Available online at http://bjas.bajas.edu.iq
https://doi.org/10.37077/25200860.2024.37.1.12
College of Agriculture, University of Basrah

Basrah Journal of Agricultural Sciences

ISSN 1814 – 5868 Basrah J. Agric. Sci., 37(1), 149-163, 2024 E-ISSN: 2520-0860

Some Amino Acids Affect the Response of Grape (*Vitis vinifera* L.) Single Nodules *In Vitro* Multiplication

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Received 17th September 2023; Accepted 25th March 2024; Available online 18th May 2024

Abstract: Organic nitrogen, which comes from amino acids, is absorbed and transported more rapidly than inorganic nitrogen by plant cells and tissues. Therefore, this study was conducted to evaluate the effect of some amino acids on the response of single nodes of grape *Vitis vinifera* L. Superior and Red globe cultivars were classified for in *vitro* multiplication to develop an efficient protocol for propagation of these two valuable species. The study included two experiments: first, the effect of adding three concentrations (0, 0.5, and 1) mg L^{-1} of Benzyl Adenine (BA) to the MS medium in the initiation stage. The second investigation utilized three amino acid types (glutamine, asparagine, and methionine) at five concentrations (0, 10, 20, 40, and 80) mg L^{-1} added to MS medium containing 2 mg L^{-1} of BA. Results indicated that the most significant newly initiated shoots (2.40 and 2.88) and the largest leaves number (7.90 and 5.88) for both cultivars, Superior and Red globe, respectively were obtained in a medium with 1 mg L^{-1} of BA. The multiplication results in eight weeks after cultivation showed that adding amino acid glutamine at 10 mg L^{-1} significantly increased the number of shoots/explant (6.60 and 6.00) and (29.50 and 38.30) leaves/explant for Red globe and Superior, respectively. The high amino acid concentrations (40 and 80) mg L^{-1} significantly reduced the studied growth parameters, especially the number of newly formed shoots. The results obtained from the present study exhibited the possibility of applying this modified protocol to the propagation of selected grape cultivars to encourage the expansion of the grape-growing industry in Iraq.

Keywords: Asparagine, Glutamine, Methionine, Micropropagation, Red globe, Superior.

Introduction

Grape *Vitis vinifera* L. belongs to the Vitaceae family, Rhamnales order, and Vitis genus. It is classified among European grapes and under the type *V. vinifera* subsp. *Sativea* includes all cultivated grape varieties and spreads globally or locally in any production area. The source of these varieties is direct selection from wild grapes, the subsequent crossing of varieties with each other, or as a result of mutations (Reynolds, 2017). It is a perennial plant that grows in the tropics, subtropics, and temperate regions (Al-Saeedi, 1982). The Superior variety is considered one of the very early varieties. The cluster is medium in size, complete with wings, and its shape is short and compressed. The bean is large, round, tending to oval, greenish-white in color when ripe, without seeds, and has a chewy texture. It has a distinct taste and a light muscat flavor. It ripens in early July. Grape rearing is usually by reed thrones. To obtain good growth for grape vines, 12-14 eyes and 4-6 regenerative rotors are left on 6-8 stalks for rearing on cubes or by the Y method (Al-Saeedi, 1982).

Red globe was produced from a cross between Noceea, Hunisa, and Emperador. Its vines are not vigorous, medium to late maturing. It ripens in mid-September. Its kernels contain 3-5 seeds; the cluster is large, complete, and conical in shape (1 kg), and the seeds are medium density, with a long cluster holder (Al-Saeedi, 1982).

The bean is enormous, round, pinkish-red in color, has a sweet taste, and has stable acidity when stored. It is resistant to transportation and storage conditions. Furthermore, the Red globe cultivar is suitable for the Kardon breeding method by leaving 12-14 stems of two eyes in length, and the basal shoots are very fertile. Plants are densely growing with high yields that need summer pruning to improve, it is characterized by its high commercial value (Al-Saeedi, 1982).

