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In Vitro Micropropagation of the Medicinal Plant *Physalis angulata* L.

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Abstract

Physalis angulata L. is an important medicinal herb. An efficient direct adventitious plant regeneration protocol was developed for large scale propagation using leaf disc as explants. The explants were cultured on MS basal medium supplemented with 0.25-3.0 mg/L 6-benzyl amino purine (BAP) for primary shoot proliferation. Inclusion of indole-3-acetic acid (IAA) and gibberellic acid (GA_3) in the culture medium along with BAP promoted a higher rate of shoot multiplication. The maximum number of shoots was produced in MS + BAP (1.0 mg/L) + IAA (0.5 mg/L) + GA_3 (0.20 mg/L) after the third subculture. An average of 152.8 ± 0.40 shoots were produced from each leaf disc. For root induction the shootlets were transferred to MS medium supplemented with different concentrations of indole-3-butyric acid (IBA). The highest percentage of root induction was observed in 1.0 mg/L (IBA). Rooted plants were successfully established in the soil after hardening. The survival percentage of rooted plants on soil was found to be 85%. This result will facilitate the conservation and propagation of the important medicinal herb *Physalis angulata* L.

Keywords: direct regeneration, green house, leaf disc, microshoots, subculture

Introduction

Physalis angulata L. belongs to the family Solanaceae. In India, it is commonly known as Mulluca. *P. angulata* is an annual herb that generally grows in warm weather conditions, with a height up to 3.5 m. The plant is of high economically importance because of its medicinal properties, used as the whole plant as well as parts of the plant (Mahalakshmi *et al.*, 2014). The extract of *Physalis* exhibits *in vitro* antimicrobial action against some bacterial and fungal pathogenic organisms (Osho *et al.*, 2010).

Nowadays, *P. angulata* L. is facing a rarity in India, since the plant species are generally considered weeds in agricultural crops; hence the farmers weeding the plant from the fields contribute towards its rarity.

To overcome its rarity due to the over exploitation of land by agricultural means, an efficient method for rapid propagation of this species is highly desirable. Reports on *in vitro* propagation of this species with leaf disc explants are not available till date. However, Aniel Kumar *et al.* (2015) reported *in vitro* propagation of *Physalis* species using axillary meristem explants. On the other hand, there are few reports on adventitious shoot regeneration through direct organogenesis using leaf explants of some medicinally important plant species such as *Pothomorphe umbellata* (Pereira *et al.*, 2000), *Tanacetum cinerariifolium* (Hedayat *et al.*, 2009), *Withania somnifera* (L.) Dunal (Aniel Kumar *et al.*, 2011), *Pogostemon cabin* (Hua *et al.*, 2014). Direct regeneration from explants without an intervening callus phase has several advantages, including production of true type progenies (Manickavasagam *et al.*, 2004).

Keeping in mind the importance of the *P. angulata* L., the present study was aimed at developing a large scale propagation protocol through direct plant regeneration using leaf disc explants.

Materials and Methods

Juvenile leaves collected from young branches of mature *Physalis angulata* L. growing within the Herbal Garden of Botany Department, Andhra University, India, were washed thoroughly with 5% labolean solution, under running tap water for 15 min. After several rinses with distilled water, the leaves were surface disinfected with 0.1% $HgCl_2$ (w/v) for 5-7 min and then rinsed with sterile double distilled water.

MS (Murashige and Skoog, 1962) media was used for the study. The sucrose content of the media was 3% and the pH of the media was adjusted at 5.8 before autoclaving at 15 pound pressure for 15 min. Gelling of the media was done with 0.8% agar powder (tissue culture grade). The cultures were maintained under cool fluorescent light intensity of 2,000-3,000 lux for 16-8 h light-dark period and temperature of 22 ± 2 °C. Each treatment consisted of 25 replicates and was repeated twice. The effect of cytokinin on propagation was observed singly or in combination with IAA and GA_3. Twelve different concentrations of BAP (0.25, 0.50, 0.75, 1.0, 1.25, 1.50, 1.75, 2.0,

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2.25, 2.50, 2.75 and 3.0 mg/L) were tested for shoot multiplication. The microshoots were repeatedly subcultures for four times at a constant three-week interval on one concentration of BAP (1.0 mg/L) with three concentrations of IAA (0.25, 0.50 and 0.75 mg/L) and three concentrations of GA_3 (0.10, 0.20 and 0.30 mg/L) for adventitious shoot development.

