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Optimization of *in vitro* Propagation of Purple Passion Fruit (*Passiflora edulis*), an Important Medicinal and Ornamental Plant

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Abstract
Tissue culture techniques can be beneficial for quick clonal propagation and production of disease-free plants of purple passion fruit as one of the most important medicinal and ornamental plants. These techniques are essential tools for the production of transgenic plants and high-value phytochemicals. The present study was planned to introduce an efficient *in vitro* propagation for purple passion fruit (*Passiflora edulis* Sims.). To do so, the effect of different plant growth regulators was investigated on micropropagation medium of purple passion fruit. For proliferation stage, nodal segments were cultured in media supplemented with various combinations of different plant growth regulators including: BA (0, 2.2, 4.4 and 8.9 µM), TDZ (2.3, 4.5 and 9.1 µM), GA_3 (0 and 2.9 µM) and IBA (0 and 0.5 µM). For rooting, shoots with about 1.5 cm long originating from explants were removed and cultured in half-strength MS medium containing different concentrations of auxin-based plant growth regulators including: IAA (0, 1.1, 2.9, 5.7 and 11.4 µM), IBA (1, 2.5, 4.9 and 8.9 µM), and NAA (1.1, 2.7, 5.4, 10.7 µM). For the proliferation stage, the best plant growth regulator combination was 8.9 µM BA+2.9 µM GA_3+0.5 µM IBA, resulting in the maximum shoot proliferation, number of shoots per explants, and shoot length. Half-strength MS medium supplemented with 5.4 µM NAA or 8.9 µM IBA was the most effective treatment for the rooting of shoots. Gradual acclimatization of the rooted plantlets was performed and the plantlets were established in the soil successfully. The micropropagated plants did not exhibit any visually detectable variation to their mother plants.

Keywords: Nodal explant, plant growth regulator, proliferation, rooting.

Introduction

Passiflora is the genus belonging to the family Passifloraceae, which is comprised of about 500 varieties distributed all around the tropical and subtropical regions (Garcia et al., 2011). This genus possesses a sizeable economic value due to the nutritional value of its fruits, medicinal properties of its leaves, and ornamental value of its flowers. The purple passion fruit (*Passiflora edulis* Sims.) is the most important and most widespread species in many of the countries, and it is precious due to its edible and sweet fruits as well as its ornamental properties (Huh et al., 2017).

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The purple passion fruit is propagated by seeds, cuttings, air layering, and grafting. The majority of the purple passion fruit growers in many countries use seedlings to establish orchards because the plants produced by seeds prevent the spreading of woodiness virus. However, the plants produced from seeds show huge genetic diversity. Cutting and grafting are also occasionally used to propagate this plant, but these methods have the risk of virus outbreak. In addition, cutting and grafting depend on some other factors such as plant age, physiological conditions, and horticultural practices (Nakasone and Paull, 1998). Therefore, *in vitro* tissue culture techniques for purple passion fruit can be very useful for quick clonal propagation and for the production of disease-free plants. Moreover, *in vitro* propagation of purple passion fruit is an essential stage in the success of transgenic experiments and determines the efficiency of a transformation protocol (Aldwinckle and Malony, 2009).

There are many protocols regarding *in vitro* propagation of passion fruit using different explants including shoot nodal segments, leaf, hypocotyl, and cotyledon (Reis et al., 2003; Pinto et al., 2010; Silva et al., 2015; Huh et al., 2017). However, each of these protocols has its advantages and disadvantages (such as low proliferation rate, high contamination, difficulties in *in vitro*-rooting, and browning of the culture media or the explants due to leaching of the phenolic materials). Therefore, it is essential to make efforts to remove the disadvantages and to achieve a protocol that can lead to maximum regeneration. Among the tissue culture methods, direct regeneration is an efficient method for micropropagation due to its high potential to produce large numbers of plants similar to the mother plants in a short time (George et al. 2008). Various factors, including the concentration of plant growth regulators, are useful for micropropagation of plants under *in vitro* conditions (Valizadeh Kaji et al., 2013; Huh et al., 2017). Many studies have been carried out to identify the optimal composition of plant growth regulators for *in vitro* propagation of various species of passion fruit (Ozarowski and Thiem, 2013). The most important plant growth regulators that were used in plant tissue culture are auxins and cytokinins (Valizadeh Kaji et al., 2013; Huh et al., 2017). Therefore, in the present study, the effects of auxin and cytokinin-based plant growth regulators (BA, TDZ, GA_3, IAA, and IBA) were investigated on the *in vitro* propagation of passion fruit plant. To the best of our knowledge, this is the first report on *in vitro* propagation of an Iranian purple passion fruit landrace. The purple passion fruit landrace that was used, 'Mazani', is a leading Landrace in Mazandaran province with white and purple flowers and purple fruits. Reduced quality seeds and lack of disease-free plants can inhibit adequate production and supply of purple passion fruit. Thus, availability of healthy planting materials for the growers could sustain the production and benefits of purple passion fruit. The major objective of this study was to develop a rapid micropropagation protocol for generating healthy, uniform, and inexpensive passion fruit planting materials.