Grapes are often propagated traditionally by vegetative methods, such as cuttings, layering, or grafting. However, such propagated plants commonly carry disease-causing agents [microbes, mites, insects, nematodes, fungi, bacteria, viruses especially the Phylloxera insect] (Alizadeh *et al*., 2010.). Moreover, the vegetative methods, are time-consuming, seasonally restricted, stressful for the plant, and limited produced plants (Hartmann *et al*, 2011). On the other hand, *in vitro* propagation technique provides several advantages such as the possibility of large-scale production within a short period at low production cost for the desired genotypes (Rathore *et al*., 2005). Moreover, *in vitro* propagation allows for high quality plant production, free of diseases and pathogens in a limited space without being restricted to the season (Melyan *et al*., 2015). Numerous studies on grapes micropropagation have been conducted over the past years on selected genotypes of the genus Vitis using the cultivation of shoot apices, axillary shoots, or adventitious bud formation (Kizi *et al*., 2022; Sammona, 2022;). Although most plants can synthesize their essential amino acid requirements for cell reproduction and regeneration, adding amino acids to culture media is essential in cell growth and tissue formation (Hamdeni *et al*., 2022). Plant cells and tissues can absorb and transport organic nitrogen in amino acids more easily and rapidly than inorganic sources (Yang *et al*., 2020).

Numerous studies have reported using amino acids as a source of organic nitrogen during *in vitro* propagation of many plant species such as orange, strawberry Chandler variety, Asian rice, rose, papaya, and hibiscus plants to promote the growth of plant tissues and increase their proliferation (Siwach *et al*., 2012; El-Sharabasy *et al*., 2015; Pawar *et al*., 2015; Akhtar *et al*., 2016; Greenwell & Ruter, 2018). Alzubi *et al*., (2012) reported that *in vitro* propagation efficiency of *Vitis spp*. vary significantly based on used plant growth regulator concentration and the genotype of the crop. Earlier research has not considered the amino acids' impact on grape growth despite their importance in the regeneration of propagated plants *in vitro*. Amino acids represent a significant source of organic nitrogen (Lehmann & Ratajczak, 2008), and their role in proteins and enzymes synthesis that are major in controlling cell differentiation

(Miflin & Habash, 2002). Due to the lack of studies on the effect of different amino acid types and their optimal concentrations on the propagation of grape plants in general and the two varieties Superior and red globe in particular, this study aimed to investigate the effect of adding different amino acids types at various concentrations on grapes *in vitro* single nodes growth and multiplication.

Materials & Methods

The study was conducted in the plant cell and tissue culture laboratory, Department of Horticulture, Agricultural Sciences Engineering College, Dohuk University, Kurdistan Region, Iraq. Single nodes of Superior and Red globe Grapevines varieties planted in a private orchard in Bagirat, north of Duhok (Longitude 43.09E, latitude 36.57N, and an altitude of 881 meters above sea level), were selected as explants.

Nutrient media preparation

The nutritional medium MS (Murashige & Skoog, 1962) (prepared by Caisson LTD, U.S.A.) was used in all study stages. To prepare 1 liter of MS, 4.43 grams of ready-made medium salts were dissolved in 100 ml of distilled water, 7 grams of agar for medium hardening, and 30 grams of sucrose were added to the medium. The pH has been adjusted to 5.75. The solution was dispensed at 20–25 ml in 200-ml glass vessels and closed with aluminum foil. The glass vessels were sterilized in an autoclave for 20 minutes at 121 °C and 1.04 bar.

Surface sterilization and culture establishment stage

Apical shoots growing in spring, containing 5-7 buds, were placed under running tap water for one hour to remove dirt and dust. Under aseptic conditions inside the laminar, the sterilization process was carried out. The plant parts were immersed in 96.6% ethyl alcohol for 10 seconds and then immersed for 10 minutes in a 20% commercial bleach solution containing 5.25% sodium hypochlorite (NaOCl). The explants were rinsed three times in sterilized distilled water to remove the harmful effect of the disinfectant material. After The damaged shoot ends were removed, the aseptic parts were cut into nodal segments approximately 1 cm long, containing at least one bud, the single sterilized nodules were cultured on a full-strength MS basal medium (Murashige & Skoog, 1962) containing 0, 0.5, and 1 mg L^{-1} Benzyl Adenine (BA).

Multiplication stage

Amino acids affect shoot multiplication

At this stage, five concentrations (0, 10, 20, 40, and 80) mg.L^{-1} of three amino acid types (glutamine, asparagine, and methionine) were added to MS basal medium containing 2 mg L^{-1} BA. Eight weeks later the measurements were taken.

