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EFFECT OF GROWTH REGULATORS AND EXPLANT TYPES ON THE CALLUS INDUCTION IN GRAPE VINE (*VITIS VINIFERA* L.)

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SUMMARY

This study presents the use of plant tissue culture technology in inducing callus from different plant parts and varying concentrations of growth regulators in common grape vine (*Vitis vinifera* L.). The research, carried out in 2022–2023 at the Tissue Culture Laboratory-College of Agriculture, University of Kerbala, Kerbala, Iraq, comprised two stages, including sterilizing the explants using various concentrations of sodium hypochlorite (0%, 0.5%, 1%, 1.5%, and 2%) for 5, 10, and 15 minutes. The second stage included establishing callus types by growing the explants on MS medium prepared with different concentrations of 2,4-D (0, 1, 2, 3, and 4 mg L^{-1}) and benzyl adenine (BA) (0, 0.1, 0.2, and 0.4 mg L^{-1}). The results showed the sterilizing agent (1.5%) with 10 minutes gave the lowest contamination without affecting the vitality of explants. The interaction of 2,4-D (3 mg L^{-1}) and BA (0.4 mg L^{-1}) emerged superior by showing the highest response rate (80%) to callus induction from shoots. The growing apex with 2,4-D (2 mg L^{-1}) achieved the maximum fresh and dry weights of callus (3.32 and 1.03 mg, respectively).

Keywords: Grape vine (*V. vinifera* L.), explants, growth regulators, 2,4-D, benzyl adenine, fresh and dry weights of callus

Key findings: In the common grape vine (*V. vinifera* L.), the sodium hypochlorite (1.5%) with a duration of 10 minutes showed the lowest percentage of contamination without affecting the vitality of explants. The interaction of 2,4-D (3 mg L^{-1}) and BA (0.4 mg L^{-1}) was superior in most of the studied traits.

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INTRODUCTION

The common grape vine (*Vitis vinifera* L.) is one of the deciduous fruits of the temperate regions belonging to the family Vitaceae, with 14 genera and more than 100 species. It grows worldwide under a wide range of environmental conditions in subtropical, warm temperate, and cold temperate regions (Martín-Gómez *et al.,* 2024). Grape cultivation began in the Central Asian region between the Southern Black Sea and the Caspian Sea. Most botanists agreed this region was the origin of European grapes, and the grape genotypes originated before the discovery of the North American continent, where its cultivation spread in the East and West (McGovern *et al.,* 2017).

Grapes' fruits have the highest nutritional value and greater economic importance. Their fruits contain sugar, vitamins, organic acids, mineral salts, proteins, and fats. The grape crop has received special care in its cultivation and production because of its prominence in food and medical uses. The ripe fruits of grapes contain the compound resveratrol, which inhibits the growth of prostate cancer cells (Hudson *et al.*, 2007). Grapes have also become one of the most beneficial and profitable fruits. The grapes have an effective role in building the body, strengthening it, restoring its tissues, and treating many diseases and ailments, in addition to their availability year-round. Grapes are easily digestible fruits with high nutritional value and a rapid metabolism in the body's cells (Andrushia *et al.,* 2024).

Plant tissue culture is the science of growing plant cells, tissues, and organs isolated from the parent plant and from cell protoplasts on nutrient media (Ozden, 2024). Tissue culture plays an imperative role in serving humanity, especially in the field of plant propagation and obtaining huge numbers of plants free of pathogens similar to the mother plant in a relatively short time. Additionally, the said technology also helps in the formation of adventitious buds, stimulating the growth of axillary buds, the development of asexual embryos (somatic embryology), and the study of basic aspects of plant growth and development and secondary metabolism (Hesami and Jones, 2020).

Callus, defined as undifferentiated parenchyma cells that arise in areas of cuts and wounds in plant parts, has the process of inducing it on the plant part by growing in the appropriate nutrient medium through three stages—stimulation, division, and differentiation. In the stimulation phase, important variations occur in the cells, such as the construction of proteins and DNA and preparing them for the division process. The second stage witnesses successive cell division and the formation of a mass of callus cells covering the plant part. Following it is the differentiation stage through the expansion of certain meristematic cells and their differentiation to form asexual embryos, provided that appropriate growth factors are available (Kulus and Tymoszuk, 2020).

