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IN VITRO CLONAL PROPAGATION OF VULNERABLE MEDICINAL PLANT *PHYSALIS MINIMA* L.

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ABSTRACT

In vitro method for mass propagation of valuable medicinal plant Physalis minima L. belongs to the family Solanaceae, direct from shoot tip, nodel and leaf explants. In the present study direct organogenesis shoot multiplication in to nodal explants was higher than shoot tip explants and maximum percentage of response and number of shoots were noticed on the medium containing 2.0 mg/l of BAP and KN. Leaf explants failed to produce multiple shoot even on repeated subcultures. Callus induction and organogenesis of three different explants (shoot tip, node and leaf) showed positive response for callus induction on MS medium containing auxin (NAA) and cytokinins (BAP & KN). The regenerated shoots from all the explants were responded well for rooting on MS medium supplemented with IBA or IAA (2.0 mg/l). During the process of hardening, the rooted plantlets were transferred to the sterilized mixture of garden soil, farmyard manure, and sand (2:1:1) showed 80% survival and were identical to the donar plant with respect to growth characteristics as well as floral features. These plants grew normally without showing any morphological variations.

KEY WORDS

Physalis minima L, In vitro propagation, organogenesis, growth hormone and medicinal uses.

INTRODUCTION

Physalis minima Linn. (Solanaceae) is considered to be one of rare herbaceous medicinal plant commonly known as Sodakku thakkali in Tamil. *P. minima* L. commonly found on the bunds of the fields, wastelands around the houses, on roadsides etc., where the soil is porus and rich in organic matter. It occurs throughout in India, Baluchistan, Afghanistan, Tropical Africa, Singapore, Malaysis and Australia, and is reported as one of the important medicinal plants in Indian system of medicines.The parts of *P. minima* Linn. are bitter, appetizing, tonic, diuretic, laxative, useful in inflammations, enlargement of the spleen and abdominal troubles. The fruit is considered to be a tonic, diuretic and purgative in Tamilnadu, (Kirtikar and Basu, 1975). Further, *in vitro* propagation through shoot tip, node and leaf culture is an easy and economical way for obtaining large number of consistently uniform and true-to-type plants within a short span of time.

Tissue culture methods offer an important option for effective multiplication and improvement of plants within a limited time frame. It offers novel approaches to plant production, propagation, and preservation (Anis and Faisal, 2005). Recently, these techniques have been increasingly applied to many medicinal plants in particular for their mass propagation and conservation for production of bioactive compounds and genetic improvement. The *in vitro* propagated medicinal plants furnish a ready source of uniform, sterile, and compatible plant material for biochemical characterization and identification of active constituents (Miura *et al*., 1987; Ruby Priskilla, 2011). In addition, compounds from tissue cultures may be more easily purified because of simple extraction procedures and absence of significant amounts of pigments, thus

possibly reducing the production and processing costs (Lozovaya *et al*., 2006).

This study were aimed to find an appropriate system to regenerate plants through shoot tip, node and leaf explants and followed by successful establishment of regenerated plantlets were transferred to potting soil and adopted to greenhouse conditions.

MATERIALS AND METHODS:

Shoot tip, node and leaf explants*P. minima* L. grown in wild were collected from healthy plants near Vaduvur village in Thiruvarur district of Tamilnadu, India. The explants were excised from the well grown plants and washed with running tap water for 30 minutes followed by treatment with an aqueous solution of 2% (w/v) Teepol (Reckitt Benckiser, India) and ethanol for 15 seconds and washed with sterile distilled water for three to five times. The explants were then disinfected with 0.1% (w/v) aqueous mercuric chloride ($HgCl_2$) solution for a period of 5minutes and finally rinsed with autoclaved distilled water (five to seven changes). The disinfected plant segments were then trimmed at both ends prior to inoculation on culture media.

