



Direct Somatic Embryogenesis and Organogenesis of The Passion Fruit Plant *Paassifora edulis Sims. F. Flavcarpa in Vitro*

Zainab M. Abdul – Hussein and Lamiaa Kh. Jawad*

Dept. of Horticulture and landscape Gardening, College of Agricultural Engineering Sciences, University of Baghdad, Iraq

*Correspondence: lamiaa.khlifa@coagri.uobaghdad.edu.iq

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ABSTRACT

This study was conducted in the Plant Tissue Culture Laboratory, College of Agricultural Engineering Sciences, University of Baghdad, from 2022 to 2024, aiming to develop an efficient micropropagation protocol for passion fruit (*Passiflora edulis Sims. f. flavicarpa*) through direct organogenesis and somatic embryogenesis under in vitro conditions. Several experiments were performed, including seed germination, culture initiation, and shoot development and multiplication. The seed germination experiment evaluated the effects of medium physical state (liquid and solid) and various dormancy-breaking treatments and their interactions. The initiation stage assessed the influence of explant type (roots, embryos with cotyledons, hypocotyls, and cotyledonary leaves), 2,4-dichlorophenoxyacetic acid (2,4-D), and benzyl adenine (BA). The organ development and shoot multiplication stage examined the effects of BA, gibberellic acid (GA₃), and silver nitrate (AgNO₃). All experiments were arranged in a completely randomized design. Results indicated that the interaction between liquid medium and seed tip excision produced the highest germination rate (30%). Explant type played a critical role in inducing organogenesis and somatic embryogenesis. The highest direct organogenesis response (80%) was obtained from cotyledonary leaf explants cultured on medium containing 1 mg/L BA without 2,4-D. Direct somatic embryogenesis was maximized (40%) using cotyledonary leaf explants with 0.5 mg/L BA. The highest callus formation rate (50%) was observed in hypocotyl explants treated with 1 mg/L BA and 4 mg/L 2,4-D. For shoot multiplication, the combination of 2 mg/L BA, 1 mg/L GA₃, and 4 mg/L AgNO₃ significantly enhanced shoot number, length, and biomass.

توالد الاعضاء والاجنة الجسمية المباشر لنبات الباشن فروت *Passiflora edulis Sims.f.flavicarpa* خارج الجسم الحي

زينب مهدي عبد الحسين ولمياء خليفة جواد
قسم البستنة وهندسة الحدائق – كلية علوم الهندسة الزراعية ، جامعة بغداد ، العراق

الخلاصة

نفذت التجربة في مختبر زراعة الأنسجة النباتية بكلية علوم الهندسة الزراعية، جامعة بغداد، خلال المدة 2022–2024، بهدف توظيف تقانة زراعة الأنسجة النباتية في الإكثار الدقيق لنبات الباشن فروت (*Passiflora edulis Sims. f. flavicarpa*) من خلال التوالد المباشر للأعضاء والأجنة الجسمية تحت ظروف الزراعة خارج الجسم الحي. تضمنت الدراسة عدة تجارب شملت مرحلة إنبات البذور، ومرحلة النشوء، ومرحلة تطور وتضاعف الأفرع الخضرية. تضمنت تجربة إنبات البذور عاملين هما الحالة الفيزيائية للوسط الغذائي (سائل وصلب) ومعاملات كسر السكون، إضافة إلى التداخل بينهما. أما مرحلة النشوء فقد درست تأثير نوع الجزء النباتي (الجذور، الجنين مع الفلقتين، السويقة تحت الفلقية، والأوراق الفلقية) وتركيز من منظمي النمو D-2,4 و BA والتداخل بينهما. في حين شملت مرحلة تطور وتضاعف الأفرع الخضرية دراسة تأثير تراكيز BA و GA₃ ونترات الفضة AgNO₃ والتداخل فيما بينها. صُممت جميع التجارب وفق التصميم العشوائي الكامل (CRD). أظهرت النتائج تفوق معاملة التداخل بين الوسط السائل وقرط طرفي البذرة في نسبة الإنبات، إذ بلغت 63%. كما تبين أن نوع الجزء النباتي يعد عاملاً حاسماً في استحداث التوالد المباشر للأعضاء والأجنة الجسمية. سجلت الأوراق الفلقية أعلى نسبة استجابة لتكوين الأفرع الخضرية المباشرة (80%) عند معاملتها بـ 1 ملغم لتر⁻¹ من BA دون إضافة D-2,4، في حين بلغت أعلى نسبة لاستحداث الأجنة الجسمية المباشرة 40% عند استخدام 0,5 ملغم لتر⁻¹ من BA. كما حققت السويقة تحت الفلقية أعلى نسبة لتكوين الكالس (50%) عند معاملتها بـ 1 ملغم لتر⁻¹ من BA و 4 ملغم لتر⁻¹ من D-2,4. وأظهرت مرحلة تضاعف الأفرع الخضرية تفوق معاملة 2 ملغم لتر⁻¹ من BA مع 1 ملغم لتر⁻¹ من GA₃ و 4 ملغم لتر⁻¹ من AgNO₃ في عدد الأفرع وطولها والوزن الرطب والجاف.

الكلمات المفتاحية: الإكثار الدقيق، نبات الباشن فروت الاصفر، تكوين الأفرع العرضية، الاجنة الجسمية.

