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In vitro propagation of *Physalis alkekengi* L. using axillary buds

Ebrahim Dorani* and Zahra Sadeghi

Department of Plant Breeding and Biotechnology, University of Tabriz, Tabriz, Iran
*Corresponding author; dorani@tabrizu.ac.ir

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Abstract

Objective: Chinese lantern (*Physalis alkekengi* L.) is an important medical herb due to its physalin. This plant is widely used to treat various diseases. Therefore, developing a repeatable micropropagation method for this plant would be valuable.

Methods: In this study, shoot tips and axillary buds of Chinese lantern were treated with different growth regulators (IAA, NAA, BAP, GA3, and IBA) with various concentrations. In another experiment, the effect of salt concentration onthe MS medium was investigated.

Results: The results showed that using axillary buds as an explant in the full-strength MS, supplemented with 2 mg/L BAP and 0.5 mg/L IAA, resulted in a higher number of shoots per explant (3±1.22) and the highest number of nodes (13±1.22) and stem length (8.04±1.92). Compared with other strengths of MS salts, the full-strength MS medium, supplemented with 2 mg/L BAP and 0.5 mg/L IAA, or 0.5 mg/L BAP alone, produced the highest number of shoots per explant, number of nodes, and stem length. However, the MS salt supplemented with a low concentration of BAP (0.5 mg/L) alone looks economically feasible. Decreasing the salt concentration of the basal medium decreased all micropropagation characteristics and in most cases, the decrease was statistically significant. The addition of GA3 to the combination of 2 mg/L BAP and 0.5 mg/L IAA, significantly improved stem elongation at the concentration range of 0.5 – 1.5 mg/L The number of roots was higher (2±00) after 30 days on the MS basal medium when supplemented with 1 and 2 mg/L NAA or 2 mg/L IAA. The well-rooted plantlets were isolated and transplanted to paper cups for hardening and the well-established plants were transferred to the field for acclimatization.

Conclusion: Our results showed that the full strength of MS medium, supplemented with 2 mg/L BAP plus 0.5 mg/L IAA and 0.5–1.5 mg/L GA3 improved micropropagation characteristics of *P. alkekengi.*

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Introduction

Herbal medicine is gaining *more* popularity *in* today's medical profession. According to a report by the World Health Organization (WHO), 80% of people in developing countries use medicinal plants for primary health care (Behrens 2004). *Physalis* is of great commercial importance because it is rich in antioxidants, carotenoids, and some other important compounds (Lima *et al.* 2020). *Physalis alkekengi* L. (Ground cherry) belonging to Solanaceae is an indigenous plant in Iran and some other countries. The extracts of this plant may be used as a remedy for diseases such as inflammation, rheumatism, arthritis, etc. *P. alkekengi* contains citric acid, physalins, and vitamin C (Raj *et al.* 2015). *Physalis* fruits and shoots may treat intestinal and digestive issues and are applied as antimutagenic, anticoagulant, antispasmodic, and antileukemic agents (Shariff *et al.* 2006; Helvaci *et al.* 2010). Thus, this herb has gained the interest of the pharmaceutical industry.

Micropropagation is the process of vegetative growth and propagation from all types of plant tissues, even seeds (Zhou *et al.* 2006). This method vastly leads to fast propagation, conservation, and improved production of secondary metabolites in medicinal plants (Manzoor *et al.* 2019). Propagation of *P. alkekengi* through seed is difficult because of seed dormancy. Therefore, *in vitro* tissue culture may serve as an alternative for propagation of this plant (Zhang *et al.* 2019). Efforts for micropropagation by shoot regeneration have not been reported in *P. alkekengi* yet. However, some reports are available on *in vitro* shoot proliferation and plant regeneration of other *Physalis* species (Afroz *et al.* 2009; Ramar *et al.* 2014; Guney *et al.* 2016; de Jesús Romo-Paz *et al.* 2021). Thus, this study was conducted to efficiently develop an *in vitro* regeneration protocol for *P. alkekengi* L. through the internode culture.

