An efficient plant regeneration system through callus for *Pseudarthria viscida* (L.) Wright and Arn., a rare ethnomedicinal herb

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Abstract The purpose of this study was to develop a protocol to induce high frequency of callus and subsequent plantlet regeneration for *Pseudarthria viscida*; an important medicinal plant. The cotyledonary node and young leaf pieces (1×0.5 cm, length $\times$ breadth) were used as explants for callus induction and subsequent shoot regeneration and adventitious roots induction from the shoots. The best results were achieved on the following media: (1) 96 % callus induction from cotyledonary node explants on MS medium supplemented with 1.5 mg l^{-1} 2, 4 dichlorophenoxyacetic acid (2, 4-D) and 0.5 mg l^{-1} 1-naphthalene acetic acid (NAA), (2) 97 % shoot regeneration from cotyledonary node derived calli with an average of 44.9 shoots per explant on MS medium fortified with 3.0 mg l^{-1} N_6 -benzylaminopurine (BA) and 1 mg l^{-1} NAA, (3) 98 % rooting with an average number of 3.3 roots per shoot on MS medium containing indole-3-butyric acid (IBA) or NAA ($0.5\text{--}4 \text{ mg l}^{-1}$) after 45 days. The plantlets were transferred to the field after acclimatization. Of the 40 plantlets transplanted to the soil, 29 survived (72.5 %).

Keywords Callus · Cotyledonary node · Ethnomedicinal · *Pseudarthria viscida* · Red-listed plant

Abbreviations

BA	N_6 -benzylaminopurine
2, 4-D	2, 4- dichlorophenoxyacetic acid
GA_3	Gibberellic acid
IBA	Indole-3-butyric acid

Kn	Kinetin
MS medium	Murashige and Skoog medium
NAA	1-naphthalene acetic acid

Introduction

Pseudarthria viscida is a valuable ethomedicinal plant of Papilionaceae. It is a perennial shrub with slender branches, trifoliate leaves and is one among the “Dasamoola” (10 roots) of Ayurveda, mentioned in most of the ancient scriptures. It is a major component in many Ayurvedic preparations like Dasamoolarishta, Agastya haritaki rasayana, Anu thaila, Brahma rasayana, Sudarshana churna, Narayana thaila and Dhanuanthara ghrita (Ansari and Jeeja 2006; Warriar et al. 1996).

The whole plant is used to treat a vast range of diseases; fever, rheumatism, bronchial asthma, hemorrhoids (Nadkarni 1982; Warriar et al. 1996) and diabetes mellitus (Shaw et al. 2004; Masirkar et al. 2008; Rajendran et al. 2010). As it effectively arrests bleeding and alleviates edema, it is a valuable panacea for heart diseases and blood disorders (Rajendran et al. 2007). Other properties like antihypertensive (Klaus et al. 1995), antioxidant (Mathew and Sasikumar 2007; Vijayabaskaran et al. 2010a), antiulcer (Babu et al. 2008), antifungal (Deepa et al. 2004), antidiarrheal (Rajendran et al. 2007; Vijayabaskaran et al. 2010b) and antitumor (Vijayabaskaran et al. 2007; Rajendran et al. 2010) have also been reported from *P. viscida*.

Since *P. viscida* has high commercial and medicinal values, it has been included in the group of high trade sourced medicinal plants from Western Ghats (Ved and Goraya 2007) and is also incorporated in the 100-Red listed medical plants of conservation concern (Ravikumar and

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Ved 2000). The excessive collection has resulted in gradual disappearance of this plant from its natural habitat and at present its number is highly reduced in the wild.

Despite its vast economic and pharmaceutical potential, this taxon has not been cultivated for commercial purposes due to low seed viability, germination rate and high mortality of seedlings in young stages (Oommen et al. 2000). To conserve the genetic stocks of this plant in vitro method of propagation can be utilized successfully. There is only a single report on in vitro studies of *P. viscida* (Vinothkumar et al. 2009), but it is of preliminary nature. The present work was carried out with the objective of inducing calli from cotyledonary node and immature leaf explants of *P. viscida*, shoot regeneration, rooting of shoots and transfer of plantlets to natural habitat.