Growth regulators play a major role in stimulating the emergence and growth of callus tissues. It has been evident that the balance between auxins and cytokinins has the greatest role in this field. It requires adding them in specific concentrations depending on the genetic makeup of the plants, the type of plant part grown, and its internal hormone content (Fahmy, 2003). Based on the grape therapeutic nutritional standpoint, the tissue culture technology's significance in micropropagation, callus culture production, and its stimulation to produce metabolic compounds, this study aimed to assess the plant parts' ability added with different growth regulators in MS medium in inducing and growing callus.

MATERIALS AND METHODS

The study on grape transpired from 2022 to 2023 at the University of Kerbala, Kerbala, Iraq. The research used the grape variety Halwani, grown in plastic bags at the age of two years in the wooden canopy, as prepared from specialized nurseries for deciduous fruits.

Experiment on sterilizing explants

Selecting stem cuttings (20–30 cm long) came from recent growth. With the outer leaves surrounding the nodes removed, the cuttings' chopping into small pieces (2 cm long) ensued for each piece to contain one node, as well as the growing apex (1 cm long) (Sekhi *et al.,* 2023). The plant parts' sterilization comprised sodium hypochlorite with five concentrations (0%, 0.5%, 1%, 1.5%, and 2%) for periods of 5, 10, and 15 minutes. Afterward, planting the sterilized cultures in glass bottles continued, which contained the MS nutrient medium free of growth regulators, with 10 replicates for each concentration and duration. The incubation of cultures proceeded in the growth room with an intense light of 1000 lux for 16 hours. At day 1, at the temperature of 25 °C ± 2 °C, the recording of results represented by the percentage of contamination ensued after two weeks of plantation (Kadhim and Abdulhussein, 2021).

Experiment of callus induction

Based on the sterilization experiment, the plant parts (growing tips and nodes) sustained planting on the MS nutrient medium prepared for callus induction. Each medium has different concentrations of 2,4-D (0, 1, 2, 3, and 4 mg L^{-1}) and BA concentrations (0, 0.1, 0.2, and 0.4 mg L^{1}), with 10 replications for each concentration. The plant parts incubated in the dark had a temperature of 25 °C ± 2 °C for six weeks. The recorded results were on the percentage of response to callus induction and the standards of fresh and dry weights of callus to determine the plant parts. The study also recorded the best concentration of growth regulators to induce callus for propagation and obtaining grape callus culture.

Statistical analysis

All the recorded data's analysis was according to the completely randomized design (CRD) with factorial arrangement. Analyzing the data used the SAS statistical program (AL-Sahuki and Waheeb, 1990), with the means further compared according to the least significant difference ($LSD_{0.05}$) test.

RESULTS AND DISCUSSION

Surface sterilization of explants

The effect of sterilization duration and hypochlorite concentration on the percentage of contamination in common grape vine (*V. vinifera* L.) appears in Table 1. The 15-minute time recorded the lowest rate of contamination (24%) compared with the control treatment (48%). With increasing concentrations of the substance, significant differences were notable in the percentage of contamination. The comparison treatment provided the highest concentration (100%), and then the percentage of contamination decreased by increasing the concentration to 1.5%, reaching 6.66%, which also did not differ significantly from the concentration of 2%, showing the same percentage.

As for the effect of the interaction between the duration and concentration of the sterilizing agent, the 10-minute period with the concentrations of 1.5% and 2% achieved the lowest rate of contamination (0%). Likewise, the duration of 15 minutes at concentrations of 1.5% and 2% attained the same rate. However, the plants in this case were pale and had no response to growth, and the reason could refer to high concentrations of sodium hypochlorite having a toxic effect on plant parts. Lazo-Javalera *et al.* (2016) observed contamination of plant parts exposed to low concentrations, while parts exposed to high concentrations lost their vitality. Thus, the time and concentration of 10 minutes depended on the sterile material (1.5%) in carrying out subsequent experiments. The effect of sodium hypochlorite and its work as a sterilizing substance for plant tissue was due to the hypochlorous acid (HOCl), which is a strong oxidizing substance. The acid formation was a result of chlorine dissolving in water, and these results agreed with the findings of Naeem and Almukhtar (2023).