Rooting stage:

The shoots of 1.5- 2 cm in length from the two cultivars, Red globe and Superior, were planted for 4 weeks on MS medium with a half concentration of media salts, 1 mg L^{-1} of Indole-3- Acetic Acid (IBA) at this stage (Fig. 1, C1 and C2).

Acclimatization stage:

Plantlets lengths of approximately 3 cm were taken out from vessels, and their roots were washed with running water to remove the remnants of agar. Then, the rooted plants were planted on a sterilized culture medium containing a mixture of 1:1:1 (V/V/V) peat moss, perlite, and sand. Then, the seedlings were covered with polyethylene to maintain the humidity. It was gradually raised until completely lifted after 10 days and then successfully transferred to the greenhouse (Fig. 1, D1 and D2).

Cultivation conditions & Experiment design

The cultures were incubated during all study stages under a light intensity of 2500-3000 lux, for 16 hours of cold white light period supplied by fluorescent lamps, 8 hours of darkness, and 1 ± 23℃ temperature, according to the required period for each experiment. The experiment was carried out as a factorial trial in a completely randomized design (CRD), and the comparison between the means was performed according to Duncan's multiple range test at a test level ($p < 0.05$).

Results

Establishment response percentage, average number of shoots/explants, and average number of leaves/explants

The presented result in table (1) (Fig. 1, A1 and A2) after four weeks of explants cultivation on MS medium indicated no significant differences in response to growth for the two cultivars. At the same time, there was an increase in the average number of formed shoots on the explants with BA concentrations increased. Media containing 1 mg L^{-1} of BA recorded an increase in shoots' number, reaching 2.55 shoots/explant, compared to 0.0 mg L^{-1} and 0.5 mg L^{-1} which gives 1.15 and 1.33 shoots/explant, respectively. It was also observed that cultivar has a significant effect on this trait, as the Red globe cultivar recorded an increase in shoot number of 2.10 bud/explant compared to the Superior cultivar which recorded 1.43 shoots explant^{-1}.

As for the interaction effect between the BA concentrations and the cultivars, both cultivars recorded the highest values (2.88 and 2.40 bud/ explant), respectively. Table (1) also shows the number of formed leaves on the explants which scored a significant increase on explants that grown in 1 mg L^{-1} BA-media 6.80 leaves/ explant and 0.5 mg L^{-1} BA-media 4.90 leaves/ explant compared to 0 mg L^{-1} BA -media 2.20 leaves/ explant. While the variety had no significant effect on this trait. The overlapping treatments of the cultivar and BA concentrations, the highest values for the average number of leaves (7.90 and 5.88 leaves/ explant) were recorded of both cultivars Superior and Red globe with 1 mg L^{-1}, respectively compared to both cultivars with 0 mg L^{-1}, of BA, which recorded the lowest values (2.00 and 2.40 leaf/ explant, respectively).

Table (1): The effect of different concentrations of BA on the percentage of viable parts responsive to growth, shoot number, and the leaves number of grape cultivars Superior and Red globe during the initiation stage after four weeks of cultivation on MS medium.

BA con. Mg L^{-1}.	(%) Response			mean number of shoots/ explants			mean number of leaves/ explants		
	Superior	Red globe	Con. effect	Superior	Red globe	Con. effect	Superior	Red globe	Con. effect
0.00	0.90a	1.00a	0.95a	0.70d	1.60b-d	1.15b	2.00c	2.40c	2.20b
0.5	0.80a	1.00a	0.90a	1.20cd	1.90 a-c	1.33b	5.80ab	4.00bc	4.90a
1.0	0.90a	1.00a	0.95 a	2.40ab	2.88 a	2.55a	7.90a	5.88ab	6.80a
Cultivars effect	86.6b	100 a		1.43 b	2.10 a		5.23 a	4.03 a	

* Numbers with similar letters are not significantly different within each factor and interaction according to Dunkin's multiple range test at the 5% probability level.

