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EFFECT OF DROUGHT STRESS ON THE METABOLIC PATHWAY AND SURVIVAL OF TWO GRAPE (*Vitis Vinifera* L.) CULTIVARS GROWN UNDER *In Vitro* CONDITIONS

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ABSTRACT

The study aimed to harden two grape cultivars, Superior and Red globe shoots grown *in vitro* by adding different PEG concentrations (0, 5, 10, 20, and 40 g L^{-1}) to the growth medium (MS + 2 mg L^{-1} BA) for inducing *in vitro* drought stress. Biochemical compounds directly impacted by drought were assessed 30 days after planting to determine the modifications of their metabolic pathways to adapt to the drought and shoots survived. Compared to the control treatment, the addition of PEG resulted in a significant increase in the antioxidants produced in the explant (anthocyanin, proline, total sugars, and catalase enzyme) as well as a significant decrease in the explant content of malondialdehyde and chlorophyll. The results also demonstrated that the cultivars differed significantly in all the studied traits. Likewise, the interaction coefficients between the cultivars and the added PEG concentrations achieved significant differences from the control for each cultivar separately. The largest anthocyanin concentration for the Red globe and Superior cultivars (24.46 and 13.30 mg 100 g^{-1}) proline (25.08 and 20.75 mmol g^{-1}), respectively, obtained from shoots growing in media supplied with 20 g l^{-1} PEG While the most significant percentage of total sugars (11.17 and 10.58%), respectively, were obtained from shoots grown in media content 40 gm L-1 PEG, While the highest catalase enzyme concentration for the two cultivars Red globe and Superior (9.01 and 8.21 units mg-1) and was obtained from shoots grown in the presence of 40 and 20 g l-1 PEG, respectively. The study reveals that the Red Globe variety is more resilient to droughts than the Superior variety, making it ideal for water-scarce regions.

INTRODUCTION

Abiotic stresses, such as drought, salinity, and heat, caused by climate change and global warming significantly threaten agricultural production, biodiversity, plant genetic resources, and global food safety (Ahuja *et al*., 2010; Alalaf and Fekry, 2020). And as Smith *et al*., (2009) stated that “stressed environments” provide less than optimal conditions for plant growth, thus quantitatively and qualitatively reducing their lifespan and production. Whenever a plant is subjected to environmental stressors, such as drought stress, it produces intracellular reactive oxygen species (ROS), active oxygen derivatives with a lack of electrons in their outer orbits so they are unstable and more effective at triggering biological molecules' oxidation processes (catabolism), like proteins, sugars, lipids, and nucleic acids. This leads to a severe disruption of nutritional metabolism and irreversible functional damage (Abogadallah, 2010). Drought stress occurs when the percentage of water available to the plant decreases. Therefore, it becomes difficult for the plant to absorb moisture (Shashidhar *et al*., 2013), leading to morphological, physiological, chemical, biological, and molecular changes that negatively affect plant growth and productivity (Jiao *et al*., 2023). Wang-xia *et al*. (2003) mention that the spread of drought and salinity in the Mediterranean region could lead to the loss of 50% of the total arable land by the year 2050. On the other hand, Iraq is on the verge of severe climate changes during the short term, taking it to the level of dry areas (Al-Maliki *et al*., 2022; Walid, 2023).

Many methods have been used to improve plant growth under drought conditions, which have proven that the most successful approach is implementing plants hardening programs by exposing them to mild drought stress and selecting the most resistant to obtain drought-resistant cultivars (Bolat *et al*., 2016). Previous studies demonstrated that breeding programs are more effective than other approaches in producing drought-resistant plants because plants are highly adaptive to unfavorable environmental conditions; this is achieved by altering their genetic activity and their functional training to withstand drought by activating specific genes that produce enzymes or antioxidant chemicals beneficial to the plants to resist active oxygen derivatives and lessen or stop the harm they cause to the plant (Pourghayoumi *et al*., 2017).

Viticulture is an important branch of agriculture worldwide (Al-Atrushy and Ibrahim, 2023). Therefore, it is expected to be significantly affected by potential global warming effects and resulting stresses, including drought stress, and thus, significant changes in the physiological activities of the vine and its yield (Marín *et al*., 2021).

Some in *vivo* studies were conducted to determine the effects of drought stress on the physiological activities of grapevines (Sabır, 2016; Tangolar *et al*., 2016; Sucu *et al*., 2018), but they were characterized by taking a long time, requiring a large area, and being greatly affected by the environmental conditions of the region. On the other hand, tissue culture provides better possibilities for determining the effects of drought stress and the extent of plant tolerance to it, away from the field cons mentioned above.

A high molecular weight, water-soluble polyethylene glycol (PEG 6000) has been frequently used to induce *in vitro* drought conditions as a simulation of field drought conditions so that plants tolerance to drought can be assessed *in vitro*, and the most resistant one selected (Lawlor, 1970).

Polyethylene glycol is a safer agent for inducing drought stress *in vitro* than soluble mannitol and other sugars that are commonly used for this purpose because the doses used in the tests have no adverse effects on the plant and do not interfere with other vital processes in the plant (Hohl and Schopfer, 1991; Verslues *et al*., 1998). Furthermore, it does not affect the path of water entry into the cell via the apoplast, so it simulates drought conditions similar to soil drought conditions compared to mannitol and other sugars commonly used previously (Nepomuceno *et al*., 1998).

Many previous studies have been conducted *in vitro* using polyethylene glycol to evaluate the efficiency of some grape cultivars in tolerating water stress conditions (Cui *et al*., 2016; Saygac *et al*., 2019; Alebidi *et al*., 2024). However, no studies are available to evaluate the drought tolerance of the two grape cultivars, Superior and Red Globe, which are valuable and desired locally. Therefore, this study aimed to verify the resistance of these two cultivars to *in vitro* drought stress and study its effect on metabolic pathway changes in the plant by estimating some traits directly related to drought, such as chlorophyll pigment content, anthocyanin pigment, protein, the amino acid proline, total sugars, catalase enzyme activity and malondialdehyde concentration (MDA) in order to harden plants and determine the most effective concentration for hardening and the most tolerant variety to recommend its application in future breeding initiatives aimed at producing drought-tolerant cultivars suitable for the permaculture or water shortage environments that prevail in most of Iraq's regions.

MATERIALS AND METHODS

The study was conducted in the Plant Cell and Tissue Culture Lab / Horticulture Department/ Agricultural Engineering Sciences College / Dohuk University. Superior and Red Globe grape vines were selected from a private vineyard in the Bagirat area, north of Dohuk/Iraq. from 1/4/2023 until 5/9/2023. Newly young shoot tips containing 5-7 buds growing in the spring were collected and placed under running tap water for an hour. Shoots were sterilized under the Laminar by immersing them for ten seconds in 96.6% ethyl alcohol, followed by immersing for 10 minutes in a 20% commercial bleach solution containing 5.25% sodium hypochlorite (NaOCl), and three times in sterile distilled water for 15 minutes each. Once, the shoots were divided into stem segments approximately 1 cm long containing at least one bud (Single node).

