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In vitro PROPOGATION OF *Physalis peruviana* (L.) USING APICAL SHOOT EXPLANTS

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Abstract. *Physalis peruviana* L. is belongs to Solanaceae family and commonly known as Cape gooseberry. More recently it is very popular and widely used as medicinal plant to treat malaria, asthma, hepatitis, dermatitis and rheumatism and has diuretic and anti-inflammatory properties. In this study, it was aimed to develop *in vitro* propagation protocol for *P. peruviana* L. using apical shoots as an explant sources. Regenerated plants were evaluated based on their multiplication rate and shoot length using various concentration of BAP (1, 2, 3 mg l^{-1}) in combination with IBA (0, 0.1, 0.2, 0.4 mg l^{-1}) and NAA (0, 0.1, 0.2, 0.4 mg l^{-1}). In addition, efficiency of various auxin concentrations of (1 and 2 mg l^{-1} IBA and NAA) was also applied on root formation of *P. peruviana* L. The highest shoot numbers were obtained from 2 mg l^{-1} BAP with 0.4 mg l^{-1} IBA (6.00) combinations and shoot length obtained in 2 mg l^{-1} BAP with 0.2 mg l^{-1} IBA combinations (3.30 cm). As for the effects of BAP and NAA combinations; the highest shoot length were obtained from 2 mg l^{-1} BAP without NAA combinations (3.33 cm) while the lowest one was in 3 mg l^{-1} BAP with 0.4 mg/I NAA combinations. The highest root numbers were obtained from NAA application (2 mg l^{-1} and 1 mg l^{-1}, respectively). *In vitro* derived plants were acclimatized to the soil smoothly. The present study highlights the importance of plant tissue culture in order to be used for large-scale production of *P. peruviana* (L.) due to the elimination of sexual propagation.

Key words*:* Apical shoots, *Solanaceae* L., plant growth regulators, rooting

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INTRODUCTION

Horticulture concerned with plants that are used by people for food, either as edible products, or for culinary ingredients, for medicinal use or ornamental and aesthetic purposes. They are genetically very diverse group and play a major role in modern society end economy. Fruits and vegetables are an important component of traditional food, but are also central to healthy diets of modern urban population [Bajpai et al. 2014, Feng et al. 2014, Ruttanapraset et al. 2014, Mlcek et al. 2015].

Physalis peruviana (L.) (golden berry) is belongs to the family Solanaceae, a small herbaceous annual plant grown as weed in the crop field [Ramirez et al. 2013]. According to the previous reports, this plant has a tremendous medicinal value for curing out different diseases. Although some medicinal plants have toxicity effects on human but toxicity of golden berry was demonstrated just in high concentrations only in males [Perk et al. 2013]. The golden berry fruit tastes like a sweet tomato and includes high levels of vitamin C, vitamin A and the vitamin B-complex. The fruit was demonstrated to have both anti-inflammatory and antioxidant properties [Wu et al. 2006, Pardo et al. 2008]. Furthermore, *P. peruviana* extracts were reported to have anti-hepatoma activity due to the induction of apoptosis in a human hepatocellular carcinoma (Hep G2) cell line [Wu et al. 2004]. Yen et al. [2010] reported that golden berry-derived 4βHWE is a potential DNA-damaging and chemotherapeutic agent against lung cancer. Furthermore, leaf and shoot extracts possess cytotoxic effect on HeLa cells when applied as 100 μg/mL concentration and can alter antiapoptotic genes [Cakir et al. 2014]. Golden berries usually propagated by seeds. Sometimes the growers has faced to inadequate propagation material when used seeds. Furthermore, there may be a germination problem with seeds and this brings about time consuming. In this way, micropropagation is an effective approach to conserve such germplasm. Furthermore, genetic improvement is another approach to enhance the yielding capacity of the plant. Therefore, it is important to develop an efficient micropropagation technique for *P. peruviana* to rapidly disseminate superior clones once they are identified. Tissue culture technique can play an important role in the clonal propagation of elite clones and germplasm conservation of this medicinal hurb. In recent years, numerous studies on *in vitro* propagation of different plant species have shown that this technique may be a solution for rapid propagation of selected plant species [Boulay 1987, Chalupa 1987]. *In vitro* techniques are important tools for modern plant improvement programs introducing new traits into selected plants, to multiply elite selections and to develop suitable cultivars in the short time also [Taji et al. 2002]. Used in conjunction with classical breeding methods, an efficient *in vitro* shoot proliferation and regeneration system could accelerate cultivar development programs. The ability to regenerate plants is crucial to the successful application of *in vitro* methods [Cao and Hammerschlag 2000]. This technique has been widely used especially in Europe and USA [Zimmermann 1981, Boxus et al. 1989]. Morever, the capability for regenerating and propagate plants from cultures cells and tissues is one of the most useful aspects of *in vitro* cell and tissue culture. Till now the highest regeneration from leaf and nodal explants in golden berry tissue culture was observed at the highest concentration of BAP and Kinetin [Otroshy et al. 2013].

