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IMPROVING *IN VITRO* CULTURE RESPONSES IN PASSIFLORA 'POSSUM PURPLE' (*Passiflora edulis Sims f. edulis*)

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Abstract

Micropropagation of *Passiflora* species remains challenging due to inherent morpho-physiological constraints, including limited regeneration capacity and poor adventitious root formation. Although the role of plant growth regulators has been extensively investigated, increasing attention has been directed toward the use of supplementary additives. In this study, the effects of 6-benzylaminopurine (BAP) combined with either silver nitrate ($AgNO_3$) or peptone were evaluated for shoot regeneration, growth, and proliferation in the cultivar 'Possum Purple'. Rooting responses were subsequently assessed using different concentrations of indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) under different pre-incubation conditions. In addition, the influence of substrate type on plantlet survival during the acclimatization phase was investigated. Nodal explants were cultured on full-strength MS medium during the initiation and multiplication phases, whereas half-strength MS medium was used for rooting. Shoot development and rooting performance were evaluated at defined culture intervals. Although $AgNO_3$ had no significant effect on shoot regeneration, it significantly reduced tissue browning, with the lowest browning observed at 17.7 µM $AgNO_3$. In contrast, the greatest shoot growth and proliferation during the multiplication phase were achieved on culture media supplemented with 8.88 µM BAP and 11.8 µM $AgNO_3$. Peptone provided only limited benefits for shoot regeneration and development, particularly in BAP-free media, and had no statistically significant effect on shoot multiplication. Rooting was maximized in microshoots cultured on medium containing 4.92 µM IBA following a 10-day dark pre-incubation. During acclimatization, the highest survival percentage (72%) was obtained in plantlets transferred to the Jiffy-7 substrate.

Keywords: Dark treatment. Micropropagation. Passion fruit. Peptone. Rooting. shoot regeneration. Silver nitrate.

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1. Introduction

The family Passifloraceae includes the genus *Passiflora*, which comprises approximately 500 species and is of considerable commercial importance (He et al. 2020). Although native to South America, *Passiflora* species are naturally distributed and cultivated throughout tropical and subtropical regions including the Caribbean, South Africa, southern Florida, and several parts of Asia (Yuan et al. 2017; He et al. 2020). Several species, including *P. edulis* f. *edulis*, *P. edulis* f. *flavicarpa*, and *P. quadrangularis*, are commercially valuable for their edible fruits (Cazarin et al. 2015). Among these, cultivars of *P. edulis* f. *edulis* are the most widely cultivated worldwide (Huh et al. 2017).

Passiflora species are propagated by both sexual (seed) and vegetative (cuttings, grafting, air-layering) methods (Khas et al. 2020). In many countries, seed propagation is preferred for establishing commercial plantations because it is simple and cost-effective. However, seed-derived plants exhibit high genetic variability and do not produce true-to-type progeny, making them unsuitable for commercial production (Faleiro et al. 2019; Khas et al. 2020). Among vegetative propagation methods, cutting and grafting are the most commonly used. Nevertheless, these approaches are labor-intensive and carry a relatively high risk of virus transmission (Alexandre et al. 2009; Machado et al. 2015). In contrast, plant tissue culture offers several advantages, including rapid clonal propagation, production of disease-free planting material, and facilitation of international germplasm exchange while meeting phytosanitary requirements (Aldwinckle and Malony 2009; Khas et al. 2020).

In vitro propagation of *Passiflora* species, as with many woody plants, is frequently constrained by genotype-dependent physiological factors (Isutsa 2004; Trevisan and Mendes 2005; Jafari et al. 2022; Oros et al. 2022). In particular, the limited morphogenic capacity of certain genotypes represents a major obstacle, affecting shoot initiation and the subsequent stages of micropropagation. Among the factors contributing to this limitation, tissue browning caused by oxidative processes remains one of the most persistent challenges. This phenomenon typically occurs during the early stages of culture and is primarily associated with the release of phenolic compounds and other secondary metabolites following explant excision and tissue injury. Oxidation of these compounds impairs cellular development and ultimately reduces explant viability. In addition to tissue browning, *in vitro* propagation of *Passiflora* is frequently hindered by other physiological disorders such as apical necrosis, leaf drop, and poor root formation (Khas et al. 2020).

These morpho-physiological disorders substantially reduce the efficiency of micropropagation and hinder the development of commercially applicable propagation protocols (Khas et al. 2020). Many of these disorders are associated with ethylene accumulation resulting from restricted gas exchange within sealed culture vessels. To overcome this limitation, silver nitrate ($AgNO_3$) has been widely used to counteract ethylene-related effects and is recognized as an effective inhibitor of ethylene action in plant tissue culture (Kumar et al. 2009; Oluwasegun et al. 2025).