The effect of amino acids on shoot multiplication:

Shoot number (shoots/explant)

The results presented in table (2) (Fig. 1, B1 and B2) eight weeks after the cultivation of Superior and Red globe nodal segments on the studied medium indicated no significant difference between the two cultivars in a newly formed shoot number. on the other hand, the amino acid type had a significant effect on this trait, explants that grown on media equipped with the amino acid glutamine recorded the highest values (3.80) compared to explants that grown in media equipped with asparagine (3.45), which outperformed on explants that grown in methionine-containing media. Also, amino acid concentrations showed significant effects in shoot numbers, as a 10 mg L^{-1} BA-medium was Superior to all other study concentrations, with an average shoot number of 4.35 shoot/explant. In contrast, the 20 mg L^{-1} concentration recorded a 3.75 shoot/explant value, which did not differ significantly from the value (3.80) of the control.

For the combination between the cultivars and amino acid types, it is clear from the table that Red globe explants recorded the biggest shoot number (3.90) when planted on media equipped with glutamine. Similarly, Superior explants that grew on the same media gave 3.70 shoots/explants. In contrast, the lowest number of shoots (2.00) was recorded by the Red globe cultivar with methionine amino acid. For the effect of the combination between cultivars and amino acid concentration, both cultivars with 10 mg L^{-1} recorded the highest number of shoots (4.26 and 4.43 shoot/explant, respectively) with significant differences. Still, they were significantly superior to all other studied interactions. In contrast, the two cultivars (Superior and Red globe) with 80 mg L^{-1} of amino acids concentration recorded the least shoot numbers (1.33 and 1.16, respectively). The interaction of the amino acid type with the concentration significantly affected the shoots formed number, as glutamine interacted with a concentration of 10 mg L^{-1} recorded the highest value (6.30) for this trait, with clear significant superiority over all other study interactions. In contrast, the rest of the interaction treatments decreased significantly compared to the control, especially those in which the amino acid methionine was utilized in all its concentrations, as well as glutamine or asparagine at high concentrations (40 and 80 mg L^{-1}).

For the combination of three study factors, the data in the same table indicate that both cultivars explant (Superior and Red globe) were grown on media containing the amino acid glutamine at a concentration of 10 mg L^{-1} gave the most significant values (6.00 and 6.60 shoot/explant, respectively). Followed by the treatment of Red globe with the amino acid glutamine at a concentration of 20 mg L^{-1}, recorded 5 shoots/explant and Superior variety with the amino acid asparagine at a concentration of 20 mg L^{-1}, which achieved an average shoot number of 4.80 shoots/explant.

Table (2): Effect of cultivars, amino acids type, concentration, and their interaction on the **number of new shoots/explants formed on the grape explant in the multiplication stage eight weeks after cultivation on the MS medium.**

cultivars	amino acid type	amino acid con. Mg L^{-1}.					Cultivars + amino acid	amino acid	Cultivars
		00	10	20	40	80			
Superior	glutamine	3.90 d-f	6.00 a	4.00 d-f	3.00 gh	1.60 jk	3.70 ab		3.14 a
	Asparagine	3.90 d-f	4.10 d-f	4.80 bc	3.10 gh	1.40 jk	3.46 b		
	methionine	3.90 d-f	2.70 h	2.40 hi	1.40 jk	1.00 k	2.28 c		
Red globe	glutamine	3.70 e-g	6.60 a	5.00 b	3.10 gh	1.10 jk	3.90 a		3.11 a
	Asparagine	3.70 e-g	4.20 c-e	4.50 b-d	3.40 fg	1.40 jk	3.44 b		
	methionine	3.70 e-g	2.50 h	1.80 ij	1.00 k	1.00 k	2.00 d		
Cultivars + con.	Superior	3.90 b	4.26 a	3.73 b	2.50 c	1.33 d			
	Red globe	3.70 b	4.43 a	3.76 b	2.50 c	1.16 d			
amino acid + con.	glutamine	3.80 d	6.30 a	4.50 bc	3.05 e	1.35 h		3.80 a	
	Asparagine	3.80 d	4.15 cd	4.65 b	3.25 e	1.40 h		3.45 b	
	methionine	3.80 d	2.60 f	2.10 g	1.20 h	1.00 h		2.14 c	
Concentration-effect		3.80 b	4.35 a	3.75 b	2.50 c	1.25 d			

* Numbers with similar letters are not significantly different within each factor and interaction according to Dunkin's multiple range test at the 5% probability level.

