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Clonal propagation and synthetic seed production from nodal segments of Cape gooseberry (*Physalis peruviana* L.), a tropical fruit plant

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Abstract: *Physalis peruviana* L. contains polyphenols and carotenoids with antiinflammatory and antioxidant activities used against diabetes. To establish an efficient regeneration system using nodal segments excised from 4-week-old germinated seedlings, direct plant regeneration, without additional rooting stage, was achieved on LS medium containing 0.5 mg/L 6-benzylaminopurine (BAP), kinetin (KIN), thidiazuron (TDZ), or gibberellic acid (GA_3), alone or in combination with 0.25 mg/L indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA), after 2 weeks of incubation. The highest mean numbers of shoots and well-developed roots were obtained on LS medium containing solely 0.5 mg/L TDZ, producing 5.3 shoots and 3.3 roots per explant after 2 weeks of incubation. Direct shoot and root formation were also recorded on LS medium containing no plant growth regulators. Due to the high regeneration capacity of nodal segments, synthetic seed production was also investigated using the sodium alginate (NaAlg) encapsulation technique. Four different matrix compositions, including NaAlg with or without LS medium containing 3% (w/v) sucrose alone or in combination with 0.5 mg/L abscisic acid (ABA) as a growth retardant were tested for the regrowth performance of synthetic seeds after storage at 4 °C up to 70 days. The highest regrowth (100%) was observed at 28 days of storage for all matrix compositions. All plantlets were acclimatized to the soil and then progressively transferred to the field. The fruits were harvested after 5 months. This study might provide a new insight through protocol development for micropropagation and synthetic seed production of many solanaceous species with economical relevance.

Key words: Clonal plant propagation, Cape gooseberry, encapsulation, *Physalis peruviana* L., synthetic seed, thidiazuron

1. Introduction

Physalis peruviana L. is a species from the family Solanaceae and genus *Physalis*, commonly known as Cape gooseberry (also known as physalis, golden berry, etc.). It is an herbaceous, semishrub, perennial plant, native to tropical Peru and other warm temperate and subtropical regions throughout the world (Ramirez et al., 2013). Cape gooseberry resembles tomato in flavor and appearance, though the sour and sweet taste is richer in this tropical fruit. This plant has increasing popularity due to its nutritional and medicinal values. Cape gooseberry is a source of health-related compounds found in the fruits and other parts of the plant, including leaves and stems. It has been widely used for treating diabetes, hepatitis, ulcers, and several other diseases (Mayorga et al., 2002; Arun and Asha, 2007). It also contains high levels of vitamins A, C, and B-complex, as well as compounds with antiinflammatory and antioxidant properties (Strik, 2007). Wu et al. (2009) reported that supercritical carbon dioxide extracts of *P. peruviana* leaves induced cell cycle arrest and apoptosis in human lung cancer cells. Similarly, Çakir et al. (2014) reported that phenolic content of ethanolic leaf extracts (100 µg mL^{-1}) possessed high cytotoxic effects on HeLa cells (an immortal cell line).

The use of tissue culture methods for the selection of the best cultivars for agricultural practices and clonal propagation might be conducive for micropropagation, especially when taking into account high consumption due to value-added natural compounds, limited plant production, and requirements of manpower, transport, and storage (Rodrigues et al., 2013a). Though *P. peruviana* was reported as recalcitrant for shoot organogenesis through adventitious regeneration from leaf explants (Torres, 1991), there are a limited number of in vitro regeneration studies concerning the micropropagation of *P. peruviana* (Rodrigues et al., 2013b), not containing information about the synergistic effects of plant growth regulator (PGR) combinations on growth parameters. Several studies on in vitro propagation of other *Physalis* species, including *P. minima*, *P. ixocarpa*, and *P. pruinosa*,

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were also reported (Bapat and Rao, 1977; George and Rao, 1979; Gupta, 1986; Ramirez-Malagón and Ochoa-Alejo, 1991; Rao et al., 2004).

Methods on propagation and conservation of plant genetic resources remain a major challenge. From this point of view, encapsulation technology has provided a new understanding in recent years, because of its wide use in germplasm conservation and delivery of tissue-cultured plants for commercial and research purposes. Calcium alginate hollow beads are used frequently for plant cells, shoot tips, nodal segments, or somatic embryos for conservation purposes, and especially for synthetic seed production (Patel et al., 2000; Rai et al., 2009; Benelli et al., 2013; Sharma, 2013; Kocak et al., 2014). Synthetic seed production in *Physalis* might also provide year-round productivity, because most plants produce seeds only during certain months of the year. Since Cape gooseberry is recalcitrant to adventitious regeneration, synthetic seed production concerning germ plasm preservation, propagation, storage, and transportation issues might be promising not only for this aforesaid fruit plant, but also for a wide range of solanaceous species. In response to these shortcomings, we developed an efficient clonal propagation system testing different medium formulations, as well as verifying the viability of synthetic seeds that were stored at 4 °C for different time periods (up to 70 days) in terms of germination, regrowth, and development in the sowing medium in vitro.

2. Materials and methods

2.1. Preparation of plant materials and germination

Seeds of *P. peruviana* were purchased from commercial sellers in October 2012 in Bolu, Turkey. Seeds were washed thoroughly with tap water and then surface-sterilized by dipping them into 50 mL of 20% commercial bleach [Domestos; ~5% (v/v) sodium hypochlorite] for 10 min, followed by rinsing with sterile distilled water 5 or 6 times. For germination, an average of 15–20 seeds were aseptically cultured in disposable petri dishes (90 × 15 mm) containing 25 mL of LS medium (Linsmaier and Skoog, 1965) supplemented with 3% (w/v) sucrose. The medium was solidified with 0.8% (w/v) agar prior to autoclaving at 121 °C and 1.06 kg/cm^2 pressure for 20 min, after adjusting the pH to 5.7. Nodal segments (~5 mm) were isolated from 4-week-old seedlings and subcultured in the glass jars (330 mL volume) with transparent plastic lids containing LS medium without growth regulators for the multiplication of stock sources, by recycling the regenerated shoots through nodal segments at 2-week intervals.