To our knowledege, there is very limited previously published paper on *in vitro* propogation of *P. peruviana* L. The present paper deals with the development of a protocol for *in vitro* asexual multiplication in *P. peruviana* L. derived from apical shoots using various plant growth regulators.

MATERIALS AND METHODS

Plant material. The apical shoots of *P. peruviana* were used as an explant sources. Explants were washed in running tap water approximately 5 min. and then washed again thoroughly by adding a few drops of Tween-20. Surface sterilization were applied using 6% sodium hypochlorite for 8 min. and then rinsed several times in sterile double distilled water inside the Laminar Air Flow Chamber. Subcultures were done every 3–4 weeks intervals.

The explants of *P. peruviana* L. were maintained *in vitro* on basal medium (McCown's woody plant basal salts) and including vitamins 20 g l^{-1} sucrose and 7.5 g l^{-1} agar [Lloyd and McCown 1981]. All medias were adjusted to pH 5.8 before autoclaving. All cultures were propagated at 25^{o}C under 16 h light, 8 h dark cycle photoperiod by cool fluorescent lamp at 1500 lux.

At the beginning, the apical shoot explants were taken the medium consisting of basal medium without adding plant growth regulators to inhibit phenolic release to the medium until the whole plant formation (approximately 2 weeks). These plants were transferred including various BAP (0, 1, 2 and 3 mg l^{-1}) with IBA (0, 0.1, 0.2 and 4 mg l^{-1}) and NAA (0, 0.1, 0.2 and 0.4 mg l^{-1}) combinations to detect multiplication efficiency. The number of shoots per explant and average shoot length was determined. Rooting performance of intact plants was also investigated using various auxin types and levels. NAA and IBA were used as an auxin sources consisting, 1 and 2 mg l^{-1}.

Statistical Analysis. Obtained data were recorded after 40 days for number of shoots per explant. Fourth explants were used per treatment and each treatment repeated three times on the same medium composition. All experiments were conducted in a completely randomized design. All recorded data were analyzed using JMP soft pack programme at 5% significant level ($p \leq 0.05$) and mean separation by LSD.

RESULTS AND DISCUSSION

A multiple shoot culture and standardization of media and plant growth regulators may provide a mass propagation of *P. peruviana* L. which is a very important source of pharmacologically active plant constituents. The results of multiple shoot cultures of *P. peruviana* L. derived from single apical shoots were given in Table 1 and Table 2. As seen in Table 1, the differences of shoot numbers were not found statistically significant when used 1 mg l^{-1} BAP and 3 mg l^{-1} BAP with various IBA combinations. Among the 1 mg l^{-1} BAP and various combinations of IBA, the highest shoots detected in 1 mg l^{-1} BAP + 0.2 mg l^{-1} IBA combinations while the lowest in 1 mg l^{-1} BAP + 0 mg/L IBA combinations. The combination of 2 mg l^{-1} BAP and various IBA combinations were

found to be higher than the 1 mg l^{-1} and 3 mg l^{-1} BAP combinations. The highest shoot numbers were obtained from 2 mg l^{-1} BAP with 0.4 mg l^{-1} IBA (6.00) combinations (fig. 1). As for the 3 mg l^{-1} BAP and various IBA combinations, the shoot numbers were ranged between 2.00 (3 mg l^{-1} BAP + 0.4 mg l^{-1} IBA) to 3.33 (3 mg l^{-1} BAP + 0.1 mg l^{-1} IBA).

