



EFFECT OF PLANT PART TYPE AND GROWTH REGULATOR ON THE INITIATION AND MULTIPLICATION OF VEGETATIVE SHOOTS OF YELLOW PASSION FRUIT (*PASSIFLORA EDULIS SIMS. F. FLAVICARPA*) IN VITRO

Zainab Mahdi Abdul – Hussein^{1*}, Lamia Khalifa Jawad²

¹Dept.of Horticulture and Landscape Gardening, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq, Zainab.abd2105p@coagri.uobaghdad.edu.iq

²Professor PhD. Dept.of Horticulture and Landscape Gardening, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq, Lamiaa.khlifa@coagri.uobaghdad.edu.iq

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ABSTRACT

Experiments were conducted at the Plant Tissue Culture Laboratory - Postgraduate Studies Section of the Department of Horticulture and Landscape Engineering, College of Agricultural Engineering Sciences, University of Baghdad, during 2022–2024 using a Completely Randomized Design (CRD) Each treatment had 10 replicas in each experiment. The aim was to study the effect of plant part type and growth regulators on the initiation and multiplication of vegetative shoots of in vitro cultures of yellow passion fruit (*Passiflora edulis Sims. f. flavicarpa*), Initiation Stage involving two experiments: The first experiment examined two factors: plant parts and BA. The single node treatment combined with 4 mg L⁻¹ BA resulted in the highest number of shoots and the highest response percentage of plant parts, The second experiment examined two factors: plant parts and a combination of Kin + BA. The apical bud combined with 2 mg L⁻¹ Kin + 2 mg L⁻¹ BA showed significant superiority, providing the highest shoot number and plant part response percentage, For the multiplication stage, two experiments were conducted: The first experiment involved a single factor (BA), where the treatment of 2 mg L⁻¹ BA gave the highest number of shoots and shoot lengths, The second experiment involved a single factor (mT), where the treatment of 1 mg L⁻¹ mT achieved the highest shoot number and lengths.

Keywords: passion fruit, plant part, metatopolin (mT), benzyl adenine (BA), kinetin (Kin).

تأثير نوع الجزء النباتي ومنظم النمو في نشوء وتضاعف الافرع الخضرية لنبات الباشن فروت الاصفر *Passiflora edulis Sims. f. flavicarpa* خارج الجسم الحي

زينب مهدي عبد الحسين¹, لمياء خليفة جواد²

¹قسم البستنة وهندسة الحدائق، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق، Zainab.abd2105p@coagri.uobaghdad.edu.iq
²الأستاذ الدكتور، قسم البستنة وهندسة الحدائق، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق، Lamiaa.khlifa@coagri.uobaghdad.edu.iq

الخلاصة

نفذت التجارب في مختبر زراعة الانسجة النباتية- الدراسات العليا التابع الى قسم البستنة وهندسة الحدائق كلية علوم الهندسة الزراعية / جامعة بغداد من 2022 - 2024 وفق التصميم العشوائي الكامل (CRD) وبواقع عشر مكررات لكل معاملة بهدف دراسة تأثير نوع الجزء النباتي ومنظم النمو في نشوء وتضاعف الافرع الخضرية للمزارع النسيجية لنبات الباشن فروت الاصفر *Passiflora edulis Sims.f.flavicarpa* خارج الجسم الحي ، مرحلة النشوء شملت

*This article is taken from the doctoral dissertation of the first researcher.



تجربتين الأولى تضمنت عاملين (الأجزاء النباتية و البنزل ادنين BA) اذ اعطت معاملة العقدة المفردة بالتداخل مع معاملة التركيز 4 ملغم لتر⁻¹ اعلى معدل لأعداد الأفرع والنسبة المئوية لاستجابة الأجزاء النباتية، اما التجربة الثانية تضمنت عاملين (الأجزاء النباتية و BA + Kin) اذ تفوقت معنوياً معاملة القمة النامية بالتداخل مع معاملة التركيز 2+2 من Kin + BA بأعطائها اعلى معدل لعدد الأفرع والنسبة المئوية لاستجابة الأجزاء النباتية، اما مرحلة التضاعف فقد شملت تجربتين التجربة الأولى تضمنت عامل واحد (BA) حيث اعطت معاملة التركيز 2 ملغم لتر⁻¹ من BA اعلى معدل لعدد الأفرع واطوالها، اما التجربة الثانية تضمنت عامل واحد (mT) حيث اعطت معاملة التركيز 1 ملغم لتر⁻¹ اعلى معدل لعدد الأفرع واطوالها.

