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Original Research Article

In Vitro Shoot Multiplication and Plant Regeneration of *Physalis peruviana* L. An Important Medicinal Plant

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A B S T R A C T

Physalis peruviana(L) is an important medicinal plant which belongs to the family Solanaceae. It is widely used as folk medicine and treating for many diseases. The efficient *in vitro* regeneration of *Physalis peruviana* was achieved from node, internode and leaf explants on MS medium with B5 vitamins and different concentrations and combinations of PGRs like BAP,GA_3 and 2,4-D.The maximum numbers of multiple shoots were achieved from nodal and internodal explantson 2.0 mg/l BAP + 1.0mg/l GA_3 + 1.0mg/l 2, 4-D. The high frequency of shoot multiplication observed from leaf explants on 3.0 mg/l BAP+1.0mg/l GA_3+ 1.0mg/l 2, 4-D.The regenerated shoots were transferred in to half strength MS medium fortified with IBA for root induction. Rooted plantlets were successfully acclimatized. The current study showed efficient in vitro shoot multiplication and regeneration capabilities of *Physalis peruviana* L.

Keywords

In vitro regeneration; Shoot multiplication; MS medium; BAP; GA_3; IBA; 2, 4-D.

Introduction

Physalis peruviana is a medicinal plant and it is commonly known as Cape gooseberry or golden berry. It belongs to the family Solanaceae, in which the genus *Physalis* includes about 100 species, which form their fruits in an inflated calyx (Legge, 1974).*Physalis peruviana* L. (Cape gooseberry) originates from the Andean highlands of South America. After Christopher Columbus the Cape gooseberry was introduced into Africa and India (Popenoe, 1990).This plant fruits is smooth berry, resembling a miniature, spherical, yellow tomato. The seeds are bright yellow to orange in color, and it is sweet when ripe, with a characteristic, mildly tart flavor, making it ideal for snacks, pies, or jams. Cape gooseberryis commercially produced in Ecuador, South Africa, Kenya, Zimbabwe, Australia, New Zealand, Hawaii, India, Malaysia, Colombia, and China. Many researchers indicate that *Physalis peruviana* are widely used medicine foranticancer, ant mycobacterial, antipyretic, immunomodulatory and treatingmalaria,

asthma, hepatitis, dermatitis, as rheumatismand diuretic (Pietro *et al*. 2000 and Soares *et al*. 2003) (Perry, 1980).The ethanol extracts from *Physalis peruviana* contain well an antioxidant activity and highest (95%) antioxidant properties (Chun-Ching Lin, 2005). *Physalis peruviana* showed main elements of K, Mg, Ca and Fe in its mineral composition and the lipidic fraction presented predominance of the linoleic acid (72, 42%) in its composition (Rodrigues *et al.* 2009). Some researchers noted that *Physalis peruviana* has a several physalin compounds (Kawai *et al.* 1992, Sen and Pathak, 1995). Some physalin compounds like physalin B and F were noted to have great potential for treating tumour (Antoun *et al.* 1981, Chiang *et al*. 1992a, b and Sunayama *et al.*, 1993).

For this above medicinal purposes, this plant is highly focused in many countries and pharmaceutical industries. Tissue culture plays an important key role for medicinal plants in rapid propagation, conservation and enhanced the production of secondary metabolites. The secondary metabolites production can be possible through *in vitro* plant cell culture (Barz and Ellis, 1981) (Deus and Zenk, 1982).In this present investigation was undertaken with an objective to develop an efficient *in vitro* regeneration protocol for important medicinal plant *Physalis peruviana* L. through nodal, internodal and leaf explants.

Materials and Methods

Healthy plants of *Physalis peruviana* were collected from Samuthiram, Tiruchirappalli district, Tamilnadu. Live specimens were planted in the Botanical Garden, National College (Autonomous), Tiruchirappalli in green house conditions. Nodal and leaf explants of *Physalis peruviana* L.were collected from two months old greenhouse grown plants. The sterilization of explants was done by dipping them in 70% ethanol for 10 seconds followed by continuous shaking. Then the explants were washed with detergent Tween-20 for 5 mins and after that explants were surface sterilized by 0.1% mercuric chloride ($HgCl_2$) for 1 min then finally rinsed for 3 times with sterilized distilled water. All the process of sterilization and transfer were carried out inside the laminar air flow with proper sterilization techniques. The nodal and leaf explants were inoculated to the MS medium (Murashige and Skoog, 1962) with B5 vitamins (Gamborg *et al*., 1968) and different concentrations and combinations of plant growth regulators like BAP (0.5-4.0mg/l), GA_3(0.5-2.5mg/l)& 2, 4-D (0.5-2.0mg/l).The cultures were maintained at 25± 2^0C under a 16 hour photoperiod of 35µmol m^{-2} s^{-1} irradiance provided by cool white fluorescent light with 55-65% relative humidity. Observations were recorded after an interval of four weeks. For root induction, *in vitro* elongated shoots were excised and transferred to half strength MS basal medium supplemented with IBA (1.0 mg/l). After the rooted plantlets were transferred to pots containing cow- dung, sand and red soil (1:1:1) for hardening.

