

## Effect of Growth Regulators and Exogenous Arginine on the In Vitro Micropropagation of 'Sweet Sapphire' Grape

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**Abstract.** This study aimed to optimize an in vitro micropropagation protocol for the grapevine cultivar Sweet Sapphire (Witch Fingers) by determining the optimal concentrations of plant growth regulators during the establishment, multiplication, rooting, and acclimatization stages. The experiments were conducted at the Plant Tissue Culture Laboratory, Al-Musayyib Technical College, from March to October 2025, using nodal explants approximately 1 cm in length. The results of the establishment stage showed that the interaction between 2-isopentenyl adenine (2iP) at 2 mg·L<sup>-1</sup> and naphthalene acetic acid (NAA) at 0.5 mg·L<sup>-1</sup> produced the highest number of leaves and the longest shoots. During the multiplication stage, the combination of 2iP at 2 mg·L<sup>-1</sup> with NAA at 0.25 mg·L<sup>-1</sup> resulted in the highest number of shoots. In the rooting stage, the interaction between benzyl adenine (BA) at 2 mg·L<sup>-1</sup>, arginine at 200 mg·L<sup>-1</sup>, and activated charcoal at 400 mg·L<sup>-1</sup> achieved the greatest root length. For acclimatization, the substrate composed of peat moss and perlite at a ratio of 1:2 recorded the highest plant survival rate (90%). These results demonstrate the effectiveness of the identified concentrations in producing uniform, disease-free grapevine plantlets with high efficiency, supporting their application in grapevine micropropagation programs.

**Keywords:** Exogenous arginine; Growth regulators; In vitro culture; Micropropagation; Sweet Sapphire grape.

### 1. INTRODUCTION

Grapevine is one of the oldest and most important fruit crops known to humans. It belongs to the genus *Vitis* within the family Vitaceae, and its scientific name is *Vitis vinifera* L. Grapevine is characterized by its wide varietal diversity and multiple uses, including fresh consumption and processing into juices, jams, raisins, and wine, making it an economically important fruit crop. Grapevines are cultivated worldwide over an estimated area of approximately 7.3 million hectares [1]. Grapes are a rich source of bioactive compounds, particularly in their skins and

seeds, and are well known for their high content of beneficial compounds such as resveratrol and anthocyanins. These natural constituents possess significant biological activities, functioning as potent antioxidants and anti-inflammatory agents, in addition to their promising role in combating cancer cells [2]. Due to their high nutritional value and diverse industrial applications, grapes are considered a crop of major agricultural and economic importance. The expansion of grape cultivation and advancements in production techniques have contributed significantly to the development of key economic sectors in many countries. Viticulture represents an integrated agricultural and industrial practice that is essential for social and economic sustainability worldwide [3]. Despite this importance, the grapevine sector faces numerous challenges, including climate variability [4], socio-economic factors affecting wine production and marketing [5], and the spread of pests and [6],[7]. Climate change is increasingly impacting grapevine production. To maintain strong and competitive grape production, it is essential to cultivate grapevine plants that are completely free from viruses and diseases. In vitro culture techniques represent a fundamental approach to achieving this objective; however, their success depends largely on identifying the most suitable nutritional media and growth conditions for plant tissue development [8]. The major value of plant tissue culture techniques lies in their ability to produce a large number of disease-free plants from a very small amount of initial plant material. This capability makes tissue culture a promising tool for rapid and reliable mass propagation of many plant species. For example, up to 12,000 grapevine plantlets can be produced from a single bud measuring 3–5 mm within four months [9]. Grapevine micropropagation begins with determining the optimal concentration and exposure time for surface sterilization of tender buds to eliminate microbial contaminants that may hinder growth under in vitro conditions. Subsequently, the most effective combinations of plant growth regulators are identified to stimulate bud break, shoot growth, and multiplication within the culture medium, leading to the production of a large number of healthy plantlets. Following the multiplication stage, root formation is induced by transferring regenerated shoots to suitable rooting media to ensure successful establishment. Finally, the regenerated plantlets are acclimatized in a greenhouse under conditions similar to the field environment, allowing gradual adaptation before transfer to open-field conditions and ensuring successful establishment and production.