Table 1. Effect of various levels of BAP and IBA combinations on shoot numbers and shoot length responce of *Physalis peruviana*

BAP + IBA	Shoot numbers	Shoot length (cm)
BAP 1 mg l^{-1} + 0 mg l^{-1} IBA	1.66	1.80 b
BAP 1 mg l^{-1} + 0.1 mg l^{-1} IBA	2.00	180 b
BAP 1 mg l^{-1} + 0.2 mg l^{-1} IBA	2.66	2.83 a
BAP 1 mg l^{-1} + 0.4 mg l^{-1} IBA	2.33	3.16 a
Average	1.630	0.577
BAP 2 mg l^{-1} + 0 mg l^{-1} IBA	3.33 b	1.50 c
BAP 2 mg l^{-1} + 0.1 mg l^{-1} IBA	3.66 b	2.83 ab
BAP 2 mg l^{-1} + 0.2 mg l^{-1} IBA	4.0 ab	3.30 a
BAP 2 mg l^{-1} + 0.4 mg l^{-1} IBA	6 a	2.66 b
Average	2.033	0.504
BAP 3 mg l^{-1} + 0 mg l^{-1} IBA	2.66	1.76 a
BAP 3 mg l^{-1} + 0.1 mg l^{-1} IBA	3.33	1.80 a
BAP 3 mg l^{-1} + 0.2 mg l^{-1} IBA	2.33	2.23 a
BAP 3 mg l^{-1} + 0.4 mg l^{-1} IBA	2.00	2.83 a
$MSD_{(\%5)}$	1.331	1.129

Table 2. Effect of various levels of BAP and NAA combinations on shoot numbers and shoot length of *Physalis peruviana*

BAP	Shoot numbers	Shoot length
BAP 1 mg l^{-1} +0 mg l^{-1} NAA	1.00 b	1.5 c
BAP 1 mg l^{-1} +0.1 mg l^{-1} NAA	3.00 a	1.33 c
BAP 1 mg l^{-1} +0.2 mg l^{-1} NAA	1.33 ab	3.16 a
BAP 1 mg l^{-1} +0.4 mg l^{-1} NAA	1.33 ab	2.33 b
$MSD_{(\%5)}$	0.768	0.787
BAP 2 mg l^{-1} +0 mg l^{-1} NAA	1.66 b	3.33 a
BAP 2 mg l^{-1} +0.1 mg l^{-1} NAA	3.33 a	2.33 b
BAP 2 mg l^{-1} +0.2 mg l^{-1} NAA	3.33 a	2.16 b
BAP 2 mg l^{-1} +0.4 mg l^{-1} NAA	2.33 ab	1.83 b
$MSD_{(\%5)}$	1.087	0.760
BAP 3 mg l^{-1} +0 mg l^{-1} NAA	2.73 a	2.40 a
BAP 3 mg l^{-1} +0.1 mg l^{-1} NAA	2.66 a	2.66 a
BAP 3 mg l^{-1} +0.2 mg l^{-1} NAA	2.90 a	2.90 a
BAP 3 mg l^{-1} +0.4 mg l^{-1} NAA	1.30 b	1.30 b
$MSD_{(\%5)}$	0.714	0.551