الكلمات المفتاحية: الباشن فروت، الجزء النباتي، ميتاتوبولين (mT)، بنزل ادنين (BA)، كاينتين (kin).

INTRODUCTION

Yellow passion fruit belongs to the genus *Passiflora* in the family *Passifloraceae*. Its original habitat is tropical and subtropical regions of the world, encompassing 20 genera and approximately 500 species widely distributed. Among them, 400–500 species are found in the Americas, with 50–60 species bearing edible fruits. Additionally, around 20 species of this genus are cultivated as ornamental plants due to their beautiful flowers (Heywood, 1998).

The origin of *Passiflora edulis* is Brazil, and it spread to tropical and subtropical regions worldwide during the 19th century (Duarte *et al.*, 2021).

Passion fruit is a perennial woody climbing plant with tendrils, reaching up to 15 meters in height. The stems and tendrils are green, emerging in the axils of leaves, cylindrical in shape, and extending beyond the length of the leaf. There are two forms: *P. edulis edulis* with purple fruit and *P. edulis flavicarpa* with yellow fruit (Purseglove, 1968). The genus *Passiflora* contains many bioactive compounds, including alkaloids, phenolics, flavonoids, and glycosides. The fruits of *Passiflora edulis* are rich in carbohydrates, glycosides, flavonoids, resins, alkaloids, and phenolic compounds while the leaves are rich in tannins, flavonoids, terpenoids, steroids, and saponins (Freitas *et al.*, 2020; Santos *et al.*, 2022).

The growing interest in using passion fruit as a table fruit or juice due to its pleasant flavor and health benefits has increased in recent years, Passion fruit is also known as the "king of fruits," "maracuja," or "fruit of passion." Various products have been developed from passion fruit, including cakes, ice cream, jams, yogurt, beverages, tea, vinegar, sauces, and soups. It is also used as traditional herbal medicine and a cosmetic moisturizing agent in many countries (Baseggio *et al.*, 2022).

Many pharmacological studies conducted in vitro have revealed numerous bioactivities of the *Passiflora* genus, such as antioxidant, antimicrobial, anti-inflammatory, antihypertensive, hepatoprotective, antidiabetic, and anxiolytic effects (Silva *et al.*, 2015). These align with traditional uses of *Passiflora* in herbal and folk medicine. The pharmacological effects are believed to result from bioactive components such as polyphenols, triterpenes, and polysaccharides (Khuwijitiaru *et al.*, 2020; Ling *et al.*, 2022).

Tissue culture technology has many applications in vegetative propagation, particularly in clonal micropropagation with the ability to retain genetic traits identical to the mother plant. Many studies have investigated micropropagation techniques for various *Passiflora* species, using different explants and media (Vieira *et al.*, 2014). Micropropagation of *Passiflora* species represents a significant technological advancement, especially for passion fruit as an



ornamental plant, fruit crop, or medicinal source, preserving genetic traits such as flower colors, numbers, and sizes, as well as secondary metabolite production (AL-Shugeairy *et al.*, 2021; Ibrahim *et al.*, 2024).

One of the major challenges in the initiation stage of passion fruit tissue culture is the difficulty in seed germination, even when following established protocols (Martinez *et al.*, 2020). The primary cause of seed dormancy in passion fruit is the seed coat. (Hall *et al.*, 2000) suggested that the only successful method for breaking dormancy is completely removing the seed coat. While scarification may succeed, its use is challenging due to the small and robust nature of passion fruit seeds.