Silver ions have been reported to inhibit ethylene perception, thereby enhancing the regeneration capacity and promoting shoot development *in vitro* (Tahoori et al. 2018; Kaur and Kumar 2020). In addition, $AgNO_3$ has been suggested to exhibit an antioxidant-like effect by indirectly reducing the oxidation of phenolic compounds and thereby minimizing tissue browning (Kumar et al. 2009). However, excessive concentrations of $AgNO_3$ may be phytotoxic and adversely affect plant growth. The effectiveness of $AgNO_3$ in reducing tissue browning and enhancing regeneration has been demonstrated in several species, including pistachio (Ozden-Tokatli et al. 2005), coffee (Sridevi and Giridha 2014), finger lime (Mahmoud et al. 2020), banana (Shekhar et al. 2021), and date (Eldawayati et al. 2025). Similarly, previous studies have shown that supplementing the culture medium with $AgNO_3$ reduces phenolic oxidation in *Passiflora* explants and consequently improves shoot growth and development (Trevisan and Mendes 2005; Huh et al. 2017; Oros et al. 2022).

Peptone is another supplementary additive known to promote morphogenetic development under *in vitro* conditions. As a water-soluble protein hydrolysate, it consists primarily of peptides, amino acids, and inorganic salts, and may also contain small amounts of lipids, vitamins, and carbohydrates (Salsabila et al. 2022). Plant response to peptone varies considerably among species and genotypes. Previous studies have demonstrated that peptone supplementation enhances explant growth and development in several plant

species, such as avocado (Nhut et al. 2008) and orchid (Alam et al. 2010; Salsabila et al. 2022). However, its potential role in the micropropagation of *Passiflora* species remains largely unexplored.

In micropropagation, root formation is as critical as shoot regeneration and multiplication and is often regarded as one of the most challenging stages of the process (Khas et al. 2020). Several studies have shown that exposing auxin-supplemented media to a defined period of dark pre-incubation during the *in vitro* rooting phase enhances root induction, as reported in apple (Podwyszyńska and Cieślińska 2018), walnut (Dirlik et al. 2022), and hazelnut (Pincelli-Souza et al. 2023). Nevertheless, rooting remains particularly challenging in *Passiflora* species as demonstrated in studies involving *P. edulis* Sims (Isutsa 2004), *P. caerulea* (Jafari et al. 2017), *P. quadrangularis* (Oros et al. 2022), *P. foetida* L. (Nguyen et al. 2024). To our knowledge, however, no studies has been investigated the interaction between auxin treatments and dark pre-incubation on adventitious root formation in *Passiflora edulis* f. *edulis* Sims.

In this present study, the independent effects of BAP combined with either $AgNO_3$ or peptone on *in vitro* shoot regeneration, growth, and proliferation of *Passiflora edulis* were evaluated. Additionally, the interactive effects of auxin types, auxin concentrations, and pre-incubation conditions (10-day dark versus light treatment) on *in vitro* rooting of microshoots were evaluated. Finally, the effects of different substrates on plantlet survival during acclimatization were assessed.

2. Material and Methods

Plant material

Five-year-old plants of *Passiflora edulis* f. *edulis* 'Possum Purple' were used in this study. Mother plants were maintained in the greenhouse of the Plant Transformation Center at the University of California, Riverside. Nodal segments containing one to two axillary buds, excised from one-year-old shoots were used as explants.

Culture media and incubation conditions

Murashige and Skoog (MS) medium (M519, PhytoTech Labs, USA) was used as basal medium during the initiation and shoot proliferation phases, whereas half-strength MS was used during the rooting phase. For the initiation and shoot proliferation experiments, MS medium was supplemented with different concentrations of 6-benzylaminopurine (BAP) (B800, PhytoTech Labs, USA), 0.0, 4.44, and 8.88 µM in combination with silver nitrate ($AgNO_3$) (S169, PhytoTech Labs, USA), 0.0, 5.9, 11.8, and 17.7 µM, and peptone (P775, PhytoTech Labs, USA), 0.0, 1.0, 2.0, and 3.0%. For the rooting experiment, the culture medium was supplemented with indole-3-butyric acid (IBA) (I460, PhytoTech Labs, USA), 0.0, 2.46, 4.92, and 9.85 µM, and naphthaleneacetic acid (NAA) (N600, PhytoTech Labs, USA), 0.0, 2.68, 5.37, and 10.74 µM. Plant growth regulators (PGR) and peptone were added to the culture medium before sterilizing. The $AgNO_3$ solution was sterilized by filtration through a 0.22 µm membrane filter and added aseptically to the sterilized medium after it had cooled to approximately to 55°C. All culture media were supplemented with 3.0% (w/v) sucrose (Thermo Fisher Scientific, USA) and 0.7% (w/v) agar (A038, Caisson Labs, USA). The pH was adjusted to 5.7 before the addition of agar, and the medium was sterilized at 121 °C for 20 min.