INTRODUCTION

Passion fruit (*Passiflora edulis*) belongs to the family Passifloraceae and is a widely distributed plant in tropical and subtropical regions around the world. The genus *Passiflora* includes nearly 500 species, of which about 50-60 species bear edible fruits. Some of these species are also cultivated as ornamental plants due to the beauty and fragrance of their colorful flowers. The passion fruit plant can grow up to 15 meters in height and climbs with green tendrils, producing beautiful flowers in multiple colors and a pleasant fragrance (Weyya *et al* 2024). The fruit is small with a leathery texture, containing sweet juice. It is used in the production of cosmetics, perfumes, as well as in preparing sweets and beverages. The origin of *Passiflora edulis* is from Brazil and spread to all tropical and subtropical regions of the world during the 19th century (Zeng *et al.*, 2023; Chuang *et al* 2024, Yu *et al* 2024). It exists in two forms: *P. edulis edulis*, with purple fruits that are 4-5 cm in diameter and sweet-flavored pulp, growing at high altitudes of around 2000 meters above sea level; and *P. edulis flavicarpa*, with yellow fruits of 5-6 cm in diameter and sour pulp, which thrives in lowland areas (Zhao *et al.*, 2023).

The genus *Passiflora* contains many bioactive compounds, including alkaloids, phenols, flavonoids, and glycosides. Chemical analysis of *Passiflora edulis* fruits has shown that they are rich in carbohydrates, glycosides, flavonoids, resins, alkaloids, and phenolic compounds while passion fruit leaves are rich in tannins, flavonoids, terpenoids, steroids, and saponins (Oliveira *et al.*, 2022). Seed germination and seedling production of the *Passiflora* genus through traditional methods suffer from low germination rates, negatively affecting traditional vegetative propagation (Ozarowski, 2013). Several sources related to the micropropagation of different species of the genus *Passiflora* have indicated that the ability to form organs and both direct and indirect somatic embryos has been successfully achieved using various plant tissues and different types of media (Faria *et al.*, 2020; Khas *et al.*, 2020).

Organogenesis is a developmental or morphogenic process that occurs in the plant kingdom, involving the formation of vegetative growths such as roots and shoots from tissues of various plant parts. Organogenesis can occur in two forms: direct and indirect (Kothari *et al.*, 2010). New shoots can form directly from non-meristematic cells of the cultured plant part, which may emerge from the leaf petiole, a root piece, or the internodes of the stem. This ensures genetic stability for plants propagated in this manner, except for plants arising from chimeras, which are referred to as "chimeric plants." (Hieu *et al.*, 2019). To utilize the direct organogenesis ability, the culture medium is prepared with a suitable combination of growth regulators, nutrients, and hormones to control organ formation outside the living organism, depending on the plant part, its age, and growth conditions (Ramoat, 2004). Direct organogenesis is induced in *Passiflora* species mainly through the use of BA (Benzyl aminopurine) at concentrations ranging from 2.2 to 8.8 micromolar (Garcia *et al.*, 2011; Trevisan *et al.*, 2005; Vieira *et al.*, 2014; Mikosvki *et al.*, 2019).

Somatic embryogenesis is considered an important scientific and practical application for plant propagation *ex situ*, as it provides a broad avenue for vegetative multiplication of plants. Through the use of bioreactors, somatic embryos can be produced on a large scale, followed by encapsulation for the production of synthetic seeds, it is noted that somatic embryos derived from young or meristematic plant parts may either be single or proliferate into new embryos from a single embryo, forming a cluster of embryos, which are referred to as secondary somatic embryos. This is of significant importance in increasing the number of plants to be propagated, and both types of embryonic formation are considered forms of direct embryo formation (Direct Embryogenesis) (Al-Samadi, 2017). Therefore, the study aimed to establish tissue cultures of the passion fruit plant through organogenesis and the induction of somatic embryos.

MATERIALS AND METHODS

All tools used, including forceps, blade holders, glassware, Petri dishes, culture vessels, and distilled water, were sterilized in an autoclave at 121°C and 1.04 kg/cm² for 30 minutes (Salman, 1988). Additionally, ethyl alcohol at a concentration of 99% was used for sterilizing the forceps and blades and for burning them using a Glass Bead Sterilizer at 200°C after each planting operation inside the laminar air flow cabinet, which was sterilized, along with hands, before and after each planting operation with 70% ethyl alcohol. Culture vessels containing the medium were sterilized in the autoclave at 121°C and 1.4 kg/cm² for 15 minutes for all experiments, using the ready-made Murashige & Skoog (1962) (MS) medium, weighing 4.91 g L⁻¹ from Himedia India. Sucrose was added at a concentration of 30 g L⁻¹ at all stages, and plant growth regulators were added according to each experiment. The Potenz Hydrogen (PH) was adjusted to 1±5.7 for all experiments using sodium hydroxide (NaOH) and hydrochloric acid (HCl) 1N (Rahim *et al.*, 2021).

Effect of the Physical State of MS Medium and Dormancy Breaking Treatments on the Percentage of Seed Germination (%) of Passion Fruit Plant *in Vitro* after Two Months of Cultivation: Seeds were obtained from yellow passion fruit *Passiflora edulis flavicarpa* from local markets in the Kurdistan region. The seeds were extracted from the fruits, washed with liquid soap, and placed under running water for 5 minutes. They were then spread on filter paper and left to dry at room temperature for one day.