To initiate rooting, 2 to 2.5 cm microshoots were transferred to MS full strength medium with four concentrations of IBA (0.50, 1.0, 1.50 and 2.0 mg/L). The rooted plantlets were transferred to MS basal liquid medium after two weeks and were kept in the laboratory environment for one week. After one week the plantlets were transferred to sterile double distilled water and kept for 2 to 4 days in the culture room. The plantlets were further transferred into sterile soil in pots and kept in culture room whereas regular watering was followed. After attaining a considerable height, the plantlets were first transferred to green house environment and finally transferred to the natural environment.

Fig. 1. Direct *in vitro* micropropagation of *Physalis angulata* L. using leaf disc explants; (a) Direct induction of multiple shoots at the cut ends of the leaf disc; (b, c & d) Proliferation of multiple shoots at first, second and third subcultures, respectively, on MS + BAP (1.0 mg/L) + IAA (0.50 mg/L) + GA_3 (0.20 mg/L); (e) rooting *in vitro* grown plantlet in MS + IBA (1.0 mg/L); (f) Acclimatized plant in earthen pot

Table 1. Effect of BAP on leaf disc explants of *Physalis angulata* L.

S.No.	BAP (mg/L)	*In vitro* response	
		Shooting (%)	Shoot no/ explants
01.	-	00.0 ± 0.00	0.0 ± 0.00
02.	0.25	74.8 ± 0.15	4.0 ± 0.14
03.	0.50	80.6 ± 0.20	5.4 ± 0.10
04.	0.75	85.0 ± 0.24	10.2 ± 0.25
05.	1.00	100.0 ± 0.00	16.0 ± 0.20
06.	1.25	96.0 ± 0.20	13.8 ± 0.13
07.	1.50	91.8 ± 0.31	11.2 ± 0.22
08.	1.75	88.2 ± 0.14	9.2 ± 0.32
09.	2.00	85.4 ± 0.33	6.4 ± 0.24
10.	2.25	83.6 ± 0.18	6.0 ± 0.18
11.	2.50	78.4 ± 0.26	5.1 ± 0.30
12.	2.75	70.1 ± 0.31	3.8 ± 0.16
13.	3.00	66.4 ± 0.25	3.3 ± 0.21

Values are mean ± SE of twenty five replicates

Results and Discussion

The establishment of a vast and a rapid micropropagation system in plant tissue culture has best results by direct plant regeneration through adventitious multiple shoot induction from the explants; thus, a protocol of such manner was adopted for *Physalis angulata*, as it does not involve an intermediate callus phase, and tends to produce genetically similar plants (Bao *et al*., 2001). However, plant regeneration using callus system requires a longer period to establish the culture and to obtain embryogenic calli, along with the risk of somaclonal variation in the plants regenerated from callus (Bregitzer and Tonks, 2003; Kishore *et al*., 2006; Yookongkaew *et al*., 2007).