Cultures were incubated in a controlled growth chamber at 25 ± 1°C under a 16-h light/8-h dark photoperiod provided by cool-white fluorescent lamps with a photosynthetic photon flux density (PPFD) of 40 µmol m^{-2} s^{-1}.

Sterilization phase

Nodal segments containing one to two axillary buds, excised from the greenhouse-grown plants were washed under running tap water at approximately 20°C for 20 min immersed in 70% ethanol for 1 min, and rinsed with sterile distilled water. The explants were then surface-sterilized in 10% commercial sodium hypochlorite (Clorox®, 6% active chlorine, USA) for 10 min, followed by three rinses (5 min each) with sterile distilled water in a laminar-flow chamber.

Initiation phase

The effects of BAP applied alone or in combination with $AgNO_3$ or peptone on shoot regeneration were evaluated during the initiation phase. Sterilized nodal segments were cultured individually in test tubes (120 × 25 mm) containing 10-mL of MS medium. Four weeks after culture initiation, shoot regeneration (%) and tissue browning (%) were recorded.

Shoot proliferation phase

The effects of BAP applied alone or in combination with $AgNO_3$ or peptone on shoot growth and proliferation were evaluated during the proliferation phase. Following the initiation stage, nodal segments were transferred to 250-mL culture jars containing 50-mL of MS medium. Explants were maintained on the same medium treatment used during the initiation phase and subcultured every four weeks for a total of three subcultures. At each subculture, explants were transferred to fresh MS medium of the same composition. After the third subculture (12 weeks), the number of shoots, the number of leaves, and shoot height (mm) were recorded.

Rooting phase

The interactive effects of auxin treatments and pre-incubation conditions (dark or light) on *in vitro* rooting were evaluated. Cultures were pre-incubated for 10 days either in complete darkness or under a 16 h photoperiod, after which all cultures were maintained under a 16-h photoperiod. The duration of dark pre-incubation was selected based on previous reports demonstrating its positive effect on auxin-mediated rooting (Chevreau and Bell 2005). Healthy microshoots obtained from the optimal proliferation treatment were transferred to 250-mL culture jars containing 50-mL of rooting medium. After six weeks on the rooting medium, rooting percentage (%), number of roots, and root length (cm) were recorded.

Acclimatization of plantlets phase

Following *in vitro* rooting, plantlets were transferred either to Jiffy-7 peat pellets or to a perlite–peat mixture (1:1, w/w) and maintained under the same controlled-environment conditions described above (25 ± 1°C, a 16-h light/8-h dark photoperiod, and a PPFD of 40 µmol m^{-2} s^{-1}). To facilitate acclimatization, the containers were covered with transparent plastic bags during the first week to maintain relative humidity above 90%. During the following weeks, humidity was gradually reduced to approximately 80%. Plantlets were subsequently transferred to greenhouse conditions (25 ± 2°C and 50–60% relative humidity), irrigated every 2–3 days and grown for approximately one month. Plantlet survival was recorded weekly over a six-week acclimatization period.

Statistical analysis

The experiments were conducted using a completely randomized design (CRD). Data were analyzed using one- or two-way analysis of variance (ANOVA), depending on the experimental design, using XLSTAT software. Mean comparisons were performed using Duncan's multiple range test at $p \leq 0.05$. Percentage data were subjected to arcsine transformation before analysis. Graphs were generated using RStudio (version 2025.05.1+513).

For the initiation experiment, 25 individual explants, each cultured in separate tubes, were used per treatment. For the shoot proliferation and rooting experiments, five culture jars, each containing five shoots, were used per treatment. During the acclimatization experiment, 25 plantlets were evaluated for each treatment.

3. Results

Effect of BAP, $AgNO_3$ and peptone on *in vitro* shoot regeneration and tissue browning

In this experiment, the effects of BAP combined with $AgNO_3$ and BAP combined with peptone on shoot regeneration and tissue browning were evaluated using two separate factorial experiments (Table 1). Regardless of the presence or absence of $AgNO_3$ or peptone, the highest regeneration rates were consistently obtained in media supplemented with 8.88 µM BAP. These results indicate that BAP concentration significantly influenced shoot regeneration ($p \leq 0.05$).

In contrast, $AgNO_3$ concentration alone, as well as its interaction with BAP, did not significantly affect shoot regeneration ($p > 0.05$). However, $AgNO_3$ concentration had a significant effect on tissue browning ($p \leq 0.05$). Increasing $AgNO_3$ concentrations resulted in a progressive reduction in tissue browning with the lowest browning percentage (12%) recorded at 17.7 µM $AgNO_3$ (Table 1; Figure 1a).

Regarding peptone, its concentration alone did not significantly affect shoot regeneration; however, the interaction between BAP and peptone significantly influenced regeneration. The highest regeneration percentage (72.0%) was achieved with the combination of 8.88 µM BAP combined with 2.0% peptone (Table 1; Figure 1b). In contrast, neither the main effect of peptone concentration nor its interaction with BAP had a statistically significant effect on tissue browning ($p > 0.05$).

