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Establishment of a Rapid Plant Regeneration System in *Physalis angulata* L. through Axillary Meristems

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Abstract

An optimal plant propagation method of *Physalis angulata* L., a medicinally important herbaceous plant species has been developed using axillary meristem explants. Shoot bud proliferation was initiated from axillary meristem explants cultured on MS medium supplemented with various concentrations of 0.5-2.5mg/L/(BAP)/(Zeatin)/(KIN). The maximum *in vitro* response of shooting frequency of explants (88.1%) and shoots per explant (42) was achieved with medium containing 1.0mg/L BAP. Multiple shoot culture was established by repeated subculturing of the shoot buds of axillary meristems on shoot multiplication medium. Among the subculture media BAP in combination with 1.5mg/L (IAA)+0.25mg/L(GA_3) produced maximum shoots per explant (128±0.29) after two weeks of culture. Effective *in vitro* shoot elongation and rooting was achieved on 1.0mg/L(GA_3) and 1.0mg/L(IBA), respectively. Most of the generated shoots were successfully transferred to soil under field conditions. The survival percentage of the transferred plants on soil was found to be 90 per cent. This protocol can be used for commercial propagation and for future genetic improvement studies.

Keywords: axillary meristem, conservation, multiple shoots, *Physalis angulata*, rhizogenesis, zeatin

Abbreviations: BAP (Benzyl amino purine), IAA (Indole acetic acid), IBA (Indole butyric acid), KIN (Kinetin), PGR (Plant growth regulator)

Introduction

Medicinal plants play a crucial role in health care needs of people around the world especially in developing countries (Rao *et al.*, 2004; Bekalo *et al.*, 2009). About 80% of the population from developing countries still depends on the use of traditional medicine (Cunningham, 1993). *Physalis angulata* L. belongs to *Solanaceae* family and it is distributed throughout tropical countries (Sultana, 2008). The plant have multimedicinal applications against various diseases such as bladder, spleen and liver inflammations (Pinto *et al.*, 2010), sedative (Agra *et al.*, 2007), depurative, antirheumatic, relief of earache, diabetes and hepatitis (Lawal *et al.*, 2010), asthma (Rathore *et al.*, 2011), malaria (Ruiz *et al.*, 2011), cancer (Hseu *et al.*, 2011), antipyretic and antinociceptive (Bastos *et al.*, 2006), antidiuretic (Nanumala, 2012), sore throat, abdominal pain and cervicitis (Mahalakshmi *et al.*, 2014). Besides this, plant also shows good antibacterial and antifungal activities (Osho *et al.*, 2010). Conventionally this plant is propagated through seeds. Natural population of this plant species is decreasing due to indiscriminate exploitation and this has been the trend for decades in developing countries (Navdeep *et al.*, 2015). Therefore, it is necessary that a systematic propagation to be introduced in order to conserve biodiversity and protect these highly medicinal plant species. The plant regeneration from meristems is considered to be one of the most promising ways for multiplying a selected variety true to its type. Such individuals are genetically similar showing the same agronomic characteristics. To our knowledge there are no published reports on multiplication of *Physalis angulata* L. However, plant regeneration studies have been reported in different species of *Physalis* viz., *P. peruviana* (Otroshy *et al.*, 2013), *P. minima* (Farhana *et al.*, 2009; Arvind *et al.*, 2011), *P. pubescence* (Rao *et al.*, 2004; Mona. 2013), *P. ixocarpa* (Guzman *et al.*, 2009). Hence the *in vitro* propagation of this medicinally important species was undertaken. The present study describes the maximization of shoot multiplication through *in vitro* regeneration of *Physalis angulata* L. by axillary meristem explants using standard culture medium fortified with different plant growth regulators. The protocols presented here should become a valuable part of future efforts in genetic improvement of *Physalis angulata*.

Materials and Methods

Plant material and explant source

One month old plants of *Physalis angulata* L. grown in the Experimental Farm of Botany, Andhra University were selected as stock plants. The leaves and roots were discarded and shoots were washed thoroughly under running tap water for 20min. Axillary portion was used as an explant.

Surface sterilization

Axillary meristem explants were washed thoroughly in running tap water for 10min, then treated with two drops of

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aqueous surfactant Tween 20 (5% v/v) for 5min, followed by repeated rinsing with distilled water. Further, sterilization was done under aseptic conditions in Laminar Air Flow cabinet. Explants were surface sterilized with 50%(v/v) ethyl alcohol (1min) followed by 0.1% (w/v) $HgCl_2$ (3min). Finally, the explants were washed thoroughly (3 to 5 times) with sterilized distilled water to remove traces of $HgCl_2$.