The two cultivars' shoots were planted on the MS basal medium (Murashige and Skoog, 1962) enriched with 1 mg L^{-1} benzyl adenine (BA). pH was adjusted to 5.7-5.8 before adding agar. The medium was distributed in (250 ml) vessels and autoclaved at 121°C for 20 minutes. The cultures have been preserved at 23 ± 1°C and 16-h photoperiod, equipped with white fluorescent tubes in a growth chamber. After three weeks, the formed shoots were separated into sections one node size and replanted on fresh media for another three weeks to obtain uniform shoots in length (2-3 cm) and leave number (2-3 leaves $shoot^{-1}$).

The newly formed shoots from the second subculture were planted in fresh MS medium supplemented with 2 mg L^{-1} BA (6-benzyl adenine), 30 g L^{-1} sucrose, 7 g L^{-1} agar, and different levels of polyethylene glycol (0, 10.5, 20 and 40 g L^{-1}) (PEG 6000) as a simulation of inducing water stress conditions *in vitro*. At the end of the stress exposure period (30 days), shoots were removed from the culture medium and gently washed with running tap water to estimate stress-related biochemical indicators. Including the total chlorophyll pigment concentration, mg g^{-1} fresh weight (Knudson *et al*. 1977), anthocyanin pigment (Ranganna, 1986), total protein (Lowyer *et al*., 1951), proline (Bates, 1973). Total sugars (carbohydrates) (Ibrahim, 2010). The activity of the catalase enzyme (Beers and Sizer, 1952). Peroxidation of lipids in plant tissue samples by estimating the amount of malondialdehyde (MDA) (Li *et al*., 2011). Also, the Damaged shoots Percentage (%) 30 and 60 days after exposure to drought conditions was measured.

Statistical analysis

The experiment was conducted with completely randomized design (CRD). The means were then compared using Duncan's multiple range test at the 0.05 confidence level for all parameters studied (Al-Rawi and Khalfallah, 1980). SAS (V.9) computer software was used for statistical analysis.

RESULTS AND DISCUSSION

Total chlorophyll pigment concentration (mg 100g^{-1} Fresh Weight)

The results in Table (1) After 30 days of two grape cultivars, Superior and Rose Globe shoots, planted in MS medium prepared with different PEG concentrations showed a significant difference in the total chlorophyll content in both cultivar's shoots, as well as between the studied PEG concentration. The Superior cultivar outperformed the red globe cultivar in its total chlorophyll content (0.28 and 0.15) mg.100g^{-1} Fw, respectively, while the control treatment (PEG-free) significantly outperformed the rest of the treatments. It was noted that adding PEG led to a significant decrease in the shoot's chlorophyll content.

As for the interaction of cultivar with PEG concentrations, an apparent decrease was recorded in the total chlorophyll shoot content as a comparison with the control treatment, which recorded high values (0.60 and 0.32 mg 100 g^{-1} FW, respectively) for both cultivars (Superior and Red globe), while the lowest values were recorded for the two interference treatments with 40 mg L^{-1} (0.08 and 0.06 mg. 100 g^{-1} FW, respectively).

Table (1): Effect of cultivar, PEG concentration, and their interaction in total chlorophyll content in explant 30 days after exposure to drought conditions.

Cultivars	Total chlorophyll (mg 100 g^{-1} FW)					Effect of cultivars
	PEG concentration g L^{-1}					
	Zero	5	10	20	40	
Superior	0.60 a	0.37 b	0.20 c	0.17 cd	0.08 d	0.28 a
Red globe	0.32 b	0.15 cd	0.12 cd	0.09 d	0.06 d	0.15 b
Concentration effect	0.46 a	0.26 b	0.16 c	0.13 cd	0.069 d	

*Numbers with similar letters are not significantly different within each factor and interaction according to Duncan's multiple range test at the 0.05 probability level.

Anthocyanin pigment Concentration (mg 100 g^{-1} fresh weight)

The results in Table (2) after 30 days of two grape cultivars, Superior and Rose Globe shoots, planted in MS medium enriched with different PEG concentrations showed the superiority of Red Globe over the Superior cultivar in its shoot's anthocyanin pigment content with an amounted of 17.41 and 10.16 mg 100g^{-1} FW respectively. The results also showed an increase in the explant content of the anthocyanin pigment with the increase of The PEG concentrations from 0 to 20 gm liter^{-1}, and it ranged between (10.31 and 18.88 mg 100 g^{-1} FW, respectively). Then, the value decreased at the high PEG concentration (40 g L^{-1}) to give 13.25 mg 100g $^{-1}$ FW. The results also refer to significant differences between the cultivar interaction treatments with PEG concentrations, as the treatment of the Red globe and the Superior cultivar with 20 g l^{-1} PEG achieved the highest accumulation of anthocyanin pigment, 24.46 and 13.30 mg 100 g^{-1} FW, respectively, with significant superiority over all other studied interference treatments, while the lowest values reached 7.36 mg 100g^{-1} FW obtained from shoots of the Superior cultivar grown on a PEG-free medium (control).

Table (2): Effect of cultivar, PEG, and their interaction on the anthocyanin explant content 30 days after exposure to drought conditions.

Cultivars	Anthocyanin pigment (mg 100 g $^{-1}$ FW)					Effect of cultivars
	PEG concentration g L^{-1}					
	Zero	5	10	20	40	
Superior	7.36 g	9.16 f	11.94 e	13.30 d	9.06 f	10.16 b
Red globe	13.26 d	15.09 c	16.84 b	24.46 a	17.43 b	17.41 a
Concentration effect	10.31 e	12.12d	14.39 b	18.88 a	13.25 c	

* Numbers with similar letters are not significantly different within each factor and interaction according to Duncan's multiple range test at the 0.05 probability level.

Protein percentage (%)

Table (3) shows the effect of adding different PEG concentrations to the medium on the protein percentage shoot content of the Red globe and Superior grape cultivar 30 days after planting. It is noted that the Red globe cultivar is significantly superior in its protein percentage content (0.403%) over the Superior cultivar (0.392%). It is also noted that adding PEG caused a significant reduction in the percentage of protein in the shoots, as the highest values reached (0.424%) and were obtained from the control treatment. While the lowest percentage (0.374%) was obtained from the treatment of adding 40 g L^{-1} PEG.

The interactions of the cultivar with the studied PEG levels also recorded significant differences. Still, none recorded higher values than the comparison treatment for each cultivar individually, noting that the highest percentage of protein reached 0.431%. It was achieved from the interaction treatment of the Red Globe cultivar with no addition of PEG, with significant superiority. On all other interference treatments, it was followed by the interference treatment of the Superior cultivar with no addition of PEG, which amounted to 0.417%. while the lowest values (0.386% and 0.362%) were recorded for the Red Globe and the Superior cultivar grown in media containing 40 mg. L^{-1} PEG.

Table (3): The effect of cultivar, PEG, and their interaction on the Total protein (%) explant content 30 days after exposure to drought conditions.