To overcome seed dormancy, several treatments were applied following the methods described by Amir *et al.* (2018) and William *et al.* (2022). Seeds were treated with concentrated sulfuric acid (H_2SO_4 , 96%) for 3 min. Fermentation was conducted using a 10% sucrose solution for 7 days. Hydrogen peroxide (H_2O_2) at a concentration of 15% was applied for 24 h, while gibberellic acid (GA_3) at 4000 ppm was used for 6 h. Mechanical scarification was performed using wood sandpaper (grade 100) to abrade the seed coat. In addition, seeds were soaked in 3% sodium hydroxide (NaOH) solution for 7 h or immersed in hot water at 40°C for 24 h. Seed tip excision was also carried out as a physical dormancy-breaking treatment. After the treatments, the seeds were placed under running tap water for 30 minutes and then transferred to a laminar flow cabinet for sterilization using a commercial bleach (Fass) at a concentration of 6% NaOCl, mixed at 30 ml per 100 ml of distilled water with a drop of liquid soap for 15 minutes. The seeds were then rinsed with sterile distilled water four times for 5 minutes each to remove the fungicide effects. Afterward, the seeds were planted on the MS medium in two forms: liquid form (without agar, using medical gauze) and semi-solid form (with the addition of agar at 7 g L^{-1}), with sucrose added at a concentration of 30 g L^{-1} . There were 10 replications with one seed per replication. The culture vessels were incubated in a growth chamber under 2000 lux light intensity with a light/dark cycle of 16/8 hours per day at $25^\circ\text{C} \pm 2$. The germination percentage was recorded after 2 months of planting (Al-Obaidi, 2021).

Effect of Benzyl adenine (BA) and 2,4-D Concentrations on the Type and Response Percentage of Plant Parts (Roots, Embryo + Cotyledons, Hypocotyl, Cotyledon Leaf) in Passion Fruit Plant in Organ Formation and Differentiation of Somatic Embryos and Callus Formation: The plant parts (roots, embryo + cotyledons, hypocotyl, and cotyledon leaf) were planted on full-strength MS medium supplemented with 2,4-D at two concentrations: $0,4 \text{ mg L}^{-1}$ and varying concentrations of benzyl adenine (0, 0.25, 0.50, 0.75, and 1 mg L^{-1}). Measurements were taken, including the type of response and the percentage of response, after 2 months of planting. Replanting was done with the same medium components, and microscopic examination was performed every 10 days to observe the stages of formation and differentiation (Jafari *et al.*, 2017; Silva *et al.*, 2021).

Effect of Benzyl adenine (BA), Gibberellin (GA_3), and Silver Nitrate (AgNO_3) Concentrations on the Development and Biomass Increase of (Green Shoots): Green shoots were planted on full-strength MS liquid medium, supplemented with benzyl adenine at concentrations of $0,2 \text{ mg L}^{-1}$ and gibberellin at $0,1 \text{ mg L}^{-1}$ (Mostafa *et al.*, 2020) varying concentrations of silver nitrate 0,1, 2, 4 mg L^{-1} . The number and length of green shoots, along with fresh and dry weight, were measured after 3 months of planting (Glaucia *et al.*, 2017).

RESULTS AND DISCUSSION

The results in Table 1 shows the effect of both the physical state of the medium and the type of dormancy-breaking treatment, as well as their interaction, on the germination percentage of seeds, the physical state of the medium had a significant effect on the average germination percentage. The liquid medium gave the highest germination rate, reaching 3.75%, whereas the solid medium treatment did not result in any germination, Regarding the effect of dormancy-breaking treatments on the germination percentage, the treatment involving cutting the seed tips gave the highest germination rate of 15%, while the other

treatments showed no response. As for the interaction between the physical state of the medium and the dormancy-breaking treatments, the combination of liquid medium with seed tip cutting yielded the highest germination percentage of 30%, whereas the other combinations showed no germination at all.

Table (1) Effect of the physical state of MS medium and dormancy-breaking treatments on seed germination percentage of passion fruit plant in vitro after two months of cultivation

Physical State of the Medium	Treatment Type								Mean Physical State of the Medium
	Sulfuric Acid	Fermentation	H ₂ O ₂	GA ₃	Scarification	Hot water	NaOH	Tip cutting	
Liquid Medium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	30.00	3.75
Solid Medium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LSD 0.05				10.68					3.74
Mean Treatment Type	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.0	
LSD 0.05				7.55					



Figure (1) Passion Fruit Seed Germination

The response of the seeds to germination when treated by cutting the seed tips in combination with the liquid medium may be attributed to the hardness of the seed coat, which is considered one of the main factors that keeps seeds of many plant species in a dormant state. This dormancy may occur through one or more of the following mechanisms: impermeability to water, impermeability to gases, or mechanical restriction of embryo growth and expansion (Salman, 1988). Many studies on passion fruit seeds have reported a decrease in germination percentage, irregular germination, and an extended period from the beginning to the end of germination. This leads to uneven seedlings, which

is one of the major problems facing the cultivation of various types of passion fruit (William *et al.*, 2022).

The results presented in Table 2 indicate that the type of plant tissue used significantly influences the induction of somatic embryos and organ development in the studied plant species. Among the different treatments, the use of cotyledon leaves treated with 1 mg L⁻¹ of BA and 0 mg L⁻¹ of 2,4-D yielded the highest rate of direct organogenesis (shoot formation), with green organ development observed in 80% of the explants. In contrast, other plant parts under their respective treatments exhibited markedly lower response rates, with a maximum of 10%. For somatic embryo induction, cotyledon leaves treated with 0.5 mg L⁻¹ of BA and 0 mg L⁻¹ of 2,4-D achieved the highest response rate of 40%, followed by hypocotyl segments treated with 0.5 mg L⁻¹ of BA and 4 mg L⁻¹ of 2,4-D, which showed a 20% response rate. Regarding callus formation, the hypocotyl explants treated with 1 mg L⁻¹ of BA and 4 mg L⁻¹ of 2,4-D exhibited the highest response rate at 50%, while root explants under the same treatment conditions showed the lowest rate, at just 10%.

From the previous results, it is evident that the plant organ plays an important role in *in vitro* cultivation of any plant species. The variation in the response of plant parts may depend on the interaction between endogenous and exogenous growth regulators. *In vitro* growth and morphogenesis are regulated by the interaction and balance between the growth regulators added to the medium and those produced internally by the cultured cells (Son *et al.*, 2011). (Figure 2).