Shoots length (cm)

The data in table (3) shows that the cultivars had no significant effect on the average length of newly formed shoots. In contrast, the amino acid type had a significant effect in this trait, explants that grew on asparagine --media recorded an average length (2.94 cm) compared to explants that grew on glutamine and methionine media which reached 2.59 and 1.88 cm, respectively. On the other hand, amino acid concentration has no impact on shoot length of explants.

As for the interaction effect of cultivar and amino acid type in this trait, the results showed that the interaction of each two cultivars (Superior and Red globe) with asparagine recorded the highest values (2.95 and 2.93 cm, respectively). While the Superior variety with the amino acid methionine recorded the lowest values (1.80 cm). None of the amino acids' concentrations added to the culture media caused an increase in the average shoot lengths of both cultivars. The lowest shoot lengths (1.82

cm) were recorded in the Superior variety with a concentration of 80 mg L^{-1}. The control treatments were better for all investigated interactions, and the interactions of amino acid type and concentrations exhibited the same behavior as the preceding interaction in terms of their inability to elongate the shoots.

The combination of three agents, cultivar, amino acids type, and their concentrations do not affect shoot length. The treatments in which the Superior variety explants grown on devoid media of amino acids recorded the highest values (3.84 cm) and were significantly superior to all studied interaction treatments, except for the interaction treatment of the Red globe variety with asparagine at 40 mg L^{-1} concentration which recorded 3.53 cm.

Table (3): Effect of cultivar, type, and concentration of amino acids and their interaction on the average lengths of new shoots formed on the grape explant in the multiplication stage eight weeks after cultivation on the MS medium.

cultivars	amino acid type	amino acid con. Mg L^{-1}.					Cultivars + amino acid	amino acid	cultivars
		00	10	20	40	80			
Superior	glutamine	3.84 a	2.52 f-h	2.36 g-j	2.78 d-f	1.99 j-l	2.69 b		2.48 a
	Asparagine	3.84 a	3.20 bc	3.11 cd	2.38 g-i	2.26 h-j	2.95 a		
	methionine	3.84 a	1.43 mn	1.28 n	1.25 n	1.21 n	1.80 e		
Red globe	glutamine	3.01 c-e	1.75 k-m	2.70 e-g	2.98 c-e	2.05 i-k	2.49 c		2.46 a
	Asparagine	3.01 c-e	2.71 e-g	2.80 d-f	3.53 ab	2.64 e-g	2.93 a		
	methionine	3.01 c-e	1.67 lm	2.03 i- l	1.56 mn	1.52 mn	1.95 d		
Cultivars + con.	Superior	3.84 a	2.38 de	2.25 ef	2.13 f	1.82 g			
	Red globe	3.01 b	2.04 f	2.51 cd	2.69 c	2.07 f			
amino acid + con.	glutamine	3.42 a	2.31 d	2.53 c	2.88 b	2.02 d		2.59 b	
	Asparagine	3.42 a	2.95 b	2.95 b	2.95 b	2.45 c		2.94 a	
	methionine	3.42 a	1.55 of	1.65 e	1.40 f	1.36 f		1.88 c	
concentration effect		3.42 a	2.21 c	2.38 b	2.41 b	1.94 d			

* Numbers with similar letters are not significantly different within each factor and interaction according to Dunkin's multiple range test at the 5% probability level.

Leaves number (leaf.explant^{-1})

Data in table (4) eight weeks after explants planting indicate that the Red globe variety was significantly superior to the Superior variety in the number of leaves (15.88 and 14.20, respectively). As for the effect of amino acid type, a clear significant superiority is noted for the amino acid glutamine (19.68 leaves) over asparagine (16.88 leaves), which was significantly superior on amino acid methionine (8.58 leaves). The concentrations of amino acids showed a significant effect in this characteristic, especially in 10 mg L^{-1}, which achieved 21.16 leaves.explant^{-1} and was significantly superior over the rest studied concentrations.