Materials and methods

Plant material and culture initiation

The mature seeds of *P. viscida* were collected from the plants maintained in the green house of the botanical garden. Because of hard seed coat, the healthy seeds were immersed in 50 % sulphuric acid for 20 min. The seeds were washed thoroughly with distilled water and then soaked in distilled water for 12 h. The soaked seeds alone were selected for further experiments and washed with Teepol solution for 5 min and Bavistin 1 % for 10 min and then rinsed seeds in distilled water. The seeds were again surface sterilized with 20 % v/v sodium hypochlorite (NaOCl) for 5 min followed by 3 rinses in distilled water. The seed coat was removed just before inoculation in sterilized culture tubes containing MS basal medium (Murashige and Skoog 1962).

Callus initiation

The cotyledonary node (1.0–1.5 cm) was excised from 7-day-old germinating seedling after removing the apical meristem and the radicle portion. Immature leaf was isolated from 3-week-old plantlets and cut into small pieces each measuring 1×0.5 cm (length × breadth). Both the explants were cultured on MS medium supplemented with 2, 4-D (0.5–2.0 mg l⁻¹) or GA₃ (0.5–2.0 mg l⁻¹) alone or in combination with NAA (0.5 mg l⁻¹). The calli were maintained on MS medium supplemented with 2, 4-D (1.5 mg l⁻¹) and NAA (0.5 mg l⁻¹).

Plant regeneration, rooting and field transfer

The calli obtained from cotyledonary node and immature leaf piece was employed for shoot regeneration assessment on MS

medium supplemented with various concentrations of BA or kinetin (1.0–4.0 mg l⁻¹) alone or in combination with NAA (0.5 or 1.0 mg l⁻¹). The number of explants with shoot buds and average shoot length was scored after 45 d. For shoot elongation, low concentration of GA₃ (0.5 mg l⁻¹) was added in the subculture medium. Shoots above 2.5 cm were transferred to rooting medium containing ½ strength MS medium supplemented with different concentrations of IBA or NAA (1.0–4.0 mg l⁻¹).

The rooted shoots were washed thoroughly in sterile distilled water to remove traces of agar. The plantlets were transferred to small plastic cups (6.0 cm diameter) containing sterilized garden soil and sand in 1:1 ratio. The cups were covered with polythene bags to retain 70–80 % relative humidity and maintained at 25±2 °C with 16 h/d photoperiod for 4 weeks in culture room. The plants were then transferred to shade for 3 weeks and eventually to field.

MS medium supplemented with 30 gm/l sucrose and 8 gm/l agar was used as basal medium and the pH was adjusted to 5.8 prior to autoclaving at 1.06 kg/cm² and 121 °C for 20 min. The culture tubes were incubated at 25±2 °C under 16 h photoperiod with a photosynthetic photon flux density of 60 μmol m⁻² s⁻¹ supplied by Philips TL 40 W fluorescent tubes. Each treatment consisted of at least

Table 1 Effect of 2, 4-D, GA₃ alone or in combination with NAA on callus induction from cotyledonary node and leaf explants of *P. viscida*. Medium: MS; Culture period: 45 days

Plant growth regulators (mg l ⁻¹)			Percent explants forming callus ^a	
GA ₃	2, 4-D	NAA	Cotyledonary node	Leaf
0.0	0.0	0.0	0.0	0.0
0.5			38±2.8e	31±2.2d
1.0			44±3.1d	43±4.3c
1.5			57±3.6d	48±2.7c
2.0			61±4.2c	44±3.4c
	0.5		41±3.8d	34±3.7d
	1.0		56±4.2d	47±4.3c
	1.5		69±4.1c	48±4.0c
	2.0		61±4.2c	46±3.5c
0.5		0.5	74±2.8b	48±3.3c
1.0		0.5	81±3.4b	52±4.2c
1.5		0.5	85±4.5b	56±5.2c
2.0		0.5	80±4.6b	49±4.8c
	0.5	0.5	78±3.5b	69±4.6b
	1.0	0.5	89±4.1b	73±3.2b
	1.5	0.5	96±3.2a	78±4.3a
	2.0	0.5	87±4.6b	71±3.6b

^a The values represent the means (±SE) of three independent experiments. At least 10 cultures were raised for each experiment. Mean values within a column followed by the same letter are not significantly different by Duncan's multiple range test ($P \geq 0.05$)

24 tubes and all experiments were repeated 3 times. Data were subjected to analysis of variance (ANOVA) and comparisons of means were carried out with Duncan's multiple range test at 0.05 % significance.