2.2. Direct plantlet regeneration and acclimatization

To attain plantlet regeneration, the nodal explants excised from 4-week-old stock plants were cultured on LS medium supplemented with 0.5 mg/L kinetin (KIN), 6-benzylaminopurine (BAP), thidiazuron (TDZ), or gibberellic acid (GA_3), either alone or in combination with 0.25 mg/L indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA), in disposable petri plates for 2 weeks. After 2 weeks, the regenerated shoots with well-developed roots were transferred from the petri plates to jars (330 mL volume) containing PGR-free LS medium with 3% (w/v) sucrose and 0.8% (w/v) plant agar for further growth and development. All cultures grown in disposable petri dishes and jars were kept at 24 ± 1 °C under a 16/8-h light/dark photoperiod provided by cool-white fluorescent light with an irradiance of 35 μmol^{-2} s^{-1} and at a relative humidity of 65%. For each treatment concerning plantlet regeneration, 5 nodal explants were used per petri plate, while 4 plantlets were placed into each jar; all treatments were established in triplicate. Mean numbers of shoots and roots per explant were recorded after 2 weeks of culture. Mean shoot length was recorded measuring the distance (cm) between shoot base and shoot tip of all plantlets, while mean leaf length was also recorded by measuring the distance between apex and base of the leaf lamina of the first 3 leaves. No additional rooting stage was required, because almost all the regenerated plantlets produced sufficient numbers of roots during their respective treatments. After 3 weeks of culture, plantlets were randomly selected and transferred to pots containing a mixture of soil, manure, moss, and sand at a ratio of 1:2:2:1. Finally, they were kept under greenhouse conditions at 23–25 °C with low humidity (25%–35%) prior to transfer to the field.

2.3. Encapsulation of nodal explants

Nodal explants (~3-mm segments) were excised from the stock plantlets prior to the encapsulation process. Encapsulation in sodium alginate (NaAlg) beads was carried out using the modified protocol of Winkelmann et al. (2004). The explants were encapsulated in 250 μL of 4 different 1.5% (w/v) NaAlg solutions using a micropipette (1000 μL) with a cut tip as follows: NaAlg as a control group, NaAlg together with LS medium with or without 3% (w/v) sucrose, and NaAlg matrix augmented with LS medium, 3% (w/v) sucrose, and 0.5 mg/L abscisic acid (ABA). Each droplet containing one randomly selected nodal explant was dropped into 250 mL of 1% (w/v) calcium chloride ($CaCl_2$) solution in a 500-mL beaker. The solution mix and gel beads were stirred at 100 rpm for 25 min in an orbital shaker at room temperature in order to induce further polymerization. Soon after washing all gel beads with autoclaved distilled water several times in order to remove $CaCl_2$, they were collected on a metal sieve, placed into sterile 50-mL Falcon tubes, and kept at 4 °C for 7, 14, 21, 28, 35, or 70 days before they were recultured onto LS basal medium for 10 days. Data concerning percentage of regrowth and length of shoots

were recorded after 10 days of culture for each treatment. For the observation of regrowth patterns, 5 gel beads were placed into LS medium in petri plates in triplicate (i.e. a total of 15 gel beads per treatment). All materials used in the encapsulation protocol were autoclaved as mentioned above.

2.4. Data analysis

Data obtained from tissue culture experiments were statistically analyzed by SPSS 17.0 (SPSS Inc., Chicago, IL, USA). An analysis of variance (ANOVA) was conducted to calculate the statistical significance of all data presented, and mean ± standard error (SE) values that differed significantly were determined using Duncan's multiple range test at $P < 0.05$.

3. Results

3.1. Plant regeneration and acclimatization

Germination was successfully established (data not shown) giving rise to shoot growth (~7 cm) with 3 nodal explants within 4 weeks. The highest mean number of shoot regenerations was obtained from LS medium supplemented with 0.5 mg/L TDZ producing 5.3 shoots per explant with the highest shooting frequency of explants (93.3%) after 2 weeks of cultivation (Table 1). However, the effects of auxins (0.25 mg/L IAA or IBA), especially in combination with TDZ, sharply decreased the mean number of shoots per explant from 5.3 to 1.5 shoots. In addition, BAP treatments were also moderately effective for shoot formation, producing 2.4 to 2.2 shoots per explant with over 90% shooting response of explants. The ANOVA analysis revealed that the mean number, which ranged from 2.2 to 2.4 shoots per explant, did not significantly differ in any of the treatments involving BAP. Similarly, the mean number of shoots did not significantly differ in KIN-containing treatments (~1.6 shoot per explant with 86.7% of shooting response; Table 1).

For root formation, no additional rooting stage was needed (Figure 1a), since almost all regenerated shoots produced sufficient number of roots for acclimatization (Table 1). However, the effects of auxins (IAA or IBA) were found to be promising for root formation. For example, while LS medium containing 0.5 mg/L KIN produced 1.8 roots (Figure 1b), 0.5 mg/L KIN in combination with 0.25 mg/L IAA produced 2.6 roots with a rooting frequency of 86%. Similarly, the mean number of roots increased from 3.3 to 4.0 once 0.25 mg/L TDZ was combined with 0.25 mg/L IAA. Nevertheless, there was no significant difference in terms of root formation in the TDZ and IBA

Table 1. Effect of different growth regulators on mean number of shoots and roots per explant, and mean length of shoots and leaves of golden berry after 2 weeks of culture.