Table 1. Effects of BAP, $AgNO_3$, and peptone on shoot regeneration and tissue browning in *Passiflora* 'Possum Purple' explants.

BAP (µM)	$AgNO_3$ (µM)	Shoot regeneration (%)	Tissue browning (%)
0.00	0.0	0.0	80.0
	5.9	0.0	48.0
	11.8	0.0	36.0
	17.7	0.0	24.0
4.44	0.0	56.0	48.0
	5.9	60.0	28.0
	11.8	64.0	20.0
	17.7	56.0	8.0
8.88	0.0	72.0	56.0
	5.9	72.0	20.0
	11.8	80.0	12.0
	17.7	60.0	4.0
p value			
BAP		< 0.0001	< 0.0001
$AgNO_3$		0.538*	< 0.0001
BAP x $AgNO_3$		0.931*	0.940*
BAP (µM)	**Peptone (%)**	**Shoot regeneration (%)**	**Tissue browning (%)**
0.00	0.0	0.0 c	80.0
	1.0	24.0 bc	80.0
	2.0	48.0 ab	76.0
	3.0	44.0 ab	80.0
4.44	0.0	56.0 ab	48.0
	1.0	56.0 ab	44.0
	2.0	48.0 ab	52.0
	3.0	48.0 ab	48.0
8.88	0.0	64.0 ab	56.0
	1.0	64.0 ab	56.0
	2.0	72.0 a	56.0
	3.0	60.0 ab	64.0
p value			
BAP		< 0.0001	< 0.0001
Peptone		0.217*	0.964*
BAP x Peptone		0.041	0.994*

Different letters indicate statistically significant differences among treatments ($p \leq 0.05$).
* Indicates no statistically significant difference among treatments ($p > 0.05$).

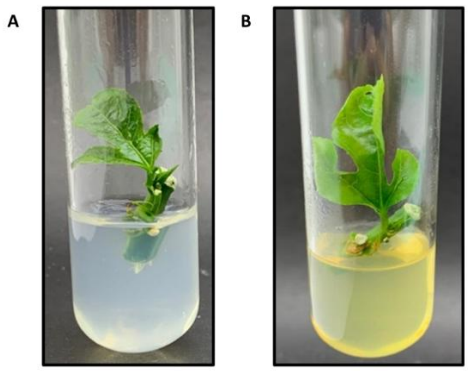

Figure 1. Effect of BAP, $AgNO_3$ and peptone on shoot development. **(A)** MS medium supplemented with 8.88 µM BAP and 17.7 µM $AgNO_3$. **(B)** MS medium supplemented with 8.88 µM BAP and 2.0% peptone (b). Scale bars = 1 cm.

Effect of BAP, $AgNO_3$, and peptone on *in vitro* shoot proliferation

During the shoot proliferation phase, BAP concentration significantly influenced the morphogenetic response. Increasing the BAP concentration to 8.88 µM significantly increased shoot height, number of leaves, and number of shoots, regardless of the $AgNO_3$ or peptone concentration ($p \leq 0.05$).

Regarding the main effect of $AgNO_3$, the highest values for shoot height (19.37 mm), number of leaves (3.00), and number of shoots (2.49) were obtained at 11.8 µM $AgNO_3$. However, $AgNO_3$ concentrations above this level significantly reduced all evaluated shoot proliferation parameters ($p \leq 0.05$). Analysis of the BAP × $AgNO_3$ interaction further showed that the combination of 8.88 µM BAP and 11.8 µM $AgNO_3$ produced the greatest shoot development, resulting in the highest shoot height (27.26 mm), number of leaves (3.52), and number of shoots (3.40) (Figure 2).

The main effect of peptone concentration significantly influenced both shoot height and the number of leaves ($p \leq 0.05$), whereas shoot multiplication was not significantly affected. The greatest shoot height (17.10 mm) and number of leaves (2.93) were obtained in media supplemented with 2.0% peptone. Analysis of BAP × peptone interaction revealed a significant effect only for the number of leaves ($p \leq 0.05$). The highest number of leaves (3.36) was obtained with the combination of 8.88 µM BAP and 0.0% peptone, whereas increasing the peptone concentration to 3.0% reduced the number of leaves to 2.80.

No significant BAP × peptone interaction was observed for shoot height or shoot multiplication ($p > 0.05$).