Table 1. Effect of sodium hypochlorite concentration and duration on the percentage of contamination of explants of grape after 10 days of planting.

Sterilization duration (min)	Sodium hypochlorite Con. (%)					Means (%)
	0	0.5	1	1.5	2	
5	100	60	40	20	20	48
10	100	30	20	0	0	30
15	100	10	10	0	0	24
Means (%)	100	33.33	23.33	6.66	6.66	
$LSD_{0.05}$ Sterilization duration: 4.41, Sodium hypochlorite Con.: 5.69, Interactions: 9.86						

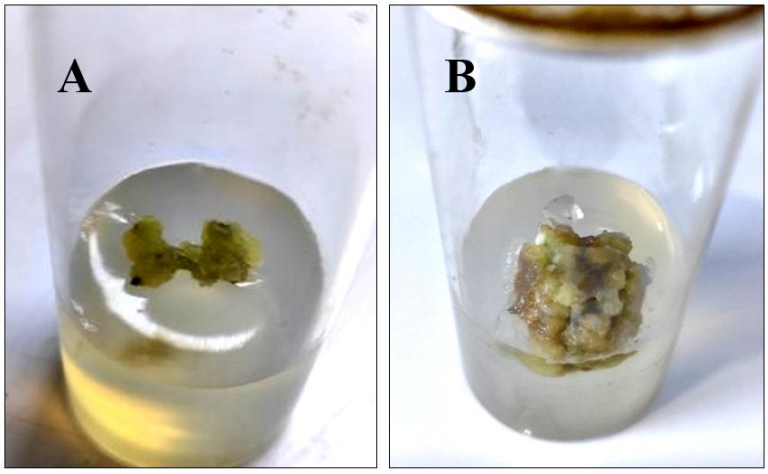

Figure 1. A: Callus of buds, B: Callus from tip shoot.

2,4-D effect on the response percentage

The results indicated the effect of different concentrations of Auxin 2,4-D and Cytokinins BA on the percentage of callus induced from the growing shoots in common grape vine (Table 2, Figure 1). The growing shoot response was visible at concentrations of 2,4-D (1, 2, and 3 mg L^{-1}) in interaction with all the concentrations of BA reaching 100%. However, the response decreased at the concentration of 2,4-D (4 mg L^{-1}) in interaction with BA (0, 0.1, 0.2, and 0.4 mg L^{-1}), reaching 70%, 80%, 80%, and 80%, respectively, while the control treatment did not achieve any significant response.

2,4-D effect on the bud response

The findings exhibited the effect of different concentrations of Auxin 2,4-D and Cytokinins BA on the percentage of callus induced by the shoots in *V. vinifera* L. (Table 3, Figure 1). However, the response of shoots was notable at the 2,4-D concentration (3 mg L^{-1}) in interaction with BA concentrations (0, 0.1, 0.2, and 0.4 mg L^{-1}), reaching 50%, 60%, 60%, and 80%, respectively. The other auxin concentrations in interaction with cytokinins did not show any significant response.

2,4-D and BA effect on the fresh weight

The results showed significant differences by adding 2,4-D with various concentrations to the nutrient medium prepared for the induction of callus from the growing tips of the common grape plants (Table 4). The 2,4-D concentration (2 mg L^{-1}) emerged superior and gave the highest average weight. The freshness of the callus reached 3.32 mg, which significantly differed from the rest of the treatments. However, the response decreased by increasing the auxin (3 mg L^{-1}) amounting

Table 2. Effect of 2,4-D and BA concentrations on the percentage response of tip shoot to callus induction after six weeks of cultivation on MS medium.

2,4-D con. (mg L^{-1})	BA con. (mg L^{-1})	Number of replicates	Number of successful replications	Response rate (%)
0	0	10	0	0
	0.1	10	0	0
	0.2	10	0	0
	0.4	10	0	0
1	0	10	10	100
	0.1	10	10	100
	0.2	10	10	100
	0.4	10	10	100
2	0	10	10	100
	0.1	10	10	100
	0.2	10	10	100
	0.4	10	10	100
3	0	10	10	100
	0.1	10	10	100
	0.2	10	10	100
	0.4	10	10	100
4	0	10	7	70
	0.1	10	8	80
	0.2	10	8	80
	0.4	10	8	80

Table 3. Effect of 2,4-D and BA concentrations on the percentage of bud response to callus induction after six weeks of cultivation on the MS medium.