## 2. MATERIALS AND METHODS

This study was conducted at the Plant Tissue Culture Laboratory, Department of Plant Production Technologies, Al-Musayyib Technical College, during the period from March to October 2025. The grapevine cultivar Sweet Sapphire (Witch Fingers) was used in this experiment. This cultivar is a hybrid resulting from a cross between 'Bita Mouni' and 'C 22-121', and is characterized by large, relatively loose clusters bearing elongated, finger-like berries, which distinguish it from other grape cultivars. The berries exhibit a color gradient ranging from yellow-green at the base to dark red or purple at the tip, giving the clusters a unique and attractive appearance, classifying this cultivar among premium grape varieties [10]. The plant material consisted of approximately four-year-old grapevine plants grown in plastic pots with a capacity of 6 kg under wooden shade conditions prior to their transfer to the laboratory. Tender shoots approximately 5 cm in length were collected from three-year-old grapevine plants. The shoots were transferred to the laboratory and subjected to successive

cleaning procedures, including washing under running tap water for 30 min, followed by treatment with a diluted liquid soap solution to remove dust and adhering organic materials. The shoots were then prepared by removing the leaves and cutting them into nodal segments approximately 1 cm in length, each containing at least one axillary bud. The explants were surface-sterilized using commercial sodium hypochlorite (NaOCl) solution (FAS®) at a concentration of 6%. Three different concentrations were prepared by dilution with sterile distilled water in 100 mL volumetric flasks. The explants were immersed in the sterilizing solution with continuous agitation. After sterilization, the explants were rinsed three times with sterile distilled water to remove residual disinfectant. Establishment Stage Data were recorded after 30 days of culture, including number of leaves and shoot length. The culture media were supplemented with naphthalene acetic acid (NAA) at concentrations of 0, 0.25, and 0.5 mg·L<sup>-1</sup>, and 2-isopentenyl adenine (2iP) at concentrations of 0, 1, and 2 mg·L<sup>-1</sup>. Multiplication Stage Based on the results obtained from the establishment stage, shoots derived from bud growth were used for multiplication experiments. Shoots were cut into segments approximately 1.5 cm in length, and one shoot was cultured per test tube with ten replicates per treatment. Measurements were recorded after 30 days of culture. The effect of the interaction between NAA and 2iP was evaluated using the same concentrations applied during the establishment stage, and the number of shoots per explant was recorded. Rooting Stage Shoots obtained from the multiplication stage were selected and transferred to test tubes containing rooting media, with one shoot per tube (approximately 10 cm in length) and ten tubes per treatment. Results were recorded after one month of culture. The effects of Activated carbon was applied at concentrations of 0, 200, and 300 g·L<sup>-1</sup>. Arginine was applied at concentrations of 0, 50, and 100 g·L<sup>-1</sup>, while the growth regulator BA (Benzyladenine) was applied at concentrations of 0, 1, and 2 mL·L<sup>-1</sup>. Root length was recorded at this stage. Acclimatization Stage Uniform plantlets obtained from the rooting stage were transferred to the acclimatization medium. The plantlets were removed from the culture vessels and washed with tap water to remove agar residues from the roots. Subsequently, the plantlets were treated with a benomyl fungicide solution at a concentration of 1 mL·L<sup>-1</sup> for 10 min to prevent fungal infection. The plantlets were then transplanted into plastic pots (5 cm in diameter) filled with a mixture of peat moss and perlite at a ratio of 1:1 after sterilization of the growth medium in an autoclave at 121 °C and a pressure of 1.04 kg·cm<sup>-2</sup> for 20 min. Additional substrate mixtures of peat moss and perlite at ratios of 1:2 and 2:1 were also prepared. Plant survival percentage was calculated after one month of acclimatization.