Murashige and Skoog (1962) (MS) medium consisting of agar (0.8%) and sucrose (3%) was used (Table-1). Various growth regulators viz., BAP, 2,4-D, NAA, IAA, IBA, KN, were fortified according to the nature of ensuring experiments. The pH of the medium was adjusted to 5.8 with the help of 0.1N NaOH and 0.1N HCl before autoclaving at 121^{o}c for 15 minutes. Shoot tip, leaf and node explants (1cm size) were inoculated in different culture tubes in vertical position on the medium while other explants like inter node were placed in horizontal position. The leaf explants (1x1 cm) were cultured with their adaxial and abaxial surface touching the medium.

Based on the preliminary experiments the culture medium was supplemented with appropriate growth regulators for different growth response. Shoot tip, node and leaf explants were cultured on MS basal medium containing sucrose 3% (w/v) agar 0.8% supplemented with different cytokinins (0.5 to 3.0mg/l) such as BAP and KN, were used either individually or in combination. Shoot tips, node and leaf segments were taken from 2 months old seedlings and optimum size of 15 to 30 mm length and 20 mm width were selected and cut length wise into 1x1cm size. They were inoculated into the culture tubes containing MS medium supplemented with different combinations BAP and KN and sucrose (3%w/v) for shoot multiplication The cultures were incubated at 25±2^{o}C with a photoperiod of 16 hours and 2000 lux light intensity. Number of multiplies shoot were excised in different explants inoculated. Care was taken to seen that the explants was in contact with medium either by the adaxial or abaxial side. Subsequent subculture media were formulated corresponding to organogenesis.

Multiples shoots of 6 to 7 cm in length after three weeks were excised and transferred to the rooting medium containing both IAA and IBA (0.5 to 2.5 mg/l) in different concentrations. After 30 days of cultures the well rooted plantlets were removed from culture tubes and washed thoroughly with running tap water to remove the traces of agar medium and treated with 0.1% bavistin (fungicides) by immersion and planted in small plastic cups containing sterilized vermiculite and nourished with half strength MS basal medium. The plastic cups were covered with transparent polythene bags with punch holes to maintain humidity. Fifty percent of the plantlets were maintained under culture room. The remaining plantlets were placed under shade with short exposure (2-3 hr/day) to sunlight every day for acclimatization. The well grown plants from both conditions were used for further studies.

RESULT AND DISCUSSION

Shoot tip, node and leaf explants were cultured on different concentrations of BAP and KN (0.5-3.0 mg/l) supplemented MS-B5 medium. High rate of shoot induction i.e 33.0 was noticed at 2.0 mg/l BAP and 29.3 were noticed 2.0 mg/l KN after four weeks. Multiple shoot initiation was noticed from 0.5 and 2.0 mg/l BAP and KN. In the present study, MS basal (Murashige and Skoog, 1962) medium with B5 vitamins (Gamborg*et al.,* 1968) was supplemented with various concentrations and combinations of different growth regulators were used. MS media has also found to be more effective than other media by other investigations (Tefera and Wannakrairaj, 2004; Komalavalli and Rao, 2000; Ndoye *et al.,* 2003). BAP has been successfully used in many tissue culture protocols of other cereals (Vasil and Vasil, 1981, Oldach *et al*., 2001). BAP is more effective than KN for multiple shoot induction in *Sesame* (Ahmed, *et al*., 2007) and the efficiency of BAP over KN was agreed on in different plant regeneration systems (Bhojwani,

1980; We Lander *et al*., 1989; Devi *et al*., 1994; Baskaran and Jayabalan, 2003; Baskaran and Jayabalan, 2005).

Shoot multiplication rate of nodal explants was higher than shoot tip explants and maximum percentage of response and number of shoot were noticed on the medium containing 2.0 mg/l of BAP (32.7) and KN (23.9). BAP and KN at higher concentration 2.5 mg/l and 3.0 mg/l suppressed the proliferation of shoot, from the nodal explants and induced the basal callus (Table 1). There are numerous examples in the literature to show that nodal explants are most effective, these include *Asclepias curassavica* (Pramanik and Datta 1986), *Gymnema sylvestre* (Komalavalli and Rao, 1997) and *Tylophora indica* (Bera and Roy 1995), *Balisopermum montanum* (Sasikumar *et al.,* 2009). BAP or KN alone induced a higher frequency of multiple shoots. Similar results were obtained by Verma and Kant (1996), in *Emblica officinale* and Deka *et al*., (1999) in *Withania somnifera.*

