JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2024, 25(4), 184–202
https://doi.org/10.12911/22998993/184183
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Received: 2024.02.01
Accepted: 2024.02.20
Published: 2024.03.01

In Vitro Drought Tolerance of Some Grape Rootstocks

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ABSTRACT

Climate change is increasing the frequency and severity of drought strain, which poses a first-rate task to grapevine production. This have a look at investigated the response of four grape rootstocks (Richter, Salt Creek, Freedom, and Dogridge) to water deficit prompted by way of polyethylene glycol (PEG). Two pressure induction techniques had been as compared: surprise remedy, related to direct exposure of cultures to growing PEG concentrations (0%, 2%, 4%, 6%, 8%, and 10%); and step-clever long-time period remedy, steadily increasing PEG concentrations (0%, 6%, 8%, and 10%) through the years. The essential findings were as follows: drought strain negatively impacted all rootstocks, leading to decreased morphological tendencies (shoot number, period, and root number), survival %, and biochemical parameters (chlorophyll a and b, carotenoids, stomata popularity, RWC content material). It additionally led to reduced nutrient accumulation (N, P, K, Mg, Ca) in leaves. However, all rootstocks exhibited increased Proline content and antioxidant enzyme hobby under all PEG concentrations. In phrases of rootstock-unique responses, Richter and Salt Creek showed the maximum sturdy performance, maintaining better shoot and root growth, nutrient content, and photosynthetic hobby compared to Freedom and Dogridge. On the alternative hand, Freedom and Dogridge exhibited extra sensitivity to drought stress, experiencing stronger discounts in boom, biochemical parameters, and nutrient accumulation. In conclusion Richter and Salt Creek rootstocks could be valuable equipment for reinforcing drought tolerance in grapevines. The two carried out PEG remedies provide valuable methods for screening and deciding on drought-tolerant grape rootstocks.

Keywords: grape; peg; photosynthetic pigments; stomata; enzyme activity.

INTRODUCTION

Global warming is drastically altering the characteristics linked to vineyard production worldwide (Gutiérrez-Gamboa et al., 2021). Most Mediterranean regions are known to experience extremely hot summers and mild winters, with peak temperatures during the growing season frequently rising above the 40 °C threshold (Van Leeuwen et al., 2019). They include the emergence of heat and water stress simultaneously with high radiation loads, which results in leaf photo inhibitions and necrosis, a major decrease in yields, spoiled fruit quality, and vineyard loss, especially in young plantings (Gutiérrez-Gamboa et al., 2021). Water is the scarcest resource in crop production on a global scale (Brauman et al., 2013). In comparison to other factors, drought is one of the most significant ones that affects plant productivity and probably results in the biggest loss (Kapoor et al., 2020; Seleiman et al., 2023). In addition, it erodes food security around the world (Gomiero, 2016). From germination to maturity, drought stress impacts plants. As a result,