Cultivars	Total Protein%					Effect of cultivars
	PEG concentration g L^{-1}					
	Zero	5	10	20	40	
Superior	0.417 b	0.395 c	0.408 b	0.378 d	0.362 e	0.392 b
Red globe	0.431 a	0.411 b	0.397 c	0.390 c	0.386 cd	0.403 a
Concentration effect	0.424 a	0.403 b	0.402 b	0.384 c	0.374 d	

* Numbers with similar letters are not significantly different within each factor and interaction according to Duncan's multiple range test at the 0.05 probability level.

Amino acid Proline concentration (mg 100g^{-1} fresh weight)

Data in Table (4) shows the significant superiority of the Red Globe cultivar shoots in the content of the amino acid proline (15.83 mg 100 g^{-1} FW) compared to the Superior cultivar (13.38 mg 100 g^{-1} FW). The levels of PEG added to the nutrient medium had significant differences in this characteristic, as the concentration of 20 g L^{-1} achieved the highest values (22.91 mg. 100 g-1 FW), significantly superior to rest treatments. In contrast, the lowest values were 4.84 mg 100 g^{-1} FW was achieved in the control treatment (PEG-free).

As for the interaction coefficients between the cultivar and PEG concentrations, the data indicated that the highest proline accumulation reached 25.08 mg 100 g^{-1} FW, was obtained from the interaction treatment of the Red Globe cultivar with 20 g L^{-1} of PEG, which was significantly superior to all other studied interference treatments, followed by the treatment of the Superior cultivar with the same concentration of PEG, which amounted to 20.75 mg. 100 g^{-1} FW, while the lowest values were recorded in the control treatment (PEG-free) for both Red Globe and Superior cultivars (4.08 and 5.61 mg 100 g^{-1} FW, respectively).

Table (4): Effect of cultivar, PEG concentration, and their interaction on the explant amino acid proline content 30 days after exposure to drought conditions.

Cultivars	Determination of proline (mg 100 g^{-1} FW)					Effect of cultivars
	PEG concentration g L^{-1}					
	Zero	5	10	20	40	
Superior	5.61 g	9.07 f	14.73 d	20.75 b	16.76 cd	13.38 b
Red globe	4.08 g	11.45 e	17.12 c	25.08 a	21.41 b	15.83 a
Concentration effect	4.84 e	10.26 d	15.94 c	22.91 a	19.09 b	

* Numbers with similar letters are not significantly different within each factor and interaction according to Duncan's multiple range test at the 0.05 probability level.

Total Soluble sugars percentage (%)

The data in Table (5) indicate the superiority of the Superior cultivar shoots over the Red Globe cultivar in its total sugars containing (7.35%), compared to (6.75%) for the Red Globe cultivar. As for the effect of the studied PEG levels, it was noted that There was a significant increase in the total sugars in shoots in association with an increase in the stress levels, and the highest percentage of total sugars (10.87%) was recorded in shoots grown in medium enriched with a high concentration of PEG (40 g L^{-1}). The lowest values (4.42%) were recorded in the control treatment.

The interaction coefficients between the cultivar and the PEG concentrations showed a significant increase in the shoot's total sugar content simultaneously with the rise in PEG concentrations added to the medium to reach their highest value (11.17 and 10.58%) in the interaction coefficients of the two cultivar Red Globe and Superior with the concentration of 40 g L^{-1}, respectively. Meanwhile, the lowest value of total sugars was recorded in the control treatment (PEG-free).

Table (5): The effect of cultivar, PEG, and their interaction on the explant total sugars (carbohydrate) (%) content 30 days after exposure to drought conditions.

Cultivars	Total Soluble sugars percentage (%)					Effect of cultivars
	PEG concentration g L^{-1}					
	Zero	5	10	20	40	
Superior	4.72 e	5.19 de	7.26 c	9.02 b	10.58 a	7.35 a
Red globe	4.13 e	4.85 de	6.14 cd	7.46 c	11.17 a	6.75 b
Concentration effect	4.42 d	5.02 d	6.70 c	8.24 b	10.87 a	

* Numbers with similar letters are not significantly different within each factor and interaction according to Duncan's multiple range test at the 0.05 probability level.

The effective concentration of catalase enzyme: (IU mg^{-1})

Data in Table (6) 30 days after the Red Globe and Superior grape shoots planting shows that the Red Globe cultivar was significantly superior to the Superior cultivar in its content of the active catalase enzyme (5.88 and 5.45 units mg^{-1}) respectively. Adding PEG concentrations to the medium also caused significant increases in the content of the shoots of the active catalase enzyme simultaneously, with its concentration rising to 40 g L^{-1} (7.89 units mg^{-1}). At the same time, the control treatment recorded the lowest values (2.04 units mg^{-1}). Also, all interaction coefficients between the variety and the levels of PEG recorded a significant superiority over the comparison treatment for both cultivars, Red Globe and Superior, which recorded the lowest values (2.40 and 1.69 units mg^{-1}), respectively. On the other hand, the highest effective concentration of the active catalase enzyme reached 9.01 unit Mg^{-1} was achieved in treating the Red Globe cultivar with 40 g L^{-1} of PEG, significantly superior to all other studied interference treatments except for the treatment of the Superior variety with 20 g L^{-1} PEG (8.21 units mg^{-1}).

Table (6): The effect of cultivar, PEG, and their interaction on the catalase enzyme content in explant after four weeks of exposure to Drought stress conditions.

Cultivars	Catalase IU mg^{-1}					Effect of cultivars
	PEG concentration g L^{-1}					
	Zero	5	10	20	40	
Superior	1.69 f	4.65 e	5.93 cd	8.21 a	6.78 bc	5.45 b
Red globe	2.40 f	4.86 e	5.88 d	7.28 b	9.01 a	5.88 a
Concentration effect	2.04 d	4.75 c	5.90 b	7.75 a	7.89 a	

* Numbers with similar letters are not significantly different within each factor and interaction according to Duncan's multiple range test at the 0.05 probability level.

Lipid oxidation (malondialdehyde, MDA) (µmol g^{-1})

Results presented in Table (7) show the significant effect of cultivars on grape explant's MDA content when grown under different drought stress conditions Induced by PEG, as the Superior cultivar explant was significantly preferable in their content of MDA than the Red Globe cultivar (1.09 and 0.85 nmol g^{-1}), respectively. It was also noted that increasing PEG concentrations from zero to 40 gm L^{-1} caused a significant increase in the content of the MDA as the highest value reached (1.445 nmol g^{-1}) obtained from the treatment of adding 40 g L^{-1} PEG. In contrast, the lowest values (0.585 nmol g^{-1}) were recorded in the control. As for the interaction effect of cultivars with the PEG levels added to the medium, it is noted from the results of the same table that there is a significant increase in MDA accumulated in shoots of both Superior and Red Globe cultivars, with an increase in the stress substance level, as it reached its highest values in the interaction of the two cultivars with 40 gm l^{-1} PEG (1.58 and 1.31 nmol g^{-1}), respectively, while the lowest values (0.59 and 0.58 nmol g^{-1}), respectively, were obtained from the control treatments for both cultivar.