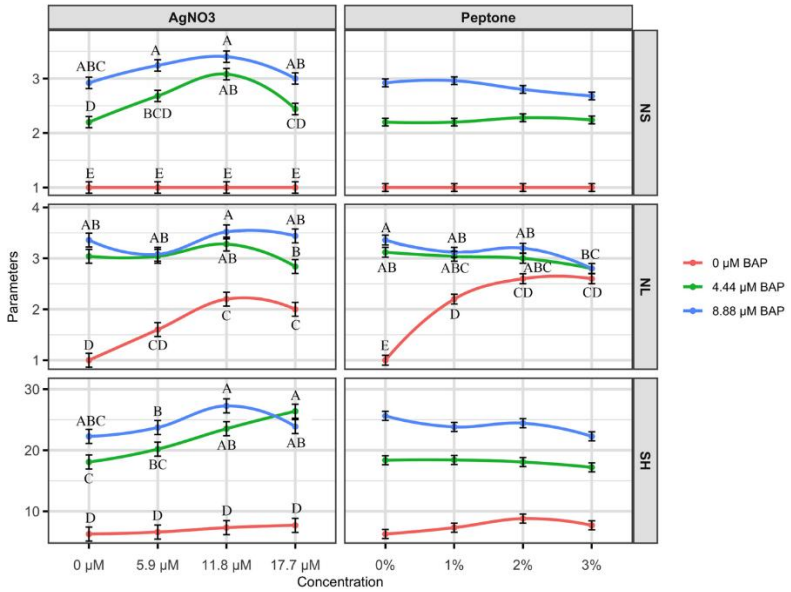

Figure 2. Effects of different BAP concentrations in combination with $AgNO_3$ or peptone on the number of shoots (NS) per explant, number of leaves (NL) per explant, and shoot height (SH) (mm) during *in vitro* propagation of 'Possum Purple'. Error bars represent the standard error of the mean. Different letters indicate statistically significant differences among BAP × $AgNO_3$ and BAP × peptone treatment combinations according to Duncan's multiple range test ($p \leq 0.05$).

Effects of auxin type, auxin concentration, and dark/light pre-incubation on *in vitro* rooting of microshoots

In the rooting experiment, pre-incubation conditions (dark and light), auxin type and concentration, and their interactions had statistically significant effects on rooting performance ($p \leq 0.05$) (Table 2). Dark pre-incubation significantly improved all rooting parameters compared with the light conditions (16-h photoperiod) ($p \leq 0.05$). The highest rooting percentage (35.62%), number of roots (2.07), and root length (2.01 cm) were obtained following dark pre-incubation, whereas the corresponding values under light pre-incubation were 22.50 %, 1.59 roots per shoot, and 1.59 cm, respectively.

Auxin treatments also significantly affected rooting responses ($p \leq 0.05$). Overall, IBA was statistically more effective than NAA in promoting root induction and development. The highest rooting percentage (55.00%), number of roots (3.32), and root length (3.22 cm) were achieved with 4.92 µM IBA. However, increasing the IBA concentration to 9.85 µM resulted in a statistically significant reduction in all rooting parameters. In contrast, NAA treatments showed a progressive improvement in rooting responses with increasing auxin concentration, although their performance remained inferior to that of 4.92 µM IBA. No root formation was observed in the auxin-free control treatment (Table 2).

Table 2. Effects of auxin type, auxin concentration, and pre-incubation conditions (dark or light) on *in vitro* rooting percentage, number of roots, and root length of 'Possum Purple' microshoots.

Conditions	Rooting (%)	Number of roots per shoot	Root length (cm)
Pre-incubation			
Dark	35.62 ^{a}	2.07 ^{a}	2.01 ^{a}
Light	22.50 ^{b}	1.59 ^{b}	1.59 ^{b}
p value	0.0096	0.0007	0.0039
Auxin type × concentration			
IBA/NAA x 0.00 µM	0.00 ^{d}	0.00 ^{e}	0.00 ^{f}
IBA x 2.46 µM	27.50 ^{bc}	2.55 ^{b}	2.36 ^{cd}
IBA x 4.92 µM	55.00 ^{a}	3.32 ^{a}	3.22 ^{a}
IBA x 9.85 µM	45.00 ^{ab}	2.55 ^{b}	2.81 ^{b}
NAA x 2.68 µM	20.00 ^{cd}	1.50 ^{d}	1.38 ^{e}
NAA x 5.37 µM	40.00 ^{abc}	2.25 ^{c}	2.10 ^{d}
NAA x 10.74 µM	45.00 ^{ab}	2.47 ^{bc}	2.51 ^{c}
p value	< 0.0001	< 0.0001	< 0.0001
Auxin treatment × Pre-incubation			
0.00 µM IBA/NAA x Light	0.00 ^{e}	0 .00 ^{i}	0.00 ^{g}
2.46 µM IBA x Light	20.00 ^{de}	2.15 ^{ef}	2.30 ^{d}
4.92 µM IBA x Light	40.00 ^{bcd}	2.80 ^{bc}	2.85 ^{b}
9.85 µM IBA x Light	30.00 ^{cd}	2.35 ^{de}	2.48 ^{cd}
2.68 µM NAA x Light	20.00 ^{de}	1.35 ^{h}	1.12 ^{f}
5.37 µM NAA x Light	35.00 ^{bcd}	1.95 ^{fg}	1.82 ^{e}
10.74 µM NAA x Light	35.00 ^{bcd}	2.10 ^{ef}	2.19 ^{d}
0.00 µM IBA/NAA x Dark	0.00 ^{e}	0.00 ^{i}	0.00 ^{g}
2.46 µM IBA x Dark	35.00 ^{bcd}	2.95 ^{b}	2.42 ^{d}
4.92 µM IBA x Dark	70.00 ^{a}	3.85 ^{a}	3.54 ^{a}
9.85 µM IBA x Dark	60.00 ^{ab}	2.75 ^{bc}	3.15 ^{b}
2.68 µM NAA x Dark	20.00 ^{de}	1.65 ^{g}	1.68 ^{e}
5.37 µM NAA x Dark	45.00 ^{abcd}	2.55 ^{cd}	2.45 ^{cd}
10.74 µM NAA x Dark	55.00 ^{abc}	2.84 ^{bc}	2.80 ^{bc}
p value	< 0.0001	< 0.0001	< 0.0001

