Journal of Horticultural Research 2014, vol. 22(1): 35-39
DOI: 10.2478/johr-2014-0004

MICROPROPAGATION OF *PLUCHEA LANCEOLATA* (OLIVER & HIERN.) USING NODAL EXPLANT

Mafatlal M. KHER[1], Dimpal JOSHI[1], Sureshkumar NEKKALA[1], M. NATARAJ[1]*, Dharmesh P. RAYKUNDALIYA[2]
[1]B.R. Doshi School of Biosciences, Sardar Patel Maidan, Sardar Patel University
Vadtal Road, Post Box No. 39, Vallabh Vidyanagar-388120, India
[2]Department of Statistics
Sardar Patel University
Vallabh Vidyanagar 388 120, Gujarat, India

Received: October 17, 2013; Accepted: April 16, 2014

ABSTRACT

Pluchea lanceolata is an important medicinal plant of Asteraceae family known for its anti-arthritic and anti-inflammatory activity. A protocol was established for micropropagation of *P. lanceolata* using nodal explants. Nodal explants were inoculated onto Murashige and Skoog (1962) – MS medium supplemented with 6-benzylaminopurine (BAP), kinetin (Kin), thidiazuron (TDZ) and 2iP (2-isopentenyladenine) at various concentrations (0.0, 0.5, 1.0, 1.5 and 2.0 mg·dm^{-3}). The highest multiplication rate was obtained for nodal explants cultured on MS medium, supplemented with 0.5 mg·dm^{-3} thidiazuron (TDZ). *In vitro* raised shoots were successfully rooted on ½ mineral salt concentration of MS medium supplemented with 1.0 mg·dm^{-3} IBA.

Key words: *Pluchea lanceolata*, micropropagation, TDZ, BAP, Kin, 2iP

INTRODUCTION

Pluchea lanceolata, is a perennial herb belonging to the family Asteraceae grown in warm climatic regions of India and known as Rasana. This plant is prized for its medicinal importance. Ethanolic extract of aerial parts of *P. lanceolata* has anti-inflammatory activity (Srivastava et al. 1990), and leaves are known to contain compounds of immunosuppressive activity (Bhagwat et al. 2010). Because of indiscriminate harvesting and lack of proper cultivation methods along with poor seed viability, the wild population of *P. lanceolata* has declined rapidly (Arya et al. 2008). Plant tissue cultures are widely used for clonal propagation of many medicinally important plants (Inamdar et al. 1990, Varghese et al. 1992, 1993a, b). Due to increased exploitation, plant tissue cultures offer a viable option for the conservation of this plant.

In vitro regeneration systems for *P. lanceolata* - as callus mediated organogenesis from leaf explants (Arya et al. 2008, Kumar et al. 2004), and shoot proliferation from shoot tip explants (Arya & Patni 2013) were described. Mass propagation through *in vitro* cloning of plants from seedling-based explants and callus-mediated organogenesis can result in somaclonal variation. *In vitro* propagation through axillary shoot proliferation is a rapid method for large-scale production of true-to-type planting material of selected genotypes. Nodal explant culture is a popular method for micropropagation of plants (Faisal et al. 2005, Noshad et al. 2009, Sivanesan et al. 2011). The aim of this study was to develop an *in vitro* protocol for propagation of *P. lanceolata* using nodal explants.

MATERIALS AND METHODS

Nodal explants were collected from Anand Agriculture University, Gujarat, India. The explants were surface sterilised with 0.1% (w/v) mercuric chloride solution for 5 min under aseptic condition

*Corresponding author:
e-mail: mnatarajspu@gmail.com

and washed 3-4 times with autoclaved distilled water. In order to initiate *in vitro* cultures of axillary shoots, surface sterilised nodal cuttings 1.5-2.0 cm with one node inoculated on Murashige and Skoog (1962) (MS) medium containing 30 $g \cdot dm^{-3}$ sucrose, 0.25% phytagel, and 6-benzyaminopurine (BAP) or kinetin (Kin) or 2-isopentenyladenine (2iP) or thidiazuron (TDZ) and pH of the medium adjusted to 5.8.before autoclaving. Initial explants were cultured in borosilicate glass tubes (25 × 150 mm) containing 20 ml of medium. Data concerning bud breaking (%), shoot number per initial bud and shoot length were recorded after 45 days of culture.

For shoot multiplication, single nodes from elongated shoots were excised and were regularly subcultured at intervals of 45 days on MS medium supplemented with 0.5 $mg \cdot dm^{-3}$ TDZ, and on the same time shoots (minimum 3.0 cm in length) were excised from culture and transferred to rooting medium containing mineral MS salts diluted by half, supplemented with 1.0 $mg \cdot dm^{-3}$ IBA and solidified with 0.25% phytagel.