Benzyl adenine (BA) is a commonly used cytokinin in tissue culture to stimulate adventitious shoot formation and growth of axillary buds. However, BA should be used in low concentrations to allow proper elongation and growth of buds (Ozarowski *et al.*, 2013). High BA concentrations can cause abnormalities like vitrification, necrosis of shoot tips, poor acclimatization, and rooting difficulties, leading to tissue instability in various species (Rahim *et al.*, 2021; AL-Jubori *et al.*, 2022; Nawal *et al.*, 2024).

Meta-topolin (mT) or [6-(3-hydroxybenzylamino)purine] is a naturally aromatic cytokinin derived from poplar leaves (Strnad *et al.*, 1997). It resembles BA in structure, except for the hydroxyl group on the benzene ring. Studies have highlighted the potential of mT as an effective substitute for BA, addressing issues like vitrification, necrosis, poor acclimatization, and rooting difficulties while achieving higher multiplication rates for vegetative tissues cultured in vitro (Naaz *et al.*, 2019; Ying *et al.*, 2020; Kucharka *et al.*, 2020; AL-Amery *et al.*, 2021; Abed *et al.*, 2021; Manokari *et al.*, 2021; Nawsupe *et al.*, 2021; Saurabh *et al.*, 2024; Facundo *et al.*, 2024). The research aims to determine the optimal concentration of cytokinins for its effect on the multiplication of vegetative shoots of tissue-cultured passion fruit (*Passiflora edulis*) for micropropagation..

MATERIALS AND METHODS

The seeds were extracted from the yellow passion fruit, washed with liquid soap (Zahi), 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. Afterward, they were placed under running tap water for 30 minutes.

The seeds were then transferred to a laminar airflow cabinet (hood) and sterilized using commercial bleach solution containing sodium hypochlorite (NaOCl) at a concentration of 6%. Different concentrations of the bleach solution (0, 10, 20, and 30 mL per 100 mL of distilled water) were used, with a drop of liquid soap added. The sterilization process lasted for 15 minutes.

After sterilization, the seeds were rinsed four times with sterile distilled water, each rinse lasting 5 minutes, to remove the effects of the bleach. The seeds were then cultured on a semi-solid and liquid MS medium (using medical gauze), with 10 replications, one seed per replicate. The culture containers were incubated in a growth chamber under a light intensity of 2000 lux for 16 hours of light and 8 hours of darkness per day at a temperature of 25°C ±2. The percentage of contamination was recorded after 10 days of cultivation.



Effect of MS Strength, BA, and GA₃ Concentrations on Germination Percentage

Previously sterilized seeds were planted on liquid MS medium at different strengths (full strength and half strength) with benzyl adenine (BA) at concentrations of 0 and 1 mg/L and gibberellic acid (GA₃) at concentrations of 0 and 1 mg/L. The interaction of these factors was evaluated, and the germination percentage was calculated after two months using the following formula:

Effect of Different BA Concentrations on Vegetative Tissue Response

Explants of single nodes, shoot tips, or nodes with two cotyledon leaves (1–1.5 cm long) were cultured on MS medium supplemented with different concentrations of BA (0, 1, 2, and 4 mg/L).

Effect of BA and Kinetin (KIN) on Vegetative Tissue Response

Explants (single nodes, shoot tips, or nodes with two cotyledon leaves, 1–1.5 cm in length) were cultured on MS medium containing various combinations of BA and kinetin (KIN) as follows:

- 0.5 mg/L BA + 0.5 mg/L KIN
- 1 mg/L BA + 1 mg/L KIN
- 2 mg/L BA + 2 mg/L KIN

Effect of BA and Meta-topolin (mT) on Biomass Accumulation of Shoots

Based on the results of the initiation stage, the appropriate explant (1.5 cm in length) was selected and cultured on half-strength liquid MS medium supplemented with different concentrations of BA and meta-topolin (mT) (0, 0.5, 1, and 2 mg/L), each tested separately. Each treatment consisted of 10 replicates, with one shoot per replicate. Cultures were incubated in a growth chamber under the previously described conditions, and data on the number and length of shoots were recorded after two months.