Different letters indicate statistically significant differences among treatments ($p \leq 0.05$).

The interaction between auxin treatments and pre-incubation conditions significantly affected rooting performance ($p \leq 0.05$). The highest rooting response was achieved with 4.92 µM IBA following dark-preincubation, which was statically superior to all other treatment conditions. This treatment produced the highest rooting percentage (70%), number of roots (3.85), and mean root length (3.54 cm) ($p \leq 0.05$) (Table 2). In contrast, when the same IBA concentration (4.92 µM) was applied after light pre-incubation, the rooting percentage decreased to 40%, indicating that the dark pre-incubation enhanced the root-inducing capacity effect of 4.92 µM IBA by approximately 75%.

For IBA treatments, all rooting parameters increased significantly with increasing concentration up to 4.92 µM under both pre-incubation conditions ($p \leq 0.05$). However, increasing the IBA concentration to 9.85 µM resulted in a statistically significant decline in rooting performance. Under dark pre-incubation, for example, the number of roots decreased from 3.85 to 2.75 per shoot, accompanied by corresponding reductions in rooting percentage and root length (Table 2).

A similar, although less pronounced, response was observed for NAA. The highest rooting percentage (55%) was obtained with 10.74 µM NAA following dark pre-incubation, compared with 35% under light pre-incubation. Although dark pre-incubation enhanced rooting in both auxin treatments, the response of IBA was more strongly influenced by pre-incubation conditions than that to NAA. No root formation was observed in the auxin-free control, irrespective of whether microshoots were pre-incubated in darkness or under light (Table 2).

Acclimatization of plantlets

During acclimatization, both substrate type and duration significantly affected plantlet survival ($p \leq 0.05$), whereas their interaction was not significant ($p > 0.05$). Regardless of the evaluation time, plantlets grown in Jiffy-7 exhibited significantly higher survival rates than those grown in the perlite–peat (1:1) substrate. Plantlet survival gradually declined over the six-week acclimatization period in both substrates, with the most pronounced reduction occurring after the fourth week. At the end of the sixth week, the survival percentage remained at approximately 72% in Jiffy-7 but declined to about 52% in the perlite–peat (1:1) substrate (Figures 3 and 4).

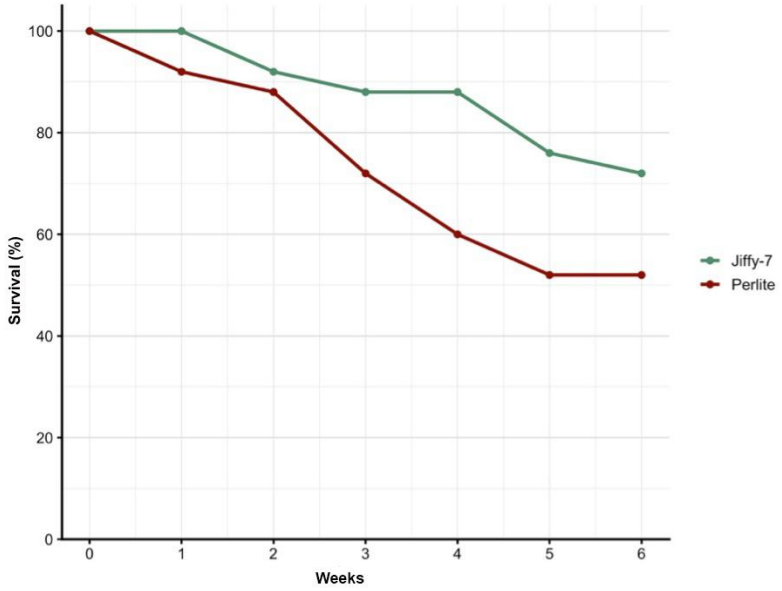

Figure 3. Survival of *Passiflora* 'Possum Purple' plantlets cultured in two different substrates (Jiffy-7 peat pellets and perlite–peat) over a six-week acclimatization period.