Materials and Methods

The present study was conducted in the laboratory of tissue culture and greenhouse of the Agriculture and Natural Resources Faculty of Arak University, Arak, Iran in 2017-2018. Purple passion fruit landrace 'Mazani', obtained from a research greenhouse in Mazandaran (north of Iran), was used. Explants were taken from two-year-old plants grown in 15-liter pots in the greenhouse with normal daylight and a temperature range of 35–25 °C during day/night. For proliferation experiments, a completely randomized design (CRD) with various combination of BA (0, 2.2, 4.4 and 8.9 μM), TDZ (2.3, 4.5 and 9.1 μM), GA_3 (0 and 2.9 μM) and IBA (0 and 0.5 μM) was used. For rooting experiments, a completely randomized design (CRD) with

13 concentrations of auxin (IAA, IBA, and NAA) was used.

Explant preparation

Nodal segments (about 0.5 cm long) were excised from two-year-old plants. After eliminating the leaves, explants were washed with running tap water for 20 min, then were disinfected for 15 min in a 0.1 w/v calcium hypochlorite solution with 2-3 drops Tween 20 and rinsed four times in autoclaved distilled water. The sterilized explants (four explants/jar) were vertically cultured in the induction culture medium.

Culturing conditions

In this study, Murashige and Skoog (MS) medium (Murashige and Skoog 1962) was used. Sucrose was applied at 30.0 g L^{-1} and myo-inositol at 0.1 g L^{-1}. The pH of the prepared media was then adjusted to 5.6–5.8 with 0.1 N NaOH, before adding 6 g L^{-1} agar.

Culture media were then decanted in 200 mL jars; media volume for each jar was about 20 mL. The jars having media were autoclaved at 121°C and 1.5 kg cm^{-2} pressure for 15 min and left to be air-cooled for media solidification. For culture, the basal end of the node inserted a few mm into the culture medium. For growth, media containing explants were placed at 25±1 °C with white light (40 µmol m^{-2} s^{-1}) and a 16 h photoperiod. Each treatment involved seven jars as seven replicates with four explants in each and repeated thrice. After three months of culture, the growth characteristics of shoots developed from nodal segments of purple passion fruit were calculated.

In vitro rooting of shoots

Shoots with 1-1.5 cm long originating from explants were removed and cultured in half-strength MS medium containing different concentration of IAA (0, 1.1, 2.9, 5.7 and 11.4 µM) or IBA (1, 2.5, 4.9 and 8.9 µM) or NAA (1.1, 2.7, 5.4, 10.7 µM). Each auxin treatment consisted of seven jars as seven replicates with two or three shoots in each. After three months of culture, rooting percentage, number, length, and diameter of roots were evaluated.

Acclimatization of regenerated plantlets

Explants with well-developed roots were separated from the culture media. The roots were washed gently with tap water to eliminate agar, and then transferred to small plastic pots having autoclaved cocopeat-perlite mixture. The pots were enclosed with polyethylene bags to maintain high humidity and kept at 25±1°C under artificial light (50 µmol m^{-2} s^{-1}) provided by white fluorescent tubes for about four weeks. To harden the plants, polyethylene bags opened gradually, from a few min per day until normal conditions. Plants were then transferred to larger pots (15 cm diameter) containing garden soil (soil: compost, 1:1), kept under shade for another two weeks, and then transferred to direct sunlight condition. The survival rate was examined three months after translocation.

Data analysis

The data were analyzed using the GLM procedure of SAS software (Version 9.1), and significant differences were tested at $P \leq 0.05$ using Duncan's multiple ranges. Before statistical analysis, the expressed data as percentages were subjected to arcsine transformation, and the original values of all transformed data were presented.

Results

The effect of plant growth regulators on in vitro propagation of purple passion fruit

The results of the variance analysis showed that plant growth regulators had a significant impact on the percentage of shoot proliferation ($P = 0.0008$), number of induced shoots ($P \leq 0.0001$), shoot height ($P = 0.0034$) and the number of node ($P = 0.0101$). While, no considerable influence ($P = 0.3024$) was found for the effects of plant growth regulators on the number of leaves (Table 1). The explants were capable of growing on all the culture media (Fig. 5a,

b). Based on the obtained results, proliferation was observed in all of the treatments with some of them exhibiting the highest rate of proliferation (100%). The lowest proliferation percentage (44%) was obtained in 2.3 µM TDZ+2.9 µM GA3+0.5 µM IBA and 4.5 µM TDZ+2.9 µM GA3 treatments. However, no significant difference was found between this treatment and some other treatments regarding proliferation percentage (Table 1).