Explants were incubated at 25 ºC, 16 h light/8 h dark, 35 $\mu mol \cdot m^{-2}s^{-1}$ light intensity.

For hardening and acclimatisation, rooted shoots were carefully removed from the culture tubes and washed with sterile water to remove traces of phytagel. The plantlets were then transferred to plastic pots containing cocopeat and moss (1 : 1) for 10 days. Then plantlets were transferred to thermocol cup containing soil : sand : cocopeat (1 : 1 : 1) for 15 days.

All the experiments were conducted with minimum 12 replicates per treatment and repeated twice. Each replicate represents one node per culture vessel. The results are expressed as means ± SD of two experiments. The data were analysed statistically as one-way ANOVA model using SPSS Inc. (Chicago, IL. USA). The significance of difference among means values was carried out using Duncan's multiple range test (DMRT) at $p < 0.05$.

RESULTS AND DISCUSSION

Shoot multiplication could be achieved from the nodal explants of *P. lanceolata* inoculated on MS medium supplemented with BAP or Kn or 2iP or TDZ. The ability of the nodal explants for the bud break varied depending on the plant growth regulators and their concentration. Shoots developed with all the concentrations of cytokinins investigated and bud break occurred after 7-8 days of culture. It was observed that in the cultures where shoot number was higher, the shoot length remained shorter (Table 1, Fig.1B & C).

The optimum TDZ concentration for bud break and shoot multiplication was 0.5 $mg \cdot dm^{-3}$ which produced 9.7±3.49 shoot buds per nodal segment in 95.8% of explants (Table 1, Fig. 1D). Explants inoculated on medium supplemented with 2.5 $mg \cdot dm^{-3}$ BAP produced 5.40±2.62 shoots in 83.3% cultures (Table 1, Fig. 1C). Higher concentration of TDZ caused stunting and vitrification of shoots (Fig. 1A & E). However, the increasing concentration of BAP, Kin and 2iP resulted in increase in frequency of regeneration (Table 1). Findings that TDZ induced vitrification was well documented (Huetteman & Preece 1993, Lu 1993, Murthy et al. 1998). These authors recommended for micropropagation much lower concentration of TDZ than the adenine derivative cytokinins (Huetteman & Preece 1993). BAP, Kin and 2iP were less effective in comparison with TDZ in terms of the shoot number per node and the response percentages (Table 1). The high impact of TDZ on axillary shoot proliferation has been reported in number of plant species (Faisal et al. 2005, Noshad et al. 2009, Vasudevan & van Staden 2011).

The rates of shoot multiplication increased to the seventh subculture. However, after the fifth subculture, significant reduction in shoot length along with vitrification was observed (Table 2). The increase of shoot number due to successive transfer of cultures on fresh media may be due to suppression of apical dominance during subcultures that induce basal meristematic cells to form new shoots (Shekhawat & Shekhawat 2011, Tripathi & Kumari 2010). Similarly, repeated subculture in the TDZ supplemented medium had deleterious effects on *Cassia angustifolia* nodal explants after third subculture (Siddique & Anis 2007).

Table 1. Effect of BAP, Kinetin, TDZ and 2iP on growth of shoots in nodal explants culture of *P. lanceolata* (medium MS, culture period 45 days)

Cytokinin (mg·dm^{-3})		Shoot length (cm)	Number of shoots per node	% Response
Control		6.2±0.2^{abc}	1.6±0.6^{h}	8.3
BAP	0.5	6.3±0.4^{ab}	3.6±1.3^{efg}	41.7
BAP	1.0	6.4±0.6^{ab}	4.4±1.9^{defg}	54.2
BAP	1.5	6.0±0.5^{abc}	4.3±1.5^{defg}	70.8
BAP	2.0	5.6±0.8^{cd}	4.9±2.0^{bcdef}	75.0
BAP	2.5	4.9±0.9^{e}	5.4±2.6^{bcde}	83.3
Kinetin	0.5	6.5±0.4^{a}	2.4 ±.8^{gh}	29.2
Kinetin	1.0	6.4±0.6^{ab}	3.5±1.5^{efgh}	41.7
Kinetin	1.5	6.1±0.5^{abc}	3.8±1.1^{efg}	58.3
Kinetin	2.0	5.7±0.7^{bcd}	4.1±1.3^{defg}	70.8
Kinetin	2.5	4.8±0.9^{ef}	4.7±1.8^{bcdef}	79.2
TDZ	0.5	5.1±1.6^{de}	9.7±3.5^{a}	95.8
TDZ	1.0	4.9±1.1^{e}	8.3±3.2^{a}	83.3
TDZ	1.5	4.7±1.6^{ef}	6.4±2.9^{bc}	79.2
TDZ	2.0	4.2±1.6^{fg}	6.4±1.7^{b}	70.8
TDZ	2.5	4.0±1.0	6.0±1.5^{bcd}	62.5
2iP	0.5	6.5±0.4^{a}	3.1±1.2^{fgh}	33.3
2iP	1.0	6.4±0.6^{ab}	3.5±1.2^{efgh}	41.7
2iP	1.5	6.1±0.5^{abc}	3.8±1.6^{efg}	50.0
2iP	2.0	5.7±0.7^{bcd}	3.9±1.5^{efg}	62.5
2iP	2.5	4.8±0.9^{ef}	4.5±1.7^{cdef}	70.8