### 3. Results and Discussion

**Table (1). Effect of different concentrations (mg·L<sup>-1</sup>) of NAA and 2iP and their interaction on the number of leaves produced from a single bud (1 cm in length) after 30 days of culture.**

mean	Hardening stage			
2ip	0.5	0.25	0.00	Naa 2ip
2.333	3.33	1.500	2.167	0.00
2.000	1.333	2.167	2.500	1
4.389	5.833	4.333	3.000	2

	3.500	2.667	2.556	Mean of NAA
Least Significant Difference	Naa*2ip=0.6369	2ip = 0.3677	NAA = 0.3677	LSD (0.05)

The results presented in Table (1) for the number of leaves of the grapevine cultivar Sweet Sapphire during the establishment stage showed that the plant growth regulator 2-isopentenyl adenine (2iP) exhibited a clear and significant superiority at a concentration of 2 mg·L<sup>-1</sup>, recording the highest mean value of 4.389 leaves compared with lower concentrations. Regarding naphthalene acetic acid (NAA), the results indicated that a concentration of 0.5 mg·L<sup>-1</sup> resulted in a significantly higher mean number of leaves (3.500 leaves). When the interaction between the two growth regulators was examined, the combination of 2 mg·L<sup>-1</sup> 2iP with 0.5 mg·L<sup>-1</sup> NAA was significantly superior to all other treatments. Considering the individual values rather than the overall means, the highest recorded value was 5.833 leaves under the interaction of 2 mg·L<sup>-1</sup> 2iP with 0.5 mg·L<sup>-1</sup> NAA, whereas the lowest value (1.333 leaves) was observed at the interaction of 1 mg·L<sup>-1</sup> 2iP with 0.5 mg·L<sup>-1</sup> NAA. This superiority can be attributed to the effective role of cytokinins (2iP) in stimulating cell division, breaking apical dominance, and activating lateral buds, which consequently increases the number of nodes and, therefore, the number of leaves, particularly when an optimal hormonal balance with auxins (NAA), which support tissue differentiation and elongation, is achieved. The fluctuation observed in the response to NAA concentration, characterized by an initial increase followed by a decrease and subsequent increase, may be explained by the fact that increasing NAA concentration initially promotes limited leaf growth through stimulation of cell division; however, excessive auxin levels enhance callus formation, which reduces organ differentiation. At higher concentrations, a slight improvement may occur due to a relative balance between callus formation and vegetative growth, thereby explaining the observed variability in leaf number. Several studies support the concept that plant tissue responses to auxins such as NAA do not always follow a linear trend but may fluctuate depending on internal hormonal balance and callus formation. This fluctuation in response to NAA concentration can be explained by the physiological interaction between leaf differentiation and callus induction, where intermediate concentrations may direct metabolic energy toward non-organogenic growth. These findings are consistent with those reported by Machado [11], who indicated that precise hormonal balance determines whether tissues undergo vegetative development or callus

formation. Overall, the present results are in agreement with the findings of Al-Safadi [12] regarding the micropropagation of the grapevine cultivar ‘Halawani’

**Table (2). Effect of different concentrations ( $\text{mg}\cdot\text{L}^{-1}$ ) of NAA and 2iP and their interaction on shoot length produced from a single bud (1 cm in length) after 30 days of culture**

mean	Hardening stage			
2ip	0.5	0.25	0.00	Naa 2ip
0.439	0.500	4.17	0.400	0.00
0.672	0.883	0.433	0.700	1
2.111	2.600	1.400	2.333	2
	1.328	0.750	1.144	Mean of NAA
Least Significant Difference	Naa*2ip= 0.7903	2ip = 0.4563	NAA = 0.4563	LSD (0.05)