Figure (2) The different stages of somatic embryo formation and development via the direct method in passion fruit (*Passiflora edulis*) *in vitro*:

A1 and A2: Globular stage , B: Heart-shaped stage , C: Torpedo stage , D1 and D2: Cotyledonary stage at different ages

Table (2) Effect of Benzyl adenine (BA) and 2,4-D Concentrations on the Type and Response Percentage of Plant Parts (Roots, Embryo + Cotyledons, Hypocotyl, Cotyledon Leaf) of Passion Fruit in Organ Formation, Somatic Embryo Development, and Callus Formation after 3 Months of Cultivation

Plant Parts	2,4-D	BA	Type of Response	Response Percentage
Roots	0	0.00	-	0
		0.25	-	0
		0.50	+++	10
		0.75	+++	10
		1.00	-	0
	4	0.00	-	0
		0.25	-	0
		0.50	-	0
		0.75	-	0
		1.00	++	10
Embryo + Cotyledons	0	0.00	+	20
		0.25	+++	30
		0.50	+++	40
		0.75	+++	30
		1.00	+++	20
	4	0.00	+++	10
		0.25	-	0
		0.50	-	0
		0.75	-	0
		1.00	+++	20
Hypocotyl	0	0.00	-	0
		0.25	+++	10
		0.50	++++	30
		0.75	+++	20
		1.00	+++	30
		0.00	-	0
		0.25	+++	10
		0.50	++++	20
		0.75	-	0
		1.00	++	50
Cotyledon Leaf	0	0.00	-	0
		0.25	+++	30
		0.50	++++	40
		0.75	+++	60
		1.00	+++	80
	4	0.00	-	0
		0.25	+++	20
		0.50	-	0
		0.75	-	0
		1.00	++	40

The results of Table 3 show significant differences between the concentrations of BA, GA3, and AgNO3, as well as their interactions, in terms of shoot development and

multiplication rate. Effect of AgNO₃: The treatment with 4 mg L⁻¹ of AgNO₃ resulted in the highest average number of shoots, reaching 3.45 shoots plant⁻¹, which was significantly superior to other treatments. The control treatment had the lowest average of 2.23 shoots plant⁻¹, Effect of GA₃: The concentration of 1 mg L⁻¹ GA₃ recorded the highest average number of shoots (3.64 shoots plant⁻¹) compared to the control, which had the lowest average of 1.84 shoots plant⁻¹, Effect of BA: A concentration of 2 mg L⁻¹ BA yielded the highest average number of shoots (4.94 shoots plant⁻¹), while the control had the lowest average (0.54 shoots plant⁻¹, Two-way Interaction: The combination of 2 mg L⁻¹ BA with 1 mg L⁻¹ GA₃ recorded the highest significant difference, achieving 6.45 shoots plant⁻¹, significantly differing from other treatments. The control recorded the lowest number, 0.25 shoots plant⁻¹, The interaction between 4 mg L⁻¹ AgNO₃ and 1 mg L⁻¹ GA₃ resulted in the highest number of shoots (3.85 shoots plant⁻¹), while the control recorded the lowest (1.05 shoots plant⁻¹), 1-The interaction of 4 mg L⁻¹ AgNO₃ with 2 mg L⁻¹ BA gave the highest shoot number (6.20 shoots plant⁻¹) compared to the control (0.030 shoots plant⁻¹), Three-way Interaction: The combination of 2 mg L⁻¹ BA 1 mg L⁻¹ GA₃, and 4 mg L⁻¹ AgNO₃ yielded the highest average of 6.70 shoots plant⁻¹, while the control had the lowest at 0.10 shoots plant⁻¹.

Table (3) Effect of different concentrations of Benzyl adenine (BA) mg L⁻¹, Gibberellin (GA₃) mg L⁻¹, and Silver Nitrate (AgNO₃) mg L⁻¹, and their interaction on the growth and development of organs (vegetative Shoots) per plant Shoot of passion fruit grown on MS medium after 3 months of cultivation

MS medium after 5 months of cultivation						
BA mg L ⁻¹	GA ₃ mg L ⁻¹	AgNO ₃ mg L ⁻¹				BA * GA ₃
		0	1	2	4	
0	0	0.10	0.20	0.30	0.40	0.25
	1	0.50	0.80	1.00	1.00	0.82
2	0	2.00	3.00	3.00	5.70	3.42
	1	6.30	6.30	6.50	6.70	6.45
LSD 0.05		1.165				0.582
BA * AgNO ₃						
BA		0	1	2	4	Mean BA
0		0.30	0.50	0.65	0.70	0.54
2		4.15	4.65	4.75	6.20	4.94
LSD 0.05		0.824				0.412
GA ₃ * AgNO ₃						
GA ₃		0	1	2	4	Mean GA ₃
0		1.05	1.60	1.65	3.05	1.84
1		3.40	3.55	3.75	3.85	3.64
LSD 0.05						0.412
AgNO ₃						
AgNO ₃		0	1	2	4	
Mean		2.23	2.58	2.70	3.45	
LSD 0.05		0.582				

The results shown in Table 4 indicate that the effect of AgNO₃ concentrations did not differ significantly from each other. The treatment with a concentration of 4 mg L⁻¹ gave the highest mean shoot length of 0.247 cm, while the control treatment gave the lowest mean shoot length of 0.205 cm. As for the GA₃ concentration, the treatment with 1 mg L⁻¹