The highest number of shoots (17.67) was observed in 8.9 µM BA+2.9 µM GA3+0.5 µM IBA treatment, although no significant difference was found between this treatment and some other treatments (Table 1). Furthermore, the lowest number of induced shoots was found in 4.5 µM TDZ+2.9 µM GA3+0.5 µM IBA treatment. Nevertheless, no significant difference was found between this treatment and most other plant growth regulators for the number of induced shoots (Table 1).

The longest of shoot length (15.12 mm) was related to 8.9 µM BA+2.9 µM GA3+0.5 µM IBA treatment; whereas culture medium containing 4.5 µM TDZ+2.9 µM GA3 led to production of explants with the lowest shoot length (7.32 mm). However, no significant difference was found among many of the treatments for the height of their shoots (Table 1).

The results of the mean comparison of the data showed that the maximum number of nodes (10.44) belonged to 8.9 µM BA treatment. However, no significant difference was found between this treatment and most of the other treatments for the maximum number of nodes. On the other hand, the minimum number of nodes (3.83) was found in 2.3 µM TDZ+2.9 µM GA3+0.5 µM IBA treatment, although this treatment had no significant difference with some other treatments (Table 1).

Table 1. Effect of different concentrations of BA, TDZ, GA3 and IBA on *in vitro* growth characteristics of shoots developed from nodal segments of purple passion fruit after 3 months of culture

Treatments			Shoot proliferation (%)	Shoot number	Shoot length (mm)	Node number	Leaf number
Cytokinin (µM)	GA3 (µM)	IBA (µM)					
BA							
0	0	0	100.00±00.00 a	3.33±0.57 fgh	11.10±0.48 b-e	7.77±0.77 a-e	7.77±0.50
2.2	0	0	100.00±00.00 a	6.00±1.73 de	10.83±2.10 b-f	7.00±2.90 cde	10.11±2.79
2.2	0	0.5	77.33±19.62 abc	4.66±2.08 efg	10.18±2.34 b-g	9.89±2.77 abc	8.83±1.75
2.2	2.9	0	88.66±19.62 ab	5.33±1.52 ef	9.55±1.83 b-g	8.66±1.33 a-d	10.28±3.82
2.2	2.9	0.5	100.00±00.00 a	6.00±1.00 de	11.42±0.79 bc	9.11±0.84 abc	9.44±0.69
4.4	0	0	100.00±00.00 a	11.00±1.73 c	11.29±1.16 bcd	7.44±1.07 b-e	10.55±1.67
4.4	0	0.5	100.00±00.00 a	7.66±1.52 d	10.02±0.29 b-g	8.89±1.51 abc	8.89±3.18
4.4	2.9	0	100.00±00.00 a	11.66±0.57 c	7.90±1.36 efg	8.44±1.57 a-d	9.44±2.83
4.4	2.9	0.5	100.00±00.00 a	7.66±1.52 d	10.64±0.47 b-f	8.72±1.25 a-d	10.05±2.77
8.9	0	0	100.00±00.00 a	15.66±0.57 ab	9.34±1.00 b-g	10.44±0.76 a	9.83±3.01
8.9	0	0.5	100.00±00.00 a	15.33±1.15 b	8.54±0.98 c-g	7.11±1.16 b-e	7.44±1.95
8.9	2.9	0	100.00±00.00 a	17.00±2.00 ab	9.52±0.87 b-g	9.22±0.77 abc	9.78±0.19
8.9	2.9	0.5	100.00±00.00 a	17.66±1.15 a	15.12±1.15 a	9.00±0.30 abc	8.89±1.07
TDZ							
2.3	0	0	88.66±19.62 ab	3.00±1.00 fgh	10.36±2.49 b-g	8.39±0.97 a-d	8.33±1.45
2.3	0	0.5	77.33±19.62 abc	2.33±0.57 gh	11.95±1.84 b	8.27±1.49 a-d	9.05±2.55
2.3	2.9	0	77.66±38.68 abc	2.66±0.57 gh	11.29±1.03 bcd	7.11±0.84 b-e	8.44±2.36
2.3	2.9	0.5	44.00±19.05 bc	2.00±1.00 h	9.69±2.97 b-g	3.83±1.04 f	4.83±1.04
4.5	0	0	100.00±00.00 a	3.00±0.00 fgh	8.08±0.20 d-g	10.00±1.88 ab	10.33±2.35
4.5	0	0.5	88.66±19.62 ab	3.00±1.00 fgh	8.93±1.13 b-g	7.66±1.20 a-e	7.66±1.15
4.5	2.9	0	44.00±19.05 bc	1.76±1.15 h	7.32±2.72 g	7.00±1.41 cde	7.50±2.12
4.5	2.9	0.5	55.33±38.68 bc	1.66±1.15 h	7.95±1.61 efg	7.00±1.00 cde	8.66±2.88
9.1	0	0	66.33±33.50 abc	2.00±1.41 h	7.78±1.92 fg	7.00±1.41 cde	8.00±2.82
9.1	0	0.5	100.00±00.00 a	4.00±1.00 efgh	11.57±2.27 bc	5.33±2.08 ef	7.00±3.00
9.1	2.9	0	88.66±15.62 ab	2.62±063 gh	8.32±1.72 d-g	7.01±1.11 cde	7.30±1.06
9.1	2.9	0.5	66.50±47.37 abc	2.33±0.57 gh	7.78±0.81 fg	5.83±0.76 def	5.94±0.82
P-value			0.0008	<.0001	0.0034	0.0101	0.3024