The leaf explants were cultured on MS-B5 medium with different combinations and concentration of BAP and KN (0.5-3.0 mg/l). The explants failed to produce multiple shoot even on repeated subcultures and the explants showed positive response for callus induction on the MS medium containing different combination and concentration of auxins and cytokinins (Table-2). Callus formation can be useful for the establishment of cell suspension culture (Kumar and Kanwar 2007, Vasil *et al*., 1990). Some researchers have reported that an appropriate combination of NAA and BAP stimulated shoot formation (Tokuhara and Mii, 1993; Tisserat and Jones, 1999; Roy and Banerjee, 2003; Janarthanam and Seshadri (2008).

The regenerated and elongated shoot lets were transferred to the rooting medium containing different concentrations of auxins, IAA and IBA (0.5 to 3.0 mg/l). Maximum root initiation (51.8 %) was observed from the shoots differentiated from the leaf on the MS medium supplemented with 2.0 mg/l of IBA after 20 days of culture. In the IAA (2.0 mg/l) containing medium, 50.6 % of root formation was recorded. From the present observation it is evident that 2.0 mg/l IBA is an ideal concentration for *in vitro* root induction (Table 3). Similar observation was reported in earlier studies on *Anisochilus carnosus, Annona squamosa, Quisqualis indica* by Jeyachandran (2004), Roxana Ahmed (2005) and Poornima and Shivamurthy (2005), *Ocimum americanum, O. canum* and*O. Sanctum* (Pattnaik *et al.,* 1996), *Ocimum basilicum* like (Sahoo *et al*., 1997), *Clitoria ternatea* (Barik *et al*., 2007) and *Murraya koenigii* (Bhuyan *et al.,* 1997) and also in *Heracleum candicans* (Wakhlu and Sharma 1999) respectively. In general, IBA was found to be more effective to promote rooting as compared to the other growth regulators.

In the present study after the sufficient development of root the plantlets were successfully transplanted into small plastic pots containing vermiculite and the plantlets were successfully acclimatized without growth chamber facility. 100% plantlet survival was seen after hardening on garden soil, farmyard(manure) and soil (2:1:1) (Fig. 1). Sterilized garden soil minimized the cost of transplantation as documented by several authors (Agretious *et al*., 1996; Anand*, et al*., 1997). These results will encourage large scale *in vitro* propagation of this medicinal plant.

Table – 1 Effect of MS-B5 medium supplemented with BAP and KN on shoot proliferation from shoot tip and nodal explants of *P. minima* L.

Growth regulator (mg/l)	Shoot sprouting frequency %		Number shoots/explant		Shoot length (cm)	
	Shoot tip	Node	Shoot tip	Node	Shoot tip	Node
BAP						
0.5	48^{c}	55^{c}	14.3^{e}	15.1^{bc}	11.7^{a}	10.7^{a}
1.0	63^{bc}	69^{bc}	18.6^{c}	19.4^{b}	10.6^{b}	9.7^{ab}
1.5	75^{b}	82^{ab}	22.7^{ab}	23.6^{ab}	9.8^{c}	7.8^{bc}
2.0	85^{a}	90^{a}	33.0^{a}	32.7^{a}	7.3^{d}	6.8^{c}
2.5	69^{bc}	78^{b}	19.8^{bc}	21.9^{ab}	8.9^{c}	8.9^{b}
3.0	60^{b}	50^{d}	16.2^{d}	18.3^{b}	10.2^{b}	10.2^{a}
KN						
0.5	35^{c}	35^{d}	11.8^{f}	17.4^{b}	12.1^{a}	10.5^{a}
1.0	50^{b}	38^{cd}	15.7^{d}	21.2^{ab}	11.3^{b}	9.8^{ab}
1.5	56^{ab}	52^{b}	19.1^{ab}	25.6^{ab}	8.8^{c}	8.1^{b}
2.0	63^{a}	66^{a}	29.3^{a}	23.9^{a}	6.7^{d}	6.5^{c}
2.5	42^{bc}	58^{ab}	18.3^{b}	18.7^{bc}	5.3^{e}	7.2^{bc}
3.0	20^{d}	40^{c}	14.1^{ce}	14.1^{c}	8.2^{c}	6.8^{c}

Treatment means followed by different letters in their superscript are significantly different from each other comparison by Duncan's Multiple Range Test. Data recorded after 45 days of culture.