Table (4): Effect of cultivar, type, and concentration of amino acids and their interaction on the average number of leaves/explants formed on the grape explants in the multiplication stage eight weeks after cultivation on the MS medium.

cultivars	amino acid type	amino acid con. Mg L^{-1}.					Cultivars + amino acid	amino acid	cultivars
		00	10	20	40	80			
Superior	glutamine	16.50 fg	29.50 b	18.30 of	17.40 fg	8.60 hi	18.06 b		
	Asparagine	16.50 fg	18.40 ef	21.00 de	14.80 g	9.00 hi	15.94 c		14.20 b
	methionine	16.50 fg	10.10 h	9.10 hi	3.50 l	3.90 kl	8.62 d		
Red globe	glutamine	16.80 fg	38.30 a	25.60 c	19.70 ef	6.10 i-l	21.30 a		
	Asparagine	16.80 fg	21.20 de	23.80 cd	19.10 ef	8.20 h-j	17.82 b		15.88 a
	methionine	16.80 fg	9.50 h	7.00 h-k	5.10 j-l	4.30 kl	8.54 d		
Cultivars + con.	Superior	16.50 c	19.33 b	16.13 cd	11.90 e	7.16 f			
	Red globe	16.80 c	23.00 a	18.80 b	14.63 d	6.20 f			
amino acid + con.	glutamine	16.65 d	33.90 a	21.95 b	18.55 cd	7.35 f		19.68 a	
	Asparagine	16.65 d	19.80 c	22.40 b	16.95 d	8.60 ef		16.88 b	
	methionine	16.65 d	9.80 e	8.05 ef	4.30 g	4.10 g		8.58 c	
concentration effect		16.65 b	21.16 a	17.46 b	13.26 c	6.68 d			

* Numbers with similar letters are not significantly different within each factor and interaction according to Dunkin's multiple range test at the 5% probability level.

As for the interaction effect between cultivars and amino acid types, the largest leaf number (21.30) was achieved in the interaction of the Red globe variety with glutamine. As it is noted from the same table that the interaction of variety and amino acid concentrations had a significant effect on this characteristic, so when Red globe explants were planted in a medium containing 10 mg L-1 amino said, it recorded an average leaves number of 23.00, followed by interaction of the Superior variety with the same concentration, which gave a leaves number of 19.33, outperforming the rest of treatments except interaction treatment of the Red globe with a concentration of 20 mg L-1. Amino acid glutamine at a concentration of 10 mg L-1 was recorded as the largest leaf number (33.90), significantly superior to all other studied interaction treatments.

As for the triple interaction effect between cultivars, amino acid type, and their concentrations on leaves number, Red globe explants that grown on nutrients media equipped with the amino acid glutamine at a concentration of 10 mg L-1 achieved the highest value (38.30 leaves) with a significant and clear superiority over all other studied triple interaction treatments

Fig. (1): A1, Red globe A2, superior explant in the initiation stage. B1, Red globe B2, superior explant grown on media containing the amino acid glutamine at a concentration of 10 mg. L^{-1} in the multiplication stage. C1, Red globe C2, superior shoots in the rooting stage. D1, Red globe D2, superior plantlets in the acclimatization stage.

Discussion

Growth characteristics superiority (shoots and leaves number in initiation stage) was significant in most cytokinins enriched medium compared with control (Table 1), and this may be due to the role of cytokinins in regulating the activity of apical meristems, morphogenesis, chloroplast development, and leaf growth (Fay & Throop, 2005) The positive effect of cytokinins used in shoots multiplication comes from their role in stimulating the growth of lateral shoots by eliminating apical dominance and stimulating cell division and cell expansion, in addition, cytokinins have an important role in attracting and accumulating metabolites at lateral shoots and stimulating the transfer of nutrients and other growth materials to start bud growth (George *et al*., 2008). Also, cytokinins prevent the decomposition of chlorophyll and proteins in cells as they work to reduce the activity of the ribonuclease enzyme RNAse, which helps prevent the decomposition of RNA and results in an increase in the production of amino acids (Hönig *et al*., 2018).

The increase in some of the studied traits and the response upon raising the levels of cytokinins in the media can be attributed to the fact that these increases achieved the ideal hormonal balance in the explant to give the optimal response. While, hormonal imbalance occurred in the hormonal balance in the cells of the explants negatively affected the course of vital processes, especially cell division and elongation, and thus decreased the values of the studied traits (Pasternak & Steinmacher, 2024). Our results were consistent with Sammona's (2022) finding in terms of BA effect during the initiation stages when studying the effect of different types of cytokinins (benzel adenine BA, isopentenyl adenine 2ip, kinetin, and thiadazuron TDZ) at 1mg L^{-1} concentration was added to the MS on the response of single nodes of two grape varieties (Superior and Red globe) to multiply *in vitro*. Batukaev *et al*. (2021) investigated the best nutrient medium for the emergence of explants (shoot tips and lateral shoots) of two grape cultivars (Bart & Augustine) *in vitro,* The results showed that amino acids play a vital role in stimulating and developing the largest number of multiplying shoots, they also showed that the type and concentration of amino acid that used significantly stimulated the formation of shoots.