Results

Induction of callus

Callus regeneration from cotyledonary nodes and leaf explants was evaluated. 2, 4-D was comparatively better than

GA₃ when employed individually in the medium (Table 1). 2, 4-D at 1.5 mg l⁻¹ produced optimum result when added individually in the medium. Here, 69 % and 48 % explants produced callus from cotyledonary node and leaf explants respectively. The addition of 0.5 mg l⁻¹ NAA with 2, 4-D and GA₃ significantly improved the percentage response as well as the rate of callus induction. Optimum response was observed on MS medium supplemented with 1.5 mg l⁻¹ 2, 4-D and 0.5 mg l⁻¹ NAA. The frequency of callus induction on this medium was 96 % and 78 % in cotyledonary node and leaf explants respectively (Table 1). Comparatively, cotyledonary node responded better than leaf explants.

Table 2 Effect of growth regulators on shoot regeneration from cotyledonary node and leaf derived callus of *P. viscida*. Medium: MS; Culture period: 45 days. CN-cotyledonary node, LE-Leaf explants

Plant growth regulators (mg l ⁻¹)			Percent response ^a		Average no. of shoots ^a		Average length of shoots (cm) ^a	
BA	KN	NAA	CN	LE	CN	LE	CN	LE
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5	–	–	18±3.2g	13±1.8h	5.4±0.3h	5.3±0.7f	1.4±0.6a	1.3±0.3a
1.0	–	–	22±3.5f	16±2.2g	6.0±0.5h	5.5±0.5f	1.2±0.6a	1.6±0.6a
2.0	–	–	27±2.8f	19±3.2g	7.4±0.7h	6.2±0.5f	1.6±0.4a	1.1±0.2a
3.0	–	–	43±4.3e	21±3.6f	9.3±0.2f	6.7±0.7e	1.4±0.3a	1.3±0.6a
4.0	–	–	28±3.7f	20±4.2f	8.1±0.2g	5.3±0.6f	1.5±0.7a	1.1±0.6a
0.5	–	0.5	70±4.2c	56±2.9d	7.4±0.8g	5.8±0.7g	1.2±0.3a	1.8±0.1a
1.0	–	0.5	77±4.1c	68±3.9c	8.0±0.9g	6.3±0.8f	1.4±0.5a	1.3±0.3a
2.0	–	0.5	79±3.9c	71±3.6b	8.4±0.7g	6.9±0.8f	1.8±0.8a	1.6±0.2a
3.0	–	0.5	82±3.4b	73±4.0b	12.3±0.8e	8.4±0.9e	1.4±0.7a	1.9±0.4a
4.0	–	0.5	76±3.7c	63±3.8c	9.1±0.9f	6.2±0.7f	1.7±0.3a	1.3±0.8a
0.5	–	1.0	72±3.3c	57±2.9d	12.8±0.8e	8.3±0.9e	1.6±0.5a	1.4±0.4a
1.0	–	1.0	82±3.4b	65±4.3c	18.9±0.7c	9.3±0.3d	1.9±0.7a	1.6±0.2a
2.0	–	1.0	89±3.5b	76±3.9b	22.5±0.5b	14.8±0.6b	1.4±0.8a	1.9±0.3a
3.0	–	1.0	97±4.2a	82±2.8a	44.9±0.8a	18.8±0.8a	1.7±0.5a	1.8±0.6a
4.0	–	1.0	87±2.3b	60±2.7c	17.5±0.6d	12.1±0.2c	1.9±0.2a	1.9±0.3a
–	0.5	–	23±2.8f	16±3.0g	5.7±0.3h	5.1±0.4f	1.7±0.3a	1.7±0.5a
–	1.0	–	27±3.7f	19±3.8g	6.3±0.3h	5.5±0.6f	1.2±0.2a	1.3±0.6a
–	2.0	–	39±3.5e	26±2.9f	10.3±0.6f	5.2±0.6f	1.5±0.5a	1.1±0.4a
–	3.0	–	35±2.4e	22±3.9e	7.9±0.6h	5.7±0.7f	1.6±0.5a	1.5±0.3a
–	4.0	–	29±3.7f	20±3.9f	8.4±0.7f	5.6±0.4f	1.2±0.2a	1.2±0.3a
–	0.5	0.5	68±4.1d	52±2.8d	6.4±0.4h	5.2±0.3g	1.9±0.8a	1.6±0.5a
–	1.0	0.5	71±4.0c	55±3.8d	8.6±0.7g	7.7±0.6f	1.7±0.4a	1.6±0.5a
–	2.0	0.5	79±3.6c	58±3.8d	12.7±0.3e	8.4±0.8e	1.1±0.7a	1.7±0.3a
–	3.0	0.5	73±2.7c	62±2.8c	10.8±0.8f	6.7±0.9f	1.7±0.6a	1.6±0.2a
–	4.0	0.5	67±3.2d	53±3.5d	9.6±0.9f	6.2±0.5f	1.1±0.5a	1.2±0.4a
–	0.5	1.0	74±2.9c	66±3.2c	9.6±0.9f	7.8±0.5f	1.1±0.5a	1.2±0.2a
–	1.0	1.0	79±2.8c	72±2.8b	12.7±0.7e	9.6±0.2d	1.6±1.1a	1.4±0.3a
–	2.0	1.0	84±3.6b	78±3.4b	19.6±0.9c	13.8±0.5b	1.1±0.5a	1.2±0.5a
–	3.0	1.0	77±2.9c	68±2.8c	16.7±0.7d	10.6±0.2d	1.6±1.1a	1.4±0.3a
–	4.0	1.0	63±3.7d	54±4.6d	12.8±0.8e	9.3±0.5d	1.9±0.9a	1.7±0.4a