Mean values followed by the similar letters within a column are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). Values represent the mean ± SD.

In vitro rooting and gradual acclimatization of plantlets

- ***Rooting percentage***

The results of the mean comparison of the data showed that plant growth regulators had a significant effect on rooting percentage of purple passion fruit (Fig. 1). Out of the 13 treatments used, eight treatments induced rooting on the shoots. The highest rooting percentage (100%) was observed in 8.9 µM IBA, 5.4 µM NAA, and 10.7 µM NAA treatments. In addition, amongst the treatments that caused rooting, the lowest rate of rooting (20%) was found in 1.1 µM NAA. However, a significant difference was found between this treatment and some other treatments for the rooting percentage (Fig. 1).

- ***Root number***

Mean comparison for the effect of different levels of plant growth regulators on the number of the root indicated that the highest number of the roots (5.40) related to 10.7 µM NAA treatment, which was not significantly different from 5.4 µM NAA treatment. The lowest number of the root (0.20) was obtained for 1.1 µM NAA, although this treatment had no significant difference compared to some other treatments (Fig. 2).

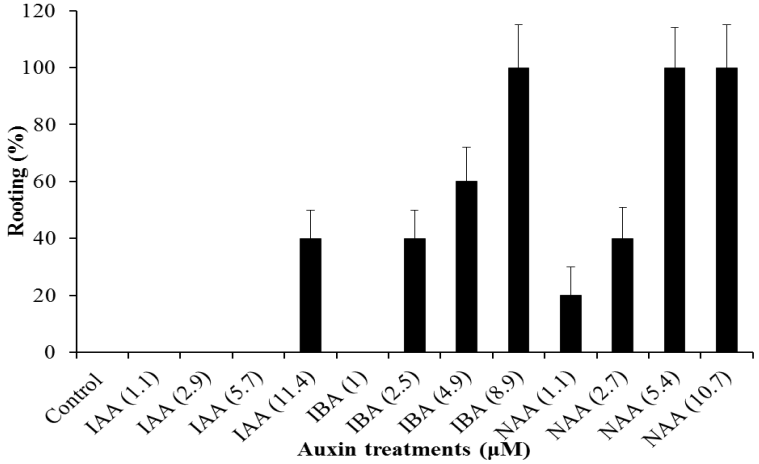

Fig. 1. Effect of different concentrations of auxin-based plant growth regulators (IAA, IBA and NAA) on *in vitro* rooting percentage of shoots developed from nodal segments of purple passion fruit after 3 months of culture. Different letters at the top of columns indicate significant differences ($P \leq 0.05$) among treatments. Vertical bars indicate mean value plus standard deviation.

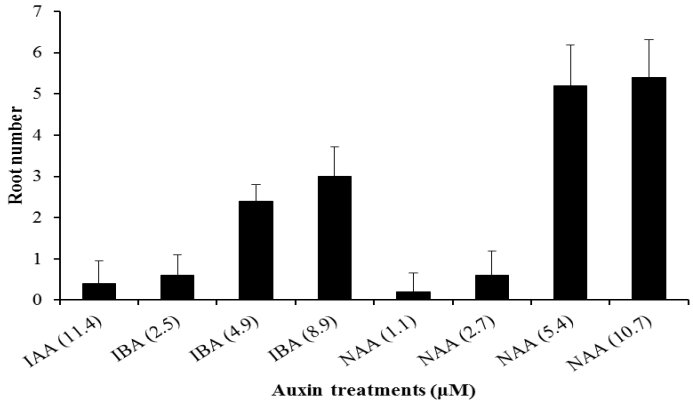

Fig. 2. Effect of different concentrations of auxin-based plant growth regulators (IAA, IBA and NAA) on *in vitro* root number of shoots developed from nodal segments of purple passion fruit after 3 months of culture. Different letters at the top of the columns indicate significant differences ($P \leq 0.05$) among treatments. Vertical bars indicate mean value plus standard deviation.