one of the main objectives of plant breeding is to increase production during drought (Khadka et al., 2020; Mohammadi, 2018). The need for water conservation and assessment of the current and/or newly created crop plant germplasm for their tolerance to drought have grown to be key concerns, claim (Molnar et al., 2022). With regard to low and high plant water potential, grapevine roots and rootstocks have drought tolerance mechanisms that include stomatal closure, a reduction in cell development and photosynthesis, the activation of respiration, and the accumulation of osmolytes and proteins in response to drought (Bhargava and Sawant, 2013). Without a doubt, choosing a rootstock resistant to drought is an essential decision when starting a new vineyard on a site where water is scarce. Although viticulture has made slow progress in rootstock breeding and selection programmes, as demonstrated by the slow rate of material released over the past century, warming trends have put pressure on the selection of genotypes that may confer a higher tolerance to abiotic stresses on the scion. Remarkably, the M4 rootstock (*Vitis vinifera* × vitis berlandieri) × *Vitis berlandieri* cv. *Resseguier* n.1) has been identified as a potentially useful material for vineyard establishment in regions susceptible to droughts during the summer. When the weather is hot or dry, vineyards usually use drought-tolerant and sturdy rootstocks (*Vitis berlandieri* × *Vitis rupestris*), such as 110 Richter, 1103 Paulsen, or 140 Ruggeri, whose performance is good in forming tap roots that can dig water from deep soil layers (Frioni et al., 2020). One of the most recent models to explore drought tolerance is polyethylene glycol (PEG) – induced drought stress (Zhang and Zhou, 2019). An easy way to evaluate the effects of drought on plant growth and development under controlled circumstances is to simulate drought stress in vitro using chemical reagents (Pradhan et al., 2020). An inexpensive synthetic polymer made from oil is called polyethylene glycol (PEG), and it is readily available (Xie et al., 2020). PEG is a naturally occurring non-ionic, water-soluble chemical having a molecular weight greater than 6000 (PEG 6000). Because they are inert and cell impermeable, the molecules are both small enough to affect osmotic pressure and large enough to avoid being absorbed by plants. According to research, PEG significantly increases water stress in plants while having no hazardous side effects (Yosefi et al., 2020). Evidence suggests that water stress causes oxidative stress in plants. According to Jan et al.,(Jan et al., 2022), free radicals (O_2^{*-}, superoxide radicals; OH^*, hydroxyl radical; HO_2^*, perhydroxy radical; and RO^*, alkoxy radicals) and non-radical (molecular) forms (H_2O_2, hydrogen peroxide, and 1 O_2, singlet oxygen) can damage proteins, lipids, carbohydrates, and nucleic acids (Dvořák et al., 2021). Plants have developed unique defense mechanisms and acclimation processes to withstand harsh growth conditions (Gechev and Petrov, 2020). In this regard, the study particularly seeks to achieve the following goals:

- compared the effects of physiological drought induced by PEG on the morphological, biochemical, and physiological traits of four genotypes of grapes that have been tested for drought susceptibility by adding different concentrations of PEG to MS media, whether by shock treatment or by stepwise long-term treatment under tissue culture conditions;
- suggest future rootstocks that will be required to graft some grape varieties onto it under drought-stress conditions In light of climate change, contemporary;
- pinpoint potential photosynthetic pathways that could distinguish and characterize the tested- grape rootstocks' responses to imposed physiological drought conditions, as well as best account for genotype variability;
- this research can advance our knowledge of the physiology of water stress, advancing the field of breeding for grape cultivars resistant to drought.

MATERIALS AND METHODS

Plant material and experimental design

This study was conducted in the tissue culture laboratory of the Horticulture Department, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt, during the three successive years: 2019, 2020, and 2021.

Sterilization of explants

From three-year-old plants, the current shoots of four grape rootstocks-Salt Creek, Freedom, Dogridge, and Richter-were removed. After cleaning the shoots with running water for 30 minutes to remove any dust, they were submerged in a 70% ethanol solution for 30

seconds. Then, they were sterilized in 0.1% mercury chloride (HgC_{l2}) for 3 minutes before being washed in distilled water (1×). Next, they were sterilized for 15 minutes in 10% sodium hypochlorite with droplets of Tween 20, and then rinsed three times in sterile distilled water. The selected 3–8 mm length one-node cuttings were prepared and cultured in jars containing 40 ml of the Murashige and Skooge (MS) basal medium supplemented with 30.00 g/L sucrose, 4 g/L agar, 1 g/L vitagel, 1.0 mg/L BA, and 0.01 mg/L NAA. After a further 30 days, uniformly formed explants were chosen and added to the same MS medium with various PEG concentrations at 0.0, 2%, 4%, 6%, 8%, and 10%. There are two ways to treat drought stress:

- stepwise long-term treatment, in which cultures are stressed out over time with progressively higher selecting agent concentrations,
- shock treatment, in which cultures are directly exposed to a high concentration shock and only those that can withstand it survive selecting agents.