2,4-D con. (mg L^{-1})	BA con. (mg L^{-1})	Number of replicates	Number of successful replications	Response rate (%)
0	0	10	0	0
	0.1	10	0	0
	0.2	10	0	0
	0.4	10	0	0
1	0	10	0	0
	0.1	10	0	0
	0.2	10	0	0
	0.4	10	0	0
2	0	10	0	0
	0.1	10	0	0
	0.2	10	0	0
	0.4	10	0	0
3	0	10	5	50
	0.1	10	6	60
	0.2	10	6	60
	0.4	10	8	80
4	0	10	0	0
	0.1	10	0	0
	0.2	10	0	0
	0.4	10	0	0

Table 4. Effect of 2,4-D and BA concentrations on the fresh weight of callus induced from the tip shoot after six weeks of cultivation on the MS medium.

2,4-D con. (mg L^{-1})	BA con. (mg L^{-1})				Means (mg)
	0	0.1	0.2	0.4	
1	0	0	0	0	0
2	1.70	2.50	2.66	2.75	2.40
3	2.25	3.40	3.80	3.85	3.32
4	2.36	1.76	1.78	1.50	1.85
Means (mg)	1.57	1.91	2.06	2.02	
$LSD_{0.05}$ 2,4-D con.: 0.09, BA con.: 0.09, Interactions: 0.18					

to 1.85 mg compared with the control treatment, which did not record any response. The results further indicated significant differences in the average callus weight based on the BA concentrations added to the medium. The BA concentration of 0.2 mg L^{-1} was remarkably superior in achieving the maximum rate (2.06 mg), which also did not significantly differ from the BA (0.4 mg L^{-1}) at 2.02 mg. The control treatment obtained the lowest rate (1.57 mg). The interaction effects between 2,4-D (2 mg L^{-1}) and BA (0.4 mg L^{-1}) provided the topmost fresh weight of callus (3.85 mg), while the control treatments for auxin and cytokinins have not shown any response.

2,4-D and BA effect on the dry weight

The outcomes exhibited significant differences in the dry weight rate of callus depending on the 2,4-D concentrations added to the nutrient medium in common grape vine (Table 5). The 2,4-D concentration (2 mg L^{-1}) appeared superior and gave the highest dry weight of callus (1.03 mg), which substantially varied from the rest of the treatments. However, the said response decreased (0.45 mg) with an increased auxin concentration (3 mg L^{-1}) compared with the control treatment not displaying any response. The results indicated significant differences in the average dry weight of callus based on the BA concentrations added to the nutrient medium. The BA concentration (0.2 mg L^{-1}) proved considerably superior in achieving the highest rate (0.61 mg), which also did not differ remarkably from the BA (0.4 mg L^{-1}) with a value of 0.59 mg compared to the control treatment with the lowest rate (0.47 mg). The interaction effects between the auxin and cytokinin concentrations on the average dry weight of callus revealed that 2,4-D (2 mg L^{-1}) and BA (0.4 mg L^{-1}) attained the highest rate (1.18 mg) versus the control treatments, which showed no response.

2,4-D and BA effect on the buds fresh weight

The 2,4-D concentrations revealed significant differences by adding to the nutrient medium prepared for inducing callus from grape buds in *V. vinifera* L. (Table 6). The 2,4-D concentration (3 mg L^{-1}) showed the highest average fresh weight (1.30 mg), while other concentrations and the control treatment showed no response. The results further indicated notable variations in the average callus weight based on BA concentrations added to the food medium. The BA concentration (0.4 mg L^{-1}) was significantly superior in achieving the highest bud weight (0.43 mg), which also did not vary relevantly from the BA concentration (0.2 mg L^{-1}) with a bud weight of 0.38 mg compared with the control treatment, acquiring the lowest bud weight (0.16 mg). According to the effect of the interaction between the concentrations of auxin and cytokinins on the average fresh weight of callus, the 2,4-D (2 mg L^{-1}) and BA (0.4 mg L^{-1}) obtained the topmost rate (1.75 mg), while 2,4-D with higher concentrations showed no response.