Materials and Methods

Healthy seeds of *P. alkekengi* L. were collected from Arasbaran, East Azarbaijan Province, Iran. The sterilization of seeds was done by dipping them in %2.5 sodium hypochlorite for 15 minutes followed by continuous shaking. The seeds were stored in the refrigerator for 14 days to break the seed dormancy in the sterile packages. After 14 days, the seeds were transferred to the culture vials containing ½ MS medium for germination. Axillary buds of *P. alkekengi* L. were collected from one-month *in vitro* plants. They were put on the MS medium (Murashige and Skoog 1962) with various combinations and concentrations of plant growth regulators such as BAP, NAA, and IAA. The sucrose content of the media was 3% and the pH of the media was maintained at 5.8 before autoclaving at 15-pound pressure for 15 minutes. Gelling of the media was done with 0.8% agar

powder (tissue culture grade).

In the next step, the effect of medium salt strength (MS, ½MS, and ¼MS) with three selected combinations of plant growth regulators (2 mg. l-1 BAP, 0.5 mg. l-1 BAP, and 2 mg. l-1 BAP plus 0.5 mg. l-1 IAA) was investigated. Finally, in the third experiment, the effect of adding GA3 in seven concentrations (0, 0.25, 0.5, 0.75, 1, 1.5, and 2 mg/L) was tested in the chosen salt stringency and cytokine-auxin combination. In all experiments, the cultures were maintained under a cool fluorescent light intensity of 2000-3000 lux for a 16-8 h light-dark period and a temperature of 22 ± 2 °C. The number of shoots, stem length, and number of nods per shoot were recorded for each explant after one month for the two first experiments and after 20 days for the third experiment.

At the final stage, for root induction, the 45-day-old shoots were cut and cultured in the MS medium containing 1 and 2 mg/ml of different auxins (IAA, IBA, and NAA). After one month, the number of shoots with roots and the number of roots per shoot were recorded. After root development, the plantlets were adapted to the external environment and plants were transferred to the soil.

Also, an experiment with different concentrations of salt was conducted as factorial based on a completely randomized design. The other three experiments were conducted as a completely randomized design. All experiments were performed in five replications. After the test for assumptions, the data were subjected to a one-way analysis of variance followed by Duncan's multiple range test.

Results and Discussion

Multiple shoots were initiated within 15 days of inoculation in almost all media. Maximum number of shoot proliferation was observed within 25-30 days. The nodal explants were first grown on the MS basal medium supplemented with different concentrations of IAA, NAA, and BAP. A 2 mg/L BAP in combination with 0.5 mg/L IAA induced a significantly higher number of nodes (13±1.22) when compared to the combination of 2 mg/L BAP with 0.5 mg/L NAA (5.8±0.89). Among different combinations of BAP with IAA or NAA, the MS basal medium, supplemented with 2 mg/L BAP and 0.5 mg/L IAA, revealed a higher number of shoots (3±1.22) and the highest number of nodes (13±1.22) and stem length (8.04±1.92) (Table 1 and Figure 1). In a study conducted by Mascarenhas *et al.* (2019), the use of 12.50 µM BAP showed the highest direct shooting from explants. According to Ahmadi *et al.* (2022), the highest shoot induction was achieved in Oregano (*Origanum vulgare* L.) in the media supplemented with 1 mg/ l BAP.

Table 1. The effect of different concentrations of plant growth regulators on multiple shoot induction from nodal explants of *Physalis alkekengi* L. in the MS medium.

