Research in Biotechnology, 3(2): 83-88, 2012 ISSN: 2229-791X
www.researchinbiotechnology.com

Regular Article

Micropropagation of *Dysophylla myosuroides* (Roth.) Benth. In. Wall. through leaf culture

N. Savithramma, *S. Ankanna, Beena Prabha and A. Sasikala

Department of Botany, Sri Venkateswara University, Tirupati – 517 502, A.P., India.
*Corresponding author Email: ankanna2010@gmail.com

A method was developed through leaf as explant of *Dysophylla myosuroides* (Roth.) Benth. for multiple shoot regeneration. The effect of Auxins (IAA, NAA, IBA) and Cytokinins (BA) were studied on shoot regeneration in clulture. On MS half strength medium light green compact calli were formed with 0.1 mg l^{-1} NAA. The cultures produced 62.57 ± 0.04 shoots on half strength medium with 0.5 mg l^{-1} BA and 0.01 mg l^{-1} NAA and also maximum mean length (0.52 ± 0.02) of shoots were achieved. *In vitro* produced shoots rooted on half strength MS medium with 1 mg l^{-1} IBA. The *in vitro* regenerated plantlets were successfully acclimatized in paper cups containing vermiculate, then transferred to green house. Hardened plants were transplanted in to sand and soil (1 : 1).

Key words: *Dysophylla myosuroides;* growth hormones; callus induction; shoot proliferation; transplantation.

Dysophylla myosuroides (Roth.) Benth. In.Wall. or *Pogostemon myosuroides* (Roth.) El.Gazzar & L. is common on open rocky crevices in hill slopes of Tirumala. It was also distributed in Kambakkam, Chandragiri fort and Sadhumallamma Kona (Madhavachetty *et al.,* 2008). It is much branched perennial herb with a woody root stock, leaves are 1.5 inches long, 0.3 inches broad, flowers blue in terminal spikes. Only local healers and tribals used to cure ailments and leaf extract is used for relieving anxiety and stimulation of brain (Savithramma, 2003). Leaves are rich in flavonoids, phenols, steroids, glycosides and volatile oils were reported from leaves (Saradvathi, 2009) this plant is not cultivating and uprooting the wild plants number has been reducing on the hill slopes. Hence development of viable micropropagation protocol is inevitable for *ex situ* conservation and sustainable utilization of selected plant species. As best of our knowledge it is the first report on micropropagation of this medicinally important plant species.

Micropropagation has many advantages over conventional methods of vegetative propagation. Plant tissue culture is the process of small pieces of living tissues (explants) isolated from a plant and grown aseptically for indefinite periods on a semi defined or defined nutrient medium (Ignacimuthu, 1997). The application of a reliable *in vitro* clonal propagation system would provide an alternative method of propagation to meet the pharmaceutical needs and for effective conservation of plant species. *In vitro* propagation of plants holds tremendous potential for the production of high quality plant based medicines (Murch *et al.,* 2000). This protocol can assure that a stable supply of this medicinally important plant irrespective of any seasonal variations and may serve as a better source for biological active compounds.

Received : April 1, 2012; Accepted : April 27, 2012

Materials and methods

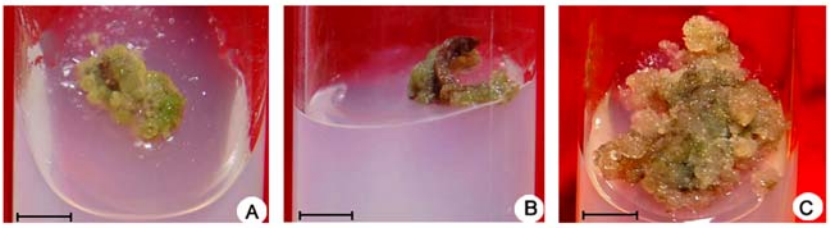
Leaves of *Dysophylla myosuroides* (Roth.) Benth. were collected on the hilly regions of Tirumala, Tirupati, Andhra Pradesh, India. Nearly 1 cm^2 leaf segments were taken as explant. These explants were initially washed under running tap water with Teepol solution (5% v/v) for 15 min. followed by 4 to 5 washings with water. Then surface sterilized with 70% ethanol for 60 sec. followed by rinsing for 3 times in sterile distilled water. Finally the explants were immersed in 0.1% $HgCl_2$ (Mercuric chloride) for 3 min. and washed thoroughly with autoclaved water. The surface sterilized explants were cultured on MS medium (Murashige and Skoog, 1962) containing 3% (w/v) sucrose and 0.8% (w/v) agar. Explants were implanted in different combinations and concentrations of growth regulators (BA, IAA, IBA, NAA and 2,4-D) singly as well as in combinations for shoot proliferation. The pH of the medium was adjusted to 5.8 by using 0.1N HCl (Hyderocloric acid) or 0.1N NaOH (Sodium hydroxide) solutions before autoclaving. All cultures were incubated in a culture room at 25 ± 2^0C with a relative humidity of 50 to 60% and 16 h photoperiod at a photon flux density of 15-20 µE m^2/s^{-1} from white cool fluorescent tubes. For each treatment 12 replicates were used and each experiment was repeated at least thrice. The cultures were examined periodically.