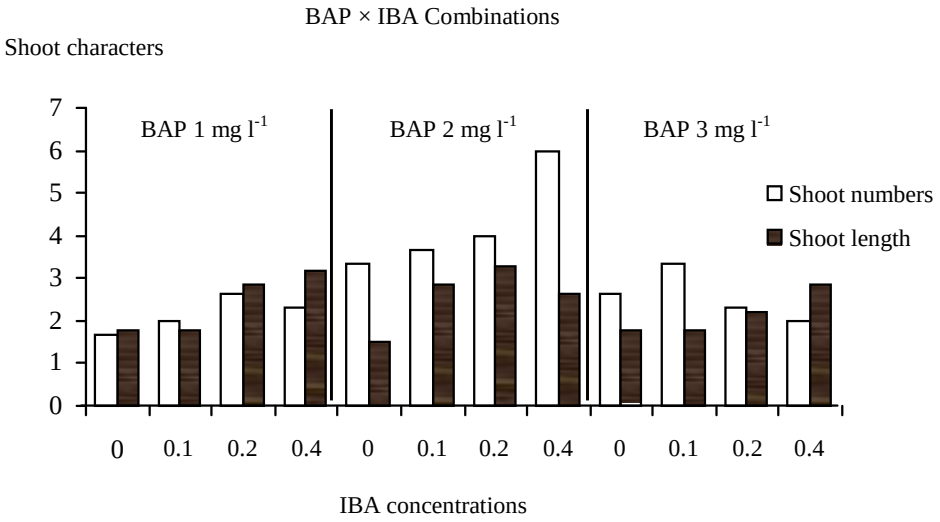

Fig. 1. Effects of different combinations of Indole Butyric Acid (IBA) (0, 0.1, 0.2, and 0.4 mg l^{-1}) supplemented with BAP (6-Benzylaminopurine) (1, 2, and 3 mg l^{-1}) concentrations on shoot numbers and shoot length (cm) of *P. peruviana* L.

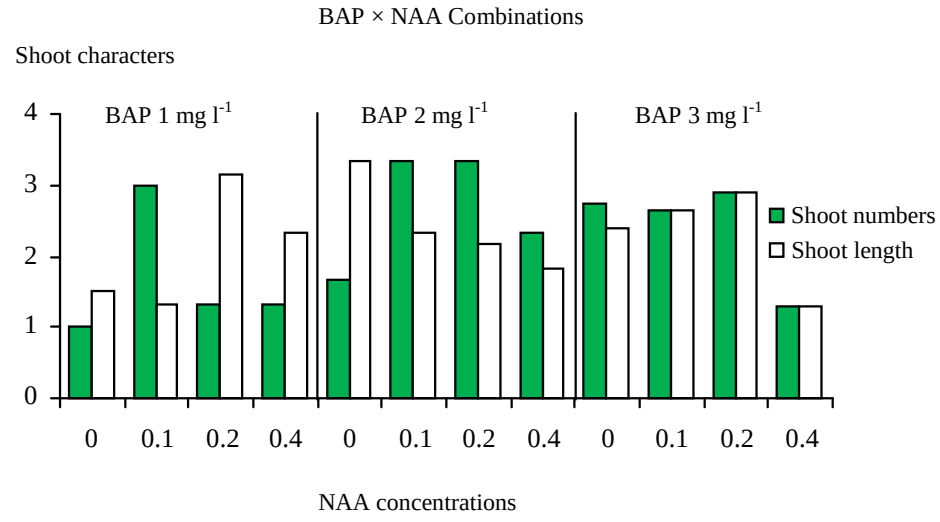

Fig. 2. Effects of different combinations of NAA (Naphthalen Acetic Acid) (0, 0.1, 0.2, and 0.4 mg l^{-1}) supplemented with BAP(6-Benzylaminopurine) (1, 2, and 3 mg l^{-1}) concentrations on shoot numbers and shoot length (cm) of *P. peruviana*

The results of various BAP and NAA combinations were give in Table 2. As seen in Table 2, the highest shoot numbers were detected in 2 mg l^{-1} BAP with 0.1 and 0.2 mg l^{-1} NAA combinations (3.33). The results indicated that when the NAA level increased to level 0.4 mg l^{-1}, the shoot numbers affected negatively in all BAP combinations (fig. 2).

The results of various level of BAP and IBA combinations on shoot length of *P. peruviana* L. were given in Table 1. As seen in Table 1, the highest shoot length were obtained in 2 mg l^{-1} BAP with 0.2 mg l^{-1} IBA combinations (3.30 cm) while the lowest shoot length was observed in 2 mg l^{-1} BAP and without IBA combination (1.50 cm). As for the effects of BAP and NAA combinations; the highest shoots were obtained from 2 mg l^{-1} BAP without NAA combinations (3.33 cm) while the lowest in 3 mg l^{-1} BAP with 0.4 mg l^{-1} NAA combinations.