Incubation Conditions:

The cultures were incubated in a growth room under a light intensity of 1000 lux for 16 hours of light and 8 hours of darkness, at a temperature of 23°C ± 2.

Statistical analysis:

The experiments were designed using Completely Randomized Design *Al-Sahouki et al (1990)* Each treatment had 10 replicas in each experiment and Significant differences between means were recorded using least significant differences (L.S.D) at 0.05 level.

RESULTS AND DISCUSSIONS

RESULTS:

Effect of Commercial Bleach Concentrations and the Physical State of MS Medium on the Percentage of Contamination in Passion Fruit Seeds Cultivated In Vitro for 15 Minutes

The results in Table (1) indicate that the type of medium had no significant effect on reducing contamination rates. The liquid medium resulted in a contamination rate of 45%, which was not significantly different from the solid medium, which showed a contamination rate of 55%, However, sodium hypochlorite concentrations had a significant effect in reducing



seed contamination. The results suggest that sodium hypochlorite is effective in minimizing seed contamination. At a concentration of 10 mL per 100 mL, the contamination rate was 70%, which was significantly different from the control treatment (0 mL), which had a contamination rate of 100%. Meanwhile, other treatments showed a further decrease in contamination rates, which were closely associated with increasing disinfectant concentrations. The contamination rate dropped to 30% and 0% at concentrations of 20 mL and 30 mL per 100 mL, respectively. Regarding the interaction between sodium hypochlorite concentration and the type of culture medium, the table shows that the highest contamination rate (100%) was recorded when both solid and liquid media were treated with 0 mL of sodium hypochlorite. In contrast, no contamination was observed when solid or liquid media were treated with 30 mL of sodium hypochlorite.

Table (1): Effect of Commercial Bleach Concentrations (mL per 100 mL) and the Physical State of MS Medium on the Percentage (%) of Contamination in Passion Fruit Seeds Cultivated In Vitro for 15 Minutes.

Medium Type	Concentration (mL per 100 mL)				Medium Type
	0	10	20	30	Average
Liquid Medium	100	60	20	0	45
Solid Medium	100	80	40	0	55
LSD 0.05	29.7				N.S.
Concentration Average	100	70	30	0	
LSD 0.05	21.0				

Effect of MS Medium Strength, BA, and GA₃ Concentrations on Seed Germination Percentage

Table (2): illustrates the effect of MS medium strength, BA, and GA₃ concentrations, as well as their interactions, on the percentage of seed germination after two months. GA₃ had a significant impact on improving the germination percentage, reaching 80%, compared to the control, which showed only 32.5%. In contrast, BA concentration did not have a significant effect. For BA at 0 mg/L, the average germination rate was 60%, while at 1 mg/L, it decreased to 52.5%.

Table (2): Effect of MS Medium Strength, BA(mg/L), and GA₃ (mg/L) Concentrations on Germination Percentage(%) After Two Months.

Interaction Percentage(%) After Two Months:				
MS * BA	GA ₃		BA	MS
	1	0		
65	100	30	0	1/2 MS
55	70	40	1	
55	90	20	0	MS
50	60	40	1	
N.S	39.17		LSD	
MS * GA ₃				

MS Mean	1	0	MS
60	85	35	1/2 MS
52.5	75	30	MS
N.S	27.70		LSD
BA * GA ₃			
Mean BA	1	0	BA
60	95	25	0
52.5	65	40	1
N.S	27.70		LSD
GA ₃			
	1	0	GA ₃
	80	32.5	GA ₃ Mean
	19.6		LSD

Many plant seeds fail to germinate even under optimal conditions due to dormancy, which is often attributed to elevated levels of abscisic acid (ABA) during this stage. Both ABA and gibberellic acid (GA₃) regulate seed dormancy. When GA₃ levels exceed ABA, germination is promoted; the reverse inhibits germination (Al-Khafaji, 2014), GA₃ plays a critical role in seed germination by increasing hydrolytic enzymes that break down food reserves in the seed endosperm. Additionally, it promotes faster division and elongation of the hypocotyl, enabling the seedling to emerge above the soil. The role of GA₃ extends to enhancing cell size, shoot elongation, and overall vegetative growth (Al-Asadi & Al-Khaikani, 2019). These findings align with those of (Figure 1).