Culture medium

Axillary meristem stem explants appropriate size (about 1 cm) were cultured on sterilized MS media (MS basal and MS with different concentrations of auxins [IAA(indole acetic acid), IBA(indole butyric acid)], cytokinins [BAP(benzyl amino purine), Zeatin, KIN(kinetin)] and GA_3(gibberellic acid) alone and in combined forms. All the media consists with 3% (w/v) sucrose and gelled with 0.8% (w/v) agar-agar (Murashige and Skoog, 1962). The pH of all media was adjusted to 5.8 and sterilized by autoclave at 121°C for 15 min. The cultures were incubated at 22± 2°C under a 16 h photoperiod (50 $\mu E^{-2}s^{-1}$ irradiance) provided by cool white fluorescent tubes.

Direct multiple shoot induction and multiplication

For direct shoot bud induction, the axillary meristem explants were cultured on MS+0.5-2.5mg/L(BAP)/(Zeatin)/(KIN) individually. After four weeks of incubation, the developed multiple shoots were subcultured on..MS+0.5-2.5mg/L(BAP)/(Zeatin)/(KIN)+0.5mg/L(IAA)+0.25gm/L(GA_3) for further shoot proliferation.

Shoot elongation and rhizogenesis

For shoot elongation, the microshoots from the multiple shoot clusters were cultured on MS medium fortified with different concentrations of GA_3 (0.5-2.0mg/L). Following shoot elongation, the elongated shoots were transferred onto MS medium supplemented with various concentration of IBA (0.5-2.0mg/L) individually for Rhizogenesis. Observations were taken at the end of the second week respectively.

Acclimatization

Well rooted plantlets were thoroughly washed to remove the adhering gel and planted in 10 cm plastic cups containing a mixture of peat moss and organic manure (1:1). Plastic cups were covered with polythene bags to maintain humidity. Plants were kept in culture room for ten days. Half strength MS macro salts was poured to the plastic cups at five day regular intervals until the new leaves developed. Plants were transferred to pots containing organic manure, garden soil and forest humus (1:1:1). The pots were watered at a two day interval and were maintained in greenhouse. The survival rate was recorded one month after transfer to pots.

Data analysis

Experiments were set up in Randomized Block Design (RBD) and each experiment was repeated thrice with fifteen replicates. The results observed at regular intervals and tabulated. Mean and Standard Error values were carried out for each treatment.

Results and Discussion

Axillary meristem explants were excised from natural plants and were cultured on different plant growth regulator (PGR) regimes. The MS medium without any PGR was failed to induce *in vitro* response (Table 1). Direct regeneration was found in all concentrations of cytokinins alone with MS medium at four weeks of culture. This indicates the necessity for supplementation of plant growth regulators externally for induction of multiple shoots (Sasikumar *et al.,* 2009). Of the three cytokinins tested, BAP treated explants achieved higher regeneration than those treated with Zeatin and KIN (Table 1). The 1.0mg/L (BAP) treatment yielded maximum regeneration (88.1%) and number of shoots per explant (42.0±0.19) (Table 1 and Fig. 1a). The ability of BAP to induce *in vitro* response is well documented (George, 1993). In general, herbaceous plants are highly responsive to BAP treatment and most cultured herbaceous species produces robust, well-formed shoots suitable for further shoot proliferation (Debergh and Zimmerman, 1999). At BAP, Zeatin and KIN concentrations being higher than 1.0mg/l, the number of shoots as well as the percentage response was lower. Similar study was also reported in species *P. minima* (Farhana *et al.*, 2009). Further the shoot buds were repeatedly subculturing on MS media fortified with 0.5-2.5mg/L/(BAP)/(Zeatin)/(KIN)+0.5mg/L(IAA)+0.25mg/L(GA_3) for inducing adventitious multiple shoots. Enhanced

Table 1. Effect of cytokinins on axillary meristems of *Physalis angulata* L. at four weeks of culture

S.No.	MS medium fortified with cytokinins(mg/L)			*In vitro* response	
	BAP	Zeatin	KIN	Shooting (%)	Shoot no./ explants
01.	-	-	-	00.0±0.00	00.0±0.00
02.	0.5	-	-	73.7±0.38	26.0±0.20
03.	1.0	-	-	88.1±0.33	42.0±0.19
04.	1.5	-	-	77.4±0.59	32.0±0.25
05.	2.0	-	-	69.4±0.68	26.0±0.36
06.	2.5	-	-	64.4±0.68	18.0±0.31
07.	-	0.5	-	68.6±0.49	16.0±0.30
08.	-	1.0	-	71.1±0.23	24.0±0.16
09.	-	1.5	-	69.7±0.50	20.0±0.10
10.	-	2.0	-	66.7±0.19	17.0±0.23
11.	-	2.5	-	58.7±0.38	12.0±0.28
12.	-	-	0.5	54.0±0.33	10.0±0.14
13.	-	-	1.0	63.1±0.33	16.0±0.29
14.	-	-	1.5	60.0±0.33	12.0±0.38
15.	-	-	2.0	52.1±0.33	08.0±0.20
16.	-	-	2.5	52.0±0.33	05.0±0.21