1 was significantly superior, giving the highest mean shoot length of 0.301 cm, while the control treatment gave the lowest mean shoot length of 0.162 cm. As for the effect of BA concentration, the treatment with 2 mg L⁻¹ gave the highest mean shoot length of 0.314 cm, which was significantly superior to the control treatment, which had a shoot length of 0.151 cm. Regarding the effect of the binary interaction between GA₃ and BA, the treatment with 2 mg L⁻¹ of BA in combination with 1 mg L⁻¹ of GA₃ gave the highest mean shoot length of 3.337 cm, which was significantly superior to the control treatment, which had a mean shoot length of 0.037 cm. As for the interaction between AgNO₃ and GA₃, the treatment with 4 mg L⁻¹ of AgNO₃ combined with 1 mg L⁻¹ of GA₃ gave the highest mean shoot length of 0.320 cm, while the control treatment gave the lowest mean shoot length of 0.150 cm. The interaction between AgNO₃ and BA gave the highest mean shoot length of 0.320 cm when 4 mg L⁻¹ of AgNO₃ was combined with 2 mg L⁻¹ of BA, which did not differ significantly from the treatment with 2 mg L⁻¹ of AgNO₃ in combination with 2 mg L⁻¹ of BA, which also gave 0.320 cm. As for the triple interaction between AgNO₃, BA, and GA₃, the treatment with 4 mg L⁻¹ of AgNO₃ combined with 2 mg L⁻¹ of BA and 1 mg L⁻¹ of GA₃ gave the highest mean shoot length of 0.350 cm, which did not differ significantly from the treatment with 2 mg L⁻¹ of AgNO₃ combined with 2 mg L⁻¹ of BA and 1 mg L⁻¹ of GA₃, which also gave 0.350 cm. The control treatment, however, gave the lowest mean shoot length of 0.010 cm.

The results of the study show that Benzyl adenine (BA) and Gibberellic acid (GA₃) had a significant and complementary effect in promoting the elongation of vegetative shoots of passion fruit under tissue culture conditions. The interactions between the effective concentrations of these two regulators contributed to enhanced growth, indicating a synergistic interaction between their mechanisms of action: BA stimulates cell division, while GA₃ activates cell elongation processes, which positively reflects on the overall vegetative growth. The interactions also demonstrated that the effectiveness of growth regulators depends on the surrounding physiological environment, particularly concerning the accumulation of ethylene, which is considered one of the common obstacles in *in vitro* culture systems. The results highlighted the role of silver nitrate (AgNO₃) as an ethylene inhibitor, as its presence was shown to enhance the plant's response to growth regulators, especially BA, confirming that the efficiency of growth regulators is linked to reducing the negative effects of ethylene within the closed culture medium. Regarding the interactions between GA₃ and AgNO₃, the results indicate that the positive effect of gibberellic acid in promoting shoot elongation was also enhanced by the presence of silver nitrate, confirming the importance of managing ethylene levels as a fundamental condition for stimulating the maximum physiological response to gibberellins. On the other hand, the upward trend in average vegetative growth with increasing concentrations of AgNO₃ confirms that this compound, although not a growth regulator, plays a significant physiological role in improving the internal growth environment, thereby enhancing the full hormonal response of the vegetative shoots. Accordingly, the results highlight the importance of adopting integrated combinations that bring together key growth regulators and supporting factors such as ethylene inhibitors, in order to achieve optimal growth responses in micropropagation systems. The integration of BA and GA₃ at carefully studied concentrations, along with physiological modulation through AgNO₃, represents a promising approach to enhancing the efficiency of vegetative shoot production of passion fruit *in vitro*.

The results presented in Tables 5 and 6 indicate the effect of BA, GA₃, AgNO₃, and their interactions on the average wet and dry weights. AgNO₃: The treatment with 4 mg L⁻¹ of AgNO₃ significantly outperformed the control, achieving a wet weight of 0.165 g and a dry weight of 0.072 g. In comparison, the control recorded 0.123 g (wet) and 0.035 g (dry). The application of 1 mg L⁻¹ of GA₃ resulted in the highest wet and dry weights (0.179 g and 0.077 g, respectively), significantly higher than the control (0.116 g and 0.050 g), A concentration of 2 mg L⁻¹ of BA led to the highest values for wet and dry weights, at 0.210 g and 0.089 g, respectively. This was significantly greater than the control treatment, which had the lowest values of 0.085 g (wet) and 0.039 g (dry). The combination of 1 mg L⁻¹ GA₃ and 2 - BA produced the highest wet and dry weights (0.228 g and 0.095 g, respectively), whereas the control showed the lowest values (0.039 g and 0.018 g), The combination of 4 mg L⁻¹ AgNO₃ and 2 mg L⁻¹ BA resulted in the highest weights (0.225 g wet, 0.093 g dry), with the control again having the lowest (0.059 g wet, 0.021 g dry), The pairing of 4 mg L⁻¹ AgNO₃ with 1 mg L⁻¹ GA₃ produced the highest weights (0.193 g wet, 0.088 g dry), compared to the control (0.083 g wet, 0.045 g dry). The highest wet and dry weights were observed with the combination of 2 mg L⁻¹ BA, 1 mg L⁻¹ GA₃, and 4 mg L⁻¹ AgNO₃ (0.235 g and 0.101 g, respectively). These values did not differ significantly from the treatment combining 2 mg L⁻¹ BA, 1 mg L⁻¹ GA₃, and 2 mg L⁻¹ AgNO₃, which yielded the same results. In contrast, the control treatment had the lowest values, with a wet weight of 0.0135 g and a dry weight of 0.010 g.

Table (4) Effect of Different Concentrations of Benzyl Adenine, Gibberellin, and Silver Nitrate and Their Interactions on the Development and Length Increase of the Green Shoot Organs (Green Shoots) of Passion Fruit Plants Grown on MS Medium After 3 Months of Cultivation.