Data collection and morphological and biochemical characteristics

Determination of morphological characteristics

After 30 days, the growth of axillary shoots under drought stress was assessed by counting the increase in shoots, shoot length (cm), leaves, fresh weight of the shoots, and survival rate. The equation presented by (1) Survival (%) = Number of explants alive at the end of the time period × 100 Number of explants alive at the start of the time period

Determination of photosynthetic pigments

The chlorophyll a, b, total chlorophyll, and carotenoid contents of the leaves were measured at the end of the experiment period, according to Lichtenthaler and Buschmann (2001), after 30 days. Total chlorophyll, chlorophyll a, and chlorophyll b were determined by abrading roughly 0.2 g of fresh plant tissue in a mortar with 15 mL of 80% acetone, and filtering it before measuring its absorbance with a spectrophotometer UV-Vis model 715 Jenway at 470, 663, and 646 nm. Acetone at 80% concentration was used to calibrate the instrument. The pigment concentrations were estimated using the following formulas:

$$\text{Chl, a} = (12.25\ A\ 663.2 - 2.79\ A\ 646.8) \quad (1)$$

$$\text{Chl, b} = (21.21\ A\ 646.8 - 5.1\ A\ 663.2) \quad (2)$$

$$\text{Car} = 1000A\ 470 - 1.8\ \text{Chl, a} - 85.02\ \text{Chl, b} \quad (3)$$

where: Chl, a, Chl, b, T, Chl, and car represent the concentrations of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, respectively. The measurements of photosynthetic pigment content were based on fresh weight.

Determination of proline content material

Bates et al. (1973) working a speedy colorimetric method to check Proline concentration. Acid-ninhydrin turned into organized by way of dissolving 1.25 g of ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid with agitation. When stored bloodless (at four °C), the reagent remains solid for twenty-four hours (7 approaches). The homogenate became filtered via Whatman #2 clear out paper after being homogenized in 10 mL of three percent aqueous sulfosalicylic acid. Then, 2 mL of filtrate had been blended with 2 mL of acid-ninhydrin and a couple of mL of glacial acetic acid in a check tube and heated for one hour at 100 °C earlier than being cooled in an ice bathtub. To extract the response aggregate, 4 mL of toluene turned into used, which became vigorously agitated for 15–20 seconds with a test tube stirrer. A toluene-containing chromophore become separated from the aqueous section, warmed to room temperature, and its absorbance at 520 nm become measured with toluene as a blank. The proline attention became determined on a clean weight foundation the usage of the subsequent widespread curve:

$$[(\mu g\ proline/mL \times mL\ toluene) / 115.5\ \mu g/\mu mol] / [(gsample)/5] = \mu moles\ proline/g\ of\ sparkling\ weight\ cloth \quad (4)$$

Leaf relative water content material

This study aimed to look at the impact of PEG at the relative water content of the four tested grape rootstocks. Leaf relative water content (RWC) became calculated based totally on the approach described by way of Mata and Lamattina (García-Mata and Lamattina, 2001). In each repetition, two leaves were randomly selected from the middle of the flora. Firstly, the leaves have been separated from the stems, and their fresh hundreds (FM) have been measured. To determine the saturation mass (TM),

they have been immersed in distilled water in closed boxes for 24 hours underneath air conditions at 22°C to reach their maximum saturation mass, after which weighed. The leaves were finally positioned in an electric powered oven at 80°C for 48 hours, and the dry mass of the leaves (DM) became decided. All measurements had been taken using precision scales with a precision of 0.001g and plugged into the following formulation:

$$\textit{Relative water content material}\ (\%) = (FW - DW) / (TW - DW) \times \textit{one hundred} \quad (5)$$

where: FW represents clean weight, DW represents dry weight, and TW represents turgid weight.

