *Hemidesmus indicus* using 5 – 20 mg/L adenine sulphate with benzyl amino purine and naphthalene acetic acid (Misra *et al*., 2003). The present study identified the concentrations of cytokinins like benzyl amino purine and adenine sulphate on *in vitro*

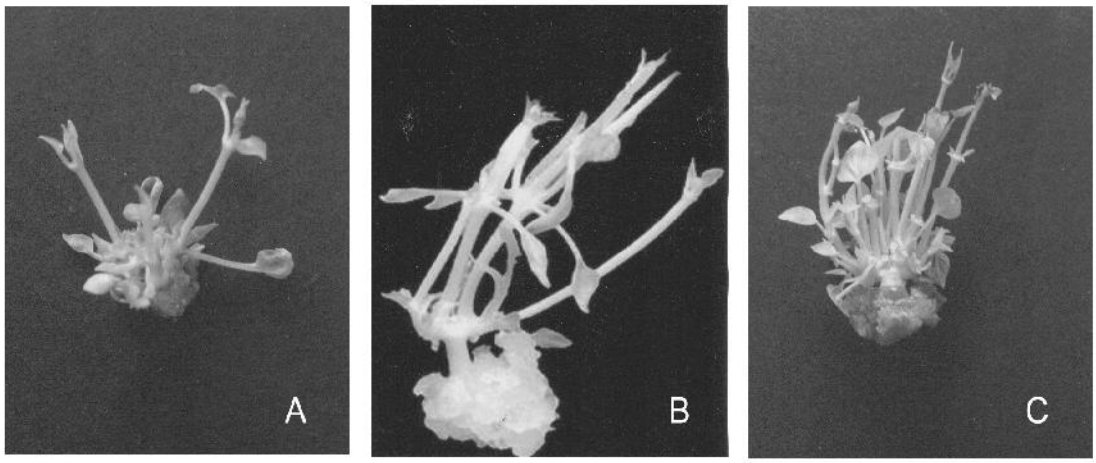

Fig. 1 : In vitro shoot and multiple shoot induction from nodal explants of Daemia extensa.
A) Shoot induction from cultured nodal explants on MS medium supplemented with 1.0 mg/L benzyl amino purine.
B) Reculture of nodalexplants excised from in vitro multiplied shoots proliferating additional shoots.
C) Multiple shoot induction from nodal explants on MS medium supplemented with 15 mg./L adenine sulphate.

shoot and multiple shoot induction from nodal explants of one of the important medicinal plants of Asclepiadaceae family.

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