Figure 4. Representative *Passiflora edulis* 'Possum Purple' plantlets six weeks after transfer to Jiffy-7 peat pellets. Scale bar = 1 cm.

4. Discussion

In the present study, nodal explants obtained from a mature *Passiflora edulis f. edulis* 'Possum Purple' plant were successfully established on MS medium supplemented with BAP in combination with either $AgNO_3$ or peptone. The establishment results clearly demonstrated that BAP is the principal regulator initiating and directing the morphogenetic response. This finding is consistent with previous studies identifying BAP as the most effective cytokinin for *Passiflora* micropropagation (Oros et al. 2020).

A major limitation in the micropropagation of *Passiflora* is tissue browning, which frequently compromises explant viability and regeneration. In the present study, $AgNO_3$ did not significantly increase shoot regeneration during *in vitro* culture establishment but effectively reduced tissue browning, thereby preserving explant viability. This observation agrees with the findings of Sales et al. (2025), who likewise reported that $AgNO_3$ has little direct effect on shoot regeneration. Ethylene has been implicated in tissue browning during *in vitro* culture through the stimulation of phenolic compounds accumulation and activation of polyphenol oxidase (PPO) (Kumar et al. 2009). Consequently, the reduction in browning observed here is likely associated with the inhibition of ethylene perception by $AgNO_3$. Previous studies have shown that $AgNO_3$ suppresses PPO activity by blocking ethylene signal, thereby limiting phenolic oxidation and reducing browning symptoms (Kumar et al. 2009). Similar reductions in tissue browning have been reported in *Passiflora* species (Huh et al. 2017; Oros et al. 2022) as well as in other phenolic-rich species, including pistachio (Ozden-Tokatli et al. 2005) and banana (Shekhar et al. 2021).

Beyond its protective effect against tissue browning, $AgNO_3$ significantly enhanced shoot proliferation during the multiplication stage. The stimulatory effect was most pronounced when $AgNO_3$ was combined with BAP, suggesting a synergistic interaction between the cytokinin and the ethylene inhibitor. Silver ions (Ag^+) interfere with ethylene perception, which may reduce apical dominance and stimulate lateral bud outgrowth, thereby increasing shoot proliferation (Kumar et al. 2009). Comparable responses have been reported in several *Passiflora* species, where $AgNO_3$ concentrations between 11.8 and 23.5 µM improved shoot development during *in vitro* propagation (Trevisan and Mendes 2005; Huh et al. 2017; Oros et al. 2022). In contrast, the decline in shoot development observed at $AgNO_3$ concentrations ≥23.5 µM is likely attributable to the phytotoxic effects of excessive silver ions, a phenomenon also reported in *Passiflora* species (Hieu et al. 2022).

The effect of peptone on shoot development depended strongly on the BAP concentration in the culture medium. Peptone significantly enhanced shoot regeneration in the absence of BAP, whereas its stimulatory effect was greatly diminished when BAP was present. These results suggest that peptone primarily functions as a supportive organic additive under conditions of limited cytokinin availability. Once cytokinin levels become sufficient to sustain shoot development, additional peptone provides little morphogenetic effect. This interpretation is consistent with the findings of Nhut et al. (2008), who reported that peptone acts mainly as a growth stimulant in hormone-free media, whereas its beneficial effect

decreases in the presence of BA or 2,4-D (Salsabila et al. 2022). Moreover, the absence of a significant effect of peptone on shoot numbers indicates that peptone primarily improves shoot quality rather than directly enhancing shoot multiplication.

To our knowledge, this is the first study to evaluate the role of peptone during *in vitro* morphogenesis of *Passiflora* species. Nevertheless, the present results are consistent with reports in other species, including *Persea americana*, *Spathoglottis plicata*, and *Hevea brasiliensis,* where peptone promoted morphogenesis at low concentrations or in the absence of exogenous plant growth regulators, whereas higher concentrations or its combination with plant growth regulators reduced morphogenetic responses (Nhut et al. 2008; Sirisom and Te-chato 2012; Salsabila et al. 2022).Although peptone alone promoted shoot development in the present study, the loss of this stimulatory effect in the presence of BAP may reflect alterations in carbon and nitrogen metabolism under *in vitro* conditions. Peptone supplies amino acids, peptides, and other organic nitrogen compounds that influence cellular metabolism and developmental responses (Nhut et al. 2008). Because the balance between carbon and nitrogen metabolism plays a central role in regulating cell proliferation and differentiation during *in vitro* morphogenesis (Thorpe et al. 2008), the additional organic nitrogen supplied by peptone together with BAP may have altered the nutritional balance required for optimal shoot morphogenesis, thereby reducing the beneficial effect of peptone.