Plant growth regulators (mg/L)			No. of shoots per explant	No. of nodes per shoot	Stem length
BAP	NAA	IAA			
0	0	0	1±0.002g✳	2.4±0.55 c	2.07±0.51ce
0.5	0	0	4.8±1.64a	6.2±0.45b	7.64±0.37a
1	0	0	2.2±0.84ef	2.8±0.84c	2.07±0.50ce
2	0.5	0	4.2±0.45ab	5.8±0.89b	7.96±1.38a
0.5	0.5	0	1±0.005g	5.6±1.34b	4.44±0.73b
1	0.5	0	1.4±0.55f	1±0.006e	1.08±0.19e
2	1	0	1.6±0.55ef	1±0.89d	1.04±0.62f
0.5	1	0	1.2±0.45f	1.4±0.55de	0.64±0.23f
1	1	0	1.6±0.55ef	1±0.006e	0.94±.47ef
2	0	0	1.8±0.45def	1±0.008e	1.2±0.38e
0.5	0	0.5	1.8±0.85def	2.6±1.78c	5.42±1.78b
1	0	0.5	2.4±1.14dc	2.2±1.92cd	4.92±0.75b
2	0	0.5	3±1.22abc	13±1.22a	8.04±1.92a
0.5	0	1	2.4±0.55cf	2.4±0.89c	4.2±0.80bc
1	0	1	2±0.70ef	2.8±0.84c	3.54±0.62c
2	0	1	2.4±0.55ce	2.8±1.09c	2.48±0.49ce
2	1	0.5	2±0.007ef	1±0.002e	2.02±2.79ce

*Data are in the form of mean ± SE. Means with different letters in each column are significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

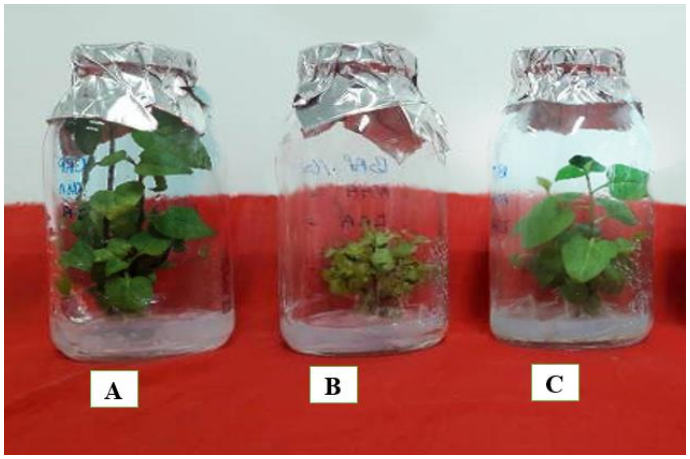

Figure 1. Multiple shoot induction of *Physalis alkekengi* L. from internode explants at the MS medium with selected different concentrations of A) 2 mg/L BAP + 0.5 mg/L IAA, B) 0.5 mg/L BAP + 0.5 mg/L IAA, and C) 0.5 mg/L BAP.

In the in-vitro propagation of plants, shoots are regenerated directly from nodal explants in the presence of meristematic tissue. This type of explant is used to produce plantlets free of bacteria or viruses and also mass propagation of plants. However, the type and concentration of plant growth regulators affect the shoot proliferation efficiency (Intzaar *et al.* 2013).

Shoot tips have always been preferred for *in vitro* studies because they can be handled easily and restore their regeneration potential over other explants. Some earlier findings showed that more shoots were produced from the nodal explants (Jain and Babbar 2003; Sheeba *et al.* 2010). The nodal explants showed an active site of positive morphogenetic response and readily developed multiple shoots.

Different types and concentrations of cytokinin led to differences in multiple shoot induction. Although many growth regulators are available for shoot multiplication, BAP is used more widely. In the present study, BAP was used to find its efficiency in shoot multiplication, because of its economic cost for commercial micropropagation. The presence of BAP in the culture medium led to an increase in the potential for shoot multiplication. Ramar *et al.* (2014) reported that BAP was highly effective in inducing bud break which led to the sprouting of a large number of shoots in *P. peruviana* L.