^a The values represent the means (±SE) of three independent experiments. At least 10 cultures were raised for each experiment. Mean values within a column followed by the same letter are not significantly different by Duncan's multiple range test ($P \geq 0.05$)

The cotyledonary node elongated initially to a length of 1.5–2.0 cm and produced fine, light yellowish friable callus at the basal cut end. The callus growth was profuse and within 3–4 weeks plenty of callus was produced. The cultured leaf developed callus at some areas of cut end with less intensity and later they developed calli all through the surface of the explant. Both calli were friable and physically similar. The calli were maintained on MS medium supplemented with 2, 4-D (1.5 mg l^{-1}) and NAA (0.5 mg l^{-1}) after 6 weeks. When subcultured, further profuse callus growth was observed.

Shoot regeneration from callus

For regeneration various concentrations of BA (0.5 – 4.0 mg l^{-1}) or Kn (0.5 – 4.0 mg l^{-1}) alone were tried. The percentage of regeneration was however, low. There was no regeneration on control medium. Of the two cytokinins, BA was comparatively better in terms of both percent response and average number of shoots per explant. Optimum response was observed on MS medium supplemented with 3 mg l^{-1} BA, 43 % and 21 % cultures responded with an average shoot number of 9.3 and 6.7 from the cotyledonary node and leaf derived callus, respectively (Table 2). The Kn concentration which produced optimum response was 2 mg l^{-1} . On this medium 39 % and 26 % cultures responded with an average number of 10.3 and 5.2 shoots per culture in cotyledonary node and leaf derived callus respectively.

The presence of low concentrations of NAA with BA or Kn resulted in further enhancement of the response and formation of more shoots during organogenesis from the callus. Hence for shoot regeneration, the callus from both explants were cultured separately on MS medium fortified with BA (0.5 – 4.0 mg l^{-1}) or Kn (0.5 – 4.0 mg l^{-1}) and NAA (0.5 and 1.0 mg l^{-1}). Of the two concentrations of NAA tried along with BA and Kn, 1.0 mg l^{-1} was optimal. MS medium containing 1.0 mg l^{-1} NAA and 3.0 mg l^{-1} BA was the most efficient plant growth regulator for shoot induction. On this medium 97 % and 82 % cultures responded with an average number of 44.9 and 18.8 shoots per explants in cotyledonary node and leaf derived callus, respectively (Table 2). In case of average length of shoots there was no significant difference among the various treatments. There was no shoot regeneration on control medium.