Table (7): The effect of cultivar, PEG, and their interaction on the malondialdehyde content in explant after four weeks of exposure to Drought stress conditions.

Cultivars	Malondialdehyde (MDA) µmol g^{-1}					Effect of cultivars
	PEG concentration g L^{-1}					
	Zero	5	10	20	40	
Superior	0.59 f	0.96 cd	1.27 b	1.05 c	1.58 a	1.09 a
Red globe	0.58 f	0.79 de	0.88 cd	0.69 ef	1.31 b	0.85 b
Concentration effect	0.585 d	0.875 c	1.075 b	0.87 c	1.445 a	

Damaged shoots Percentage 30 days after exposure to drought conditions (%):

After 30 days of exposing both cultivars' shoots to drought conditions, the percentage of damaged shoots varied Table (8). The results indicated that the conditions of drought stress achieved by adding different concentrations of PEG to the medium had a considerable effect on shoot damage, as all PEG concentrations used caused a significant damage percentage that was higher than the control treatment, which showed no damage shoots, it was noted that the damage percentage increased directly with increasing PEG concentrations in the medium.

Regarding the interference coefficients, it is observed that the shoots weren't affected by the interaction coefficients when PEG was not added to the mediums. In contrast to the control, the shoots suffered considerable harm from the interference coefficients of the PEG concentration with the cultivars.

Table (8): The effect of cultivar, PEG, and their interaction on the percentage of damaged explant after 30 days of exposure to Drought stress.

Cultivars	Damaged explant percentage (%)					Effect of cultivars
	PEG concentration g L^{-1}					
	Zero	5	10	20	40	
Superior	0.0 c	80 ab	90 ab	100 a	100 a	74 a
Red globe	0.0 c	70 b	80 ab	90 ab	100 a	68 a
Concentration effect	0.0 c	75 b	85 ab	95 a	100 a	

*Numbers with similar letters are not significantly different within each factor and interaction according to Duncan's multiple range test at the 0.05 probability level.

Damaged shoots Percentage 60 days after exposure to drought conditions (%):

The findings in Table (9), after 60 days of exposing both cultivars' shoots to drought conditions, demonstrated that the Superior cultivar outperformed the Red Globe variety in terms of damaged shoots by 55% and 35%, respectively. Regarding the PEG concentrations effect, the same table demonstrates that no damage was observed in the shoots that grew in the control treatment (the survival rate was 100%). In contrast, the shoots grown in the media enriched with PEG were damaged in different proportions directly proportional to the increasing concentration of PEG added to the medium.

Table (9): The effect of cultivar, PEG, and their interaction on the percentage of damaged explant after 60 days of exposure to Drought stress.

Cultivars	Damaged explant percentage (%)					Effect of cultivars
	PEG concentration g L^{-1}					
	Zero	5	10	20	40	
Superior	0.00 e	50 b-d	62.5 a-c	75 ab	87.5 a	55 a
Red globe	0.00 e	37.5 cd	37.5 cd	25 de	75 ab	35 b
Concentration effect	0.00 c	43.75 b	50 b	50 b	81.25 a	

*Numbers with similar letters are not significantly different within each factor and interaction according to Duncan's multiple range test at the 0.05 probability level.

As for the interaction effect of the cultivars and PEG concentrations, it is noted that the highest percentage of affected shoots for both the Superior and Red Globe cultivars was obtained from the treatment of adding 40 g L^{-1} PEG to the medium and reached 5.87% and 75%, respectively.

The comparison of Table (8) with Table (9) results demonstrated that the percentage of affected shoots for the two cultivars Superior and Red Globe decreased from 74% and 68% after 30 days to 55% and 35% after 60 days, respectively, and from 75% and 100% after 30 days of growth in media containing PEG. to 43.75% and 81.25% after 60 days, respectively, while the interference treatments caused a decrease in the percentage of affected shoots from 70% and 100% after 30 days to 25% and 87.5% respectively, after 60 days.

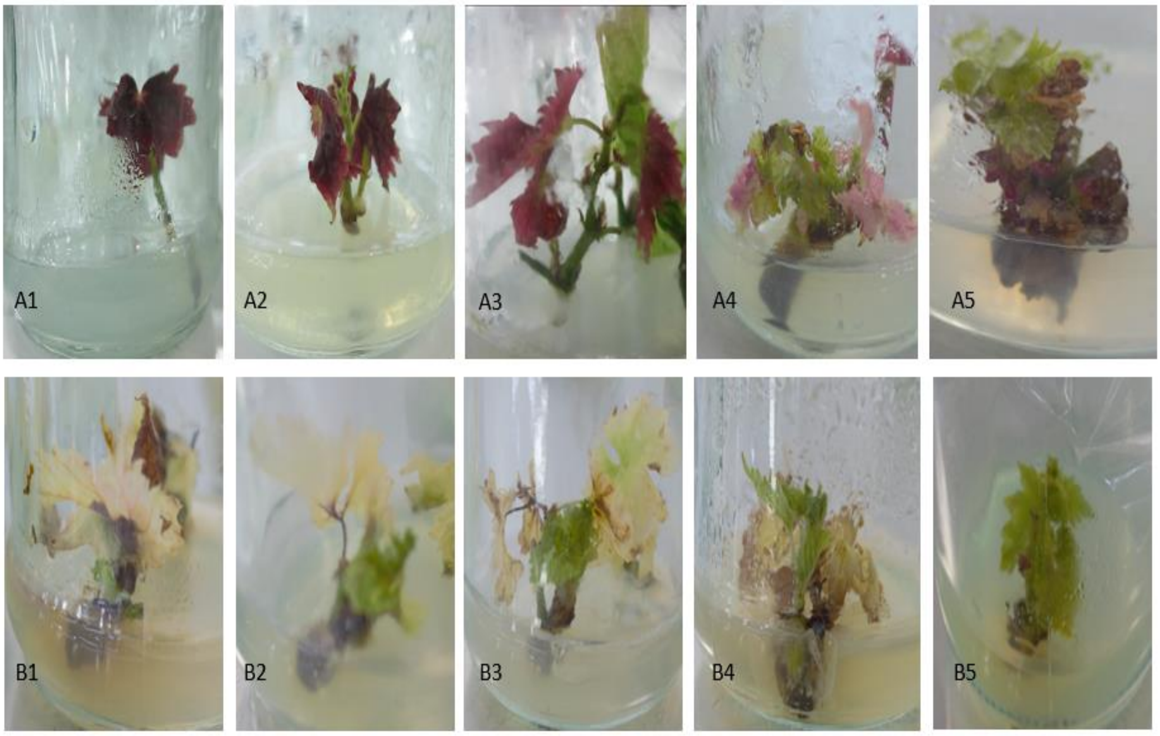

Figure (1): A1and A2 Red globe cultivar shoots during the first 30 days of exposure to drought stress. A3-A5, Red globe cultivar shoots during the second 30 days of exposure to drought stress. B1andB2 Superior cultivar shoots during the first 30 days of exposure to drought stress. B3-B5, Red globe cultivar shoots during the second 30 days of exposure to drought stress.