BA	GA ₃	AgNO ₃				BA * GA ₃
		0	1	2	4	
0	0	0.010	0.040	0.040	0.060	0.037
	1	0.210	0.280	0.280	0.290	0.265
2	0	0.290	0.290	0.290	0.290	0.290
	1	0.310	0.340	0.350	0.350	0.337
LSD 0.05			0.1340			0.067
BA * AgNO ₃						
BA		0	1	2	4	Mean BA
0		0.110	0.160	0.160	0.175	0.151
2		0.300	0.315	0.320	0.320	0.314
LSD 0.05			0.0948			0.0474
GA ₃ * AgNO ₃						
GA ₃		0	1	2	4	Mean GA ₃
0		0.150	0.165	0.165	0.175	0.164
1		0.260	0.310	0.315	0.320	0.301
LSD 0.05			0.0948			0.0474
AgNO ₃						
AgNO ₃		0	1	2	4	
Mean		0.205	0.238	0.240	0.247	
AgNO ₃						
LSD 0.05			0.0670			

Table (5) Effect of Different Concentrations of Benzyl Adenine mg L⁻¹, Gibberellin mg L⁻¹, and Silver Nitrate mg L⁻¹ and Their Interactions on the Development and Increase in Wet Weight g of the Organs (Green Shoots) of Passion Fruit Plants Grown on MS Medium After 3 Months of Cultivation.

BA mg L ⁻¹	GA ₃ mg L ⁻¹	AgNO ₃ mg L ⁻¹				BA * GA ₃
		0	1	2	4	
0	0	0.015	0.030	0.053	0.059	0.039
	1	0.102	0.114	0.152	0.152	0.130
2	0	0.152	0.200	0.202	0.216	0.192
	1	0.221	0.222	0.235	0.235	0.228
	LSD 0.05		0.061			0.031
		BA * AgNO ₃				
BA		0	1	2	4	Mean BA
0		0.059	0.072	0.102	0.106	0.085
2		0.187	0.211	0.218	0.225	0.210
LSD 0.05			0.043			0.022
		GA ₃ * AgNO ₃				
GA ₃		0	1	2	4	Mean GA ₃
0		0.083	0.115	0.127	0.137	0.116
1		0.162	0.168	0.193	0.193	0.179
LSD 0.05			0.043			0.022
		AgNO ₃				
AgNO ₃		0	1	2	4	
Mean		0.123	0.142	0.160	0.165	
AgNO ₃						
LSD 0.05			0.031			

The tables (3, 4, 5, and 6) indicate that the addition of cytokinins stimulates cell division in plant tissue cultures by increasing the rate of DNA translation, which leads to an increased rate of protein synthesis. Growth stimulation is attributed to the significant increase in the amino acid t-RNA, followed by enhanced protein synthesis activity. On the other hand, it also leads to some changes in the synthesis of other hormones such as auxin, gibberellin, and ethylene. The increase in the number of branches may also be attributed to the effect of cytokinin on apical dominance, as apical dominance results from the antagonism between cytokinin and auxin, where cytokinin inhibits the effect of auxin that contributes to apical dominance, or activates axillary buds through its influence on the development of primary xylem tissues connecting the axillary buds with the main stem. Thus, it can be concluded that apical dominance can be controlled through the balance between the internal concentrations of both cytokinin and auxin (Al-Asadi and Al-Khaikani, 2019).

Table (6) Effect of Different Concentrations of Benzyl Adenine mg L⁻¹, Gibberellin mg L⁻¹, and Silver Nitrate mg L⁻¹ and Their Interactions on the Development and Increase in Dry Weight g of the Organs (Green Shoots) of Passion Fruit Plants Grown on MS Medium After 3 Months of Cultivation.

BA mg L ⁻¹	GA ₃ mg L ⁻¹	AgNO ₃ mg L ⁻¹				BA * GA ₃
		0	1	2	4	
0	0	0.010	0.016	0.021	0.024	0.018
	1	0.032	0.057	0.075	0.075	0.060
2	0	0.080	0.082	0.082	0.086	0.083
	1	0.091	0.091	0.096	0.101	0.095
LSD 0.05			N.S			0.015
		BA * AgNO ₃				
	BA					
		0	1	2	4	Mean BA
	0	0.021	0.036	0.048	0.050	0.039
	2	0.086	0.087	0.089	0.093	0.089
LSD 0.05			0.021			0.010
		GA ₃ * AgNO ₃				
	GA ₃					Mean GA ₃
	0	0	1	2	4	
	0	0.045	0.049	0.052	0.055	0.050
	1	0.061	0.074	0.085	0.088	0.077
LSD 0.05					0.021	0.010
		AgNO ₃				
	AgNO ₃	0	1	2	4	
	Mean	0.053	0.061	0.069	0.072	
	AgNO ₃					
LSD 0.05					0.015	

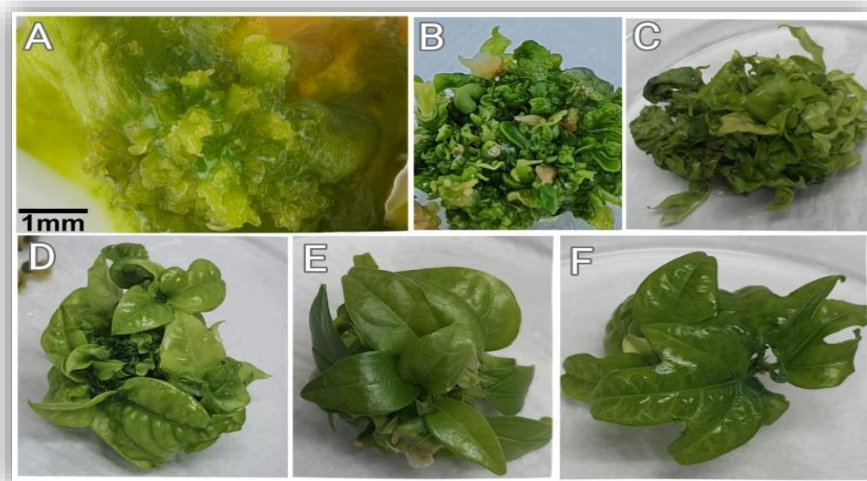


Figure (3) Different stages of growth and development of organs (vegetative branches) of passion fruit plant grown on MS medium in vitro: A – after 30 days of cultivation B – after 50 days of cultivation C – after 60 days of cultivation D – after 70 days of cultivation E – after 80 days of cultivation F – after 90 days of cultivation