Results and discussion

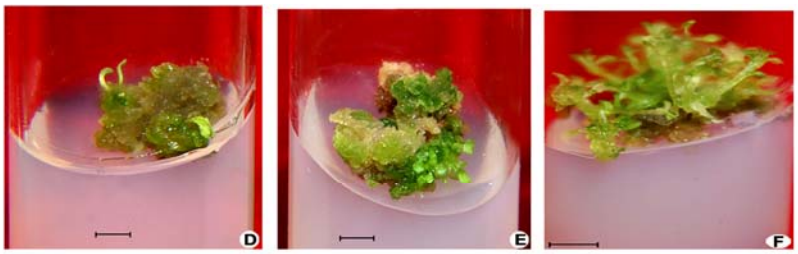
Callus initiation

Induction of callus from leaf explants in *D. myosuroides* was highly governed by the type and concentration of growth regulators. IAA and IBA showed moderate callusing from most of the explants. The best light green to cream loose calli were produced on half strength MS medium supplemented with 0.1mg l^{-1} IAA and creamy white to ash coloured compact nodular calli were produced with 0.1mg l^{-1} NAA. Whereas light brown compact hard nodular calli were developed with 0.1 mg l^{-1} 2, 4-D alone (Table -1 & Fig– 1 A, B, C). The species of *Spilanthes* produced green and white fragile calli on MS media with IAA, IBA (Chandra *et al.,* 2008) and Pandey and Agrawal – 2009) Dennis (2010) reported that 2, 4-D with Kn produced whitish yellow friable callus on MS medium in *Justicia gendarussa* using leaf explants.

Figure-1



Induction of callus from leaf culture on MS half strength medium containing A) 0.1 mg l^{-1} IAA (1 cm Bar = 5.43 mm) B) 0.1 mg l^{-1} NAA (1 cm Bar = 5.68 mm) C) 0.1 mg l^{-1} 2, 4-D (1 cm Bar = 7.57mm)



Multiple shoots regeneration from leaf culture on MS half strength medium containing D) 0.05 mg l^{-1} BA (1 cm Bar = 7.81mm) E) 0.1 mg l^{-1} BA (1 cm Bar = 7.81mm) F) 0.5 mg l^{-1} BA + 0.01 mg l^{-1} NAA (1 cm Bar = 2.90 mm)

Table 1. Effect of different plant growth regulators on callus induction from leaf explants of *Dysophylla myosuroides* on half strength MS medium.

Plant Growth Regulators (mg l^{-1})			Nature of callus response
IAA	NAA	2,4-D	
0.1			Cream nodular calli
	0.1		Light green compact calli
0.1			Light green to cream loose calli
	0.1		Creamy white to ash compact nodular calli
		0.1	Light brown compact hard nodular calli

Table 2. Effect of different plant growth regulators on indirect shoot regeneration from the callus derived from leaf explants of *Dysophylla myosuroides* on half strength MS medium.

Plant growth regulators (mg l^{-1})		Frequency of Shoot regeneration	Mean no. of shoots/ explant	Mean length of shoots (cm)
BA	NAA			
0.05	---	52.06 ±0.04^{b}	1.23 ± 0.05^{a}	0.32 ± 0.01^{a}
0.1	0.01	56.82 ± 0.01^{d}	2.87 ± 0.03^{d}	0.41 ± 0.02^{ab}
0.5	0.01	62.57 ± 0.04^{g}	5.66 ± 0.03^{g}	0.52 ± 0.02^{cd}