In this study, an in vitro hardening program was implemented for the two grape cultivars, Superior and Globe Red, which provided an opportunity to obtain more accurate results from the field because the study took place under controlled conditions inside the growth room and helped to conduct early detection of the extent of the plant’s tolerance to stress by following the nature of the change in the behavior of the metabolism of the essential substances affected by drought, including the total chlorophyll concentration, the percentage of total sugars (carbohydrates), the percentage of protein, the concentration of the amino acid proline, and the concentration of malondialdehyde.

The study revealed a significant difference between the cultivars in the values (effectiveness) of the studied parameters, as the Red Globe outperformed the Superior cultivar in the anthocyanins, proline, protein, catalase enzyme, and MDA (Tables 2, 4, 3, 6, and 7) Indicators. In contrast, the Superior outperformed the Red Globe cultivar in total chlorophyll and total sugars (carbohydrates) content (Tables 1 and 5). The significant differences between the cultivars in the values of the studied parameters can be attributed to the difference in the genetic construction of each cultivar, as the growth rate of any plant represents the result of growth factors effect (genes, hormones, enzymes, and environmental conditions) (Muhammad, 1985).

The study demonstrated that adding PEG to the growth medium significantly decreases the total chlorophyll concentration in shoots and, inversely, with increasing PEG concentrations Table (1). The significant reduction in total chlorophyll concentration in plants that grew under drought conditions can be explained based on the fact that active oxygen derivatives resulting from drought stress attacked the chloroplast membranes and caused rapid decomposition of chlorophyll molecules (Liu *et al*. 2011), Kaiser *et al*., (1981) noted swelling and deformation of carina lamellae in plants grown under drought stress. As well as, Tuna *et al*. (2008) suggested that the decrease in chlorophyll concentration in plants growing under drought conditions is due to the increased activity of proteolytic enzymes, including chlorophyllase, which breaks down the chlorophyll pigment. This result is consistent with what Alebidi *et al.* (2024) indicated, a decrease in the total chlorophyll content in grape shoots when they studied the performance of some grape rootstocks grown under drought stress induced by PEG *in vitro*. Many studies have recorded decreased chlorophyll levels under water stress conditions (Pavlousek, 2011; Haider *et al*., 2017).

The high accumulation of anthocyanin pigment in plant segments growing in a medium prepared with different PEG concentrations Table (2) could be because anthocyanin is one of the most critical elements of the plant's defense systems, as the plant increases its production under drought conditions to protect against oxidative damage and restore balance to it (Menezes-Benavente *et al*. 2004). Hatier and Gould (2008) stated that anthocyanins are non-enzymatic antioxidants that plants produce under stress conditions to scavenge active oxygen derivatives formed under drought stress, including hydrogen peroxide H_2O_2, which has a toxic effect on the plant. In addition, Hughes *et al.* (2013) mentioned that anthocyanins play a role in adjusting cell osmosis. Current study results agree with what was found by Pourghayoumi *et al*. (2017) in their study on the response of pomegranate cultivar to water stress, as well as with Bolat *et al.* (2016) in their study on the effect of drought stress on some morphological, physiological, and biochemical traits of the myrobolan 29 c plum rootstock, who indicated that a significant increase occurrence in the content of anthocyanins and phenols in plants grown under drought conditions compared to the control treatment.

The decrease in the protein percentage Table (3) and the increase in the amino acid proline concentration Table (4) in shoots exposed to drought stress induced by PEG can be due to forcing the plant to change its nutritional metabolism through activating genes for breaking down proteins, especially proteinase, and inhibiting the enzymes responsible for building proteins, especially Nitrate Reductase

(Abogadallah, 2010), and increasing the activity of enzymes that manufacture some amino acids, especially proline and glutamine, which facilitate the water entry into the plant by preserving dissolved materials in the cell and achieving osmotic balance (Yancey et al., 1982). Proline is one of the amino acids that records an immediate and significant increase in its quantity in many plants when exposed to drought conditions by activating its constructing genes and inhibiting the activity of the enzyme that oxidizes it (proline dehydrogenase) (Abogadallah, 2010).Proline maintains osmosis in plants and thus increases their tolerance to drought stress by protecting protein and the cell membrane structure and regulating the oxidation and reduction state by scavenging active oxygen derivatives (Kocsy *et al*., 2005).

The decrease in the percentage of proteins in shoots exposed to drought stress *in vitro* by adding different PEG concentrations is consistent with what was found by Munir and Aftab (2009) in Sugarcane, Singh and Kumar (2020) in eucalyptus trees. As for the increase in proline concentration in plants exposed to *in vitro* drought stress, our results agree with the results of Alebidi *et al*., (2024) on different grape cultivars as a gradual response to the increase in PEG concentration.

Regarding total sugars, the results showed that raising the levels of PEG in the nutrient medium led to a significant increase in the accumulation of total sugars in the branches of the two grape cultivars grown on media containing different concentrations of PEG Table (5). Sugars are one of the essential components of nutritional metabolism that the plant uses to resist drought conditions by converting them into forms that can retain water and protect cell membranes, thus achieving an osmotic balance in favor of water entering the plant. Among the sugars the plant maintains under stress conditions are mannitol, penton, and sucrose, the latter of which is the most accumulated in the plant under drought conditions. Bian and Jiang (2009) stated that the high concentration of total sugars in the plant can be explained based on the decomposition of starches and polysaccharides present in the plant into mono- or disaccharides in cases of water deficiency, which in turn work with proline to regulate the osmotic potential in the cells and regulate metabolic processes in plants (Xu and Huang., 2010), thus making it more adaptable to stress conditions. These findings align with earlier reports, which indicated that plants exposed to drought conditions have significantly higher soluble sugar contents (Singh and Kumar, 2020).

The results Table (6) showed increased catalase enzyme activity as a natural mechanism for resistance to drought stress and the resulting active oxygen derivatives. Hydrogen peroxide (H_2O_2) is one of the most harmful active oxygen derivatives formed in plants growing under drought stress due to its toxic effect on the plant. Under drought conditions, the plant increases the production of the enzyme catalase, which is one of the most important antioxidant enzymes that resist drought, as it works to decompose the compound hydrogen peroxide into water and oxygen, thus ending its negative effect on the plant (Das and Roychoudhury, 2014). Our results in terms of increased catalase enzyme concentration in plants ex vivo under stress conditions agree with Singh and Kumar (2020) on Eucalyptus trees, Rao and Jabeen (2013) on sugarcane callus.