During the *in vitro* rooting stage, adventitious root formation was strongly influenced by the interaction between pre-incubation conditions and auxin treatment. Dark pre-incubation on auxin-containing media markedly improved rooting efficiency most likely by enhancing tissue responsiveness to auxin, modifying endogenous hormonal balance and reducing light-mediated inhibition of root formation (Deepika et al. 2020; Li et al. 2022). This response may also be explained by the light-dependent regulation of auxin metabolism, since auxins such as IBA and IAA are susceptible to photo-oxidative degradation (Nissen and Sutter 1990). Consequently, dark incubation may improve auxin stability, facilitating its uptake and accumulation within plant tissues. Because successful adventitious root induction depends on maintaining adequate endogenous auxin levels while minimizing auxin degradation, dark pre-incubation likely favors the initiation of root primordia during the early stages of root induction (Gaspar et al. 1992).

Rooting responses were also strongly affected by the type and concentration of auxin. The failure of auxin-free controls to produce roots confirms the essential requirement for exogenous auxin during adventitious root formation. Among the auxins evaluated, IBA proved more effective than NAA, with the highest rooting percentage obtained at 4.92 µM IBA. Similar responses have been reported in several *Passiflora* species, where IBA consistently promotes superior root induction (Shekhawat et al. 2015; Jafari et al. 2017; Hieu et al. 2022). In contrast, the greater effectiveness of NAA reported for *Passiflora quadrangularis* demonstrates that rooting responses remain highly species- and genotype-dependent (Oros et al. 2022).

Although rooting occurred under both dark and light conditions, the highest rooting efficiency in 'Possum Purple' microshoots was achieved after a 10-day dark pre-incubation combined with 4.92 µM IBA. This treatment closely corresponds with the physiological sequence of adventitious root formation, in which initial cell divisions generally occur within 3–5 days, followed by the appearance of morphologically recognizable root primordia between days 7 and 10 (Naija et al. 2008). To our knowledge, this rooting strategy has not previously been reported for the 'Possum Purple' cultivar. However, comparable dark pre-incubation periods (7–14 days) combined with IBA or NAA have improved rooting in other plant species, including almond × peach rootstock (GF677), pear, and blueberry (Vaez-Livari and Salehi-Soghadi 2006; Aygun and Dumanoglu 2015; Mohamed and Saif 2023).

During acclimatization, substrate selection significantly affected plantlet survival. Among the substrates evaluated, Jiffy-7 produced the highest survival rate, most likely because its superior water-holding capacity, uniform structure, and ability to maintain a stable moisture environment around the developing root system. Its fibrous structure also promotes adequate aeration, providing a favorable balance between water retention and gas exchange that supports root establishment during the transition from *in vitro* to *ex vitro* conditions.

Peat-based substrates are known to combine high water-holding capacity with adequate air-filled porosity, owing to their unique cellular structure (Schmilewski 2008; Gruda 2009). Consistent with these characteristics, survival in the perlite–peat (1:1) mixture declined to 52% after six weeks, whereas plantlets

established in Jiffy-7 maintained a survival percentage of 72%. Similar advantages of peat-based substrates during acclimatization have been reported for other woody species, including the 'MKR1' persimmon rootstock (Hejazi et al. 2025), the 'Discolor' rowanberry cultivar (Sedlák et al. 2025), and the 'Sorrento' walnut cultivar (Gentile et al. 2023). Collectively, these findings demonstrate that substrate physical properties, particularly the balance between water retention and aeration, are critical determinants of successful ex vitro acclimatization of in vitro-derived plantlets.

5. Conclusions

The present study established an efficient *in vitro* propagation protocol for *Passiflora edulis* 'Possum Purple' by optimizing shoot regeneration, multiplication, rooting, and acclimatization. For shoot regeneration and multiplication, the combination of BAP (8.88 µM) and $AgNO_3$ (11.8 µM) is recommended, as it effectively reduced tissue browning, maintained explant viability, and enhanced shoot proliferation. In contrast, the effect of peptone was comparatively limited and strongly dependent on the hormonal composition of the culture medium. Although peptone promoted shoot regeneration under cytokinin-free conditions, its stimulatory effect was substantially reduced in the presence of BAP. For root induction, the combination of 4.92 µM IBA with a 10-day dark pre-incubation is recommended to maximize rooting efficiency. During acclimatization, Jiffy-7 proved to be the most suitable, providing the highest plantlet survival and representing a reliable option for the large-scale commercial propagation of *Passiflora edulis*.

Overall, the protocol developed in this study provides an effective platform for the rapid clonal propagation of the 'Possum Purple' and establishes a valuable foundation for future applications in genetic transformation, genome editing, and germplasm conservation of *Passiflora*.

Future research should compare the performance of $AgNO_3$ with other ethylene inhibitors to further improve shoot regeneration and tissue quality. The role of peptone also warrants additional investigation, particularly at lower concentrations and in combination with different classes of plant growth regulators. Furthermore, evaluating dark pre-incubation under a wider range of auxin treatments may provide additional opportunities to optimize adventitious root formation.

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