Treatments (mg L^{-1})	Mean of shoot numbers	% of explant-forming shoots	Mean of root numbers	% of explant-forming roots	Mean length (cm)	
					Shoots	Leaves*
No PRGs (control)	1.0 ± 0.5^{de}	60.0	5.0 ± 0.9^{a}	60.0	1.1 ± 0.2^{d}	0.5 ± 0.1^{d}
0.50 KIN	1.6 ± 0.4^{cde}	86.7	1.8 ± 0.2^{e}	86.7	1.0 ± 0.1^{d}	1.9 ± 0.2^{a}
0.50 BAP	2.4 ± 0.4^{b}	93.3	0.5 ± 0.2^{g}	26.7	1.3 ± 0.2^{cd}	0.6 ± 0.1^{d}
0.50 TDZ	5.3 ± 0.4^{a}	93.3	3.3 ± 0.2^{bc}	86.7	1.1 ± 0.1^{d}	1.0 ± 0.2^{bc}
0.50 GA_3	1.3 ± 0.4^{de}	60.0	1.8 ± 0.8^{ef}	40.0	3.8 ± 0.4^{a}	0.5 ± 0.2^{d}
0.50 KIN + 0.25 IAA	1.7 ± 0.3^{cd}	86.7	2.6 ± 0.4^{cd}	86.7	1.0 ± 0.2^{d}	1.1 ± 0.1^{a}
0.50 BAP + 0.25 IAA	2.4 ± 0.3^{b}	93.3	0.8 ± 0.2^{fg}	86.7	1.2 ± 0.2^{ab}	0.6 ± 0.1^{d}
0.50 TDZ + 0.25 IAA	1.8 ± 0.2^{cd}	86.7	4.0 ± 0.8^{ba}	86.7	1.4 ± 0.2^{cd}	1.2 ± 0.2^{b}
0.50 GA_3 + 0.25 IAA	1.1 ± 0.1^{e}	86.7	2.8 ± 0.4^{cd}	86.7	2.3 ± 0.4^{b}	0.5 ± 0.2^{d}
0.50 KIN + 0.25 IBA	1.6 ± 0.3^{cd}	86.7	2.3 ± 0.2^{e}	80.0	1.1 ± 0.2^{d}	1.0 ± 0.1^{bc}
0.50 BAP + 0.25 IBA	2.2 ± 0.3^{bc}	93.3	0.9 ± 0.2^{f}	86.7	1.1 ± 0.2^{d}	0.7 ± 0.1^{d}
0.50 TDZ + 0.25 IBA	1.5 ± 0.1^{cd}	93.3	3.2 ± 0.6^{bc}	73.3	1.5 ± 0.4^{c}	0.7 ± 0.1^{d}
0.50 GA_3 + 0.25 IBA	1.1 ± 0.3^{de}	80.0	2.1 ± 0.5^{de}	73.3	2.1 ± 0.5^{bc}	0.8 ± 0.2^{dc}

Means ± SE with the same letter within columns are not significantly different according to Duncan's multiple range at $P < 0.05$ ($n = 15$ for each treatment).

*Means of the distance between apex and base of the lamina of the first 3 leaves.