Malondialdehyde (MDA) accumulation occurs when ROS attacks cell membrane lipids and oxidizes them. Thus, the quantity of MDA in plants can be utilized to assess the oxidative stress level (Farooq et al., 2010). The significant increases in the MDA accumulation in both cultivar shoots Table (7) with increasing concentrations of the stress-causing substance PEG can be attributed to the Lipoxygenase enzyme that increased under drought stress, which oxidizes (break down) cell membranes unsaturated fatty acids and thus breaks down cell membranes (Abu Jadallah, 2010). Our results agree with Zhong *et al*. (2018) regarding significant increases in the shoot content of MDA in some grape cultivars in conjunction with increasing concentrations of PEG added to the growing medium.

The results in Table (8) showed that the two grape cultivars shoot grown on media prepared with different PEG concentrations were damaged to various degrees; this can be because PEG stimulates drought stress conditions Similar to field drought stress conditions, which produce Free radicals that attack Cell components then oxidizing and destroying them, and this was confirmed by the results of the current study, as there was a decrease in the concentration of chlorophyll Table (1), a reduction in the percentage of proteins Table (3), and an increase in the concentration of lipid peroxidation (MDA) Table (7). The plant is damaged due to the rise in active oxygen derivatives generated from drought conditions, which prompts the plant to activate its immune system by increasing antioxidant production, including the anthocyanins pigment Table (2), the amino acid proline Table (4), the percentage of total soluble sugars Table (5), and the concentration of the catalase enzyme Table (6). These results are consistent with what was indicated by (Abu Jadallah, 2010) in that active oxygen derivatives formed under drought stress conditions attack the biological molecules that make up the plant, including chlorophyll, proteins, and fats that make up cell membranes, and stimulate the formation of antioxidants such as anthocyanins, the catalase enzyme, and compounds that maintain osmosis. Such as the amino acid proline and sugars.

Results shown in Table (9) demonstrated that the produced antioxidants by the grape cultivars shoot exposure to drought stresses within thirty days, represented by anthocyanins, the amino acid proline, sugars, and the catalase enzyme Tables (2, 4, 5, 6), helped it withstand drought stress. Thus, its survival, there was no death of the shoots after 30 and 60 days of exposure to drought conditions. On the contrary, it contributed to a significant decrease in the percentage of damaged shoots between the cultivars and different PEG concentrations in addition to the binary interference treatments.

The current study Figure (1) proved that the Red Globe cultivar is more tolerant than the Superior variety to drought conditions and that exposing the shoots to drought conditions during the first 30 days changed their genetic activity and their nutritional metabolic pathway toward increasing their content of antioxidants and substances which have the ability to achieve osmotic balance, which helped the cells retain water and endure drought and reduced the percentage of damage after 60 days of Exposure to drought conditions.

CONCLUSIONS

The results of the current study demonstrated a significant decrease in the shoot content of chlorophyll and total soluble proteins in the PEG treatments compared to the control. Its content of anthocyanins, proline, soluble sugars (carbohydrates), the antioxidant enzyme catalase, and malondialdehyde (MDA) also

increased in response to the increased stress level and increase in free radicals. Based on the results obtained through this study, we can prove that the Red Globe cultivar is considered tolerant and well-adapted to drought conditions compared to the Superior cultivar. Therefore, it can be recommended that this variety be used in future breeding programs to obtain drought-tolerant grape cultivars to solve the Worsening water shortage problem, especially in Iraq.

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CONFLICT OF INTEREST

The researcher supports the idea that this work does not conflict with the interests of others.

تأثير اجهاد الجفاف في المسار الايضي والبقاء على قيد الحياة لصنفين من العنب (*Vitis Vinifera* L.) خارج الجسم الحي

وعد سعيد فيزي [1]، عمار زكي قصاب باشي [2]، روفائيل شليمون توما [3]
قسم تقنيات الإنتاج النباتي/ الكلية التقنية الزراعية / الجامعة التقنية الشمالية/ الموصل / العراق [1]
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الخلاصة

هَدفَ البحث الى تقسية فروع صنفي العنب سوبيريور ورد كلوب المنمات خارج الجسم الحي من خلال إضافة تراكيز (0، 5، 10، 20و40 غرام لتر$^{-1}$) من مادة الكلايكول متعدد الاثلين PEG الى وسط النمو (MS + 2 ملغم لتر$^{-1}$ BA) لإحداث ظروف الجفاف خارج الجسم الحي، بعد 30 يوم من الزراعة قدرت قيم بعض المركبات البايوكيمياوية ذات التأثر المباشر بالجفاف لاجل التعرف على التغير الذي احدثته الفروع في مسارها الايضي من اجل مقاومة الجفاف والبقاء حية. أظهرت النتائج ان إضافة ألـ PEG سبب انخفاض معنوي في محتوى الأجزاء النباتية من صبغة الكلوروفيل والبروتينات الكلية وزيادة معنوية في مضادات الاكسدة المنتجة في النبات (صبغة الانثوسيانين، الحامض الاميني البرولين، السكريات الكلية وانزيم الكتاليز) بالإضافة الى الدهون المؤكسدة (المالوندايالديهايد) مقارنتاً مع معاملة المقارنة، كذلك أظهرت النتائج اختلاف الأصناف عن بعضها معنوياً في جميع الصفات المدروسة، كما حققت معاملات التداخل بين الأصناف وتراكيز PEG المضاف فروقات معنوية مقارنتا مع معاملة المقارنة لكل صنف. وتحقق اكبر تركيز من الانثوسيانين للصنفين رد كلوب وسوبيريور بلغ (24.46 و 13.30 ملغم 100غم$^{-1}$) وللحامض الاميني البرولين (25.08 و20.75 ملي مول غم$^{-1}$) على التوالي في الفروع النامية في أوساط مجهزة بـ 20غم لتر$^{-1}$ من PEG في حين ان اكبر نسبة مئوية من السكريات الكلية بلغت (11.17 و 10.58%) على التوالي تم الحصول عليها من الأجزاء النباتية النامية في أوساط حاوية 40 غم لتر$^{-1}$ PEG في حين ان اعلى تركيز من انزيم الكتاليز للصنف رد

كلوب بلغ 9.01 وحدة ملغم$^{-1}$ وللصنف سوبيريور 8.21 وحدة ملغم$^{-1}$ وتم الحصول عليها من الفروع النامية بوجود 40 و 20 غم لتر$^{-1}$ PEG على التوالي. بينت نتائج الدراسة أن صنف ريد كلوب أكثر تحملا لظروف الجفاف من الصنف سوبيريور ، مما يجعله ملائما للزراعة في المناطق التي تعاني من شح المياه.

الكلمات المفتاحية: الكلايكول متعدد الاثلين؛ المالوندايالديهايد؛ برولين؛ كتاليز ؛ مضادات الاكسدة.