In the present study MS, media supplemented with different concentrations of BAP ranging from 0.25-3.0 mg/L showed effective multiplication of shoots from leaf disc explants directly (Table 1 and Fig. 1a). The frequency of shooting increased with the concentration of BAP from 0.25 to 1.0 mg/L, then decreased with further increase in the concentration among the various concentrations tested. A maximum 16 shoots per explants, with 100% induction, was recorded after four weeks of culture in MS containing BAP (1.0 mg/L). This may be due to BAP presence, since it is involved in reprogramming and differentiation of the

Table 2. Proliferation of multiple shoots of *Physalis angulata* L. at different subcultures

S.No.	Plant growth regulators (mg/L)			Subculture			
	BAP	IAA	GA_3	1st	2nd	3rd	4th
01.	1.00	0.25	0.10	26.2 ± 0.30	52.5 ± 0.15	88.2 ± 0.28	64.0 ± 0.18
02.	1.00	0.50	0.20	40.4 ± 0.17	92.4 ± 0.20	152.8 ± 0.40	122.2 ± 0.22
03.	1.00	0.75	0.30	32.8 ± 0.23	66.3 ± 0.34	100.2 ± 0.31	81.8 ± 0.16

Values are mean ± SE of twenty five replicates

Table 3. *In vitro* rhizogenesis of *Physalis angulata* L. microshoots

S.No.	MS medium with IBA (mg/L)	Rhizogenesis		
		Root induction (%)	Root no. /shoot	Root length (cm)
01.	0.00	00.0 ± 0.00	00.0 ± 0.00	0.0 ± 0.00
02.	0.50	90.0 ± 0.18	16.4 ± 0.10	11.8 ± 0.11
03.	1.00	98.2 ± 0.11	22.2 ± 0.08	15.0 ± 0.12
04.	1.50	84.0 ± 0.21	18.0 ± 0.22	10.4 ± 0.08
05.	2.00	70.2 ± 0.33	12.8 ± 0.12	6.8 ± 0.04

Values are mean ± SE of twenty five replicates

competent cells necessary for adventitious bud development, and has been reported to induce synthesis or accumulation of endogenous cytokinins (Hutchinson and Saxena, 1996).

Further, shoot multiplication and elongation were observed when the developed shoot clusters from BAP (1.0 mg/L) were transferred into fresh MS medium containing BAP (1.0 mg/L) + IAA (0.25-0.75 mg/L) + GA_3 (0.10-0.30 mg/L) and subculture regularly at an interval of every three weeks. During subcultures (e.g. shoot proliferation stage), it was noticed a decline in shoot formation capacity over repeated subcultures (Table 2). This may be due to the morphogenetic potential of the tissue that gradually decreased, which indicated that apical control increased during successive subculturing (Remphrey *et al*., 1993). BAP (1.0 mg/L) combined with IAA (0.50 mg/L) and GA_3 (0.20 mg/L) was found to be most effective and produced a maximum of 152.8 shoots per leaf disc in the third subculture (Fig. 1b,c and d).

In the present investigation, the *in vitro* response for *P. angulata* (e.g. 152.8 shoots per leaf disc) had better results when compared to the earlier reports on a related plant species achieved from culturing *in vitro* leaf explants of *Tanacetum cinerariifolium* (21.3 shoots) (Hedayat *et al*., 2009), *Pogostemon cablin* (63.8 shoots) (Hedayat *et al*., 2009) and *Withania somnifera* (68 shoots) (Aniel Kumar *et al.,* 2011).

The elongated shoots were separated and transferred to MS medium containing different concentrations of IBA for root induction. Emergence of roots occurred within a period of one week. Further incubation of one week led to a very vigorous root growth. The maximum root growth (e.g. 98.2 % root induction; 22.2 roots per shoot and 15 cm root length per root) was recorded in MS with 1.0 mg/L of IBA as a supplement (Table 3 and Fig. 1e). These results were in accordance with the findings from previous studies of Aniel Kumar *et al.* (2015). After sequential hardening, the plantlets were transferred to the greenhouse, where 85% of them survived (Fig. 1f).

Conclusions

The successful production of direct multiple shoots and *in vitro* root formation were dependent on the nutrient medium and the culture conditions. The protocol standardized hereby could be used to isolate medicinally important secondary metabolites such as Physalins from multiple shoots. The hereby proposed propagation protocol through direct plant regeneration using leaf disc explants would also have significant application in genetic transformations of this medicinally important plant.

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