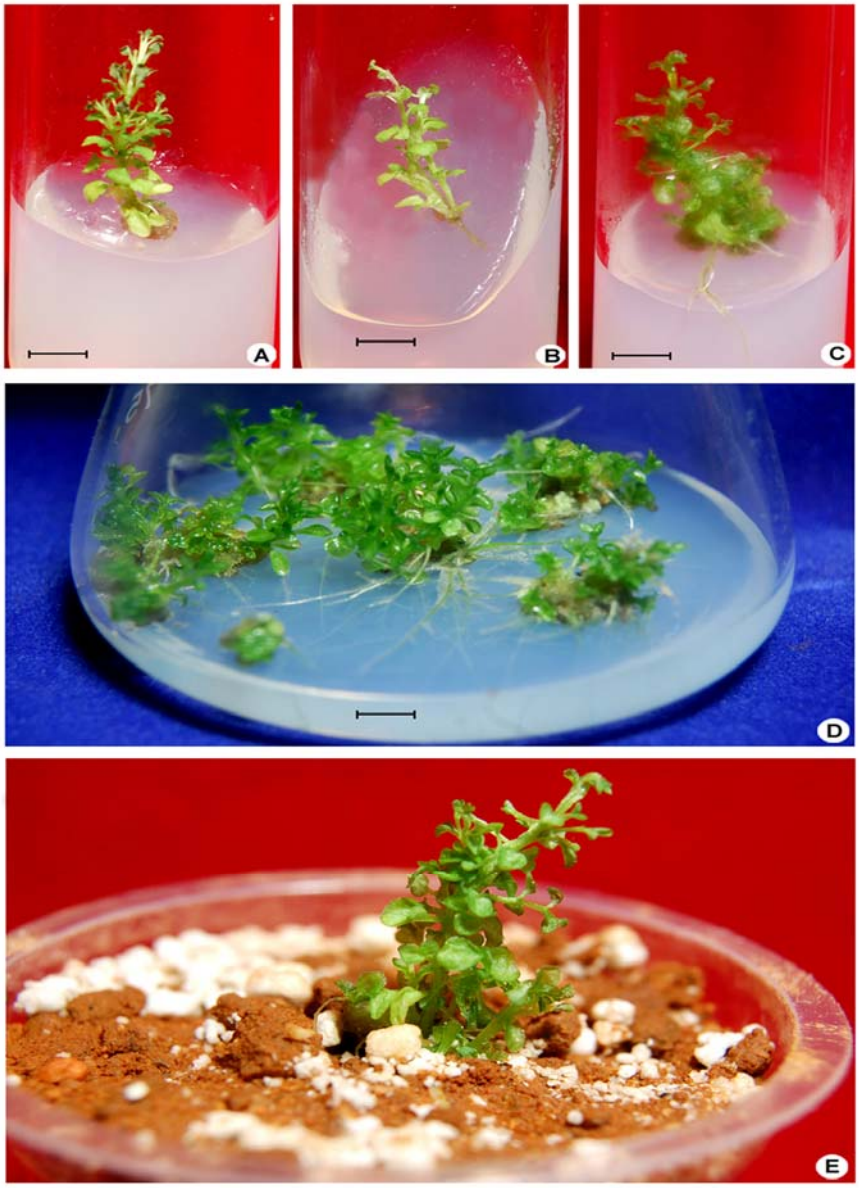
Values represented above are the means of 12 replicates. '±' indicates the standard error. Observations after 6 weeks of culture. Mean values having the same letter in each column don't differ significantly at P≤0.05 (Duncans Test).

Table 3. Effect of different auxins on root induction from *in vitro* raised shoots of *Dysophylla myosuroides* on half strength MS medium.

Plant Growth Regulators (mg l^{-1})			Frequency of root regeneration	Mean number of roots	Mean length of roots (cm)
NAA	IBA	IAA			
0.1	---	---	66.71 ± 0.01^{g}	4.21 ± 0.02^{f}	3.28 ± 0.02^{f}
0.5	---	---	58.22 ± 0.03^{e}	3.17 ± 0.03^{d}	2.76 ± 0.04^{e}
---	---	0.1	52.61 ± 0.01^{c}	3.66 ± 0.01^{e}	1.82 ± 0.02^{c}
---	---	0.5	43.47 ± 0.02^{a}	2.52 ± 0.05^{a}	1.47 ± 0.01^{b}
---	0.01	---	57.56 ± 0.03^{d}	2.84 ± 0.04^{c}	1.83 ± 0.02^{c}
---	0.1	---	65.32 ± 0.01^{f}	4.61 ± 0.02^{g}	2.23 ± 0.01^{d}
---	0.5	---	69.42 ± 0.02^{h}	7.42 ± 0.03^{h}	3.73 ± 0.03^{g}
---	1.0	---	76.38 ± 0.03^{i}	12.75 ± 0.04^{i}	3.84 ± 0.02^{h}

Values represented above are the means of 12 replicates. '±' indicates the standard error. Observations after 4 weeks of culture. Mean values having the same letter in each column don't differ significantly at P≤0.05 (Duncans Test).