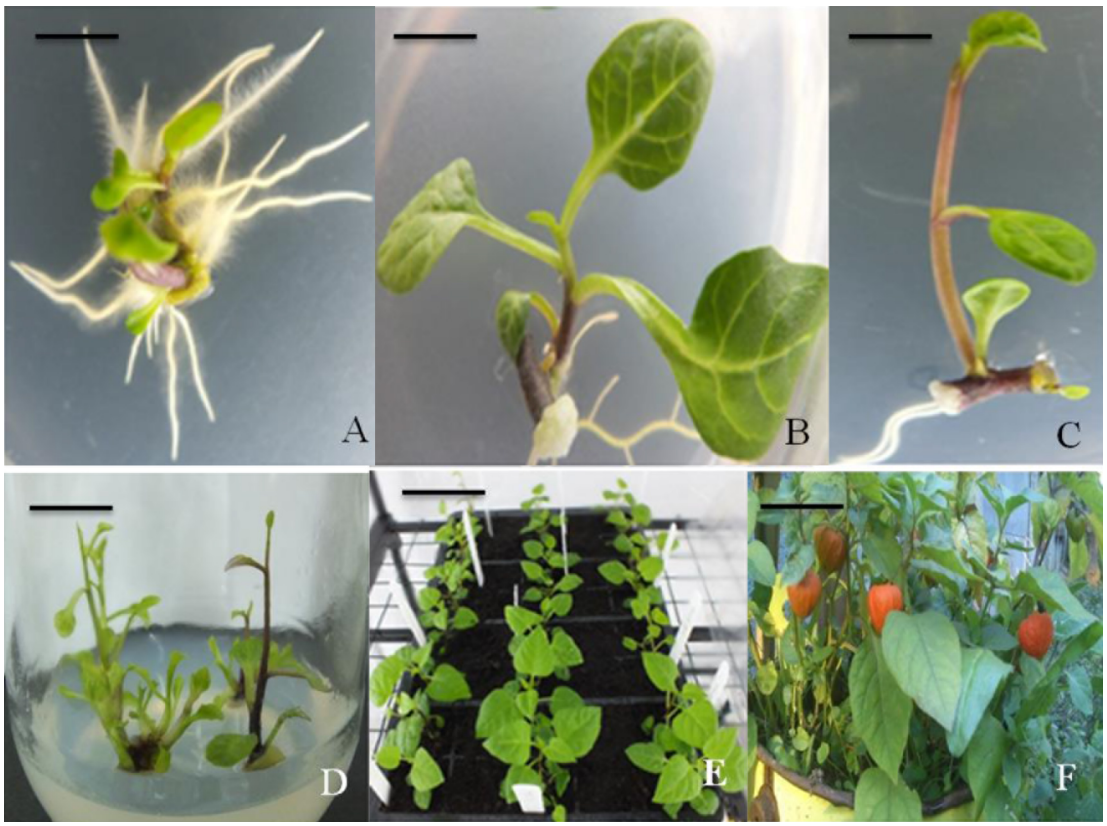

Figure 1. Steps of in vitro plant regeneration of *P. peruviana*: **(a)** more root formation with a fewer number of shoots on PGR-free LS medium, **(b)** single shoot and root formation with expanded leaves on LS medium containing 0.5 mg L^{-1} kinetin, **(c)** highest shoot elongation with smaller leaves than other treatments on LS medium containing 0.5 mg L^{-1} GA_3, **(d)** in vitro plantlets produced from LS medium containing 0.5 mg L^{-1} TDZ, **(e)** acclimatization process for the regenerants derived from LS medium containing 0.5 mg L^{-1} TDZ in greenhouse, **(f)** fruit development under ex vitro conditions 5 months after the culture initiation. Bar: a = 10 mm; b = 10 mm; c = 10 mm; d = 20 mm; e = 60 mm; f = 65 mm.

combination as compared to TDZ used alone (3.2 and 3.3 roots per explant, respectively). In spite of its low shooting response, the control group was also found highly effective for root formation, producing up to 5.0 roots per explant after 2 weeks of cultivation. However, the frequency of explant-forming roots in the control group was lower than in treatments including 0.25 mg/L IAA or IBA (60% in contrast to 73.3% or more). Among the treatments concerning the rooting capacity of explants, BAP-containing treatments were less effective at producing a single root per explant, with a maximum rooting frequency of 85.2%. Gibberellin used alone was also found inefficient for rooting response, producing 1.8 roots per explant with a rooting frequency of 40%. Although this value is similar to the rooting effect of 0.5 mg/L KIN, the percentage of explant-forming roots was quite low compared to 0.5 mg/L KIN (compare 40% and 86.7%; Table 1).

Regarding shoot length, there was no significant difference between treatments, except for GA_3-containing ones, which promoted higher shoot elongation than others. The highest mean shoot length (3.8 cm) was obtained from LS medium containing 0.5 mg/L GA_3, while producing small size leaves. This finding revealed that there was an inverse relationship between leaf and shoot length when GA_3 was used (Figure 1c). For further growth and development, randomly selected in vitro plantlets were directly transferred to PGR-free LS medium for 3 weeks (Figure 1d). All cultivars with normal growth and appearance were singled out and then successfully acclimatized under nonaxenic conditions in the greenhouse for 4 weeks before transfer to the field (Figure 1e). Plantlets (~20 cm in height) were directly transferred to the field for fruit setting. Fruit formation occurred after 5 months (Figure 1f).

3.2. Encapsulation of nodal explants

Different chemical compositions of NaAlg matrix were tested for the regrowth pattern of nodal explants on PGR-free LS medium (Table 2; Figures 2a–2d). Irrespective

Table 2. Frequency (F) of regrowth and length (L) of 10-day-old shoots derived from synthetic seeds after different periods (0, 7, 14, 21, 28, 35, and 70 days) of storage at 4 °C.

Treatments	Day 0*		Day 7		Day 14		Day 21		Day 28		Day 35		Day 70	
	F (%)	L (cm)	F (%)	L (cm)	F (%)	L (cm)	F (%)	L (cm)	F (%)	L (cm)	F (%)	L (cm)	F (%)	L (cm)
NaAlg	100	1.00 ± 0.03^{a}	100	1.04 ± 0.03^{a}	80.0	0.78 ± 0.11^{a}	80.0	0.77 ± 0.10^{ab}	67.7	0.48 ± 0.10^{bc}	20.0	0.11 ± 0.06^{d}	13.3	0.03 ± 0.02^{e}
NaAlg + LS	100	0.98 ± 0.04^{a}	100	1.02 ± 0.03^{a}	100	0.95 ± 0.04^{a}	100	0.91 ± 0.03^{a}	100	0.80 ± 0.07^{a}	73.3	0.47 ± 0.09b^{c}	40.0	0.25 ± 0.09^{bcd}
NaAlg + LS + Sucrose	100	1.10 ± 0.04^{a}	100	1.04 ± 0.03^{a}	100	1.04 ± 0.03^{a}	100	0.99 ± 0.03^{a}	100	0.87 ± 0.04^{a}	80.0	0.61 ± 0.09^{b}	60.0	0.34 ± 0.08^{bc}
NaAlg + LS + sucrose + ABA	100	1.02 ± 0.03^{a}	100	1.00 ± 0.03^{a}	73.3	0.75 ± 0.12^{a}	73.3	0.70 ± 0.11^{ab}	73.3	0.43 ± 0.10^{bc}	33.3	0.19 ± 0.08^{cd}	26.7	0.08 ± 0.04^{de}

Means ± SE (standard error) with the same letter within columns are not significantly different according to Duncan's multiple range at $P < 0.05$ (n = 15 for each treatment).
*Day 0 samples were immediately placed into a sowing medium (PGR-free LS medium).