Table - 2 Effect of auxin (NAA) and cytokinins BAP on callus induction from leaf explants of *P. minima* L.

Explants	Growth regulators mg/l		% of callus induction	Nature of callus
	NAA	**BAP**		
	0.5	2.0	43^{c}	GC
	1.0	2.0	51^{b}	FY Bulky
	1.5	2.0	67^{a}	GCN
	2.0	2.0	54^{ab}	LGC
	2.5	2.0	43^{c}	LWC
Leaf				
	NAA	**KN**		
	0.5	2.0	32^{c}	GYF
	1.0	2.0	45^{ab}	GYF
	1.5	2.0	56^{a}	DGC
	2.0	2.0	41^{b}	LGF
	2.5	2.0	34^{c}	DYF

G-Green; Y-Yellow; B-Brown; F- Friable; W-White; D-Dark; N-Nodular

Treatment means followed by different letters in their superscript are significantly different from each other comparison by Duncan's Multiple Range Test. Data recorded after 45 days of culture

Table - 3 Roots produced per shoot explants on multiplication media with various concentration of IBA/IAA (0.5 – 3.0 mg/l)

Growth regulator (mg/l)	Culture showing response (%)	Number of roots/shoots	Root length (cm)
IBA			
0.5	72^{c}	14.7^{c}	10.2^{a}
1.0	80^{b}	26.2^{b}	8.7^{b}
1.5	87^{ab}	28.9^{ab}	7.3^{c}
2.0	94^{a}	32.7^{a}	6.5^{d}
2.5	87^{ab}	30.8^{ab}	8.9^{b}
3.0	78^{bc}	28.2^{ab}	9.6^{ab}
IAA			
0.5	68^{bc}	13.4^{d}	9.9^{a}
1.0	73^{b}	19.2^{b}	8.7^{bc}
1.5	79^{ab}	23.6^{ab}	8.5^{bc}
2.0	85^{a}	27.9^{a}	7.5^{c}
2.5	76^{ab}	21.7^{ab}	8.8^{bc}
3.0	62^{c}	18.4^{c}	8.1^{b}

Treatment means followed by different letters in their superscript are significantly different from each other comparsion by Duncan's Multiple Range Test. Data recorded after 45 days of culture