Figure-2



Elongation, rooting and field acclimatization of *in vitro* raised plantlets of *Dysophylla myosuroides* on half strength MS medium A, B) Elongation of shoots after 15 days (1 cm Bar = 5.20 mm) B) after 1week (1 cm Bar = 6.41 mm), C, D, E) Rooting of *in vitro* raised shoots wih C) 0.5 mg l^{-1} IBA (1 cm Bar = 4.23 mm), D) 1 mg l^{-1} IBA (1 cm Bar = 6.15 mm), E) Hardended regenerated plant after 2 weeks.

Shoot multiplication

The calli were transferred to regenerated media, tiny green meristems were developed on surface of the calli within 4 weeks (Fig– 1 D, E, F). The combination of BA + NAA (0.5 mg l^{-1} + 0.01 mg l^{-1}) proved optimum concentration for shoot regeneration from leaf explants. Which was supported by Dhar and Joshi (2005) in *Saussuraea obvallata*. NAA in combination of BAP resulted multiple shoot buds in *Mentha piperita* (Sujana and Naidu, 2011) 0.1 mg l^{-1} BA and 0.01 mg l^{-1} NAA has given average frequency (56.82 ± 0.01) of shoot regeneration whereas increasing the BA concentration to 0.5 mg l^{-1} resulted maximum frequency (62.57 ± 0.04) (Table -2 & Fig– 2 A, B, C). Same results were

observed by Agrawal and Sardar (2006, 2007) in *Cassia angustifolia*, Echeverrigaray *et al.,* (2000) in *Chaememeleum spp* and Kumar *et al.,* (1998) in *Pauwolnia fortunei*. BA promotes shoot formation *in vitro* and minimum response were recorded when compared with combination of NAA. Where as in *Eclipta alba* BA produced maximum shoot formation (Dhaka and Kothari, 2005).

Root initiation

Different auxins (IAA, IBA and NAA) were tested at various concentrations for maximum number of roots to *in vitro* raised plantlets. IBA was found to induce a strong rooting response. Lower concentrations of IBA on half strength MS medium results in the formation of thin and long roots. By increasing the concentration of IBA, the number of roots also increased but length of the roots decreased (Table -3 & Fig– 2 D). The success of IBA in promoting efficient root induction has been reported earlier in other species by Sreekumar *et al.,* (2000) Fracaro and Echeverrigary (2001), Martin (2002), Beena *et al.,* (2003), Faisal *et al.,* (2006 and 2007) and Rani and Rana (2010).

Hardening and acclimatization

Plantlets with 4 to 6 fully expanded leaves and well developed roots were successfully acclimatized (Fig-2 E) and eventually established in soil. Thus the *Ex vitro* survival rate of the plants after transfer to fine garden soil : sand (1:1) was 75%. The *in vitro* derived plants were eventually transferred to a natural habitat. The regenerated plants did not show detectable variation in morphology and growth characteristics when compared with that of donar plant.

Results presented here showed that leaves of *Dysophylla myosuroides* have great organogenesis potential not only for shoot formation but also for the production of roots and that this ability is directly related to the presence of exogenous growth regulators in the culture media.

References

Agrawal V, Sardar PR. 2006. *In vitro* propagation through leaflet and cotyledon derived callus in Senna (*Cassia angustifolia*)—a medicinally valuable drought resistant legume. Biol Plant 50: 118–122. doi:10.1007/s10535-005-0084-8.

Agrawal V, Sardar PR. 2007. *In vitro* regeneration through somatic embryo-genesis and organogenesis using cotyledons of *Cassia angustifolia* Vahl. In Vitro Cell Dev. Biol. Plant, 43: 585–592. doi:10.1007/s11627-007-9058-1.

Beena MR, Martin KP, Kirti PB, Hariharan M 2003 Rapid *in vitro* propagation of medicinally important *Ceropegia candelabrum.* Plant Cell, Tissue & Organ Culture, 2: 285–289.