The callus cultured on MS medium with BA (0.5 – 4.0 mg l^{-1}) or Kn (0.5 – 4.0 mg l^{-1}) alone exhibited initial sign of shoot initiation after 4 weeks of culture. However, the addition of NAA (0.5 – 1.0 mg l^{-1}) resulted in rapid and enhanced regeneration frequency (Fig. 1a–d). The shoot bud regeneration was initiated within 2 weeks of culture (Fig. 1a–d). Vast number of adventitious shoots were produced from the surface of the callus within 2 weeks of

culture. Shoots further developed into vigorous plantlets within a span of 4–6 weeks of culture (Fig. 1e, f). Most of the shoots reached a size of above 1.0 cm height in about 6 weeks of time (Fig. 2a–b). However, some shoots remained less than 1.0 cm in height due to the excessive number of shoots (Fig. 2b). These shoots were excised and subcultured on MS medium supplemented with GA_3 (0.5 mg l^{-1}) for shoot elongation. On this medium the shoots reached an average height of 2.8 cm in 4 weeks.

Fig. 1 Various stages of plant regeneration from cotyledonary node and leaf derived callus of *P. viscida*. **a** Initiation of shoots from cotyledon derived callus on MS medium supplemented with 3.0 mg l^{-1} BA and 0.5 mg l^{-1} NAA. After 1 week of culture. Several minute shoot buds originated on the outer surface of the callus. Bar=0.7 cm. **b** Same as in **a** after 2 weeks of culture. The intensity of shoot buds on callus surface has increased further. Bar=0.5 cm. **c** Regenerated shoot after 3 weeks of culture of leaf derived callus on MS medium supplemented with 3.0 mg l^{-1} BA and 0.5 mg l^{-1} NAA. The number of shoots was low as compared to the cotyledonary node derived callus. Bar=0.5 cm. **d** Same as in **c** but the callus is from cotyledonary node explants. Some shoot buds reached a size of about 0.5 cm in height. Bar=0.7 cm. **e** The regenerating leaf derived callus on MS medium supplemented with 3.0 mg l^{-1} BA and 0.5 mg l^{-1} NAA after 4 weeks of culture. Bar=0.8 cm. **f** Same as in **e** but the callus was derived from cotyledonary node explants. Bar=0.8 cm

Fig. 2 Plant regeneration, rooting of shoots and acclimatization in *P. viscida*. **a** Shoot development from leaf derived callus on MS medium supplemented with 3.0 mg l^{-1} BA and 0.5 mg l^{-1} NAA. Bar=1.0 cm. **b** Shoot regeneration from cotyledonary node derived callus on MS medium supplemented with 3.0 mg l^{-1} BA and 0.5 mg l^{-1} NAA 45 days after culture. Several shoots were emerged from the callus. Bar=1.1 cm. **c** A rooted shoot on MS medium fortified with IBA (2.0 mg/l). The roots emerged from the cut end of the shoots without any callus. Bar=1.1 cm. **d** A plantlet just before transplantation to soil. The main root and its several branches are visible. Bar=1.0 cm. **e** Two transplanted plants 2 months after transfer to soil. The new leaves were already developed on the shoot. Bar=1.8 cm

Rooting and field transfer of the shoots

Our preliminary experiments with MS medium supplemented with auxins showed that full strength MS was not suitable for rooting since it promoted growth of callus from the cut end of the shoots (Data not shown). Therefore, half

MS medium fortified with various concentrations of IBA ($1.0\text{--}4.0 \text{ mg l}^{-1}$) or NAA ($1.0\text{--}4.0 \text{ mg l}^{-1}$) was used. Half strength MS medium without auxins yielded no results. Among the two auxins tested, IBA was comparatively better than NAA. Roots initiated within 2 weeks in IBA containing medium whereas it took 4 weeks in NAA

Table 3 Influence of IBA and NAA on rooting of in vitro formed shoots on half strength MS medium after 45 days