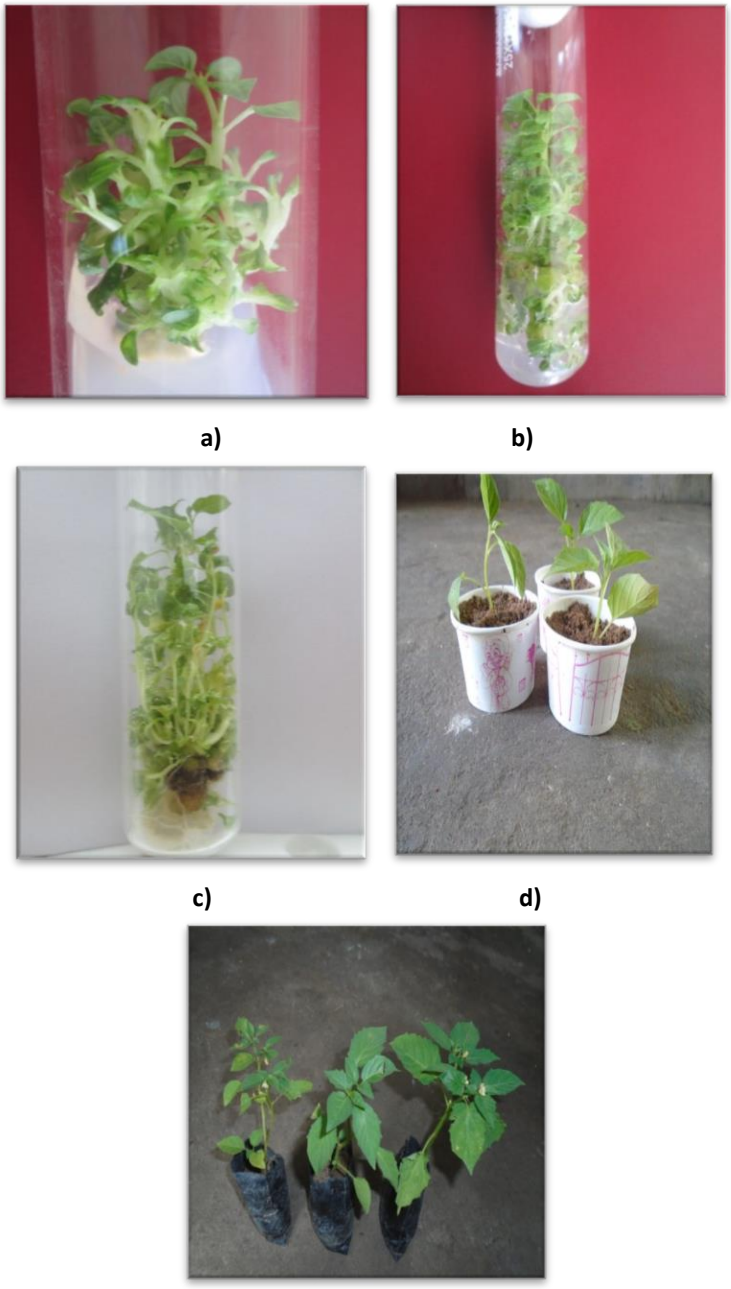

Fig. 1., a) Initiation of multiple shoots from shoot tip explants on MS medium fortified with BAP 1.0 mg/L after one week. b) Multiple shoot regeneration from nodal explants on MS medium supplemented with BAP 2.0 mg/L after three weeks. c) Direct rooting from regenerated shoots on MS medium fortified with IBA 2.0 mg/L after two weeks. d) and e) Hardened plant in poly cups containing mixture garden soil, farmyard (manure) and sand (2:1:1).