The results presented in Table (2) for shoot length of the grapevine cultivar Sweet Sapphire during the establishment stage revealed that the plant growth regulator 2-isopentenyl adenine (2iP) showed a significant superiority at a concentration of  $2 \text{ mg}\cdot\text{L}^{-1}$ , recording the highest mean shoot length of 2.111 cm compared with lower concentrations. Regarding naphthalene acetic acid (NAA), the concentration of  $0.5 \text{ mg}\cdot\text{L}^{-1}$  resulted in a significantly greater shoot length, with a mean value of 1.328 cm. Analysis of the interaction between the two growth regulators indicated that the combination of  $2 \text{ mg}\cdot\text{L}^{-1}$  2iP with  $0.5 \text{ mg}\cdot\text{L}^{-1}$  NAA was significantly superior to the other treatments. Considering the individual recorded values rather than overall means, the maximum shoot length (2.600 cm) was obtained under the interaction of  $2 \text{ mg}\cdot\text{L}^{-1}$  2iP and  $0.5 \text{ mg}\cdot\text{L}^{-1}$  NAA, whereas the minimum value (0.400 cm) was observed in the control treatment in which both growth regulators were absent ( $0.00 \text{ mg}\cdot\text{L}^{-1}$ ). The fluctuation observed in the response to NAA concentration, where shoot length was initially high (1.067 cm) in the absence of exogenous NAA, decreased at  $0.25 \text{ mg}\cdot\text{L}^{-1}$  (0.633 cm), and increased again at  $0.5 \text{ mg}\cdot\text{L}^{-1}$  (1.200 cm), may be explained by the fact that the absence of added auxin allowed endogenous auxins to function at a balanced level sufficient for shoot elongation. In contrast, the application of  $0.25 \text{ mg}\cdot\text{L}^{-1}$  NAA may have disrupted hormonal balance and directed metabolic activity toward intensive callus formation at the expense of longitudinal shoot growth. Increasing the concentration to  $0.5 \text{ mg}\cdot\text{L}^{-1}$  restored tissue elongation

capacity by establishing a new hormonal equilibrium that promoted vegetative growth. The reduction in shoot growth at lower auxin concentrations and the concurrent increase in callus formation can be attributed to the interaction between exogenous auxin levels and endogenous auxin pools, which alters the pathway of cellular differentiation. This interpretation is consistent with the physiological principles described by George [13] regarding the role of auxins in regulating growth and their concentration- and tissue-dependent dual effects. Furthermore, these findings are supported by the study of Gabr and Hegazy [14], who emphasized that optimal growth in grapevine micropropagation requires a precise balance between plant growth regulators to improve both qualitative and quantitative traits.

**Table (3). Effect of different concentrations ( $\text{mg}\cdot\text{L}^{-1}$ ) of NAA and 2iP and their interaction on the number of shoots produced from a single bud (1 cm in length) after 30 days of culture.**

mean	Multiplication stage			
2ip	0.5	0.25	0.00	Naa 2ip
1.94	2.17	2.17	1.50	0.00
3.17	3.00	3.33	3.17	1 L
4.17	4.60	4.17	4.33	2 L
	3.06	3.22	3.00	Mean of NAA
Least Significant Difference	التداخل=1.220	2ip = 0.704	NAA = 0.704	LSD (0.05)

Based on the results for the Sweet Sapphire cultivar regarding shoot length during the multiplication stage, the cytokinin 2iP showed a significant superiority at a concentration of  $2 \text{ mg}\cdot\text{L}^{-1}$ , recording the highest growth rate of 2.40 cm compared to other concentrations. As for the auxin NAA, the  $0.25 \text{ mg}\cdot\text{L}^{-1}$  concentration exhibited a significant effect, achieving a maximum shoot length of 1.94 cm. When examining the interaction between the two regulators, the combination of  $2 \text{ mg}\cdot\text{L}^{-1}$  2iP with  $0.25 \text{ mg}\cdot\text{L}^{-1}$  NAA was significantly superior to the other treatments. Considering the individual recorded values rather than the overall means, the highest shoot length (2.80 cm) was observed with the interaction of  $2 \text{ mg}\cdot\text{L}^{-1}$  2iP and  $0.25 \text{ mg}\cdot\text{L}^{-1}$  NAA, while the lowest value (1.00 cm) was recorded for  $0.25 \text{ mg}\cdot\text{L}^{-1}$  NAA in the absence of 2iP ( $0.00 \text{ mg}\cdot\text{L}^{-1}$ ). This confirms that the multiplication growth in this cultivar relies heavily on external hormonal supply to activate the latent potential of the buds. These results are consistent with Sadeghi [15], who reported that increasing cytokinin levels, especially 2iP, significantly enhances the number of shoots by stimulating the formation of lateral and axillary buds. The superiority of high 2iP concentration even in the absence of NAA in producing the maximum number of shoots directly supports the findings of Sudhir [16], which highlighted that elevated cytokinin levels (Benzyladenine or 2iP) are the most influential factor in increasing shoot proliferation in grape micropropagation.