Auxins (mg l^{-1})		Percent rooting ^a	Average no. of roots/ shoot ^a	Average root length ^a
IBA	NAA			
0.0	0.0	0.0	0.0	0.0
1.0		90±4.3b	2.5±0.6b	3.1±0.15a
2.0		98±3.9a	3.3±0.3a	3.6±0.4a
3.0		87±6.4c	2.8±0.8b	2.1±0.2b
4.0		81±5.2c	2.6±0.3b	1.9±0.7c
	1.0	71±5.7d	1.7±0.6c	1.7±0.7c
	2.0	78±4.8d	1.4±0.3c	2.6±0.7b
	3.0	81±7.7c	1.7±0.7c	2.9±0.6b
	4.0	73±6.5d	1.3±0.5c	2.1±0.8b

^aThe values represent the means ($\pm$ SE) of three independent experiments. At least 10 cultures were raised for each experiment. Mean values within a column followed by the same letter are not significantly different by Duncan's multiple range test ($P \geq 0.05$)

medium. Optimum rooting was obtained on 2.0 mg l^{-1} IBA. 98 % cultures responded with an average number of 3.3 ± 0.1 roots per shoot (Fig. 2c, d; Table 3). Plantlets with roots were transplanted to plastic cups (Fig. 2e) and hardened plants eventually shifted to clay pots. Of the 40 plantlets transplanted to the soil, 29 survived (72.5 %). The in vitro derived plants did not show any visual phenotypic variation from the parental plants.

Discussion

In the present study two explants, viz. cotyledonary node and immature leaf, were used to induce callus. Of the two, the cotyledonary node showed better result (both in terms of percent response and amount of callus) than immature leaf. The superiority of cotyledonary node as an explant in micropropagation has been documented in other plants such as *Dalbergia sissoo* (Pradhan et al. 1998), *Acacia sinuata* (Vengadesan et al. 2002) and *Tamarindus indica* (Muhammad and Amusa 2003). The explant dependent callus proliferation and inter-explant variability is a common phenomenon usually observed during the callus induction in some systems (Arockiasamy and Ignacimuthu 1998; Pereira et al. 2000; Dhar and Joshi 2005; Thomas and Maseena 2006).

Regardless of cytokinin type and concentration, the amount of callus produced increased significantly when 0.5 mg l^{-1} NAA was incorporated in the medium. Maximum callus was obtained on MS + 1.5 mg l^{-1} 2, 4-D and 0.5 mg l^{-1} NAA. 2, 4-D is a potent auxin which either alone or in combination with a cytokinin induces vigorous callusing in various plant systems (Thomas and Maseena 2006; Janarthanam and Seshadri 2008; Woo et al. 2000; Rani and Nair 2006; Rani et al. 2003; Yuan et al. 2009).

In the present study callus regeneration was obtained on MS medium supplemented with BA ($0.5\text{--}4.0 \text{ mg l}^{-1}$) or Kn ($0.5\text{--}4.0 \text{ mg l}^{-1}$) alone or in combination with 2, 4-D (0.5 and 1.0 mg l^{-1}). In an earlier investigation by Vinothkumar et al. (2009) maximum number of shoots obtained was 29.87 per explant on MS medium supplemented with 0.6 mg l^{-1} BA. However, in our study we obtained highest response on MS medium supplemented with 3.0 mg l^{-1} BA and 1.0 mg l^{-1} NAA. The average number of shoots was 49.9 per explant and this is far better than the previous report. Comparing BA and Kn, the former was more effective than the latter. This is in concordance with the results in other systems like *Pterocarpus marsupium* (Chand and Singh 2004), *Vitex negundo* (Rani and Nair 2006), etc. Our results indicate that a combination of auxin and cytokinin is essential for getting best response for regeneration in *P. viscida*. Auxin-cytokinin combination has been widely

employed for shoot regeneration in various systems (Baker et al. 1999; Zhao et al. 2001; Li et al. 2010).

In the present study IBA was superior over NAA in rooting. IBA proved to be most effective for induction of roots in legumes as was observed in members like *Pterocarpus marsupium* (Chand and Singh 2004), *Mucuna pruriens* (Faisal et al. 2006) and *Clitoria ternatea* (Barik et al. 2007).