REFERENCES

Abu Jadallah, J. M. (2010). Physiology and Molecular Biology of Plants under Water Stress, Department of Botany, Faculty of Science, Damietta University, Egypt, pp. 207-208. https://n9.cl/lhgem

Ahuja, I., De Vos, R. C. H., Bones, A. M. & Hall, R. D. (2010). Plant molecular stress responses face climate change. *Trends in Plant Science*, 15(12):664–674. https://doi.org/10.1016/j.tplants.2010.08.002

Alalaf, A., & Fekry, W. (2020). Effect of saline stress on growth of fruit plants (Review Article). *Mesopotamia Journal of Agriculture*, *48*(4), 53-61. https://doi.org/10.33899/MAGRJ.2020.128155.1070

Al-Atrushy, S., & Ibrahim, R. (2023). Impact of pruning severity, chicken manure, and foliar application of kare combi on productivity and quality of zaitouni grape cultivar (Vitis vinifera L.). *Mesopotamia Journal of Agriculture*, *51*(4), 1-12. https://doi.org/10.33899/mja.2023.139718.1231

Alebidi, A. I., Al-Saif, A. M., F. Abdel Aziz, H. F., Abdrabboh, G. A., Elnaggar, I. A., Abd El-wahed, N. A. E., Farouk, M. F. & Hamdy, A. E. (2024). In Vitro Drought Tolerance of Some Grape Rootstocks. Journal of Ecological Engineering, 25(4), 184-202. https://doi.org/10.12911/22998993/184183

Al-Maliki, L. A., Al-Mamoori, S. K., Al-Ansari, N., El-Tawel, K., & Comair, F. G. (2022, December). Climate change impact on water resources of Iraq (a review of literature). In *IOP Conference Series: Earth and Environmental Science* (Vol. 1120, No. 1, p. 012025). IOP Publishing. https://doi.org/10.1088/1755-1315/1120/1/012025

Al-Rawi, K. M. & khalaf Allah, M. A. (1980). Designs and Analysis of Agriculture Experiments. (eds). Ministry of High Education and Scientific Research, *Books House for Typography and Publication*. Mosul University, 448 l- (In Arabic).

Bates, L., Walderen, R. & Teare, I. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*. 39: 205 – 207. https://doi.org/10.1007/BF00018060

Beers, R. F. & Sizer, I. W. (1952). A Spectrophotometric Method for Measuring the Breakdown of Hydrogen Peroxide by Catalase. *The Journal of biological chemistry*, *195*(1), 133–140. https://pubmed.ncbi.nlm.nih.gov/14938361/

Bian, S. & Jiang, Y. (2009). Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of kentucky bluegrass in response to drought stress and recovery. *Scientia Horticulturae*, 120:264–270. https://doi.org/10.1016/j.scienta.2008.10.014

Bolat, I., Dikilitas, M., Ikinci, A., Ercisl, S. I. & Tonkaz, T. (2016). Morphological, physiological, biochemical characteristics and bud success responses of myrobolan 29 c plum rootstock subjected to water stress. *Canadian Journal of Plant Science*. 96: 485–493. https://doi.org/10.1139/cjps-2015-0260

Cui, Z. H., Bi, W. L., Hao, X. Y., Xu, Y., Li, P. M., Walker, M. A. & Wang, Q. C. (2016). Responses of *In Vitro*-Grown Plantlets (*Vitis Vinifera*) to Grapevine Leaf-Rollassociated Virus-3 and Peg-induced Drought Stress. *Front Physiol*. Jun 2; 7:203. https://doi.org/10.3389/fphys.2016.00203

Das, K. & Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Science*, 2:53. https://doi.org/10.3389/fenvs.2014.00053

Farooq, M., Wahid, A., Lee, D.-J., Cheema, S. A. & Aziz, T. (2010). Drought Stress: Comparative Time Course Action of the Foliar Applied Glycinebetaine, Salicylic Acid, Nitrous Oxide, Brassinosteroids and Spermine in Improving Drought Resistance of Rice: Improving rice drought tolerance *Journal of Agronomy and Crop Science*. 196, 336–345. https://doi.org/10.1111/j.1439-037X.2010.00422.x

Haider, M.S., Zhang, C., Kurjogi, M. M., Pervaiz, T., Zheng, T., Zhang, C. & Fang, J. (2017). Insights into grapevine defense response against drought as revealed by biochemical, physiological and RNA-Seq Analysis. *Scientific Reports*, 7(1): 13134. DOI https://doi.org/10.1038/s41598-017-13464-3

Hatier, J-H. B. & Gould, K. S. (2008). Foliar anthocyanins as modulators of stress signals. *Journal of Theoretical Biology*. 7;253(3):625-7. https://doi.org/10.1016/j.jtbi.2008.04.018

Hohl, M. & Schopfer, P. (1991). Water relations of growing maize coleoptiles: comparison between mannitol and polyethylene glycol 6000 as external osmotica for adjusting turgor pressure. Plant Physiology, 95(3): 716-722. https://doi.org/10.1104/pp.95.3.716

Hughes, N. M., Carpenter, K. L. & Cannon, J. G. (2013). Estimating contribution of anthocyanin pigments to osmotic adjustment during winter leaf reddening. *Journal* of *Plant Physiology.* 15;170(2):230-233. https://doi.org/10.1016/j.jplph.2012.09.006

Ibrahim, H. I. M (2010). Plant Samples Collected and Analyzed. first edition. Dar Al-Fajr for Publishing and Distribution, Egypt. (In Arabic).

Jiao, S., Zeng, F., Huang, Y., Zhang, L., Mao, J. & Chen, B. (2023). Physiological, biochemical and molecular responses associated with drought tolerance in grafted grapevine. *BMC Plant Biol* 23, 110. https://doi.org/10.1186/s12870-023-04109-x

Kaiser, W. M., Kaiser, G., Schöner, S. & Neimanis, S. (1981). Photosynthesis under osmotic stress: differential recovery of photosynthetic activities of stroma enzymes, intact chloroplasts, protoplasts and leaf slices after exposure to high solute concentrations. *Planta*. 153(5):430 -435. https://doi.org/10.1007/BF00394981

Knudson, L. L., Theodore, T. W. & Edwards, G. E. (1977). Measurement of ozone injury by determination of leaf chlorophyll concentration. *Plant Physiol*. 60:606-608. https://doi.org/10.1104/pp.60.4.606

Kocsy, G., Laurie, R., Szalai, G., Szilágyi, V., Sarkadi, L. S., Galiba, G. & De Ronde, J. A. (2005). Genetic manipulation of proline levels affects antioxidants in soybean subjected to simultaneous drought and heat stresses. *Physiologia Plantarum*, 124(2): 227-235. https://doi.org/10.1111/j.1399-3054.2005.00504.x

Lawlor, D. W. (1970). Absorption of polyethylene glycols by plants and their effects on plant growth. *New Phytologist*, v 69 issue 2, p. 501-513. https://doi.org/10.1111/j.1469-8137.1970.tb02446.x

Li, Y., Zhao, H., Duan, B., Korpelainen, H., & Li, C. (2011). Effect of drought and ABA on growth, photosynthesis and antioxidant system of cotinus coggygria seedlings under two different light conditions *Environmental and Experimental Botany*. 71, 107–113. https://doi.org/10.1016/j.envexpbot.2010.11.005