CONCLUSION

The current study showed that results revealed that the germination rate of 30% was achieved after 2 months of cultivation following the excision of the seed tip and planting it on liquid MS, The possibility of obtaining the best result for direct organogenesis through the *in vitro* culture of cotyledonary leaves of passion fruit seedlings grown on MS medium containing $1 \text{ mg} \cdot \text{L}^{-1}$ of BA (benzyladenine) The possibility of achieving the best result for direct somatic embryogenesis induction through the tissue culture of cotyledonary leaves of passion fruit seedlings on MS medium containing $0.5 \text{ mg} \cdot \text{L}^{-1}$ of BA

CONFLICT OF INTEREST

The authors declare no conflict of interest associated with this manuscript.

REFERENCES

- Al-Asadi, Maher Hameed Salman, and Ali Hussein Jassim. (2019). Plant Hormones and Their Physiological Effects. Deposit number at the National Library and Archives in Baghdad (2272 for the year 2019) / Al-Qasim Green University – College of Agriculture, Iraq.
- Al-Obaidi, M. H. A. (2021). Response of *Physalis peruviana* to nanoparticles and their effect on secondary metabolites and genetic variations *in vitro*. PhD Dissertation, College of Agricultural Engineering Sciences, University of Baghdad, Iraq.
- Al-Sahouki, M., & Wahib, K. A. (1990). *Applications in the design and analysis of experiments*. Ministry of Higher Education and Scientific Research, Iraq.
- Al-Sumaidie, K. M. I. (2017). *Applications in plant biotechnology*. Part One. Al-Nahrain University, Baghdad, Iraq.
- Amir, R., & Eric, T. S. (2018). Comparison of seed treatments on the germination of seven passion fruit species. *International Journal of Current Microbiology and Applied Sciences*, 7(11), 3074–3083. DOI:[10.20546/ijcmas.2018.711.353](https://doi.org/10.20546/ijcmas.2018.711.353)
- Chuang, Y., Xu, Y., Gu, Y., & Feng, T. (2024). Comprehensive analysis of aroma compounds in passion fruit and their application in juice beverages. DOI:[10.1007/978-3-031-38663-3_100](https://doi.org/10.1007/978-3-031-38663-3_100)
- Faria, G. A., Oliveira, C. P. M. De, Lopes, B. G., Rocha, P. S., Peron, G. M., Souza, K. S., Garcia, C. K., Furlani Junior, E., Cavichioli, J. C., & Felizardo, L. M. (2020). Establishment of a protocol for in vitro propagation of *Passiflora caerulea*. *Research, Society and Development*, 9(7), e122973629. DOI: <https://doi.org/10.33448/rsd-v9i9.7158>
- Garcia, R., Pacheco, G., Falcão, E., Borges, G., & Mansur, 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(1), 47–54.
- Gláucia Amorim ; Lucas Menezes ; Antonio Flávio Arruda ; Paula Suares ; Aline Namie ; Antônio da Silva ; Tatiana Góes ; Maria Angélica ; Ana Patricia ; Augusto Ramalho ; Beatriz Garcia ; Tiago Almeida .(2017). Concentrations of Silver Nitrate in the In Vitro Development and Conservation of *Passiflora gibertii* N. E. Brown, *American Journal of Plant Sciences*, , 8, 2944-2955. <https://doi.org/10.4236/ajps.2017.812199>