In conclusion, the present study reports an efficient protocol for plant regeneration via callus for an important ethnomedicinal plant. Cotyledonary node was the best explant for callus induction and shoot regeneration. This protocol could be vital for multiplying and field transfer of this plant. In view of its ethnomedicinal potential, there is a need for organized cultivation of this red listed plant for its conservation and sustainable utilization. An efficient system of callus regeneration is a pre-requisite for developing a transformation protocol for a given species.

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References

- Ansari R, Jeeja G (2006) Plants and plant science in Kerala. Malabar Botanical Gardens, Kozhicode, pp 34–65
- Arockiasamy S, Ignacimuthu S (1998) Plant regeneration from mature leaves and roots of *Eryngium foetidum* L. a food flavouring agent. Curr Sci 75:644–666
- Babu TD, Sasidharan N, Vijayan FP, Padikkala J (2008) Comparative phytochemical and biological analysis to detect the genuineness of substitutes of the plant Moovila in drug preparations. J Basic Clin Physiol Pharmacol 19:119–130
- Baker MC, Munoz-Fernandez N, Carter CD (1999) Improved shoot development and rooting from mature cotyledons of sunflower. Plant Cell Tissue Organ Cult 58:39–49
- Barik DP, Naik SK, Mudgal A, Chand PK (2007) Rapid plant regeneration through in vitro axillary shoot proliferation of butterfly pea (*Clitoria ternatea* L.) - a twinning legume. In Vitro Cell Dev Biol Plant 43:144–148
- Chand S, Singh AK (2004) In vitro shoot regeneration from cotyledonary node explants of a multipurpose leguminous tree *Pterocarpus marsupium* Roxb. In Vitro Cell Dev Biol Plant 40:464–466
- Deepa MA, Narmatha BV, Basker S (2004) Antifungal properties of *Pseudarthria viscida*. Fitoterapia 75:581–584
- Dhar U, Joshi M (2005) Efficient plant regeneration protocol through callus for *Saussurea obvallata* (DC) Edgew. (Asteraceae) effect of explant type, age and plant growth regulators. Plant Cell Rep 24:195–200
- Faisal M, Siddique I, Anis M (2006) An efficient plant regeneration system for *Mucuna pruriens* L. (DC) using cotyledonary node explants. In Vitro Cell Dev Biol Plant 42:59–64
- Janarthanam B, Seshadri S (2008) Plantlet regeneration from leaf derived callus of *Vanilla planifolia* Andr. In Vitro Cell Dev Biol Plant 44:84–89
- Klaus H, Nyman U, Smitt UW, Anderson A, Gudiiiksen L, Rajasekharan S, Pushpangadan P (1995) In vitro screening of