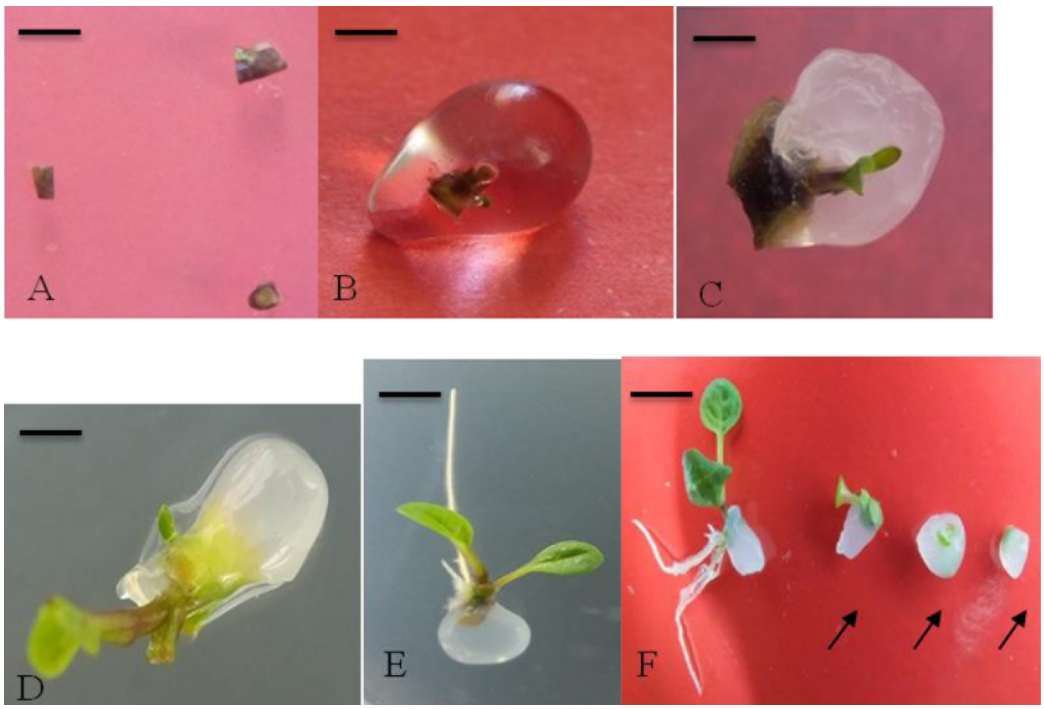

Figure 2. Synthetic seeds of golden berry: **(a)** nodal explants excised from stock plants grown in vitro, **(b)** transparent gel bead of NaAlg consisting of one single nodal explant, **(c)** shoot development of synthetic seeds encapsulated by NaAlg + LS + sucrose stored for 28 days after 5, **(d)** 10-day incubation in sowing medium, **(e)** regrowth of synthetic seeds with well-established root and shoot system, **(f)** growth retardant effect of ABA (see black arrows) in contrast to a well-regrown synthetic seed made of NaAlg + LS + sucrose. Bar: a = 10 mm, b = 5 mm, c = 5 mm, d = 10 mm, e = 15 mm, f = 15 mm.

of chemical composition, the regrowth frequency of all synthetic seeds was found to be highest (100%) until day 14 of storage (Figure 2e). Gel beads containing abscisic acid had 73% germination; however, this value gradually decreased to 26.7% on day 70 of storage (see day 70 in Table 2 and Figure 2f). The highest regrowth frequency (60%) occurred in NaAlg matrix containing sucrose and LS basal medium on day 70. In this gel bead composition, all samples performed efficient regrowth until day 28 of storage. Difference was only observed in shoot length when day 0 and day 28 were taken into account (e.g., from 1.10 to 0.87 cm in NaAlg + LS + sucrose). Hence, there is an inverse relationship between shoot length and storage prolongation. Sodium alginate matrix alone was also found effective for regrowth until day 28 of storage (Figure 2b), but the regrowth of the samples decreased sharply with longer storages. In this study, the presence of sucrose was found to be effective for prolonged storage. In samples observed on day 70, the frequency was 60% in sucrose-containing treatment and 40% in the absence of sucrose, although shoot elongation gradually decreased from 1.10 to 0.34 on sucrose-containing matrix, depending on storage periods. It was also observed that synthetic seeds did not grow on sowing medium solely containing water and agar. Instead, they grew well on LS-supplemented media (data not shown). Regardless of storage period, all plantlets exhibited new growth and normal morphological characteristics including flowering, fruit, and seed setting under ex vitro conditions.

4. Discussion

Plant tissue culture techniques offer an effective solution for true-to-type propagation compared to time-consuming and cumbersome conventional techniques such as grafting, air layering, stooling, etc. Therefore, in this study, the regeneration capacity of *P. peruviana* under in vitro conditions was investigated with underlying synthetic seed technology and an encapsulation process for medium-term storage (up to 70 days of storage) of the clones, ensuring the regrowth pattern(s) depending on storage periods, as well as the protection of explants against environmental factors such as pests, diseases, etc. Although earlier studies showed successful regeneration research about different *Physalis* species from callus cultures in *P. minima* (Bapat and Rao, 1977; Sipahimalani et al., 1981; Sheeba et al., 2010) and *P. pubescens* (Rao et al., 2004), there are only few reports on the in vitro regeneration of *P. peruviana*. Among these, Bapat and

Schieder (1981) developed a protoplast culture in several *Physalis* species including *P. peruviana*. However, none of them resulted in shoot formation. Instead, protoplasts isolated from *P. peruviana* resulted in callus formation on MS medium supplemented with a combination of 1.0 mg/L 2,4-D and BAP. Similarly, Torres (1991) reported some difficulties in terms of organogenic response in tissue culture studies, addressing the recalcitrant pattern of leaf, stem, and root explants. Recently, Rodrigues et al. (2013b) established a regeneration system using nodal explants on different concentrations of BA without testing the synergistic effects of other PGRs, as mentioned in this present study. In general, shoot tips and nodal segments are known as the best explants for the in vitro propagation of most plants species without any intervening callus formation (Otroshy et al., 2013). Thus, it is still a major challenge to perform an efficient regeneration protocol for this crop. In contrast to these shortcomings, this study aimed to develop a rapid clonal regeneration system, a very simple micropropagation protocol, and an effective way of plant material preservation.