Mean ± SD in each column followed by same letters at superscripts are not significantly different at $p < 0.05$ according to DMRT (n= minimum 12 replicates)

In the previous report on micropropagation of *P. lanceolata*, maximum 14 shoots were obtained from leaf explants on Wood and Braun (1961) medium supplemented with 2% sucrose and 0.5 mg·dm^{-3} Kin (Kumar et al. 2004). Arya et al (2008) reported high multiplication rate (28 shoots from leaf explant) via callus-mediated regeneration. Regeneration of 20 shoots from one shoot tip was reported by Araya and Patni (2013). In this study up to 16.9 shoots could be obtained from single node in five subcultures. Further subculturing resulted in vitrification though the number of shoots increased (Table 2).

In vitro raised shoots were successfully rooted on MS medium with mineral salts diluted to half, supplemented with 1.0 mg·dm^{-3} IBA (data not presented). Similar effect of IBA on rooting of other plants was reported (Bernabe-Antonio et al. 2012, Phulwaria et al. 2012, Phulwaria et al. 2013, Rathore & Shekhawat 2013). Seventy percent of plantlets survived acclimatisation (Fig. 1H & I).

In conclusion, this study describes a simple protocol for micropropagation of *P. lanceolata* for mass multiplication and conservation.

Table 2. Effect of subculture order (45 days each) on shoot multiplication of *P. lanceolata* (medium MS + 0.5 mg·dm^{-3} TDZ)

Subculture	No. of shoot per node	Shoot length (cm)	Remark
1st	9.9±3.3^{e}	5.2±1.6 ^{a}	--
2nd	10.5±3.2^{de}	5.3±2.0 ^{a}	--
3rd	13.9±5.9^{cd}	4.6±1.6^{a}	--
4th	10.5±3.2^{de}	5.3±2.0^{a}	--
5th	16.9±6.6^{bc}	2.7±1.2^{b}	--
6th	19.5±9.3^{ab}	2.2±0.8^{b}	Vitrification
7th	20.7±8.9^{a}	1.9±0.6^{b}	Vitrification

Mean ± SD in each column followed by same letters at superscripts are not significantly different at $p < 0.05$ according to DMRT (n= minimum12 replicates)