Figure (1): Germination of Yellow Passion Fruit Seeds.



Experiment: Initiation Stage

Effect of Different BA Concentrations (mg/L) on the Percentage of Response (%) of Plant Parts (Single Node, Shoot Tip, Node + Two Cotyledons) of Passion Fruit Plant After Eight Weeks of Cultivation in MS Medium:

Table (3) demonstrates the significant effect of plant parts and BA concentrations on the percentage response. The highest response rates (80%) were recorded for both Node + Two Cotyledons and Shoot Tip, which were significantly higher than the response rate of Single Node (57.5%). Regarding BA concentration, 4 mg/L showed the highest response rate (100%), which was not significantly different from 2 mg/L (90%) but significantly differed from the other concentrations. The control treatment had the lowest significant response rate (20%). For the interaction between plant parts and BA concentrations: Single Node, Shoot Tip, and Node + Two Cotyledons at 4 mg/L BA exhibited the highest response rates (100%), Shoot Tips at 1 mg/L BA and Node + Two Cotyledons at 2 mg/L BA also reached 100%, The lowest response rate (10%) was observed in Single Node with 0 mg/L BA.

Table (3): Effect of Different BA Concentrations (mg/L) on the Response Percentage of Plant Parts of Passion Fruit After Eight Weeks in MS Medium.

Plant Parts	BA (mg/L)				Mean of Plant Parts
	0	1	2	4	
Single Node	10	50	70	100	57.5
Node + Two Cotyledons	30	90	100	100	80
Shoot Tip	20	100	100	100	80
LSD 0.05	27.1				13.5
Mean BA	20	80	90	100	
LSD 0.05	15.6				

Effect of Different BA Concentrations (mg/L) on the Number of Vegetative Shoots (Shoots/Plant) Growing on Plant Parts of Passion Fruit Cultivated in Liquid MS Medium After Eight Weeks:

Table (4) highlights the influence of plant parts, BA concentrations, and their interaction on the number of shoots. The highest number of shoots (2.35 shoots/plant) was observed in Node + Two Cotyledons, which was not significantly different from Single Node (2.15 shoots/plant) but significantly higher than Shoot Tip (1.75 shoots/plant). Regarding BA concentrations, 4 mg/L BA resulted in the highest number of shoots (3.467 shoots/plant), while the control treatment had the lowest (0.333 shoots/plant). The interaction results showed that Single Node at 4 mg/L BA produced the highest number of shoots (4.5 shoots/plant), while Single Node and Shoot Tip at 0 mg/L BA had the lowest (0.2 shoots/plant for both).

**Table (4):** Effect of Different BA Concentrations (mg/L) on the Number of Shoots in Passion Fruit Plant After Eight Weeks in Liquid MS Medium.

Plant Parts	BA (mg/L)				Mean of Plant Parts
	0	1	2	4	
Single Node	0.200	1.600	2.300	4.500	2.150
Node + Two Cotyledons	0.600	2.600	2.900	3.300	2.350
Shoot Tip	0.200	2.000	2.200	2.600	1.750
LSD 0.05	0.919				0.459
Mean BA	0.333	2.067	2.467	3.467	
LSD 0.05	0.530				

Effect of BA and Kin Concentrations on Passion Fruit Propagation**Effect of BA and Kin Concentrations (mg/L) on the Percentage of Response (%) of Plant Parts After Eight Weeks of Cultivation on MS Liquid Medium**

Table (5) highlights the significant effect of BA and Kin concentrations on the response percentages of plant parts: Node + Two Cotyledons exhibited the highest average response percentage (80%), significantly outperforming other treatments. In contrast, Single Node recorded the lowest average response percentage (52.5%), The combination 2+2 mg/L BA + Kin provided the highest response percentage (96.7%), significantly superior to other concentrations. The control treatment (0+0 mg/L) gave the lowest response (20%), Interaction effects showed that: Single Node and Node + Two Cotyledons with 2+2 mg/L BA + Kin reached the maximum response (100%), Single Node with 0+0 mg/L BA + Kin showed the lowest response percentage (10%).