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REFERENCES

1. Agretious, T.K., Mortin, K. and Hariharan, M., (1996). *In vitro* clonal multiplication of *Alpinia calcarata* Rosc. *Phytomorphol.,* 46: 133 – 138.
2. Ahmed, M.B., Salahin, M., Karim, R., Razvy, M.A., Sultana, R., Hossain, R., and Islam, R. (2007). An efficient method for *in vitro* clonal propagation of a newly introduced sweetener plant (*Stevia rebaudiana* Bertoni.) in Bangladesh. *Am-Euras. J. Sci. Res.,* 2(2): 121-125.
3. Anand, P.H.M. and Hariharan, M., (1997). *In vitro* multiplication of greater galangal (*Alpinia galangal* L. Wild) a medicinal plant. *Phytomor.,* 47: 45 – 50.
4. Anis, M. and Faisal, M., (2005). *In vitro* regeneration and mass multiplication of *Psoralea corylifolia*-An endangered medicinal plant. *Ind. J. of Biotechnol.,* 4: 261-264.
5. Barik, D. P., Naik, S.K., Mudgal, A. and Chand, P.K., (2007). Rapid plant regeneration through *in vitro* axillary shoot proliferation of butterfly pea (*Clioria ternatea* L.) - a twinning legume. *In vitro Cell. Devl. Biol. Plant.* 43: 144-148.
6. Bhuyan, A. K., Pattnaik, S. and Chand, P.K., (1997). Micropropagation of curry leaf tree *Murraya koenigii* (L.) Spreng.) by axillary proliferation using intact seedlings. *Plant Cell Report.,* 16: 779-782.
7. Jeyachandran, R., (2004). *In vitro* culture root formation in *Anisochilus crnosus. J. Swamy Bot. Club.* 21: 27-30.
8. Komalavalli, N. and Rao, M.V. (1997). *In vitro* micropropagation of *Gymnema elegans* Wt. and Arn. - A rare medicinal plant. *Indian J. Exp. Biol.,* 35: 1088-1092.
9. Kritikar, K.R. and Basu, B.D., (1975). Indian Medicinal Plants, Lalit Mohan Basu, Allahabbad. 3: 2225-2227.
10. Kulkarni, V. M., Varshney, L.R., Bapat, V. A. and Rao, P.S., (2002). Somatic embryogenesis and plant regeneration in a seeded banana (*Exsete superbum* (Roxb.) Cheesman). *Curr. Sci.,* 83 (8): 939-941.
11. Kumar, S. and Kanwar, J.K., (2007). Plant regeneration from cell suspension in *Gerbera jamesonii* Bolus. *J. Fru. Orn. Plant Res.,* 15: 157-166.
12. Miura. Y., Fukui, H. and Tabata, M., (1987). Clonal propagation of chemically uniform fennel plants through somatic embryoids. *Planta Medica*, 53: 92– 94.
13. Murashige, T. and Skoog, F., (1962). A revised medium for rapid growth and bioassays for tobacco tissue cultures. *Physiol. Plant,* 15: 473-497.
14. Oldach, K. H., Morgenstern, A., Rother, S., Girgi, M., Kennedy, O. M., and Lorz, H., (2001). Efficient *in vitro* plant regeneration from immature zygotic embryos of pearl millet *Pennisetum glaucum* (L.) R. Br. and *Sorghum bicolor* (L.) Moench. *Plant Cell Reporter,* 20: 416-421.
15. Pattnaik, S.K. and Chand, P.E., (1996). *In vitro* propagation of medicinal herbs. *Plantce,* 11(15): 846 – 850.
16. Poornima, D., and Shivamurthy, G. R., (2005). Root formation in *Quisqualis indica* L. *J. Swamy Bot. Club.* 22: 37-38.
17. Rossi Hassani, B.D. and Zryard, J., (1995). *In vitro* culture and plant regeneration of large flowered purslane. *Plant Cell Tiss. Org. Cult.,* 41: 281- 283.
18. Roxana Ahmed, (2005). Best root formation in *Annona squamosa*. *Asian J. Microbial. Biotech. Env. Sci.,* 7(2): 191-194.
19. Ruby Priskilla, J., (2011). Phytochemical, anti-microbial, anti-cancerous, micropropagational studies and DNA barcoding of *Physalis minima* L. Ph.D., Thesis Bharathidasan University. Tiruchirappalli.
20. Sahoo, Y., Pattnaik, S. and Chand, P.K., (1997). *In vitro* clonal propagation of an aromatic medicinal herrb *Ocimum basilicum* L. (Sweet basil) by axillary shoot proliferation. *In vitro Cell. Dev. Biol. Plant.* 33: 293-296.
21. Sasikumar, S., Raveendar, S., Premkumar, A., Ignacimuthu, S. and Agastian, P., (2009). Micropropagation of *Baliospermum montanum* (Willd.) Muell. Arg. – A threatened medicinal plant. *Ind. J. Biotechnol.,* 8: 223 – 226.
22. Vasil, V. and Vasil, I. K., (1981). Somatic embryogenesis and plant regeneration from suspension culture of pearl millet (*Pennisetum americanum). Ann. Bol.,* 47: 669-678.
23. Vasil, V., Redway, F. and Vasil, I. K., (1990). Regeneration of plants from embryogenic suspension culture protoplasts of wheat (*Triticum aestivum* L.) *Biotechnol.,* 8: 429-433.
24. Wakhlu, A.K. and Sharma, R.K., (1999). Micropropagation of Heracleum candicans. Wall a rare medicinal herb. *In vitro* cellular and developmental biology. *Plant,* 35(1): 79 – 81.

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