- ***Root length***

Results of the mean comparison showed that the longest root length (10.32 mm) belonged to 8.9 µM IBA treatment (Fig. 5c, d, and e). In addition, the shortest root length (0.2 mm) was obtained for 1.1 µM NAA treatment. Nevertheless, no significant difference was found between root length of this treatment and some other treatments (Fig. 3).

- ***Root diameter***

Based on the results of the mean comparison, the highest root diameter (1.52 mm) was observed in 5.4 µM NAA treatment. This treatment was not significantly different from 8.9 µM IBA treatment. The lowest root diameter (0.08 mm) belonged to 1.1 µM NAA treatment, albeit this treatment had no significant difference with many of the other treatments (Fig. 4).

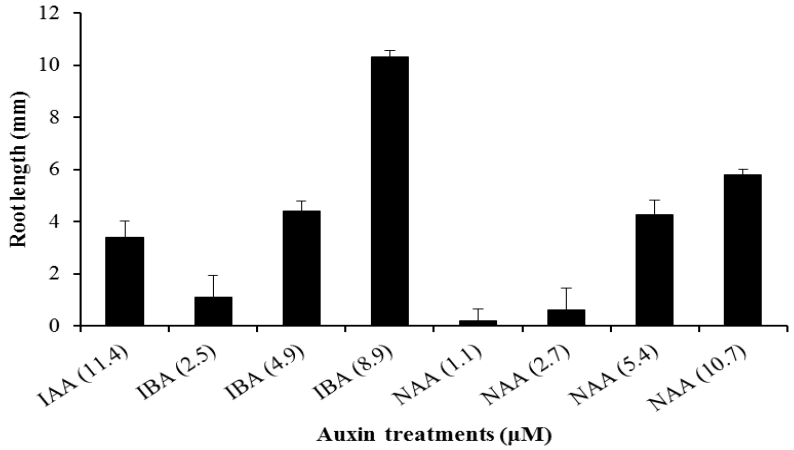

Fig. 3. Effect of different concentrations of auxin-based plant growth regulators (IAA, IBA and NAA) on *in vitro* root length of shoots developed from nodal segments of purple passion fruit after 3 months of culture. Different letters at the top of columns indicate significant differences ($P \leq 0.05$) among treatments. Vertical bars indicate mean value plus standard deviation.

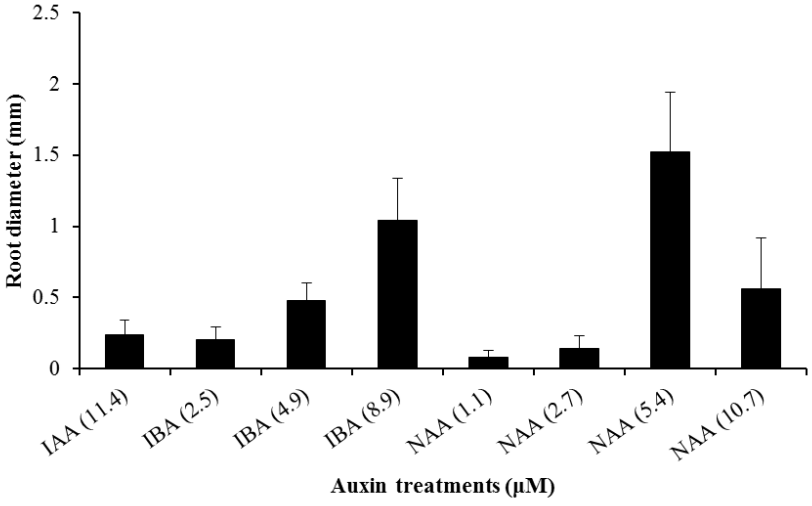

Fig. 4. Effect of different concentrations of auxin-based plant growth regulators (IAA, IBA and NAA) on *in vitro* root diameter of shoots developed from nodal segments of purple passion fruit after 3 months of culture. Different letters at the top of columns indicate significant differences ($P \leq 0.05$) among treatments. Vertical bars indicate mean value plus standard deviation.