Values are mean ± SE of Fifteen replicates

Table 2. Effect of PGR's on proliferation of shoot buds of *Physalis angulata* L. at two weeks of culture

S.No.	MS medium fortified with plant growth regulators(mg/L)					No. of multiple shoots/explant
	BAP	Zeatin	KIN	IAA	GA_3	
01.	0.5	-	-	0.5	0.25	74.00±0.40
02.	1.0	-	-	0.5	0.25	128.00±0.29
03.	1.5	-	-	0.5	0.25	98.50±0.32
04.	2.0	-	-	0.5	0.25	90.00±0.24
05.	2.5	-	-	0.5	0.25	82.80±0.19
06.	-	0.5	-	0.5	0.25	24.00±0.15
07.	-	1.0	-	0.5	0.25	74.00±0.18
08.	-	1.5	-	0.5	0.25	60.00±0.27
09.	-	2.0	-	0.5	0.25	55.80±0.20
10.	-	2.5	-	0.5	0.25	46.70±0.30
11.	-	-	0.5	0.5	0.25	15.00±0.22
12.	-	-	1.0	0.5	0.25	60.00±0.32
13.	-	-	1.5	0.5	0.25	49.50±0.23
14.	-	-	2.0	0.5	0.25	45.00±0.10
15.	-	-	2.5	0.5	0.25	22.70±0.14

Values are mean ± SE of Fifteen replicates

Table 3. Effect of GA_3 on elongation of micro shoots of *Physalis angulata* L. at two weeks of culture

S.No.	MS medium fortified with GA_3 (mg/L)	Shoot length(cm)
1.	0.00	00.00±0.00
2.	0.50	09.00±0.06
3.	1.00	12.00±0.10
5.	1.50	11.00±0.22
6.	2.00	10.00±0.14

Values are mean ± SE of Fifteen replicates

Fig. 1. *In vitro* plant regeneration of *Physalis angulata* L.
(a) Direct shoot bud induction from axillary meristem, (b) Proliferating multiple shoots, (c) Rooted plantlet, (d) *In vitro* developed plant after acclimatization

shoot multiplication in subsequent subculture is in accordance with published literature on medicinal plants like *Solanum nigrum* (Kavitha *et al*., 2012), *Catharanthus roseus* (Pandey *et al*., 2014) and *Orthosiphon stamineus* (Sheena *et al*., 2015). An average of 128 shoots was obtained from a single axillary meristem after two weeks of culture (Table 2 and Fig. 1b). According to the previous research outcomes on plant regeneration from different *Physalis* species viz., *P. pubescens* L. (Rao *et al*., 2004; Mona, 2013), *P. ixocarpa* Brot. (Guzman

Table 4. Rhizogenesis of elongated shoots of *Physalis angulata* L. at two weeks of culture

S.No.	MS medium fortified with IBA (mg/L)	Rhizogenesis		
		Root induction (%)	Root No. / shoot	Root length (cm)
01.	0.00	00.0±0.00	00.0±0.00	00.0±0.00
02.	0.50	72.0±0.18	15.0±0.18	10.2±0.23
03.	1.00	100±0.00	18.2±0.10	14.5±0.20
04.	1.50	85.0±0.29	13.6±0.22	9.0±0.11
05.	2.00	66.0±0.22	9.4±0.06	5.8±0.14

Values are mean ± SE of Fifteen replicates

et al., 2009) and *P. minima* L. (Arvind *et al*., 2011; Ramar, 2014) the present investigation proved the best regeneration capacity. The shoots lengthen from 1.0 cm to 1.5 cm were separated from multiple shoot cluster and transferred to MS medium containing GA_3 (0.5-2.0 mg/L) for shoot elongation. The maximum shoot elongation (8 cm/shoot) was achieved on MS medium fortified with GA_3 (1.0mg/L) (Table 3). 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 was recorded on MS with 1.0mg/L of IBA as a supplement (Table 4 and Fig. 1c). Similar effects of IBA were also observed in *Solanum trilobatum* (Arockiasamy *et al*., 2002) and *Physalis peruviana* (Otroshy *et al*., 2013). After sequential hardening, the plantlets were transferred to greenhouse where 90% of them survived (Fig. 1d).

Conclusions

The present study presents a rapid, efficient and reliable micropropagation protocol for *in vitro* regeneration of *Physalis angulata* L. from axillary meristem explants. The described protocol can be used for large scale propagation and may become a valuable part of strategies for *ex situ* conservation of this important medicinal herb.

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