Chandra S, Sharma HP, R Chandra, Jha S. 2008. *In vitro* propagation of various explants of *spilanthes paniculata* (DC.) Jansen. Inter J of Plant Sci, 3(2): 558-566.

Dennis T, Yoichiro H. 2010. *In vitro* propagation for the conservation of a rare medicinal plant *Justicia gendarussa* Burm. f. by nodal explants and shoot regeneration from callus. T Acta Physiol Plant 32: 943–950 DOI 10.1007/s11738-010-0482-1.

Dhaka N, Kothari SL. 2005. Micro-propagation of *Eclipta alba* (L.) Hassk. An important medicinal plant. In Vitro Cell Dev Biol Plant, 41: 770–774. doi:10.1079/IVP2005684

Dhar U, Joshi M. 2005. Efficient plant regeneration protocol through callus for *Saussuraea obvallata* (DC.) Edgew. (Asteraceae): effect of explant type, age and plant growth regulators. Plant Cell Rep, 24: 195–200 doi:10.1007/s00299-005-0932-1.

Echeverrigaray S, Fracaro F, Andrade LB, Biasio S, Atti-Serafini L. 2000 *In vitro* shoot regeneration from leaf explants of Roman chamomile. Plant Cell Tissue Organ Cult 60: 1–4.

Faisal M, Naseem A, Anis M. 2007. An efficient micropropogation system for *Tylophora indica* an endangered,

medicinally important plant. Plant Biotech Reports 1(3) : 55-161.

Faisal M, Siddique I, Anis M. 2006. *In vitro* rapid regeneration of plantlets from nodal explants of *Mucuna pruriens* a valuable medicinal plant. Annals of Applied Biology 48: 1–6.

Fracaro F & Echeverrigaray S. 2001. Micropropagation of *Cunila galioides*, a popular medicinal plant of south Brazil. Plant Cell Tissue & Organ Culture, 64: 1–4.

Ignacimuthu S. 1997 Plant Biotechnology, Oxford and IBH publishing Co. Pvt. Ltd p. 180.

Kumar PP, DimpsRao C, Goh CJ. 1998 Influence of petiole and lamina on adventitious shoot initiation from leaf explants of *Paulownia fortunei.* Plant Cell Rep, 17: 886–890. doi:10.1007/ s002990050503

Madhavachetty K, Sivaji K, Tulasi Rao K. 2008 Flowering plants of Chittoor district – Andhra Pradesh, India. Published by Students Offset Printers, Tirupati, Andhra Pradesh, India. p.282.

Martin KP. 2002. Rapid propagation of *Holostemma ada-kodien* Schult, a rare medicinal plant, through axillary bud multiplication and indirect organo-genesis. Plant Cell Reports, 21: 112–117.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15: 473-497.

Murch SJ, Krishna Raj S, Saxena PK. 2000. Tryptophan is a precursor for melatonin and serotonin biosynthesis in *in-vitro* regenerated St. John's wort (*Hypericum perforatum* L. cv. Anthos) plants. Plant Cell Rep 19: 698-704.

Pandey V, Agrawal V. 2009. Efficient micropropagation protocol of *Spilanthes acmella* L possessing strong antimalarial activity. In Vitro Cell Dev Biol-Plant, 45: 491–499 DOI 10.1007/s11627-008-9184-4.

Rani S, Rani JS. 2010 *In vitro* Propagation of *Tylophora Indica* – Influence of explanting season, growth regulator synergy, culture passage and planting substrate. Journal of American Science 6(12): 385-392.

Saradvathi J. 2009 Studies on *in vitro* propagation of selected medicinal plant taxa – *Dysophylla myosuroides* (Roth.) Benth. In. Wall. and *Talinum cuneifolium* (Vahl.) Willd. Thesis submitted to Sri Venkateswara University, Tirupati, Andhra Pradesh, India. p 90.

Savithramma N. 2003 Diversity in Phanerogams of Sri Venkateswara University Campus, Published by S.V. University, Tirupati, Andhra Pradesh, India. P.35.

Sreekumar S, Seeni S, Pushpangadan P. 2000. Micropropagation of *Hemidesmus indicus* for cultivation and production of 2-hydroxy 4-methyl benzaldehyde. Plant Cell, Tissue & Organ Culture 62: 211–218.

Sujana P, Naidu CV. 2011. High Frequency Rapid Plant Regeneration from Shoot Tip and Nodal Explants of *Mentha piperita* (L.) – An Important Multipurpose medicinal plant. Journal of Phytology, 3(5): 09-13.