The superiority of growth parameters (shoots and leaves number during the multiplication stage) was significant in some treatments containing amino acids, especially those equipped with glutamine, compared to the control (Tables 2 and 4), this related perhaps to the fact that organic sources of nitrogen could be rapidly assimilated into carbon structure during the metabolism and synthesis of protein, compared to other inorganic nitrogen sources (El-Sharabasy *et al*., 2015). As compared to inorganic sources, organic nitrogen derived from amino acids has the advantage of having lower energy requirements for mobility (Kim & Moon, 2007). Most organisms can exploit ammonia in the amino group of glutamines, which is the direct source of daughter nitrogen for constructing macromolecules and other biological compounds (Sungdae, 2002; Lehmann & Ratajczak, 2008). Vesco *et al*. (2001) indicated that adding asparagine, glutamine, or arginine to the nutrient media during in vitro propagation improved the stimulation of growth and development of somatic embryos of the Brazilian guava plant (Feijoa). Ammonia and glutamine can act as a substitute for nitrogen, although, at high concentrations, they can inhibit the growth (Zhang *et al.*, 1999).

El-Sharapasy *et al.* (2015) studied the effect of different types and concentrations of amino acids on the in vitro propagation of strawberries (*Fragaria X Ananassa* Duch cv. Chandler). The study indicated that amino acids play a vital role in the initiation and development of new shoots, the type, and concentration of amino acids have an essential role in shoot multiplication, and the best number of new shoots/explants formed on strawberry explants was in medium containing 25mg L^{-1}glutamine.

Mandal *et al.* (2021). reported a mixture of amino acids (methionine, glutamic acid, glycine, tryptophan, proline, lysine, arginine, and glutamine) at concentrations of 20 mg L^{-1} resulted in the best propagation response (100%) of *Aegle marmelos* (quince Bengal) with an average shoot number of (2.22) when grown on MS medium supplemented with 2mg L^{-1} of BAP.

Our results are consistent with the Samiei *et al*. (2021) result which indicated that the addition of 600 mg L^{-1} of casein hydrolysate to Vander Salm (VS) medium resulted in the highest shoots number of branches (4.1) while glutamic acid, at 12 mg L^{-1}, improved shoots and leaves formation in rose plant. Adding glutamine at 30 mg L^{-1} and asparagine at 20 mg L^{-1} improved the multiplication of *Orthosiphon aristatus* explants grown on MS medium supplemented with 1 mg Lmg L^{-1} BAP and 0.5 mg L^{-1} Kinetin (Jayakumar & Ramalingam, 2013).

As for amino acids inhibitory or low growth indicators effect, especially at high concentrations, Yildirim *et al*. (2019) mentioned that the effects of amino acids on inhibiting or stimulating the growth of plant tissue cultures depend on the type and concentration of amino acid and genotypes.

Our results regarding the inhibitory effect of high amino acid concentrations are consistent with what was referenced by Zamir *et al*. (2017) in their study of the effect of zeatin, glutamine, and auxins on the rooting and multiplication of guava plant (*Psidium guajava* L.) cv. Safeda that the increase of glutamine concentration in the culture medium from 250 to 500 mg L^{-1} reduced the formation percentage of new shoots from 25% to 10% with an average shoot number from 2.2 shoot/explants, to 1.1 shoot/explants.

Siwach *et al*. (2012) also mentioned in their study that the effect of adenine sulfate (ADS), glutamine (Gln), and casein hydrolysate (CH) on the multiplication and rooting of the tangerine plant (*Citrus reticulata* Blanco), at high concentrations of glutamine (100 mg L^{-1}) inhibited the reproduction and elongation of shoots.