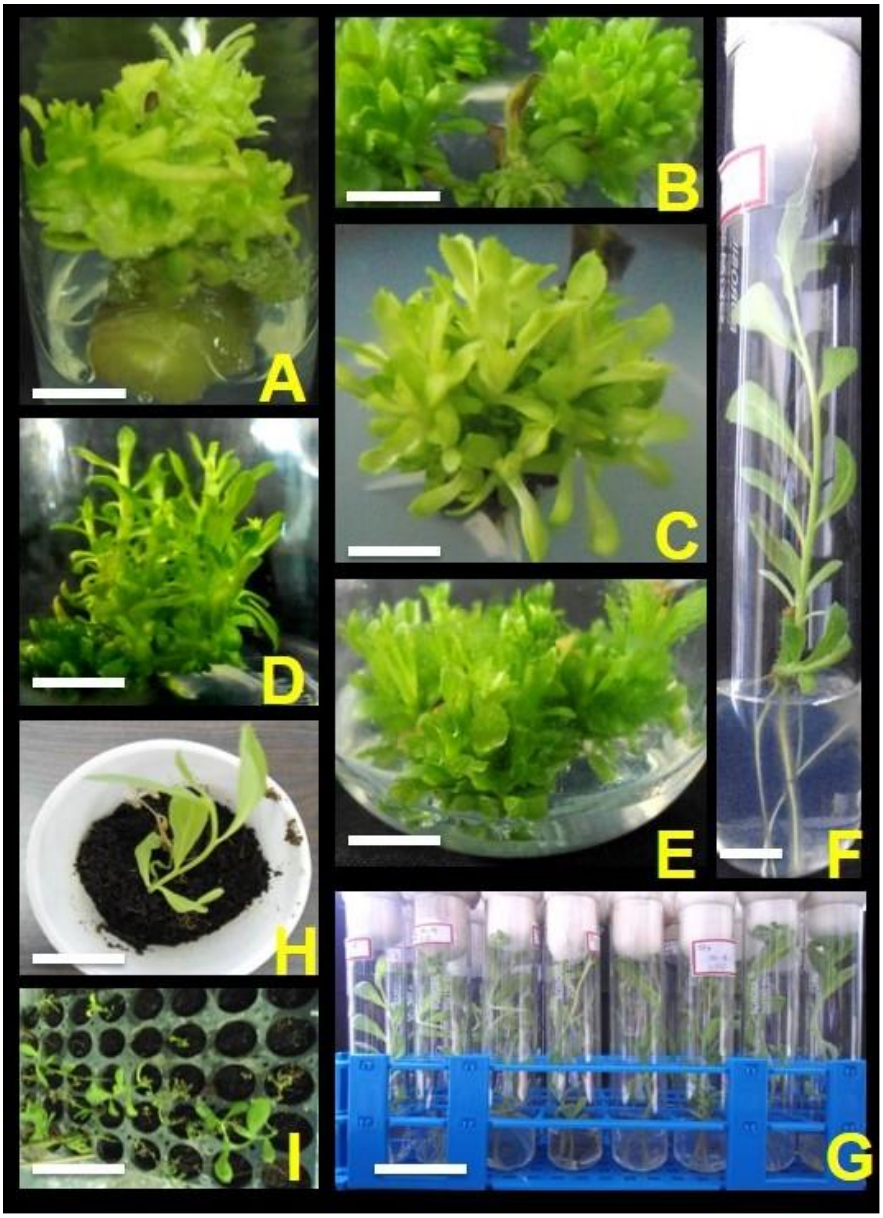

Fig. 1. Micropropagation of *P. lanceolata* from nodal explant on MS medium with:
A. 2.5 mg·dm^{-3} TDZ after 15 days (see vitrification). Bar - 1.5 cm; **B**. 0.5 mg·dm^{-3} TDZ after 28 days. Bar - 2.0 cm;
C. 2.5 mg·dm^{-3} BAP after 28 days. Bar - 2.0 cm; **D**. 0.5 mg·dm^{-3} TDZ after 45 days. Bar - 3.0 cm;
E. 2.5 mg·dm^{-3} TDZ after 28 days (see vitrification). Bar - 3.0 cm;
F. Rooting of in vitro raised shoot on MS medium supplemented with 1 mg·dm^{-3} IBA. Bar - 1.5 cm;
G. Rooting. Bar - 3.5 cm; **H**. and **I**. Acclimatisation of plantlets. Bar - 4.0 cm.

REFERENCES

Arya D., Patni V. 2013. Micropropagation of *Pluchea lanceolata* (Oliver & Hiern.) – A potent anti-arthritic medicinal herb. Int. J. Med. Arom. Plants 3: 55-60.

Arya D., Patni V., Kant U. 2008. *In vitro* propagation and quercetin quantification in callus cultures of Rasna (*Pluchea lanceolata* Oliver & Hiern.). Indian J. Biotechnol. 7: 383-387.

Bernabe-Antonio A., Santacruz-Ruvalcaba F., Cruz-Sosa F. 2012. Effect of plant growth regulators on plant regeneration of *Dioscorea remotiflora* (Kunth) through nodal explants. Plant Growth Regul. 68: 293-301. DOI: 10.1007/s10725-012-9717-z.

Bhagwat D.P., Kharya M.D., Bani S., Kaul A., Kour K., Chauhan P.S., Suri K.A., Satti N.K. 2010. Immunosuppressive properties of *Pluchea lanceolata*

leaves. Indian J. Pharmacol. 42: 21-26. DOI: 10.4103/0253-7613.62405.

Faisal M., Ahmad N., Anis M. 2005. Shoot multiplication in *Rauvolfia tetraphylla* L. using thidiazuron. Plant Cell Tiss. Org. 80: 187-190. DOI: 10.1007/s11240-004-0567-x.

Huetteman C.A., Preece J.E. 1993. Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell Tiss. Org. 33: 105-119. DOI: 10.1007/BF01983223.

Inamdar J.A., Nataraj M., Mohan J.S.S., Subramanian R.B. 1990. Somatic embryogenesis from callus cultures of *Crataeva nurvala* Buch. Ham. Phytomorphology 40: 319-322.