Table 5. Effect of 2,4-D and BA concentrations on the dry weight of callus induced from the tip shoot after six weeks of cultivation on the MS medium.

2,4-D con. (mg L^{-1})	BA con. (mg L^{-1})				Means (mg)
	0	0.1	0.2	0.4	
1	0	0	0	0	0
2	0.40	0.48	0.88	0.96	0.68
3	0.71	1.05	1.17	1.18	1.03
4	0.78	0.40	0.40	0.23	0.45
Means (mg)	0.47	0.48	0.61	0.59	
$LSD_{0.05}$ 2,4-D con.: 0.21, BA con.: 0.21, Interactions: 0.43					

Table 6. Effect of 2,4-D and BA concentrations on the fresh weight of callus induced from buds after six weeks of planting on the MS medium.

2,4-D con. (mg L^{-1})	BA con. (mg L^{-1})				Means (mg)
	0	0.1	0.2	0.4	
1	0	0	0	0	0
2	0	0	0	0	0
3	0.65	1.30	1.53	1.75	1.30
4	0	0	0	0	0
Means (mg)	0.16	0.32	0.38	0.43	
$LSD_{0.05}$ 2,4-D con: 0.05, BA con.: 0.05, Interactions: 0.11					

Table 7. Effect of 2,4-D and BA concentrations on the dry weight of callus induced from buds after six weeks of planting on the MS medium.

2,4-D con. (mg L^{-1})	BA con. (mg L^{-1})				Means (mg)
	0	0.1	0.2	0.4	
1	0	0	0	0	0
2	0	0	0	0	0
3	0.09	0.18	0.25	0.40	0.23
4	0	0	0	0	0
Means (mg)	0.02	0.02	0.02	0.02	
$LSD_{0.05}$ 2,4-D con.: 0.06, BA con.: 0.06, Interactions: 0.11					

2,4-D and BA effect on the buds dry weight

The results revealed a significant effect of 2,4-D concentration (2 mg L^{-1}) with the average dry weight of callus (0.23 mg), while the rest of the concentrations and the control treatment did not exhibit any response in the grape vine (*V. vinifera* L.) (Table 7). The effect of the BA concentration (0.4 mg L^{-1}) was significantly superior in achieving the highest dry weight of callus (0.10 mg) but did not vary significantly from the BA concentration (0.2 mg L^{-1}) with a value of 0.06 mg compared to the control treatment with the lowest bud dry weight (0.02 mg). The interaction of 2,4-D (2 mg L^{-1}) and BA (0.4 mg L^{-1}) achieved the premier rate (0.40 mg), while the 2,4-D with concentrations of 1 and 3 mg L^{-1} and the control treatment gave no response.

From this, one can infer that the growing apex is the best part for callus induction. The reason may be because its cells were active meristematic cells with a high content of auxin, of which, the spread and distribution throughout the plant was through its transfer from the meristematic areas. Therefore, the auxin concentrations constantly decreased as moving away from the growing apex. The growing apex showed superiority over other plant parts in inducing callus.

The process of callus generation on the plant cutting is manifesting that the plant piece undergoes certain changes when creating callus, such as a change in size and composition. Additionally, an increase in structural processes arises, i.e., the construction of proteins and nucleic acids. In the stimulation phase, the fundamental variations occur in the cells to prepare them for the division process. Structural processes happen in it, such as protein construction and DNA replication. The duration of completion of this stage depends mainly on several factors, including the type of tissue in the plant piece, the medium, and environmental conditions. This stage has a successive change following in the vital activities of these cells, ending with their division and the formation of a mass of callus cells that cover most parts of the plant piece (Huda *et al*., 2020). The reason for inducing callus on the surface of the plant piece may be attributable to the positive role of the growth regulator 2,4-D. It encourages the formation of callus and increased growth, as it is one of the auxins with an important role in the formation and growth of callus.