Conclusion

In vitro plant propagation is crucial to effectively produce plants that are genetically identical to the mother plant; in this study, two cultivars of grapes (*Vitis vinifera* L.) Superior and Red globe were used, to investigate the influence of adding various types and concentrations of amino acids to the MS medium during the multiplication stage of single nodes. Results revealed that the largest number of shoots (2.40 and 2.88) and leaves (7.90 and 5.88) for the Superior and Red globe were achieved in media containing 1 mg L^{-1} in the initiation stage, while the results of the multiplication stage after eight weeks of cultivation showed that the treatment with glutamine at 10 mg.L^{-1} was significantly superior to the rest of the study treatments in terms of the number of shoots/explant (6.60 and 6.00) and the number of leaves/explant (29.50 and 38.30) for the two cultivars Red globe and Superior, respectively, while the amino acids used in high concentrations (40 and 80 mg L^{-1}) caused a significant decrease in the studied growth indicators, especially in terms of the number of newly formed shoots. The micropropagation protocol developed in the present study can be applied to propagate the selected grape accessions on a large scale in programs to expand the grape-growing industry in Iraq.

Acknowledgements

We are so grateful to the College of Agricultural Engineering Sciences Deanship, Dohuk University and the Department of Horticulture for providing the laboratory and all work supplies.

Contributions of Authors

W.S.F., Carried out the experiment data collected, analyzed the results statistically, and references collecting.

A. Z. A. K. B.: Developed the idea and research plan and contributed to writing the manuscript.

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Conflicts of interest

The authors declare that they have no conflict of interests.

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تأثير بعض الاحماض الامينية في استجابة العقد المفردة للعنب (.*Vitis vinifera* L) للتضاعف خارج الجسم الحي (*In vitro*)

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المستخلص: يتم امتصاص ونقل النيتروجين العضوي الذي مصدره الاحماض الامينية بشكل أسرع من النيتروجين الغير عضوي من قبل الخلايا والانسجة النباتية، لذا تم اجراء هذه الدراسة لتقييم تأثير بعض الاحماض الامينة في استجابة العقد المفردة للعنب .*Vitis vinifera* L صنفي سوبيريور ورد كلوب للتضاعف خارج الجسم الحي بهدف تطوير بروتوكول كفوء لإكثار هذين الصنفين القييمين، تضمنت تجربتين: اختبر في الاولى تأثير تراكيز مختلفة (0، 0.5 و1ملغم لتر$^{-1}$) من البنزل ادنين (BA) مضافة الى الوسط الغذائي MS خلال مرحلة النشوء. التجربة الثانية: درس فيها إضافة أنواع مختلفة من الاحماض الامينية (كلوتامين، اسباراجين ومثيونين) وبتراكيز مختلفة (صفر، 10، 20، 40و 80 ملغم لتر$^{-1}$) الى وسط MS حاوي 2 ملغم لتر$^{-1}$ BA. اشارت نتائج مرحلة النشوء ان أكبر عدد للأفرع الجديدة (2.40 و2.88) وأكبر عدد أوراق (7.90 و 5.88) للصنفين سوبيريور ورد كلوب على التوالي تحقق في الوسط الحاوي 1 ملغم لتر$^{-1}$ بينما بينت نتائج مرحلة التضاعف بعد ثمان أسابيع من الزراعة ان المعاملة التي استخدم فيها الحامض الأميني كلوتامين بتركيز 10 ملغم لتر$^{-1}$ تفوق معنويا على باقي معاملات الدراسة في صفتي عدد الافرع/جزء نباتي (6.60و6.00) وعدد الأوراق/جزء نباتي (29.50 و38.30) للصنفين رد كلوب وسوبيريور على التوالي في حين ان الاحماض الامينية المستخدمة بالتراكيز العالية (40 و80 ملغم لتر$^{-1}$) تسببت في حدوث انخفاض معنوي في مؤشرات النمو المدروسة خاصة في صفة عدد الافرع الجديدة المتكونة. يمكن استخدام بروتوكول الاكثار الدقيق الذي تم تطويره في هذه الدراسة لإكثار أصناف مختارة مدخلة من العنب على نطاق واسع في برامج توسيع صناعة زراعة العنب في العراق.

الكلمات الافتتاحية: الاكثار الدقيق، اسباراجين، رد كلوب. سوبيريور، كلوتامين، مثيونين.

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