Table 3. Effects of NAA and IBA concentrations on rooting number and length of *Physalis peruviana*

Applications	Root numbers	Root length
Control	2.33 c	2.34 d
IBA (1 mg l^{-1})	3.00 bc	3.29 c
IBA (2 mg l^{-1})	3.33 bc	3.36 c
NAA (1 mg l^{-1})	3.66 b	4.13 b
NAA (2 mg l^{-1})	5.33 a	5.43 a
$MSD_{(\%5)}$	1.242	0.346

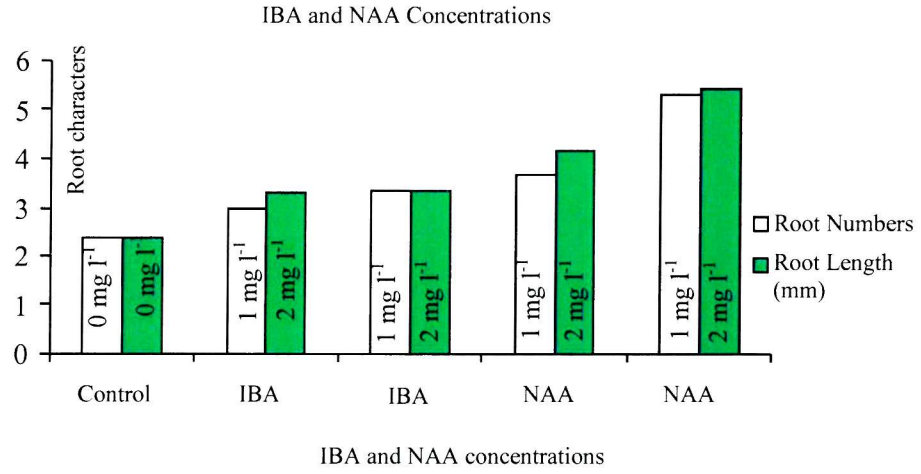

Fig. 3. Effects of different concentrations of IBA (Indole Butyric Acid) (1 and 2 mg l^{-1}) and NAA (Naftalen Acetic Acid) (1 and 2 mg l^{-1}) compared to control (0 mg l^{-1}) on root numbers and root length (cm) of *P. peruviana* L.

The results of various IBA and NAA levels on rooting numbers and length of *P. peruviana* (L.) were given in Table 3. As seen in Table 3, variation was observed among the application of various level of auxins and this differences were found to be statistically significant. The highest root numbers were obtained from NAA application with 200 mg l^{-1} and 100 mg l^{-1}, respectively (fig. 3).

A multiple shoot culture and standardization of of *P. peruviana* L. derived from single apical shoots shown the shoot numbers were lower when the 1 mg l^{-1} BAP used alone. However, Afroz et al. [2009] reported a protocol for high frequency plant regeneration of nodal segments and shoots of *Physalis minima*. About 29 and 32 shoots were found to be induced from nodal segment and shoot tip explants, respectively, cultured on MS medium supplemented with 1.0 mg l^{-1} BAP. The highest shoot numbers were detected in 2 mg l^{-1} BAP with 0.1 and 0.2 mg l^{-1} NAA combinations (3.33). Therefor when the NAA level increased to level 0.4 mg l^{-1} the shoot numbers affected negatively in all BAP combinations.

Tissue culture techniques could allow its massive propagation in a short time. Oscar et al. [2011] reported the effect of Murashige and Skoog (MS) salts concentrations with (BAP 1.7 mg l^{-1}, NAA 0.1 mg l^{-1}) on *in vitro* enlargement and rooting of this *P. peruviana* L. and also the traditional acclimation substrate of plantlets was compared with a hydroponic acclimation substrate. The best rooting results were obtained by using a 50% concentration of MS salts, producing higher shoots (14.4 cm), increase in root size (6 cm), number of leaves (4.3), and number of buds (3.3). Rooting took place simultaneously with *in vitro* shoots enlargement, and no significant differences were observed between acclimation systems. Use of auxin single or in combination for rooting was also reported by different authors [Sahoo and Chan 1998, Hassan and Roy 2005, Rahman et al. 2006, Vadawale et al. 2006, Baksha et al. 2007, Usha et al. 2007]. Afroz et al. [2009] reported that shoots can be rooted well when they are excised individually and implanted on half-strength MS medium with 0.3 mg l^{-1} NAA, and 98% of shoots rooted within 12–15 days. This may be due to using different basal medium and plant genotypes.