Increasing the concentration leads to the formation of callus; however, by surpassing the optimum level, it leads to adverse effects in grapevine (*V. vinifera* L.). Adding growth regulators to the nutrient medium stimulates the continued division of the callus tissue, and the cellular tissue, after being grown on the nutrient medium, will be able to establish an internal hormonal system. This system determines the direction of subsequent development by interacting with the growth regulators added to the medium, vital in maintaining the continuity of cell division (Cavallaro *et al*., 2022). Auxins in general are necessary for the induction of callus tissue from plant parts grown outside the body. The treatment with auxin affects the physiological state of the cells and changes the pattern of differentiation in the cells responding to it. It was evident that treatment with auxin made the cells differentiate for plant parts. It suffers from dedifferentiation and accelerates division to form callus tissue (Hesami and Jones, 2020). The reason for these effects (increasing the fresh and dry weight of callus) may be due to cytokinins, especially benzyl adenine (BA), encouraging the attraction of nutrients to the cells treated with them and stimulating cell division. Similarly, they impede protein catabolism, whose effects manifest in encouraging the division process, especially when reaching the ideal state of balance. It also leads to an increase in the construction of RNA, proteins, and enzymes inside the cell (Al-Rifai and AL-Shoubaki, 2002).

Past research also indicated the increase in the fresh and dry weights of the callus is the reflection of changes in various cell contents of cultivated plant parts, depending on its growth in the nutrient medium used. The process of callus cell division is simultaneous with an increase in the important contents to sustain cell division and growth, such as carbohydrates, proteins, and amino acids, with internal changes leading to cell division (Kadhim *et al.,* 2020). The failure of callus formation at some concentrations and in the control treatment may be because the hormones were low in said plant parts and did not help the continuation of cell division and callus production. The BA indicated that a state of hormonal balance was successful by adding the growth regulators and the internal hormones found within the plant parts. The growth regulator helped initiate cell division in the area where the plant part comes into contact with the nutrient medium, especially the external ones, and with the availability of nutrients and oxygen that aid in the respiration process. It also provided the energy necessary for the division process, as these were the main factors for the formation of callus (Park *et al.,* 2020).

The darkness prevents the oxidation of some light-sensitive compounds, such as auxins, as light activates the oxidase-IAA enzyme. Moreover, the incubation in the dark may lead to inhibition of the oxidation of phenolic substances by oxidation enzymes stimulated by light. Many researchers have also pointed out the relevance of incubation in darkness (Nazir *et al.,* 2020). It was also a belief that incubating plant parts in the dark leads to an increase in the thinness and thickness of cell walls. Consequently, it causes an increase in the permeability of substances, especially growth regulators, into the transplanted tissues and then the response of these parts to callus induction. These results were also consistent with past findings on studying the influence of explants and growth regulators in the induction of callus in plants (Hamad and Majid, 2017; Almukhtar, 2017, 2018).

CONCLUSIONS

The results enunciated using the sodium hypochlorite in low concentrations for a shorter time achieves the highest rate of sterilization of the vegetative parts without affecting their vitality in the common grape vine (*Vitis vinifera* L.). The growing apex was evidently the best plant part for inducing callus, and adding an appropriate combination of growth regulators helped obtain the best results for callus induction.

REFERENCES

Almukhtar SA (2017). Effect of growth regulators and irradiation and methyl jasmonate in the induction and production of flavonoids in callus of *Vitis vinifera* in vitro. *J. Global Pharmacy Technol*. 12(09): 446-45.

Almukhtar SA (2018). Influence of polyethylene glycol and tyrosine on morphine alkaloids production from callus of *Papaver somniferum* in vitro. *J. Pharm. Sci. Res.* 10(2): 282-287.

Al-Rifai ART, Al-Shoubaki SAR (2002). 21st Century Techniques for Plant Improvement Using Tissue Culture. Dar Al-Fikr Al-Arabi for Printing and Publishing, First Edition, Cairo, Egypt. pp. 358.

Al-Sahuki M, Waheeb KA (1990). Applications in Designing and Analyzing Experiments. Ministry of Higher Education and Scientific Research - Iraq. pp. 400.

Andrushia AD, Neebha TM, Patricia AT, Sagayam KM, Pramanik S (2024). Capsule network-based disease classification for *Vitis vinifera* leaves. *Neural Comput. Appl*. 36(2): 757-772.

Cavallaro V, Pellegrino A, Muleo R, Forgione I (2022). Light and plant growth regulators on in vitro proliferation. *Plants* 11(7): 1-12.