- Hieu, T., Tung, H. T., Nguyen, C. D., & Nhut, D. T. (2019). Efficiency of shoot regeneration and micropropagation of purple passion fruit (*Passiflora edulis Sims.*) via internodal longitudinal thin cell layer culture. *Vietnam Journal of Biotechnology*, 17(4), 699–708. DOI:[10.15625/1811-4989/17/4/14021](https://doi.org/10.15625/1811-4989/17/4/14021)
- Ibrahim, E. J., Aldabbagh, A. R., & Kayat, F. (2024). Establishment of an efficient micropropagation protocol for three kenaf (*Hibiscus cannabinus L.*) cultivars. *Iraqi Journal of Agricultural Sciences*, 55(3), 1064–1074. <https://doi.org/10.36103/dhxnja59>
- Khas, M. E., Abbasifar, A., & Valizadehkaji, B. (2020). Optimization of in vitro propagation of purple passion fruit (*Passiflora edulis*), an important medicinal and ornamental plant. *International Journal of Horticultural Science and Technology*, 7(3), 305–314. <https://doi.org/10.22059/ijhst.2020.297194.342>
- Kothari, S. L., Joshi, A., Kachhwaha, S., & Ochoa-Alejo, N. (2010). Chilli peppers — A review on tissue culture and transgenesis. *Biotechnology Advances*, 28(1), 35–48. DOI: [10.1016/j.biotechadv.2009.08.005](https://doi.org/10.1016/j.biotechadv.2009.08.005)
- Jafari, M., D., M. H., & Lotfi, A. (2017). In vitro shoot proliferation of *passiflora caerulea*. Via cotyledonary node and shoot tip explants. *Biotechnologia*, 98(2), 113–119. DOI:[10.5114/bta.2017.68310](https://doi.org/10.5114/bta.2017.68310)
- Miksovski, A. I., Silva, N. T., Souza, C. S., Machado, M. D., Otoni, W. C., Carvalho, I. F., Rocha, D. I., & Silva, M. L. (2019). Tissue culture and biotechnological techniques applied to passion fruit with ornamental potential: An overview. *Ornamental Horticulture*, 25(2), 189–199. DOI:[10.14295/oh.v25i2.2036](https://doi.org/10.14295/oh.v25i2.2036)
- Mostafa E. K., Ahmadreza A. and Babak V.(2020). Optimization of in vitro Propagation of Purple Passion Fruit (*Passiflora edulis*), an Important Medicinal and Ornamental Plant. *International Journal of Horticultural Science and Technology*, Vol. 7, No. 3; September, pp 305-314 Print ISSN: 2322-1461 . <https://doi.org/10.22059/ijhst.2020.297194.342>
- Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant.*, 15, 473–497. DOI: [10.1111/j.1399-3054.1962.tb08052.x](https://doi.org/10.1111/j.1399-3054.1962.tb08052.x)
- Oliveira, C. C. R., Feitosa, B. R., Souza, V. B., Tulini, F. L., & Martins, M. V. (2022). Physicochemical sensory analyses of sequilhos produced with non-conventional food plants: arrowroot, licuri and wild passion fruit shell. *Food Sci. Technol.*, 42, 721. DOI:[10.1590/fst.42721](https://doi.org/10.1590/fst.42721)
- Osama, S. (2022). Micropropagation of grapevine (*Vitis vinifera L.*) cvs. Red Globe and Superior. *Iraqi Journal of Agricultural Sciences*, 53(4), 833–849. <https://doi.org/10.36103/ijas.v53i4.1596>
- Ożarowski, M., & Thiem, B. (2013). Development and optimization of a low-cost system for micropropagation of valuable medicinal plants of *Passiflora* species. Conference poster.
- Rahim, Z. H. A., & Jawad, L. K. (2021). The role of growth regulators in the multiplication of *Stevia rebaudiana* Bertoni shoot and callus induction in vitro. *Diyala Agricultural Sciences Journal*, 13(2), 24–31. DOI:[10.52951/dasj.21130204](https://doi.org/10.52951/dasj.21130204)
- Ramawat, K. G. (2004). *Plant Biotechnology*. Printed in India, pp. 1–265.

- Salman, M. A. (1988). *Propagation of Horticultural Plants*. Dar Al-Kutub for Printing and Publishing, University of Baghdad, Ministry of Higher Education and Scientific Research, Iraq.
- Silva, L. N., Lima, L. K. S., dos Santos, I. S., Sampaio, S. R., & de Jesus, O. N. (2021). Multivariate strategies for selection of organic growing media to produce yellow passion fruit seedlings. *Organic Agriculture*, 12, 445–459. DOI:[10.1007/s13165-022-00401-6](https://doi.org/10.1007/s13165-022-00401-6)
- Van Son, N. G. U. Y. E. N. (2007). Response of gerbera (*Gerbera jamesonii* Bolus) varieties to micro-propagation. Master of Science Thesis in Horticulture (Agriculture). Univ. of Agri. Sci., Dharwad, India.
- Yu, C., Xu, Y., Gu, Y., Feng, T. (2024). Comprehensive analysis of aroma compounds in passion fruit and their application in juice beverages. In: mérillon, jm., riviere, c., lefèvre, g. (eds) natural products in beverages. *Reference series in phytochemistry. Springer, cham*. DOI:[10.1007/978-3-031-38663-3_100](https://doi.org/10.1007/978-3-031-38663-3_100)
- Trevisan, F., & Mendes, B. M. J. (2005). Optimization of in vitro organogenesis in passion fruit (*Passiflora edulis* f. *flavicarpa*). *Scientia Agricola*, 62(4), 346–350. DOI:[10.1590/S0103-90162005000400007](https://doi.org/10.1590/S0103-90162005000400007)
- 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 and Developmental Biology – Plant*, 50(6), 738–745. <https://doi.org/10.1007/s11627-014-9650-0>
- Weyya, G., Belay, A., & Tadesse, E. (2024). Passion fruit (*Passiflora edulis* Sims) by-products as a source of bioactive compounds for non-communicable disease prevention: extraction methods and mechanisms of action: a systematic review. (*Journal name missing—please confirm*). doi.org/10.3389/fnut.2024.1340511
- William V., Takashi S. , Atsushi S. , Naoki T. , Lenin R. and Kaihei K. (2022). Seed germination and seedling growth of yellow and purple passion fruit genotypes cultivated in Ecuador. *Horticulturae*, 8, 754, pp:1-16. <https://doi.org/10.3390/horticulturae8080754>
- Zeng, Y., Zhou, W., Yu, J., Zhao, L., Wang, K., and Hu, Z. (2023). Products of fruit and vegetables: antioxidant properties of extractable and non-extractable phenolic compounds. *Frontiers in Plant Science*, 12, 418. <https://doi.org/10.3390/antiox12020418>
- Zhao, L., Wu, L., Li, L., Zhu, J., Chen, X., Zhang, S., et al. (2023). Physicochemical, structural, and rheological characteristics of pectic polysaccharides from fresh passion fruit (*Passiflora edulis* f. *flavicarpa* L.) peel. *Food Hydrocolloids*, 136, 108301. <https://doi.org/10.1016/j.foodhyd.2022.108301>