In this study, TDZ was found to be the most effective PGR concerning direct shoot and root organogenesis of Cape gooseberry, inducing more shoot formation when used at 0.25 mg/L in LS medium at the end of the second week of cultivation (5.3 shoots per explant). It is also noteworthy that 2 weeks were adequate for shoot and root formation in this plant. The promoting effect of TDZ has been recently reported for many economically important plant species (Yucesan et al., 2007; Kumar and Reddy, 2012; Lata et al., 2013). The effectiveness of TDZ for shoot formation in several tissue culture studies might be attributed to its stability (nondegradable) in culture medium and its high persistence in growth media, demonstrated by a carbon isotope study (Mok and Mok, 1985). Due to this high stability, Huetteman and Preece (1993) reported that low concentrations of TDZ (ranging from 0.01 to 0.2 mg/L) have been found to be useful for micropropagation, whereas higher concentrations induce callusing in woody plants. In this finding, the synergistic effect of TDZ and endogenous auxins might have also played a critical role in altering the auxin:cytokinin ratio within the plant tissue and eventually enhancing axillary regeneration. Unlike natural cytokinins, TDZ has the potential to fulfill the auxin requirements of different regenerative responses (Guo et al., 2011). Therefore, it might be suggested that 0.5 mg/L TDZ used alone was sufficient not only for axillary regeneration but also for root formation in Cape gooseberry. In spite of the high popularity of TDZ, its exact biological role remains unknown due to its metabolic roles, including primary signaling event, storage, and passage of endogenous plant signals, which may or may not be established as organogenesis (Guo et al., 2011).

When shoot growth was taken into account, GA_3 played a crucial role in shoot growth up to 3.9 cm within 2 weeks, whereas it was less effective in terms of leaf number (observed data). Likewise, small leaflet formation was a predominant pattern due to the presence of GA_3 in this study. This might be due to the negative influence of fast internode elongation while retarding the leaf number and size (Bostrack and Struckmeyer, 1967). As to root formation, it is known that GA_3 in tissue culture mimics an inhibitor during rhizogenesis (Sutter, 1996; Yıldırım and Turker, 2009), as seen in the present study, producing 1.8 root per explant with a frequency of 40% explant-forming roots (compare root formation effects of PGR-free LS and GA_3 medium). Due to these side effects on leaf length, number of leaves, and root formation, all advantages of fast growth mediated by gibberellins were taken away in the regeneration system of Cape gooseberry.

In addition, 0.5 mg/L BAP was found to be moderately effective for shoot induction compared to 0.5 mg/L KIN and 0.5 mg/L GA_3. The difference between KIN and BAP was based not only on the production of shoot or root per explant, but also on morphological variability. For example, in KIN-containing treatments, leaf length was moderately longer than those produced in BAP-containing treatments, but with less shooting response than BAP, as mentioned. These findings show that the variability of physiological responses including shoot and root formation was dependent on specific interactions of plant growth regulators and explant source (Rout and Samantaray, 2000; Gurel et al., 2011; Gurel and Gurel, 2013). A tissue culture protocol based on the use of BAP for *P. peruviana* regeneration is not cost-effective, yet BAP resulted in a weak response for root formation. Otroshy et al. (2013) also reported a similar result concerning the effect of BAP on rhizogenesis. In contrast, PGR-free LS medium was also found to be effective, producing 5.0 roots per explant. In this contribution, all tested PGRs and their respective combinations clearly show that clonal propagation of *P. peruviana* is plausible via axillary regeneration using nodal explants. Unlike nodal segments, leaf, root, or stem segments were not effective for in vitro regeneration of Cape gooseberry (data not shown), as concurred by Torres (1991), due to the recalcitrance of the aforesaid tissues under in vitro conditions.

Synthetic seed production using nodal explants was also established via encapsulation in different NaAlg compositions. It was clearly shown that lack of nutrients and presence of ABA as a growth retardant can be ascribed as limiting factors for the regrowth pattern of the synthetic seeds after 28 days of storage at 4 °C. Although there was no significant difference in terms of shoot length among

LS with or without sucrose in NaAlg matrix, the highest frequency of synthetic seed regrowth was observed in the presence of sucrose. This finding was also consistent with the promising effect of sucrose on artificial endosperm formation, in such a way that sucrose and the meristematic tissue mimicked zygotic embryos during storage (Germanà et al., 2011; Sharma et al., 2013). Similarly, Burritt (2008) reported that dehydration and freezing damage in *Begonia* × *erythrophylla* shoots was dependent on the sugar concentration under liquid nitrogen.

Based on our literature survey, there is no report concerning the role of ABA in the encapsulation process of nodal or shoot tips. There are only few reports on the high survival rate of shoot tips pretreated with ABA before the dehydration–encapsulation technique (Bagniewska and Zenkteler, 2002; Burritt, 2008). In this study, ABA also played a critical role in slowing growth at the end of day 14 from 100% to 73.3% of regrowth. The use of ABA as a growth retardant might contribute to a new understanding of the optimization of storage conditions in certain plant species, having highly active meristematic tissues for germination within hollow gel beads soon after encapsulation. There are several reports on synthetic seed production of fruit plants based on in vitro conservation and storage at 4 °C, including pomegranate (Naik and Chand, 2006), mulberry (Kavyashree et al., 2006), raspberry (Piccioni and Standardi, 1995), and black nightshade (Verma et al., 2010). Plant genotype, explant type, concentration of mineral contents, and/or sucrose in medium storage conditions and growth regulators are crucial for testing the viability and conservation of synthetic seeds (Benelli et al., 2013; Sharma et al., 2013; Berjak et al., 2014). In this study, storage for up to 70 days might have resulted in the loss of genetic integrity, and in some cases malformed shoot tips were observed. To circumvent these drawbacks, NaAlg encapsulation containing LS medium with or without sucrose is feasible for obtaining high potential regrowth up to 28 days of storage at 4 °C. Moreover, for encapsulation, the use of in vitro-raised plantlets as an explant source rather than field-grown plants possibly minimizes recalcitrance under aseptic conditions (Rai et al., 2009). In addition, direct planting of the encapsulated tissues to the soil might also be possible by further research into encapsulation technology, as described in a *Muscari* species (Yücesan et al., 2014).