Kumar S., Narula A., Sharma M.P., Srivastava P.S. 2004. In vitro propagation of *Pluchea lanceolata*, a medicinal plant, and effect of heavy metals and different aminopurines on quercetin content. In vitro Cell. Dev. – Pl. 40: 171-176. DOI: 10.1079/IVP2003490.

Lu C-Y. 1993. The use of thidiazuron in tissue culture. In vitro Cell. Dev. – Pl. 29: 92-96. DOI: 10.1007/BF02632259.

Murashige T., Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15: 473-497. DOI: 10.1111/j.1399-3054.1962.tb08052.x.

Murthy B.N., Murch S., Saxena P.K. 1998. Thidiazuron: A potent regulator of in vitro plant morphogenesis. In vitro Cell. Dev. – Pl. 34: 267-275. DOI: 10.1007/BF02822732.

Noshad D., Miresmaili S., Riseman A., Ekramoddoullah A. 2009. *In vitro* propagation of seven *Daphne* L. species. Plant Cell Tiss. Org. 96: 201-209. DOI: 10.1007/s11240-008-9476-8.

Phulwaria M., Rai M.K., Harish, Gupta A.K., Ram K., Shekhawat N.S. 2012. An improved micropropagation of *Terminalia bellirica* from nodal explants of mature tree. Acta Physiol. Plant. 34: 299-305. DOI: 10.1007/s11738-011-0828-3.

Phulwaria M., Shekhawat N.S., Rathore J.S., Singh R.P. 2013. An efficient *in vitro* regeneration and *ex vitro* rooting of *Ceropegia bulbosa* Roxb.-A threatened and pharmaceutical important plant of Indian Thar Desert. Ind. Crop. Prod. 42: 25-29. DOI: 10.1016/j.indcrop.2012.05.013.

Rathore M.S., Shekhawat N.S. 2013. *Ex vivo* implications of phytohormones on various *in vitro* responses in *Leptadenia reticulata* (Retz.) Wight. & Arn. - An endangered plant. Environ. Exp. Bot. 86: 86-93. DOI: 10.1016/j.envexpbot.2010.05.009.

Shekhawat M.S., Shekhawat N.S. 2011. Micropropagation of *Arnebia hispidissima* (Lehm). DC. and production of alkannin from callus and cell suspension culture. Acta Physiol. Plant. 33: 1445-1450. DOI: 10.1007/s11738-010-0680-x.

Siddique I., Anis M. 2007. *In vitro* shoot multiplication and plantlet regeneration from nodal explants of *Cassia angustifolia* (Vahl.): a medicinal plant. Acta Physiol. Plant. 29: 233-238. DOI: 10.1007/s11738-007-0029-2.

Sivanesan I., Song J.Y., Hwang S.J., Jeong B.R. 2011. Micropropagation of *Cotoneaster wilsonii* Nakai – a rare endemic ornamental plant. Plant Cell Tiss. Org. 105: 55-63. DOI: 10.1007/s11240-010-9841-2.

Srivastava V., Varma N., Tandon J., Srimal R. 1990. Anti-inflammatory activity of *Pluchea lanceolata*: isolation of an active principle. Pharm. Biol. 28: 135-137. DOI:10.3109/13880209009082796.

Tripathi M., Kumari N. 2010. Micropropagation of a tropical fruit tree *Spondias mangifera* Willd. through direct organogenesis. Acta Physiol. Plant. 32: 1011-1015. DOI: 10.1007/s11738-010-0484-z.

Varghese S.K., Inamdar J.A., Kalia K., Subramanian R.B., Nataraj M. 1993a. *In vitro* organogenesis in *Enicostema hyssopifolium* (Willd.) Verdoorn. Proc. Natl. Acad. Sci. India B 63: 219-221.

Varghese S.K., Inamdar J.A., Kalia K., Subramanian R.B., Nataraj M. 1993b. Micropropagation of *Aegle marmelos* (L.) Corr. Phytomorphology 43: 87-92.

Varghese S.K., Subramanian R.B., Nataraj M., Kalia K., Inamdar J.A. 1992. *In vitro* studies on *Vitex negundo*. Indian Botanical Contactor 9: 147-150.

Vasudevan R., van Staden J. 2011. Cytokinin and explant types influence in vitro plant regeneration of Leopard Orchid (*Ansellia africana* Lindl.). Plant Cell Tiss. Org. 107: 123-129. DOI: 10.1007/s11240-011-9964-0.

Wood H.N., Braun A.C. 1961. Studies on the regulation of certain essential biosynthetic systems in normal and crown gall tumor cells. Proc. Natl. Acad. Sci. USA 47: 1907-1913.