

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

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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

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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 $30 \text{ g} \cdot \text{dm}^{-3}$ sucrose, 0.25% phytigel, and 6-benzylaminopurine (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 ($25 \times 150 \text{ mm}$) 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 $0.5 \text{ mg} \cdot \text{dm}^{-3}$ 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 $1.0 \text{ mg} \cdot \text{dm}^{-3}$ IBA and solidified with 0.25% phytigel.

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

For hardening and acclimatisation, rooted shoots were carefully removed from the culture tubes and washed with sterile water to remove traces of phytigel. The plantlets were then transferred to plastic pots containing cocopeat and moss (1 : 1) for 10 days. Then plantlets were transferred to thermo-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 \pm 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 $0.5 \text{ mg} \cdot \text{dm}^{-3}$ 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 $2.5 \text{ mg} \cdot \text{dm}^{-3}$ 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
1 st	9.9±3.3 ^e	5.2±1.6 ^a	--
2 nd	10.5±3.2 ^{de}	5.3±2.0 ^a	--
3 rd	13.9±5.9 ^{cd}	4.6±1.6 ^a	--
4 th	10.5±3.2 ^{de}	5.3±2.0 ^a	--
5 th	16.9±6.6 ^{bc}	2.7±1.2 ^b	--
6 th	19.5±9.3 ^{ab}	2.2±0.8 ^b	Vitrification
7 th	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= minimum 12 replicates)

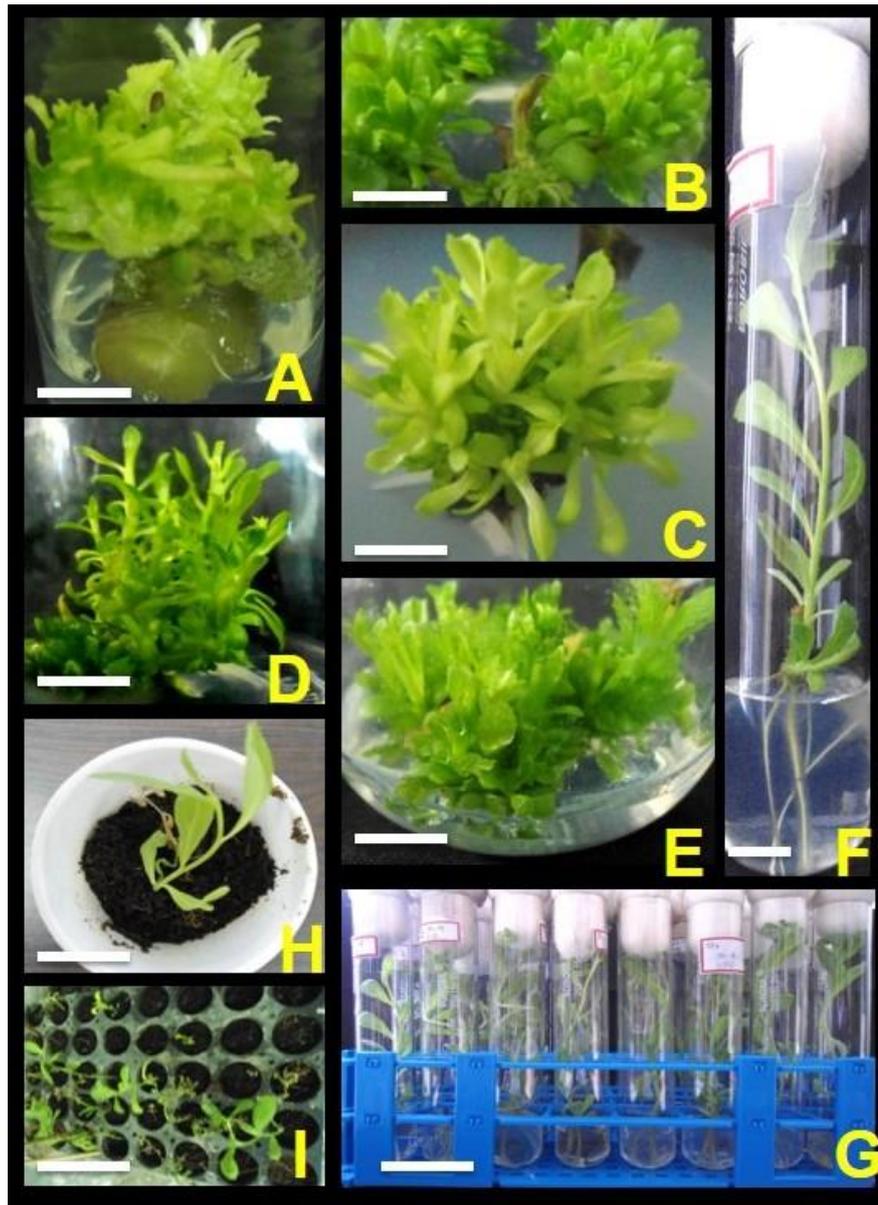


Fig. 1. Micropropagation of *P. lanceolata* from nodal explant on MS medium with:

- A. $2.5 \text{ mg} \cdot \text{dm}^{-3}$ TDZ after 15 days (see vitrification). Bar - 1.5 cm; B. $0.5 \text{ mg} \cdot \text{dm}^{-3}$ TDZ after 28 days. Bar - 2.0 cm; C. $2.5 \text{ mg} \cdot \text{dm}^{-3}$ BAP after 28 days. Bar - 2.0 cm; D. $0.5 \text{ mg} \cdot \text{dm}^{-3}$ TDZ after 45 days. Bar - 3.0 cm; E. $2.5 \text{ mg} \cdot \text{dm}^{-3}$ TDZ after 28 days (see vitrification). Bar - 3.0 cm; F. Rooting of in vitro raised shoot on MS medium supplemented with $1 \text{ mg} \cdot \text{dm}^{-3}$ IBA. Bar - 1.5 cm; G. Rooting. Bar - 3.5 cm; H. and I. Acclimatisation of plantlets. Bar - 4.0 cm.

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