The MS medium containing BAP was reported more effective compared to Kin for the induction and proliferation of axillary buds in *Solanum nigrum* L and *Hybanthus enneaspermus* (Padmapriya *et al.* 2011; Velayatham *et al.* 2012). The superior effect of BAP on shoot bud induction, shoot tip multiplication, and *ex vitro* rooting of *Centella asiatica* (Alagumanian *et al.* 2015) and *Plumbago zeylanica*. L (Velayatham *et al.* 2005) has been reported.

Figure 2 and Table 2 show growth patterns of *P. alkekengi* L. regarding the number and length of shoots taken after four weeks of *in vitro* culture. Favored shoot bud initiation from nodal explants was observed within one month of inoculation. The highest number of shoots (3±0.005) and maximum stem length (7.91±0.96) was achieved in the full-strength MS medium. By decreasing the salt strength of the MS basal medium, shoot proliferation decreased. In addition, the quality of propagated plants was very low, which was indicated by abnormal light color and appearance (Figure 2).

Investigating the effects of GA3 on the proliferation of *P. alkekengi* indicated that the addition of GA3 to the proliferation medium improved shoot length, and 1 mg/L GA3 gave the highest shoot length (6.96±0.96) followed by the 0.75 and 1.5 mg/L GA3 (Table 3). The physiological effects of GA3 on plant tissue culture are well-known. This compound is used for shoot elongation of *in vitro* propagated plants. In a study to investigate the effect of different growth regulators on *P. angulate* L.

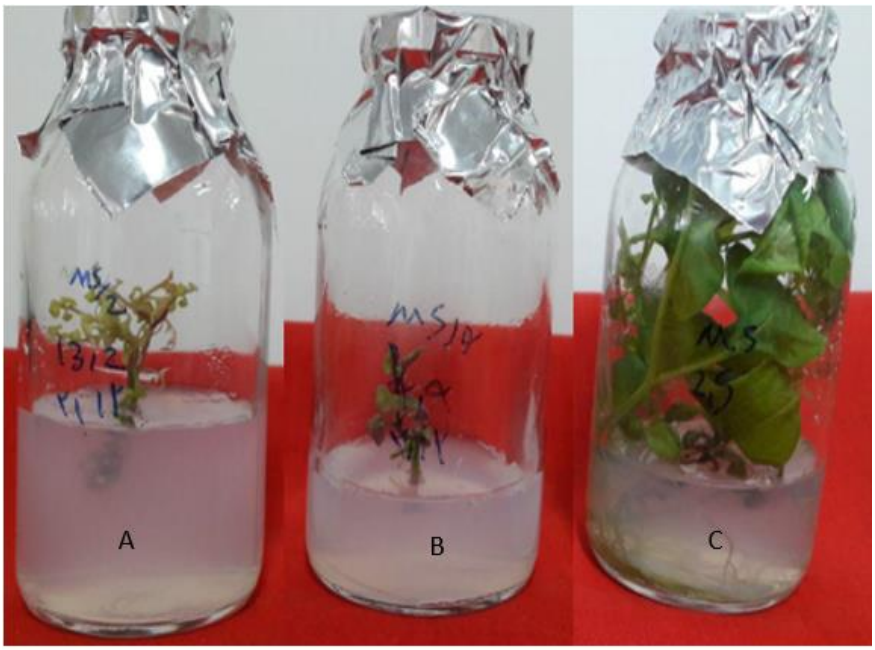

Figure 2. Effect of salt concentration of MS medium on the proliferation of *Physalis alkekengi* L. and the quality of regenerated plants: A) 1/2MS, B) 1/4MS, and C) MS.

Table 2. Effect of various concentrations of plant growth regulators and different concentrations of MS on multiple-shoot induction from nodal explants of *Physalis alkekengi* L.