able (4) shows the effect of different concentrations of BA ( $\text{mg}\cdot\text{L}^{-1}$ ), Arginine (AR), and activated charcoal (Ch,  $\text{g}\cdot\text{L}^{-1}$ ) on the number of roots produced from a single 1-cm shoot after 30 days of culture.

ba X ar	C			AR	BA
	c2	bc1	c0		
0.2555	0.3	0.3333	0.1333	ar0	ba0
0.3111	0.3666	0.3	0.2666	ar1	
0.5222	0.5666	0.5333	0.4666	ar2	
0.5111	0.5333	0.6333	0.36666	ar0	ba1
1.5111	1.76667	1.7666	1	ar1	
2.2	2.4333	2.0667	2.1	ar2	
1.7666	2.866	1.3666	1.0666	ar0	ba2
4.0778	4.7667	3.9667	3.5	ar1	
5.3556	5.7	5.3667	5	ar2	
0.23108	0.400203			lsd5%	
Mean of ba	ba X c			BA	
0.3629	0.4111	0.3889	0.28889	ba0	
1.40707	1.57778	1.48889	1.1556	ba1	
3.733	4.444	3.5667	3.1889	ba2	
0.13341	0.231058			lsd5%	
Mean of ar	ar X C			AR	
0.8444	1.2333	0.7777	0.5222	ar1	
1.9667	2.3	2.0111	1.5889	ar2	
2.6925	2.9	2.6556	2.522	ar3	
0.1334	0.231057			lsd5%	
	2.1444	1.8148	1.5444	Mean of c	
	0.133401			lsd5%	

The results of Table (4) for root length in the Sweet Sapphire grape cultivar (Root B) during the rooting stage showed that the cytokinin benzyladenine (BA) exhibited a significant effect at a concentration of  $2 \text{ mg}\cdot\text{L}^{-1}$ , achieving the highest average root length of 3.733 cm compared to lower concentrations. Regarding arginine, the  $200 \text{ mg}\cdot\text{L}^{-1}$  concentration significantly promoted root elongation, with an average length of 2.692 cm. For activated charcoal, the  $300 \text{ mg}\cdot\text{L}^{-1}$  concentration resulted in a significant increase, recording an average of 2.144 cm. When examining the combined effect of these three factors, the treatment consisting of  $2 \text{ mg}\cdot\text{L}^{-1}$  BA,  $200 \text{ mg}\cdot\text{L}^{-1}$  arginine, and  $300 \text{ mg}\cdot\text{L}^{-1}$  activated charcoal showed the highest performance, significantly surpassing all other treatments. Considering the individual observations, the maximum root length reached 5.700 cm, while the minimum was 0.133 cm in the control

treatment lacking all supplements. The superior root growth can be attributed to the synergistic role of BA in promoting tissue differentiation and cell elongation, arginine as an organic nitrogen source supporting metabolic activity and protein synthesis required for root development, and activated charcoal in adsorbing phenolic compounds and inhibitors while providing a darkened environment that stimulates root elongation. The significant enhancement of root length under the combined treatment reflects the functional integration of hormonal stimulation, organic nutritional support, and medium detoxification, consistent with the principles outlined by George [13] regarding the importance of amino acids and activated charcoal in improving in vitro rooting conditions