Results and Discussion

Node, internode and leaf explants were inoculated on MS basal medium with B5 vitamins supplemented with various concentrations and combination of BAP (0.5-4.0mg/l), GA_3(0.5-2.5mg/l)& 2, 4-D (0.5-2.0mg/l) were used for culture initiation and multiplication of shoots. After 12 days of inoculation multiple shoot induction was observed from the

Table.1 Effect of different concentrations of plant growth regulators on multiple shoot induction from nodal and internodal explants of *Physalis peruviana*

S.No	Different concentrations of Plant growth regulators (Mg/l)			Response 4 Weeks Culture	Morphogenetic Nature
	BAP	GA_3	2, 4-D	No.of shoot(M±SE)	
1	0.5	0	0	11.8 ± 0.26	Few shoots with less growth
2	1.0	0	0	12.0 ± 0.25	Few shoots with small leaves
3	1.5	0.5	0.5	13.5 ± 0.30	Multiple shoots with retarded growth and mediated leaves
4	2.0	1.0	1.0	15.4 ± 0.32	Multiple shoots with large leaves and well growth
5	2.5	1.0	0.5	12.1 ± 0.23	Few shoots with small leavesless growth
6	3.0	1.5	0.5	11.1 ± 0.26	Few shoots with small leaves and less growth
7	3.5	2.0	0.5	11.2 ± 0.27	Few shoots with small leaves and less growth
8	4.0	2.5	0.5	11.0 ± 0.24	Few shoots with small leaves and less growth

Table.2 Effect of different concentrations of plant growth regulators on multiple shoot induction from leaf explants of *Physalis peruviana*

S.No	Different concentrations of Plant growth regulators (Mg/l)			Response 4 Weeks culture	Morphogenetic Nature
	BAP	GA_3	2, 4-D	No.of shoot(M±SE)	
1	0.5	0	0	10.9 ± 0.26	Few shoots with less growth
2	1.0	0	0	11.1 ± 0.23	Few shoots with small leaves
3	1.5	0.5	0	11.1 ± 0.27	Few shoots with small leaves and less growth
4	2.0	1.0	0.5	12.0 ± 0.25	Few shoots with small leaves and less
5	2.5	1.0	0.5	13.0 ± 0.24	Multiple shoots with retarded growth and mediated leaves
6	3.0	1.0	1.0	15.3 ± 0.33	Multiple shoots with large leaves and well growth
7	3.5	1.5	1.5	12.2 ± 0.22	Few shoots with small leaves and less growth
8	4.0	2.0	2.0	11.8 ± 0.25	Few shoots with small leaves and less growth

Figure.1 [A & B] Multiple Shoot Induction of *Physalis peruviana* from Nodal explants at different concentrations of BAP (2.0 mg/l) + GA_3 (1.0 mg/l) + 2, 4-D (1.0 mg/l)

A. Multiple shoot induction from nodal explant by 14 days inoculation

B. Elongated shoot from nodal explant by after 4 weeks of inoculation

Figure.2 [C & D] Multiple Shoot Induction of *Physalis peruviana* from Internodal explants at different concentrations of BAP (2.0 mg/l) + GA_3 (1.0 mg/l) + 2, 4-D (1.0 mg/l)

C. Multiple shoot induction from internodal explant by 14 days of inoculation.

D. Elongated shoots from internodal explant by after 4 weeks of inoculation

Figure.3 [E & F] Multiple Shoot Induction of *Physalis peruviana* from Leaf explants at different concentrations of BAP (3.0 mg/l) + GA_3 (1.0 mg/l) + 2, 4-D (1.0 mg/l)

E. Multiple shoot induction from leaf explant by 14 days of inoculation.

F. Elongated shoots from leaf explant by after 4 weeks of inoculation

explants. The mean number of multiple shoots was recorded on after 4 weeks of inoculation. In nodal and internodal explants were showed the maximum number of multiple shoots on BAP (2.0mg/l) + GA_3 (1.0mg/l) + 2, 4-D (1.0mg/l) and obtained the mean value 15.4 is the best response (Table. 1) (Fig. 1. A & B) (Fig. 2. C & D).The similar results has also suggested by Ramar *et al*.,(2014) on *Solanum americanum* in BAP 3.0 mg/l+2, 4-D 0.5 mg/l+GA_3 2.0 mg/l through nodal explants.These findings are in agreement with who observed in other plant species *Aegle marmelos*(L) (Ajithkumar and Seeni, 1998). The highest number of multiple shoots was recorded from leaf explants on different concentration and combination of plant growth regulators like BAP (3.0mg/l) + GA_3 (1.0mg/l)+ 2, 4-D (1.0mg/l) showed the mean value 15.3 is the best response (Table. 2) (Fig. 3 E & F).The similar results were obtained for *Solanum americanum* where BAP(2.0 mg/l) + 2, 4-D(1.0 mg/l) + IAA(1.0mg/l) stimulated number of multiple shoots through leaf explants (Ramar *et al*.,2014). Results described by Monokesh Kumer Sen *et al.*,(2014)also in agreement with our result for using this synthetic plant growth regulator in the culture medium for *Achyranthes aspera* L. After 4 weeks the elongated shoots from nodal, internodal and leaf explants were transferred to the root induction medium containing half strength MS basal medium with IBA (1.0mg/l). In this similar results were also reported in IBA by Nayak (2013), Indrani Chandra (2013) Hassan (2012) and Osman *et al*.,(2010). The *in vitro* rooted shoots were transferred from culture medium successfully acclimatized in a cow dung, sand and red soil (1: 1) in the greenhouse with natural photoperiod conditions. The *in vitro* regeneration of medicinal plant *Physalis peruviana* revealed that the tissue culture showed good response in proliferation of multiple shoots in MS medium by supplementing with BAP, GA3 & 2, 4-D. These present study was to establish reliable regeneration protocol for *Physalis peruviana*, which can be used for easier cultivation, propagation and plant genetic studies. In this present investigation has also opened new researchers for genetic manipulation of *Physalis peruviana* for disease, pest resistance or enhancing secondary metabolites, using a rapid regeneration protocol.

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