Medium	BAP	IAA	No. of shoots	No. of nodes per shoot	Stem length
	2	0.5	2.6±0.55b*	4±1.22ab	4.1±0.92b
1/4MS	2	0	1.8±0.84bc	3.6±0.89bc	3.35±0.33bc
	0.5	0	1.6±0.55b	2.6±0.55c	2.1±0.74d
	2	0.5	2.4±0.55b	3.4±0.55bc	3.24±0.62c
1/2MS	2	0	1.4±0.55bc	0.2±0.70d	2.6±0.42d
	0.5	0	1.4±0.55bc	1.8±0.45c	1.7±0.27e
	2	0.5	3±0.005a	4.8±0.45a	4.97±0.77ab
MS	2	0	2.4±0.55a	4.2±0.84ab	3.4±0.74bc
	0.5	0	2.8±0.84a	5.4±1.34a	7.91±0.96a

*Data are in the form of mean ± SE. Means with different letters in each column are significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

through meristem culture, BAP in combination with 1.5 mg/L IAA + 0.25 mg/L GA3 produced maximum shoots per explant (Kumar *et al.* 2015). In another study on *Acacia sinuate*, the shoot was not able to elongate in the proliferation medium; however, elongation was observed after it was transferred to a GA3-supplemented medium (Vengadesan *et al.* 2002). Accordingly, the role of GA3

combined with BAP enhanced the shoot multiplication and elongation in *Melia azedarach* (Vila *et al.* 2002) and *Vitis vinifera* (Tarinejad *et al.* 2019) cultures.

Table 3. Effect of various concentrations of GA3 on shoot elongation of *Physalis alkekengi* L. in the full-strength MS medium, supplemented with 2 mg/L BAP and 0.5 mg/L IAA.

Basal medium	GA3	Stem length
MS	0	4.1±0.91$_{b*}$
	0.25	5.006±1.65$_{ab}$
	0.5	6.44±0.95$_{a}$
	0.75	6.76±0.80$_{a}$
	1	6.96±0.96$_{a}$
	1.5	6.64±0.88$_{a}$
	2	5.9±1.2$_{ab}$

*Data are in the form of mean ± SE. Means with different letters in each column are significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

In vitro rooting of micro-plantlets was successful in the full-strength basal MS medium with 8% agar within 30 days. Almost all used plant growth regulators induced root in all concentrations, except for 1 mg/L IAA. The maximum number of root initiation (2) was observed at 1 to 2 mg/L NAA and 2 mg/L IAA but IBA induced the weak rooting results. Similar results were reported in *P. peruviana* L. (Ramar *et al.* 2014). In another study, the optimal medium for rooting *P. angulata* was MS culture medium containing 1.07 μM NAA with an average of 30.51±0.94 roots (de Jesús Romo-Paz *et al.* 2021). Our observation showed that almost 95% of rooted plants adapted to soil conditions and continued their growth in the greenhouse (Figure 3).

Table 4. Effect of various concentrations of plant growth regulators on multiple root induction from internodal explants of *Physalis alkekengi* L.

Medium	IBA	IAA	NAA	No. of roots per shoot
MS	0	1	0	0±0c*
	0	2	0	2±0.003a
	0	0	1	2±0009a
	0	0	2	2±0.006a
	1	0	0	1.2±0.45b
	2	0	0	1.4±0.55b

*Data are in the form of mean ± SE. Means with different letters in each column are significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

Figure 3. Hardening of the propagated shoots ***of*** *Physalis alkekengi* L. for transferring to the soil in the greenhouse.

Conclusion

A reproducible protocol was developed using nodal explants of four-week-old plants of *P. alkekengi*. To our knowledge, this is the first work on the micropropagation of this plant with a good rate of shoot multiplication. Results indicated the possibility of optimizing plant multiplication *in vitro* by using BAP, IAA, and GA3. Propagated plants were rooted in the MS medium supplemented with NAA or IAA, and almost 100% percent of plants were adapted to greenhouse conditions. The reported protocol can be adapted for *in vitro* conservation and mass production of other ecotypes of *P. alkekengi.*

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Ethical considerations

The authors avoided data fabrication and falsification.

Conflict of interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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