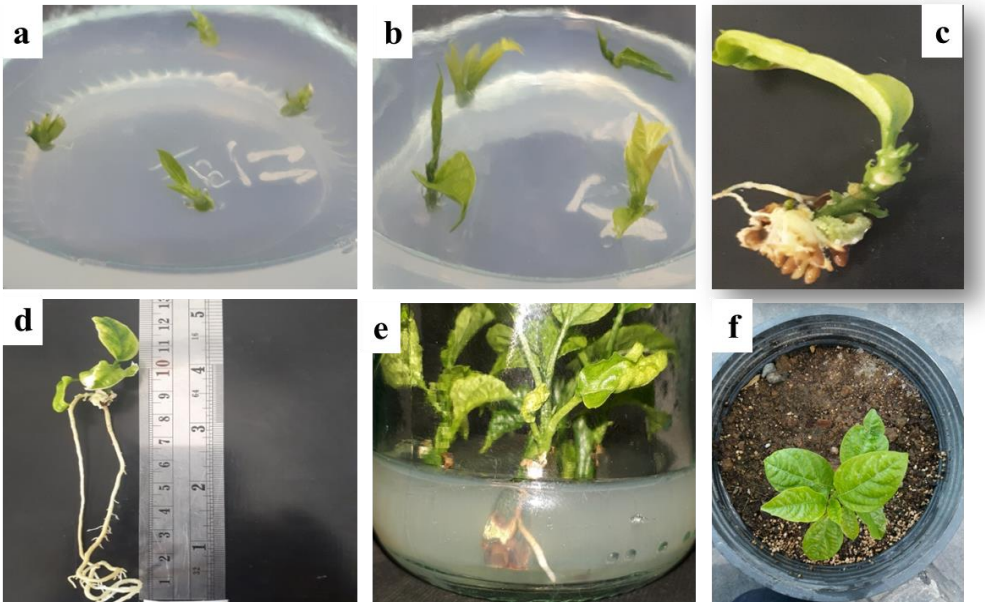

Fig. 5. Shoot development from nodal segments of purple passion fruit after 20 day of culture on Ms medium containing 8.9 µM BA + 2.9 µM GA3 + 0.5 µM IBA (a), development of shoots after 40 days of culture on the same medium (b), root development in shoot cultured on half strength MS medium containing 8.9 µM IBA after 20 days of culture (c), development of roots after 30 day of culture on the same medium (d), a rooted plantlet ready for acclimatization (e). a healthy and well-hardened plant (f).

Gradual acclimatization of the rooted plantlets occurred successfully, and the plantlets could establish in the soil (Fig. 5f). The percentage of survived plantlets was about 90%. The plantlets established in the soil did not exhibit any visually detectable variation to their mother plants.

Discussion

The major problems in the tissue culture of perennial plants are included high contamination, the recalcitrance of mature plants, hyperhydration, and browning of the culture media or the explants due to leaching of the phenolic materials (Krishna and Singh, 2007). In the present study, the nodal segments of two-year-old plants grown in the greenhouse were used as explants. The better reaction of the explants prepared from young plants to the culture medium has been reported in diverse plant species (Shukla et al., 2008; Valizadeh Kaji et al., 2013). Browning of explants due to exudation of phenolic materials has been reported as a severe problem in establishing cultures of passion flower (Huh et al., 2017). Nevertheless, in the current research, this problem was not observed because explants had been prepared from the young plants. This is probably because young plants synthesize less phenolic materials (Rai et al., 2009).

In the present study, the effect of different concentrations of BA, TDZ, IBA, and GA_3 on shoot proliferation from the nodal explants was investigated. The results indicated that the plantlets produced in culture medium containing 8.9 µM BA+2.9 µM GA_3+0.5 µM IBA were more vigorous with long shoots (Table 1), which is a critical trait in micropropagation. In accordance with the obtained results in the present study, Ozarowski et al. (2013) also reported that adding of cytokinin, gibberellic acid and auxin to the culture medium increased the efficiency of *in vitro* propagation and the shoot length of passion fruit. In addition, there are many reports regarding the effect of diverse combinations of plant growth regulators on the micropropagation of passion fruit species including *Passiflora caerulea* (Busilacchi et al., 2008; Ozarowski et al.,

2012; Prithrivaj et al., 2015), *Passiflora foetida* (Anand et al., 2012), *Passiflora setacea* (Vieira et al., 2014), *Passiflora eulis* Sims (Huh et al., 2017) and *Passiflora cincinnata* (da Silva et al., 2011). It has been reported that for each species, the certain combination of plant growth regulators is needed to achieve the maximum efficiency of *in vitro* propagation.