Fahmy FJM (2003). Plant tissue culture. Dar Al-Kutub Al-Ilmiyyah for Publishing and Distribution, Faculty of Agriculture, Assiut University, Cairo, Egypt. pp. 2.

Hamad MS, Majid NB (2017). Influence of explants and growth regulators in the induction of callus of *Annona muricata* in vitro. *Euphrates J. Agric. Sci*. 9(4): 1-12.

Hesami M, Jones AMP (2020). Application of artificial intelligence models and optimization algorithms in plant cell and tissue culture. *Appl. Microbiol. Biotechnol.* 104(22): 9449-9485.

Huda MF, Indriyani S, Widoretno W (2020). Effect of benzyl adenine concentration on callus induction of geranium plants (*Pelargonium graveolens* L'Her) from petiole and leaf explants. *J. Exp. Life Sci.* 10(1): 20-23.

Hudson TS, Hartle DK, Hursting SD, Nunez NP, Wang TTY, Young HA, Arany P, Green JE (2007). Inhibition of prostate cancer growth by Muscadine grape skin extract and resveratrol through distinct mechanisms. *Cancer Res*. 67(17): 8396- 8405.

Kadhim ZK, Abdulhussein MA (2021). Minimal media strength for in vitro conservation of strawberry (*Fragaria ananassa*) cultures. *Basrah J. Agric. Sci*. 34(2): 1-9.

Kadhim ZK, Nayyef MN, Awadh HAA, Jaafar HM, Abdulhussein MAA (2020). Impact of plant growth regulators and adenine sulfate on *Gardenia jasminoides* micropropagation. *Plant Arch.* 20(2): 71-75.

Kulus D, Tymoszuk A (2020). Induction of callogenesis, organogenesis, and embryogenesis in non-meristematic explants of bleeding heart and evaluation of chemical diversity of key metabolites from callus. *Int. J. Mol. Sci.* 21(16): 3-10.

Lazo-Javalera MF, Troncoso-Rojas R, Tiznado-Hernández ME, Martínez-Tellez MA, Vargas-Arispuro I, Islas-Osuna MA, Rivera-Domínguez M (2016). Surface disinfection procedure and in vitro regeneration of grapevine (*Vitis vinifera* L.) axillary buds. S*pringer Plus* 5: 3-9.

Martín-Gómez JJ, Rodríguez-Lorenzo JL, Gutiérrez del Pozo D, Cabello Sáez de Santamaría F, Muñoz-Organero G, Tocino Á, Cervantes E (2024). Seed morphological analysis in species of *Vitis* and relatives. *Horticulturae* 10(3): 1-14.

McGovern P, Jalabadze M, Batiuk S, Callahan MP, Smith KE, Hall GR, Lordkipanidze D (2017). Early neolithic wine of Georgia in the South Caucasus. *Proc. Nat. Acad. Sci.* 114(48): 10309-10318.

Naeem NR, Almukhtar SA (2023). Effect of explants type and plant growth regulators on the initiation and multiplication of vegetative shoots of Stevia rebaudiana Bertoni in vitro. In *IOP Conf. Ser: Earth and Environ. Sci*. 1214(1): 2-8.

Nazir M, AsadUllah M, Mumtaz S, Siddiquah A, Shah M, Drouet S, Abbasi BH (2020). Interactive effect of melatonin and UV-C on phenylpropanoid metabolite production and antioxidant potential in callus cultures of purple basil (*Ocimum basilicum* L. var. *purpurascens*). *Molecules* 25(5): 1072.

Ozden M (2024). Secondary metabolite production in callus cultures of *Vitis vinifera*: Influence of genotype and sucrose concentration in the medium on antioxidant activity. *Acta Physiol. Plant.* 46(1): 1-6.

Park JS, Seong ZK, Kim MS, Ha JH, Moon KB, Lee HJ, Kim HS (2020). Production of flavonoids in callus cultures of *Sophora flavescens*. *Plants*. 9(6): 637-688.

Sekhi Y, Kadhim Z, Hamad A (2023). Effect of sodium azide on some vegetative and biochemical properties of strawberry under polyethylene glycol of Albion variety in vitro. *Ann. Agric Biol. Res*. 28(1): 121-128.