Table (5): Effect of BA and Kin Concentrations (mg/L) on the Response Percentage (%) of Passion Fruit Plant Parts After Eight Weeks of Cultivation

Plant Parts	Kin : BA (mg/L)				Mean of Plant Parts
	0:0	0.5: 0.5	1:1	2:2	
Single Node	10	50	60	90	52.5
Node + Two Cotyledons	30	90	100	100	80.0
Shoot Tip	20	90	100	100	77.5
LSD 0.05	27.1				14.9
Mean Kin : BA	20	76.7	86.7	96.7	
LSD 0.05	15.6				

Effect of BA and Kin Concentrations (mg/L) on the Number of Vegetative Shoots (Shoots/Plant) of Passion Fruit Plant Parts

Table (6) illustrates the effect of BA and Kin concentrations and their interactions on the number of shoots: Shoot Tip showed the highest average number of shoots (3.25 shoots/plant), significantly higher than other treatments, The combination 2+2 mg/L BA + Kin achieved the highest average shoot count (3.7 shoots/plant), significantly outperforming other concentrations. The control treatment (0+0 mg/L) recorded the lowest average (0.333 shoots/plant), Interaction results: Shoot Tip with 2+2 mg/L BA + Kin yielded the highest number of shoots (4.5 shoots/plant), not significantly different from Shoot Tip with 1+1 mg/L

(4.3 shoots/plant), Both Single Node and Shoot Tip with 0+0 mg/L BA + Kin produced the lowest number of shoots (0.2 shoots/plant).

Table (6): Effect of BA and Kin Concentrations (mg/L) on the Number of Vegetative Shoots (Shoots/Plant) After Eight Weeks of Cultivation

Plant Parts	Kin : BA (mg/L)				Mean of Plant Parts
	0:0	0.5: 0.5	1:1	2:2	
Single Node	0.200	0.500	0.900	2.700	1.075
Node + Two Cotyledons	0.600	0.900	3.400	3.900	2.200
Shoot Tip	0.200	4.000	4.300	4.500	3.250
LSD 0.05	0.818				0.409
Mean Kin : BA	0.333	1.800	2.867	3.700	
LSD 0.05	0.472				

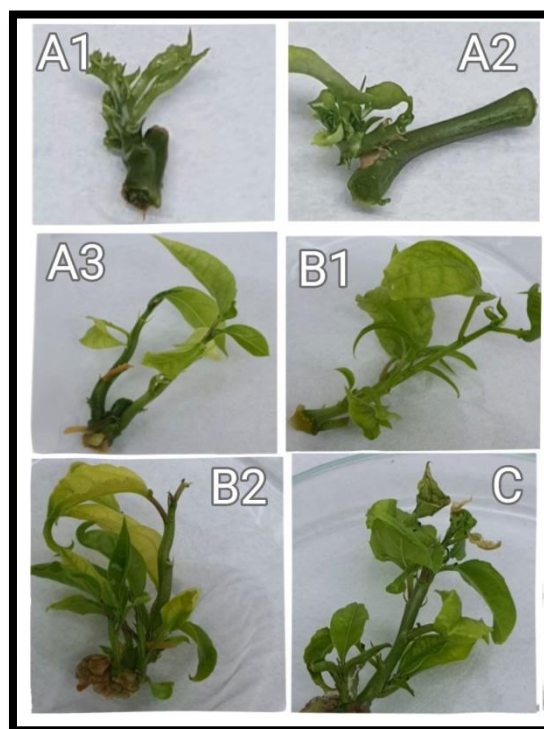


Figure (2): Emergence of Vegetative Shoots from Different Plant Parts (Single Node, Node + Two Cotyledons, or Shoot Tip) of Passion Fruit Cultivated In Vitro



Effect of BA and Meta-Topolin (mT) on Shoot Multiplication and Growth in Passion Fruit Cultivated In Vitro