In the studies on the *in vitro* propagation of diverse passion fruit species, BA has been used in a various concentrations (ranging from 2.2 to 4.4 μM). Iapichino and Airò (2009) declared that increase in BA concentration to a certain amount in the culture medium results in more shoot proliferation. In the current study, the number of shoots increased with increasing BA concentration to 8.9 μM. However, Ning et al. (2007) reported that TDZ causes shoot induction in lower level ranges than to the BA, and it exerts inhibitory effects in higher levels. This is consistent with what has been found herein. The inhibitory effect of TDZ in higher levels might be due to the high cytokinin activity of this plant growth regulator (Huetteman and Preece, 1993).

The rooting of the shoots generated from *in vitro* cultures is an essential prerequisite for the establishment in the soil. NAA and IBA are the most common auxins used for *in vitro* root induction in regenerated shoots of passion fruit. Type of auxin intensively influences the rooting of the explants (Ozarowski and Thiem, 2013). Ragavendran et al. (2012) used 1 mg L^{-1} IBA concentration for root induction in *P. foetida*, and Anand et al. (2012) applied 0.5 mg L^{-1} IBA concentration for root induction in *P. foetida*. The high levels of NAA might cause toxicity in the explants (De Klerk et al., 1997). However, in this study, high levels of all three auxins led to better rooting in the shoots. In passion fruit, the half-strength medium was used for root induction (Ozarowski and Thiem, 2013). In the present study, the half-strength MS culture medium was also efficient for root induction. It has been reported that low salt concentrations in the half-strength medium induce rooting of the shoots in several plant species (Rai et al., 2010). In some of the plant species, it is necessary to translocate the rooted shoots to auxin-free medium so that the roots can become more elongated (Naik and Chand, 2011). In this study, the roots elongated without translocating them to the auxin-free medium (Fig. 5d).

About 30 days after hardening of the plants, no problem was observed for their survival. In passion fruit, various substrate materials were used for the gradual adaptation of rooted plants, and the percentage of the survived plants ranged from 90 to 100 (Ozarowski and Thiem, 2013; Huh et al., 2017).

Conclusions

The results obtained in the present study are considered acceptable for providing a potential commercial *in vitro* propagation protocol for purple passion fruit. In conclusion, it is advised to apply 8.9 μM BA+2.9 μM GA3+0.5 μM IBA for *in vitro* proliferation and 5.4 μM NAA or 8.9 μM IBA for rooting of purple passion fruit shoots. This method may also be used to select and clone this plant, which can be further improved using genetic engineering approaches.

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Conflict of interest

The authors indicate no conflict of interest for this work.

References

1. Aldwinckle H, Malnoy M. 2009. Plant regeneration and transformation in the Rosaceae. In: Nageswara-Rao M, and Soneji J R (Eds.) Transgenic plant. Special Issue 1, pp 1-39.