Multiple shoot cultures of *Physalis minima* were established from single apical explants on MS medium supplemented with combination of hormones Kinetin (0.4 mg l^{-1}) and BAP (3.5 mg l^{-1}) [Mungole et al. 2011]. Full strength MS solid medium with 1.0 mg/l IBA without or at lower concentrations of cytokinine exhibited the best *in vitro* rooting [Otroshy et al. 2013]. In this study, the best rooting results were obtained from 200 mg l^{-1} NAA media in 2 weeks. Yücesan et al. [2015] investigated regeneration capacity of *P. peruviana* under *in vitro* conditions with underlying synthetic seed technology and an encapsulation process with four different matrix compositions, including NaAlg (sodium alginate) with or without PGR (plant growth regulator) free LS medium containing 3% (w/v) sucrose alone or in combination with 0.5 mg l^{-1} abscisic acid (ABA). The highest regrowth (100%) was observed at 28 days of storage for all matrix compositions.

CONCLUSIONS

In our study, we observed that effects of various levels of BAP with IBA and NAA combinations were differed in *P. peruviana* L. based on shoot numbers and length. The higher shoot numbers and length was obtained from BAP with IBA combinations than BAP with NAA. However, satisfactory results were obtained from NAA compare to the IBA interms of rooting of *P. peruviana* L. This study aims to develop a standard protocol to initiate multiple shoot culture and rooting media that may provide a good source of pharmacologically active plant constituents.

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ROZMNAŻANIE *in vitro Physalis peruviana* (L.) PRZY UŻYCIU EKSPLANTÓW ZE SZCZYTOWYCH PĘDÓW

Streszczenie. Gatunek *Physalis peruviana* L. należy do rodziny Solanaceae i znany jest powszechnie jako miechunka peruwiańska. Ostatnio jest bardzo popularny i używany jako roślina lecznicza w terapii malarii, astmy, zapalenia wątroby, zaplenia skóry i reumatyzmu. Posiada właściwości moczopędne i przeciwzapalne. Celem badania było opracowanie protokołu rozmnażania *in vitro* dla *Physalis peruviana* L. przy użyciu szczytowych pędów jako źródła eksplantów. Zregenerowane rośliny oceniono na podstawie ich współczynnika rozmnażania oraz długości pędów przy użyciu różnych stężeń BAP (1, 2, 3 mg l^{-1}) w kombinacji z IBA (0, 0,1, 0,2, 0,4 mg l^{-1}) oraz NAA (0, 0,1, 0,2, 0,4 mg l^{-1}). Ponadto sprawdzono też skuteczność różnych koncentracji auksyn (1 i 2 mg l^{-1} IBA i NAA) w tworzeniu korzeni *P. peruviana* L. Największą liczbę pędów otrzymano z kombinacji 2 mg l^{-1} BAP z 0.4 mg l^{-1} IBA (6.00) i dla długości pędów w kombinacji 2 mg l^{-1} BAP z 0.2 mg l^{-1} IBA (3,30 cm). Odnośnie do efektów kombinacji BAP i NAA: największą długość pędów otrzymano z kombinacji 2 mg l^{-1} BAP bez NAA (3,33 cm) natomiast najmniejszą z kombinacji 3 mg l^{-1} BAP z 0,4 mg/I NAA. Największą liczbę korzeni otrzymano przy zastosowaniu NAA (odpowiednio, 2 mg l^{-1} i 1 mg l^{-1}). Rośliny uzyskane *in vitro* dobrze aklimatyzowały się w warunkach glebowych. Badanie podkreśla znaczenie hodowli tkanek roślin w celu wykorzystania ich do produkcji *P. peruviana* (L.) na dużą skalę ze względu na eliminację rozmnażania płciowego.

Słowa kluczowe: pędy szczytowe, *Solanaceae* L., regulatory wzrostu roślin, ukorzenianie

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