- traditional medicines for anti-hypertensive effect based on inhibition of the angiotensin converting enzyme (ACE). *J Ethnopharm* 48:43–51
- Li Z, Mize K, Campbell F (2010) Regeneration of daylily (*Heemerocallis*) from young leaf segments. *Plant Cell Tissue Organ Cult* 102:199–204
- Masirkar VJ, Deshmukh VN, Jadhav JK, Sakarkar DM (2008) Antidiabetic activity of the ethanolic extract of *Pseudarthritis viscida* root against alloxan induced diabetes in albino rats. *Res J Pharmacol Tech* 1:541–542
- Mathew GM, Sasikumar JM (2007) Anti-oxidant activity of *Pseudarthritis viscida*. *Ind J Pharmacol Sci* 69:581–582
- Muhammad S, Amusa NA (2003) Effects of Sulphuric acid and hot water treatments on seed germination of tamarind (*Tamarindus indica* L.). *Afr J Biotechnol* 2:276–279
- Murashige T, Skoog F (1962) A revised medium for vapid growth and bioassays with tobacco cultures. *Physiol Plant* 15:473–497
- Nadkarni KM (1982) Indian materia medica, vol I. Popular Prakashan, Mumbai, p 1010
- Oommen S, Ved DK, Krishnan R (2000) Tropical Indian medicinal plants: propagation methods. Foundation for Revitalisation of Local Health Traditions (FRLHT). pp 232–235
- Pereira AMS, Bertoni BW, Gloria BA, Araiyo ARB, Januario AH, Loureno MV, Franca A (2000) Micropropagation of *Pathomorphe umbellata* via direct organogenesis from leaf explants. *Plant Cell Tissue Organ Cult* 60:47–53
- Pradhan C, Kar S, Pattnaik S, Chand PK (1998) Propagation of *Dalbergia Sissoo* Roxb. through in vitro shoot proliferation from cotyledonary nodes. *Plant Cell Rep* 18:122–126
- Rajendran K, Srinivasan KK, Shirwaikar A (2007) Pharmacognostical evaluation of the roots of *Pseudarthritis viscida* (Linn.). *Nat Prod Sci* 3:214–219
- Rajendran K, Sam KG, Srinivasan KK, Shirwaikar A (2010) Short term anti-diabetic studies bon alcoholic root extract of *Pseudarthritis viscida* L. *Int J Res Pharmacol* 1:382–385
- Rani DN, Nair GM (2006) Effect of plant growth regulators on high frequency shoot multiplication and callus regeneration of an important Indian medicinal plant, Nirgundi, (*Vitex nigundo* L.). *In Vitro Cell Dev Biol Plant* 42:69–73
- Rani G, Virk GS, Nagpal A (2003) Callus induction and plantlet regeneration in *Withania somnifera* (L.) Dunal. *In Vitro Cell Dev Biol Plant* 39:468–474
- Ravikumar K, Ved DK (2000) 100 Red listed medicinal plants of conservation concern in South India. FRLHT, Bangalore, pp 305–307
- Shaw LHY, Tripathi L, Bhattacharya S (2004) Antidiabetic plants used by tribals in Madhya Pradesh. *Nat Prod Rad* 3:427–428
- Thomas TD, Maseena EA (2006) Callus induction and plant regeneration in *Cardiospermum halicacabum* Linn. an important medicinal plant. *Sci Hort* 108:332–336
- Ved DK, Goraya GS (2007) Demand and supply of medicinal plants in India. FRLHT, Bangalore, pp 1–18
- Vengadesan G, Ganapathi A, Anand RP, Anbazhagan VR (2002) In vitro propagation of *Acacia sinuate* (Lour.) Merr. via cotyledonary nodes. *Agrofor Syst* 55:9–15
- Vijayabaskaran M, Venkatesaramurthy N, Arifpasha MD, Babu G, Perumal P, Jayakumar B (2007) In vitro cytotoxic effects of ethnolic extract of *Pseudarthritis viscida* Linn. *Int J Pharma Pharmaceuti Sci* 2:93–94
- Vijayabaskaran M, Venkatesaramurthy N, Babu G, Perumal P, Jayakar B (2010a) In vitro antioxidant evaluation of *Pseudarthritis viscida* Linn. *Int J Curr Pharmaceut Res* 2:21–23
- Vijayabaskaran M, Venkatesaramurthy N, Babu G, Khatale PN (2010b) Antiarrhoeal activity of *Pseudarthritis viscida* roots. *Int J Pharma Tech* 2:307–313
- Vinothkumar D, Britto SJ, Sebastianraj J, Robinson JP, Senthilkumar S (2009) Callus regeneration from stem explants of *Pseudarthritis viscida* (L.) Wight and Arn. - a valuable medicinal plant. *Afr J Biotechnol* 8:4048–4051
- Warrier PK, Nambiar VPD, Ramankutty C (1996) Indian medicinal plants: a compendium of 500 species, vol 4. Orient Longman Ltd, Hyderabad, p 336
- Woo SH, Nair A, Adachi T, Campbell CG (2000) Plant regeneration from cotyledon tissues of common buckwheat (*Fagopyrum esculentum* Moench). *In Vitro Cell Dev Biol Plant* 36:358–361
- Yuan X, Wangb Z, Liu J, She J (2009) Development of a plant regeneration system from seed-derived calluses of centipede-grass [*Eremochloa ophiuroides* (Munro.) Hack]. *Sci Hort* 120:96–100
- Zhao D, Xing J, Li M, Lu D, Zhao Q (2001) Optimization of growth and jaceosidin production in callus and cell suspension cultures of *Saussurea medusa*. *Plant Cell Tissue Org Cult* 67:227–234