2. Anand S.P, Jayakumar E, Jeyachandran R, Nandagobalan V, Doss A. 2012. Direct organogenesis of *Passiflora foetida* L. through nodal explants. Plant Tissue Culture and Biotechnology 22, 87–91.
3. Busilacchi H, Severin C, Gattuso M, Aguirre A, Di Sapio O, Gattuso S. 2008. Field culture of micropropagated *Passiflora caeruela* L. histological andchemical studies. B. Latinoam. Caribe Pl 7:257–263.
4. da Silva C.V, Loriato V.A.P, Oliveira L.S, Otoni W.C. 2011. Efeito dos brassinosteroides e da 6-benzilaminopurina na organogenese *in vitro de Passiflora cincinnata Mast. XIII Congresso Brasileiro de fisiologia vegetal XIV Reuniao Latino-Americana de fisiologia vegetal do gene a planta.* Buzios, Brasil.
5. De Klerk G.J, Arnholdt-Schmitt B, Lieberei R, Neumann K.H. 1997. Regeneration of roots, shoots and embryos: physiological, biochemical and molecular aspects. Biologia Plantarum 39, 53-66.
6. Garcia R, Pacheco G, Falcão E. 2011. Influence of type of explant, plant growth regulators, salt composition of basal medium, and light on callogenesis and regeneration in *Passiflora suberosa* L. (Passifloraceae). Plant Cell Tissue and Organ Culture 106, 47-54.
7. George E.F, Hall M.A, De Klerk G.J. 2008. Plant Propagation by Tissue Culture. Vol. 1. 3th Ed. Dordrecht, The Netherlands, Springer.
8. Huetteman C.A, Preece J.E. 1993. Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell Tissue and Organ Culture 33, 105–119.
9. Huh Y.S, Lee J.K, Nam S.Y. 2017. Effect of plant growth regulators and antioxidants on in vitro plant regeneration and callus induction from leaf explants of purple passion fruit (*Passiflora edulis* Sims). Journal of Plant Biotechnology 44, 335–342.
10. Iapichino G, Airò M. 2009. Multiplication of *Crataegus monogyna* by *in vitro* culture of nodal segments, Acta Horticulturae 812, 135–140.
11. Krishna H, Singh N.K. 2007. Biotechnological advances in mango *Mangifera indica* L. and their future application in crop improvement: a review. Biotechnology Advances 25, 223–243.
12. Murashige T, Skoog F. 1962 . A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15, 473–497.
13. Naik S.K, Chand P.K. 2011. Tissue culture-mediated biotechnological intervention in pomegranate: a review. Plant Cell Reports 30, 707–721.
14. Nakasone H.Y, Paull R.E. 1998. Tropical Fruits. CAB International, Wallingford, UK.
15. Ning G.G, Bai S.P, Bao M.Z, Liu L. 2007. Factors affecting plantlet regeneration from in vitro cultured immature embryos and cotyledons of Prunus mume "Xue mei". In Vitro Cellular & Developmental Biology-Plant 43(3), 225–230.
16. Ozarowski M, Błaszkiewicz S, Gryszczynska A, Thiem B, Budzianowski J. 2012. Search for *C*-glycosyl flavones and phenolic acids in callus and shoot *in vitro* culture of *Passiflora caerulea* L. International conference: Business meets science to cooperate in current topics. Bioconnect. Poznan, Poland.
17. Ozarowski M, Sedzik K, Gryszczynska A, Thiem B. 2013. Optimization of conditions for in vitro propagation of valuable medicinal plants of *Passiflora incarnata* L. and *P. caerulea* L. *International conference: "Facilitating dialogue between business and academia*". Bioconnect. Poznan, Poland.
18. Ozarowskia M, Thiema T. 2013. Progress in micropropagation of *Passiflora* spp. to produce medicinal plants: a mini-review. Revista Brasileira de Farmacognosia 23, 937–947.
19. Pinto A.P.C, Monteiro-Hara A.C.B.A, Stipp L.C.L, Mendes B.M.J. 2010. In vitro organogenesis of *Passiflora alata*. In Vitro Cellular & Developmental Biology-Plant 46, 28–33.
20. Prithviraj H.S, Hemanth Kumar N.K, Prakasha. Shobha J. 2015. An efficient in vitro regenaretion of multiple shoot from leaf explant of *Passiflora caerulea* L. an important medicinal plant. International Journal of Recent Scientific Research 6(11), 7263–726.
21. Ragavendran C, Kamalanathan D, Reena G, Natarajan D. 2012. *In vitro* propagation of nodal and shoot tip explants of *Passiflora foetida* L. An exotic medicinal plant. Asian Journal of Plant Science and Research 2, 707–711.
22. Rai M.K, Asthana P, Jaiswal V.S, Jaiswal U. 2010. Biotechnological advances in guava (*Psidium guajava* L.): recent developments and prospects for further research. Trees - Structure and Function 24, 1–12.
23. Rai M.K, Jaiswal V.S, Jaiswal U. 2009. Shoot multiplication and plant regeneration of guava *Psidium guajava* L. from nodal explants of in vitro raised plantlets. Journal of Fruit and Ornamental Plant Research 17, 29–38.

24. Reis L.B, Paiva Neto V.B, Toledo Picoli E.A, Costa M.G.C, Rego M.M, Carvalho C.R, Finger F.L, Otoni W.C. 2003. Axillary bud development of passion fruit as affected by ethylene precursor and inhibitors. In Vitro Cellular & Developmental Biology-Plant 39, 618–622.

25. Shukla S, Shukla S.K, Mishra S.K. 2008. In vitro plant regeneration from seedling explants of *Stereospermum personatum* D.C.: a medicinal tree. Trees - Structure and Function 23, 409–413.

26. Silva G.M, Cruz A.C.F, Otoni W.C, Pereira T.N.S, Rocha D.I, Silva M. 2015. Histochemical evaluation of induction of somatic embryogenesis in *Passiflora edulis* Sims (Passifloraceae). In Vitro Cellular & Developmental Biology-Plant 51, 539–545.

27. ValizadehKaji B, Ershadi A, Tohidfar M. 2013. In vitro propagation of two Iranian commercial pomegranates (*Punica granatum* L.) cvs. 'Malas Saveh' and 'Yusef Khani'. Physiology and Molecular Biology of Plants 19(4), 597–603.

28. Vieira L.M, Rocha D.I, Taquetti M.F, da Silva L.C, De Campos J.M.S, Viccini L.F, Otoni W.C. 2014. In vitro plant regeneration of *Passiflora setacea* D.C. (Passifloraceae): the influence of explant type, growth regulators, and incubation conditions. In Vitro Cellular & Developmental Biology-Plant 50(6), 738-745.