In conclusion, the results of in vitro multiplication, encapsulation, conservation at low temperature for different time periods, and establishment of plants to the soil confirmed the practical applicability of tissue culture as an alternative method for large scale propagation and short-term storage (up to 4 weeks) of this plant. This study established for the first time an efficient technique for multiplication, conservation, and storage of Cape gooseberry. Using the regeneration protocol presented here, it is possible to regenerate over 7000 seedlings (~3 cm) within 5 months, starting from one single nodal explant. Moreover, this protocol can provide plant material, especially in the case of stock plant scarcity, for future agricultural practices with a wide range of solanaceous plant species. Future studies will also focus on direct sowing of synthetic seeds to the soil without a nursery medium for germination purposes.

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References

Arun M, Asha VV (2007). Preliminary studies on anti-hepatotoxic effect of *Physalis peruviana* L. (Solanaceae) against carbon tetrachloride induced acute lever injury in rats. J Ethnopharmacol 111: 110–114.

Bagniewska A, Zenkteler E (2002). In vitro storage of rhizome shoot tips of *Polypodium vulgare* L. using ABA treatment before dehydration: encapsulation technique. Acta Biol Cracov Bot 41: 75–83.

Bapat VA, Rao PS (1977). Experimental control of growth and differentiation in organ cultures of *Physalis minima* LINN. Z Pflanzenphysiol 85: 403–416.

Bapat VA, Schieder O (1981). Protoplast culture of several members of the genus *Physalis*. Plant Cell Rep 1: 69–70.

Benelli C, De Carlo A, Engelmann F (2013). Recent advances in the cryopreservation of shoot-derived germplasm of economically important fruit trees of *Actinidia*, *Diospyros*, *Malus*, *Olea*, *Prunus*, *Pyrus* and *Vitis*. Biotechnol Adv 31: 175–185.

Berjak P, Cherian J, Makhathini AP, Sershen, Pammenter NW (2014). Embryonic axes of a tropical recalcitrant-seeded species: successful elimination of micro-organisms and potential for zygotic synthetic seed (synseed) production. Seed Sci Technol 42: 150–160.

Bostrack JM, Struckmeyer BE (1967). Effect of gibberellic acid on the growth and anatomy of *Coleus blumei*, *Antirrhinum majus* and *Salvia splendens*. New Phytol 66: 539–544.

Burritt DJ (2008). Efficient cryopreservation of adventitious shoots of *Begonia* × *erythrophylla* using encapsulation–dehydration requires pretreatment with both ABA and proline. Plant Cell Tiss Org 95: 209–215.

Çakir Ö, Pekmez M, Çepni E, Candar B, Fidan K (2014). Evaluation of biological activities of *Physalis peruviana* ethanol extracts and expression of Bcl-2 genes in HeLa cells. Food Sci Technol 34: 422–430.

George L, Rao PS (1979). Experimental induction of triploid *Physalis* plants trough anther culture. Protoplasma 100: 13–19.

Germanà MA, Micheli M, Chiancone B, Macaluso L, Standardi A (2011). Organogenesis and encapsulation of in vitro-derived propagules of Carrizo citrange [*Citrus sinensis* (L.) Osb. × *Poncirius trifoliate* (L.) Raf]. Plant Cell Tiss Org 106: 299–307.

Guo B, Abbasi BH, Zeb A, Xu LL, Wei YH (2011). Thidiazuron: a multi-dimensional plant growth regulator. Afr J Biotechnol 10: 8984–9000.

Gupta PP (1986). Regeneration of plants from mesophyll protoplasts of ground-cherry (*Physalis minimal* L.). Plant Sci 43:151–154.

Gurel E, Yucesan B, Aglic E, Gurel S, Verma SK, Sokmen M, Sokmen A (2011). Regeneration and cardiotonic glycoside production in *Digitalis davisiana* Heywood (Alanya Foxglove). Plant Cell Tiss Org 104: 217–225.

Gurel S, Gurel E (2013). In vitro regeneration of sugar beet (*Beta vulgaris* L.) In: Ramawat KG, Merillon JM, editors. Bulbous Plants: Biotechnology. 1st ed. Boca Raton, FL, USA: CRC Press, pp. 113–151.

Huetteman CA, Preece JE (1993). Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell Tiss Org 33: 105–119.

Kavyashree R, Gayatri MC, Revanasiddaiah HM (2006). Propagation of mulberry variety–S_{54} by synseeds of axillary bud. Plant Cell Tiss Org 84: 245–249.

Kocak M, Izgu T, Sevindik B, Tutuncu M, Curuk P, Simsek O, Kacar YA, Teixeira da Silva JA, Mendi YY (2014). Somatic embryogenesis of Turkish *Cyclamen persicum* Mill. Sci Hortic-Amsterdam 172: 26–33.

Kumar N, Reddy MP (2012). Thidiazuron (TDZ) induced plant regeneration from cotyledonary petiole explants of elite genotypes of *Jatropha curcas*: a candidate biodiesel plant. Ind Crop Prod 39: 62–68.

Lata H, Chandra S, Wang YH, Raman V, Khan IA (2013). TDZ-induced high frequency plant regeneration through direct shoot organogenesis in *Stevia rebaudiana* Bertoni: an important medicinal plant and a natural sweetener. Am J Plant Sci 4: 117–128.