Liu, C., Liu, Y., Guo, K., Fan, D., Li, G., Zheng, Y., Yu, L. & Yang, R. (2011). Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern china. *Environmental and Experimental Botany*. 71:174–183.https://doi.org/10.1016/j.envexpbot.2010.11.012

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193: 265-275. https://pubmed.ncbi.nlm.nih.gov/14907713/

Marín, D.; Armengol, J., Carbonell-bejerano, P., Escalona, J. M., Gramaje, D., Hernández-montes, E., Intrigliolo, D. S., Martínez-zapater, J. M., Medrano, H., Mirás-avalos, J. M., Palomares-rius. J. E., Romero-azorín, P., Savé, R., Santesteban, l. G. & De herralde, F. (2021). Challenges of viticulture adaptation to global change: Tackling the issue from the roots. *Australian Journal of Grape and Wine Research*, 27(1), 8-25. https://doi.org/10.1111/ajgw.12463

Menezes-Benavente L., Teixeira, K. F., Kamei, C. L. A. & Margis-Pinheiro, M. (2004). Salt stress induces altered expression of genes encoding antioxidant enzymes in seedlings of a brazilian indica rice (*Oryza sativa* L.). *Plant Science*. 166(2):323–331. https://doi.org/10.1016/j.plantsci.2003.10.001

Muhammad, A. K. (1985). Plant Physiology, Directorate of Dar Al-Kutub for Printing and Publishing, University of Mosul, Iraq. (In Arabic).

Munir, N. and Aftab, F. (2009) "The role of polyethylene glycol (PEG) pretreatment in improving sugarcane’s salt (NaCl) tolerance," *Turkish Journal of Botany*: Vol. 33: No. 6, Article 2. https://doi.org/10.3906/bot-0806-6

Murashige, T & Skoog, F. (1962). A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Plant Physiol*, 15: 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nepomuceno, A., Oosterhuis, D. & Stewart, J. (1998). Physiological responses of cotton leaves and roots to water deficit induced by polyethylene glycol. *Environmental and Experimental Botany*, 40(1): 29-41. https://doi.org/10.1016/S0098-8472(98)00018-5

Pavlousek, P. (2011). Evaluation of drought tolerance of new grapevine rootstock hybrids. *Journal of environmental biology*, 32(5): 543. https://n9.cl/njdzg

Pourghayoumi, M., Rahemi, M., Bakhshi, D., Aalami, A. & Kamgar-Haghighi, A. A. (2017). Responses of pomegranate cultivars to severe water stress and recovery: changes on antioxidant enzyme activities, gene expression patterns & water stress responsive metabolites. *Physiology and molecular biology of plants: an international journal of functional plant biology*, *23*(2), 321–330. https://doi.org/10.1007/s12298-017-0435-x

Ranganna, S. (1986). Handbook of Analysis and Quality Control of Fruit and Vegetable Products. 2nd Edition, Tata McGrow-Hill Education, New York. https://n9.cl/l9irs

Rao, S., & Jabeen, F. (2013). *In vitro* selection and characterization of polyethylene glycol (PEG) tolerant callus lines and regeneration of plantlets from the selected callus lines in sugarcane (Saccharum ofcinarum L.). *Physiol Mol Biol Plants* 19:261–268. https://doi.org/10.1007/s12298-013-0162-x

Sabır, A. (2016). Physiological and morphological responses of grapevine (*V. vinifera l.* cv. 'italia') leaf to water deficit under different rootstock effects. *Acta Scientiarum Polonorum. Hortorum Cultus*. 15(1) 2016, 135-148. https://bibliotekanauki.pl/articles/11543358

Shashidhar, H., Kanbar, A., Toorchi, M., Raveendra, G., Kundur, P., Vimarsha, H. & Bhavani, P. (2013). Breeding for Drought Resistance Using Whole Plant Architecture—Conventional and Molecular Approach Plant Breeding from Laboratories to Fields. *Vienna*, Austria.22. https://doi.org/10.5772/54983

Singh, D., Kaur, S. & Kumar, A. (2020) *In vitro* drought tolerance in selected elite clones of *Eucalyptus tereticornis* Sm. *Acta Physiol Plant* 42, 17. https://doi.org/10.1007/s11738-019-3009-4

Smith, A.M., Coupland, G., Dolan, L., Harberd, N., Jones, J., Martin, C., Sablowski, R. & Amey, A. (2009). Plant Biology. 1st Edition, Garland Science, New York/Abingdon, UK. https://doi.org/10.1201/9780203852576

Sucu, S., Yagcı, A. & Yıldırım, K. (2018). Changes in Morphological, Physiological Traits and Enzyme Activity of Grafted and Ungrafted Grapevine Rootstocks Under Drought Stress. Erwebs-Obstbau. *Erwerbs-Obstbau* 60, 127–136. https://doi.org/10.1007/s10341-017-0345-7

Tangolar, S. G., Tangolar, S., Kelebek, H. & Topcu, S. (2016). Determination of phenolics, sugars, organic acids and antioxidants in the grape cultivar kalecik karasi under different bud loads and irrigation amounts. *Korean Journal of Horticultural Science and Technology*, 34 (3): 495-509. https://doi.org/10.12972/kjhst.20160050

Tuna, A. L., Kaya, C., Dikilitas, M. & Higgs, D. (2008). The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. *Environmental and Experimental Botany*., 62(1): 1-9. https://doi.org/10.1016/j.envexpbot.2007.06.007

Verslues, P. E., Ober, E. S. & Sharp, R. E. (1998). Root growth and oxygen relations at low water potentials. Impact of oxygen availability in polyethylene glycol solutions. *Plant Physiology*., 116(4): 1403-1412. https://doi.org/10.1104/pp.116.4.1403

Walid, S. N. (2023). Climate Change: Consequences on Iraq's Environment. *Mesopotamia Journal of Agriculture*, *51*(2), 131-146. https://doi.org/10.33899/MAGRJ.2023.140391.1243

Wang-xia, W., Vinocur, B. & Altman, A. (2003). Plant Responses to Drought, Salinity and Extreme Temperatures: Towards Genetic Engineering for Stress Tolerance. *Planta*, 218(1): 1-14. https://doi.org/10.1007/s00425-003-1105-5

Xu, C. & Huang, B. (2010). Differential proteomic responses to water stress induced by peg in two creeping bentgrass cultivars differing in stress tolerance. *Journal of plant physiology*, *167*(17), 1477–1485. https://doi.org/10.1016/j.jplph.2010.05.006

Yancey, P. H., Clark, M. E., Hand, S. C., Bowlus, R. D. & Somero, G. N. (1982). Living with water stress: evolution of osmolyte systems. *Science*., 217(4566): 1214-1222. https://doi.org/10.1126/science.7112124

Zhong. Y., Zhi, L., Dan-Feng, B., Xiu-Juan, Q., Jin-Yong, C., Cui-Guo, W., Miao-Miao, L. & Jin-Bao, F. (2018). *In vitro* variation of drought tolerance in five actinidia species. *Journal of the American Society for Horticultural Science*. 143(3):226 234. https://doi.org/10.21273/JASHS04399-18