1. Effect of BA Concentration on Shoot Multiplication and Growth

Table (7) shows the impact of BA concentration on shoot number and length after 60 days: Shoot Multiplication: The concentration 2 mg/L BA gave the highest shoot number (5.40 shoots/plant), significantly outperforming other treatments, The control treatment (0 mg/L) produced the lowest shoot number (1.00 shoot/plant), Shoot Growth: Shoot lengths were not significantly affected by BA concentration. However, 2 mg/L BA yielded the longest average shoot length (1.10 cm), while the control treatment gave the shortest (1.00 cm).

Table (7): Effect of BA Concentration on Shoot Multiplication and Growth in Passion Fruit After 60 Days

BA Concentration (mg/L)	Shoot Number	Shoot Length (cm)
0	1.00	1.00
0.5	4.50	1.25
1	5.00	1.20
1.5	5.30	1.10
2	5.40	1.10
• LSD for Shoot Number: 0.644 • LSD for Shoot Length: Not Significant (N.S.)		

2. Effect of Meta-Topolin (mT) on Shoot Multiplication and Growth

Table (8) highlights the effect of mT concentration on shoot multiplication and length: Shoot Multiplication: 1 mg/L mT achieved the highest shoot number (8.00 shoots/plant), significantly superior to other concentrations, The control treatment (0 mg/L) recorded the lowest shoot number (1.00 shoot/plant), Shoot Growth: 2 mg/L mT produced the longest shoot length (1.80 cm), which did not differ significantly from 1.5 mg/L and 1.0 mg/L (both 1.70 cm), The control treatment (0 mg/L) resulted in the shortest shoot length (1.00 cm).

Table (8): Effect of mT Concentration on Shoot Multiplication and Growth in Passion Fruit After 60 Days

mT Concentration (mg/L)	Shoot Number	Shoot Length (cm)
0	1.00	1.00
0.5	4.60	1.55
1	8.00	1.70
1.5	7.70	1.70
2	7.30	1.80
• LSD for Shoot Number: 0.974 • LSD for Shoot Length: 0.316		



DISCUSSION:

The addition of cytokinins stimulates cell division in plant tissue cultures by increasing the translation rate of DNA, thereby enhancing protein synthesis. The growth activation is attributed to the significant increase in the amino acid t-RNA, followed by protein synthesis activity. Additionally, it leads to 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. Apical dominance results from the antagonism between cytokinin and auxin, where cytokinin inhibits the effect of auxin responsible for apical dominance or activates axillary buds. This occurs through their influence on the development of the primary xylem tissues connecting the axillary buds and the main stem. Hence, it can be concluded that apical dominance can be controlled by balancing the internal concentrations of both cytokinin and auxin, consistent with several studies, including those by **Al-Jabori et al., (2022)**, & **Suaad et al., (2022)**.

Cytokinins attract various substances and elements to their site of presence. These include inorganic ions and organic molecules, such as sugars, amino acids, and most of the xylem and phloem sap components. The flow of these substances is directed toward the specific location where cytokinin is present. (**Farqad et al., 2024; Safa et al., 2024**).

Metatopolin (mT) is metabolized much faster than other cytokinins and is present in all parts of the plant. As is well-known, cytokinins, at their sites of presence, act as an attractant for many substances and elements, such as inorganic ions and organic molecules like sugars, amino acids, and most of the xylem and phloem sap.

Several recent studies have highlighted the superior role of metatopolin compared to benzyl adenine (BA) in increasing the multiplication of vegetative shoots in tissue culture. Moreover, it helps eliminate the vitrification and necrosis phenomena associated with BA (Bairu et al., 2011; Mirabbasi et al., 2014). The findings align with those of Naaz et al., 2019; Ying et al., 2020; and Saurabh, 2024, on the potential use of metatopolin as a substitute for BA to achieve higher multiplication rates of vegetative shoots grown in vitro for the precise propagation of various plant species.



Figure (3): The effect of metatopolin concentrations on the development and multiplication of organs (vegetative shoots).

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