Linsmaier E, Skoog F (1965). Organic growth factor requirements of tobacco tissue cultures. Physiol Plantarum 18: 100–127.

Mayorga H, Duque C, Knapp H, Winterhalter P (2002). Hydroxyester disaccharides from fruit of Cape gooseberry (*Physalis peruviana* L.). Phytochemisty 59: 439–445.

Mok MC, Mok DWS (1985). The metabolism of [^{14}C]-tMdiaziiroii in callus tissues of *Phaseolus lunatus*. Physiol Plantarum 65: 427–432.

Naik SK, Chand PK (2006). Nutrient-alginate encapsulation of in vitro nodal segments of pomegranate (*Punica granatum* L.) for germplasm distribution and exchange. Sci Hortic-Amsterdam 108: 247–252.

Otroshy M, Mokhtari A, Mohammad S, Khodaee M, Bazrafshan AH (2013). Direct regeneration from leaves and nodes explants of *Physalis peruviana* L. Intl J Farm Alli Sci 2: 214–218.

Patel AV, Pusch I, Mix-Wagner G, Vorlop KD (2000). A novel encapsulation technique for the production of artificial seeds. Plant Cell Rep 19: 868–874.

Piccioni E, Standardi A (1995). Encapsulation of micropropagated buds of six woody species. Plant Cell Tiss Org 42: 221–226.

Rai MK, Asthana P, Singh SK, Jaiswal VS, Jaiswal U (2009). The encapsulation technology in fruit plants – a review. Biotechnol Adv 27: 671–679.

Ramirez F, Fischer G, Davenport TL, Augusto Pinzon JC, Ulrichs C (2013). Cape gooseberry (*Physalis peruviana* L.) phenology according to the BBCH phonological scale. Sci Hortic-Amsterdam 162: 39–42.

Ramirez-Malagón R, Ochoa-Alejo N (1991). Adventitious shoot formation and plant regeneration from tissues of tomatillo (*Physalis ixocarpa* Brot.). Plant Cell Tiss Org 25: 185–188.

Rao YV, Ravi Shankar A, Lakshmi TVR, Raja Rao KG (2004). Plant regeneration in *Physalis pubescens* L. and its induced mutant. Plant Cell Tiss Org 14: 9–15.

Rodrigues FA, Penoni ES, Soares JDR, Silva RAL, Pasqual M (2013a). Phenological Characterization and productivity of *Physalis peruviana* cultivated in greenhouse. J Bioscience 29: 1771–1777.

Rodrigues FA, Penoni ES, Soares JDR, Silva RAL, Pasqual M (2013b). Different Concentrations of the MS medium and BAP on multiplication in vitro of *Physalis peruviana* L. J Bioscience 29: 77–82.

Rout GR, Samantaray S, Das P (2000). In vitro manipulation and propagation of medicinal plants. Biotechnol Adv 18: 91–120.

Sharma S, Shahzad A, Teixeira da Silva JA (2013). Synseed technology – a complete synthesis. Biotechnol Adv 31: 186–207.

Sheeba E, Parvathy S, Palanivel S (2010). Direct regeneration from leaves and nodes explants of *physalis Minima* Linn. Eur J Appl Sci 2: 58–61.

Sipahimalani AT, Bapat VA, Rao PS, Chadha MS (1981). Biosynthetic potential of cultured tissues and regenerated plants of *Physalis munima* L. J Nat Prod 44: 114–118.

Strik BC (2007). Berry crops: worldwide area and production systems. In: Zhao Y, editor. Berry Fruit: Value-Added Products for Health Promotion. 1st ed. Boca Raton, FL, USA: CRC Press, pp. 3–50.

Sutter EG (1996). General laboratory requirements, media and sterilization methods. In: Trigiano RN, Gray DJ, editors. Plant Tissue Culture Concepts and Laboratory Exercises. 1st ed. Boca Raton, FL, USA: CRC Press, pp. 11–25.

Torres O, Perea M, Lopez AL, Salamanca A, Mikan J (1991). The use of *Physalis peruviana* tissue culture for breeding and selection. In: Hawkes JG, Lester RN, Nee M, Estrada-Ramos N, editors. Solanaceae III: Taxonomy, Chemistry, Evolution. Kew, UK: Royal Botanic Gardens, pp. 429–432.

Verma SK, Rai MK, Asthana P, Jaiswal VS, Jaiswal U (2010). In vitro plantlets from alginate-encapsulated shoot tips of *Solanum nigrum* L. Sci Hortic-Amsterdam 124: 517–521.

Winkelmann T, Meyer L, Serek M (2004). Germination of encapsulated somatic embryos of *Cyclamen persicum*. Sci Hortic-Amsterdam 39: 1093–1097.

Wu SJ, Chang SP, Lin DL, Wang SS, Hou FF, Ng LT (2009). Supercritical carbon dioxide extract of Physalis peruviana induced cell cycle arrest and apoptosis in human lung cancer H661 cells. Food Chem Toxicol 47: 1132–1138.

Yıldırım AB, Turker AU (2009). In vitro adventitious shoot regeneration of the medicinal plant meadowsweet (*Filipendula ulmaria* (L.) Maxim). In Vitro Cell Dev B 45: 135–144.

Yücesan B, Çiçek F, Gürel E (2014). Somatic embryogenesis and encapsulation of immature bulblets of an ornamental species, grape hyacinths (*Muscari armeniacum* Leichtlin ex Baker). Turk J Agric For 38: 716–722.

Yucesan B, Turker AU, Gurel E (2007). TDZ-induced high frequency plant regeneration through multiple shoot formation in witloof chicory (*Cichorium intybus* L.). Plant Cell Tiss Org 91: 243–250.