**Table 5 illustrates the effect of peat moss and perlite on plant survival**

Peat Moss : Perlite	Percentag of Acclimatized Plants
1 : 2	90%
1 : 1	70%
2 : 1	40%

The results indicated that the composition of the growth medium plays a direct and crucial role in determining the success rate of acclimatization. The optimal mixture, which achieved the highest success rate of 90%, was 1 part peat moss to 2 parts perlite. This suggests that a medium with a higher proportion of perlite (twice that of peat moss) is most favorable for the growth and acclimatization of tissue-cultured grape seedlings. The intermediate mixture, 1 part peat moss to 1 part perlite, resulted in a moderate success rate of 70%. The least effective mixture, 2 parts peat moss to 1 part perlite, recorded the lowest success rate of 40%. The superior performance of the perlite-rich medium (1:2) can be attributed to several physical factors, including aeration and drainage. Perlite is an inert material with high porosity, ensuring excellent aeration of the medium and rapid water drainage. This is particularly critical for tissue-cultured grape seedlings, whose roots are young and sensitive, as it prevents water accumulation around the roots and reduces the risk of root rot and suffocation. Regarding water-holding capacity, peat moss is characterized by its high ability to retain water. While this is beneficial for maintaining moisture, a high proportion of peat moss (2:1) leads to excessive water retention and reduced aeration, which can suffocate the roots and result in higher mortality, as reflected in the lowest success rate (40%). The optimal mixture (1:2) provided a balanced environment, maintaining sufficient moisture to prevent rapid dehydration of the seedlings while ensuring adequate aeration and drainage necessary for root development, leading to the highest acclimatization success. Consistency with scientific literature: These findings are largely in agreement with previous studies on optimal media for tissue culture. They confirm the superiority of media containing high proportions of perlite in supporting root growth, as reported by Vardimon [17], who demonstrated that using a perlite-rich medium

significantly enhances the survival and growth of tissue-cultured grape plants, primarily due to improved physical properties related to aeration and drainage.

#### 4. APPENDICES

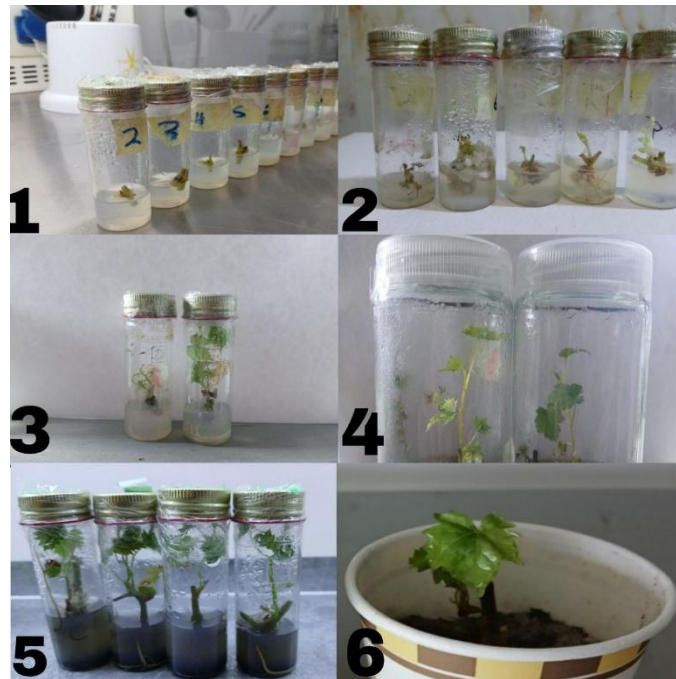


FIGURE (1):

1. nucleation of buds in nutrient media
2. Development of bud growth within the media
3. Plantlets obtained during the initiation stage
4. Plantlets obtained during the multiplication stage
5. Plantlets obtained during the rooting stage
6. Acclimatization stage

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