Ion accumulation

This study investigated the impact of MS medium supplemented with PEG at various concentrations (0.0, 2%, four%, 6%, 8%, and 10%) on ion accumulation in the leaves of the four grape rootstocks. The content material of numerous factors consisting of Na, Cl, K, Ca, P, and some hint elements in the leaves of the four grape rootstocks was predicted the usage of EDX. The samples had been evaluated at the Regional Center of Mycology and Biotechnology in Cairo, Egypt, using X-ray microanalysis (Module Oxford 6587 INCAx-sight) coupled with JEOL JSM-5500 LV scanning electron microscopy at 20 kV.

Stomatal reputation

This test became carried out to research the effect of MS media supplemented with 0.0%, 2%, four%, 6%, 8%, and 10% on stomatal conduct inside the leaves of 4 grape rootstocks. Stomatal traits measured included the quantity of stomata, internal period and width of stomata in µm, and outer duration and width of stomata in µm in the four grape rootstocks. Samples of lower floor leaves from the 4 grape rootstocks were coated with a gold sputter coater (SPI-Module). The variety of stomata/mm^2 (average of three fields) and stomatal dimensions (10 randomly selected stomata in line with discipline) had been measured for every imprint. Finally, the samples had been analyzed using scanning electron microscopy (JEOL JSM-5500 LV) in high vacuum mode at the Regional Center of Mycology and Biotechnology in Cairo, Egypt.

Determination of antioxidant enzymes

Preparation of tissue for enzymatic antioxidants: Fresh leaf samples (0.2 g) have been ground and homogenized in an ice tub in 4 mL of homogenizing answer containing 50 mM potassium phosphate buffer and 1% (w/v) polyvinylpyrrolidone (pH 7.8). The homogenate turned into centrifuged at 14,000 rpm at 4 °C for 10 min, and the ensuing supernatant became used for enzyme assays. The absorbance changed into recorded on a spectrophotometer (Jenway 6305 UV/Visible) for 60 s.

Catalase (CAT) assay

CAT action is assay according to Aebi, (1984). "BLANK" was prepared by mixing 0.05 ml of enzyme solution (supernatant) with 1.5 ml of 100 mM potassium phosphate classes buffer (pH = 7.2) and 0.5 ml of 75 mM Hydrogen Peroxide Solution (H_2O_2). The distilled water was added to the mixture up to a volume of 3 ml. The reaction occurs by the addition of H_2O_2. The absorbance recorded in decrease at 240 nm for 60s. The enzyme action was accounted by calculating the quantity of decomposed H_2O_2.

Peroxidase (POD) assay

POD hobby was determined at 420 nm. The "BLANK" changed into prepared with the aid of mixing 0.05 mL of enzyme answer with 1.07 mL of a 100 mM potassium phosphate buffer (pH 6.0 at 25°C), zero. Three mL of 5% (w/v) pyrogallol solution, 0.1 mL of 0.5% (w/w) H2O2, and 0.70 mL of water. POD activity was measured following the approach of Chance and Maehly (1955).

Polyphenol oxidase (PPO) assay

PPO hobby changed into decided at 420 nm and 25°C consistent with Duckworth and Coleman (Duckworth and Coleman, 1970). The "BLANK" become organized through mixing 0.05 mL of enzyme answer with 1.70 mL of 20 mM catechol solution (prepared in 50 mM potassium phosphate buffer, pH 6.8 at 25°C).

$$\textit{Enzyme pastime}\ (U/mL) = (\Delta Abs \times Vt \times 106) / (\Delta t \times \varepsilon \times l \times Vs \times \textit{a thousand}) \quad (6)$$

where: ΔA is the change in absorbance, Δt is the incubation time (min), ε is the extinction coefficient ($M^{-1} \cdot cm^{-1}$), l is the cuvette diameter (1 cm), Vt is the entire assay

extent, and V*s* is the volume of the enzyme sample (mL).

- The ε420 nm of pyrogallol is 2640 $M^{-1} \cdot cm^{-1}$.
- The ε420 nm of catechol is 2450 $M^{-1} \cdot cm^{-1}$.
- The ε240 nm of hydrogen peroxide is 43.6 $M^{-1} \cdot cm^{-1}$.

Statistical analysis

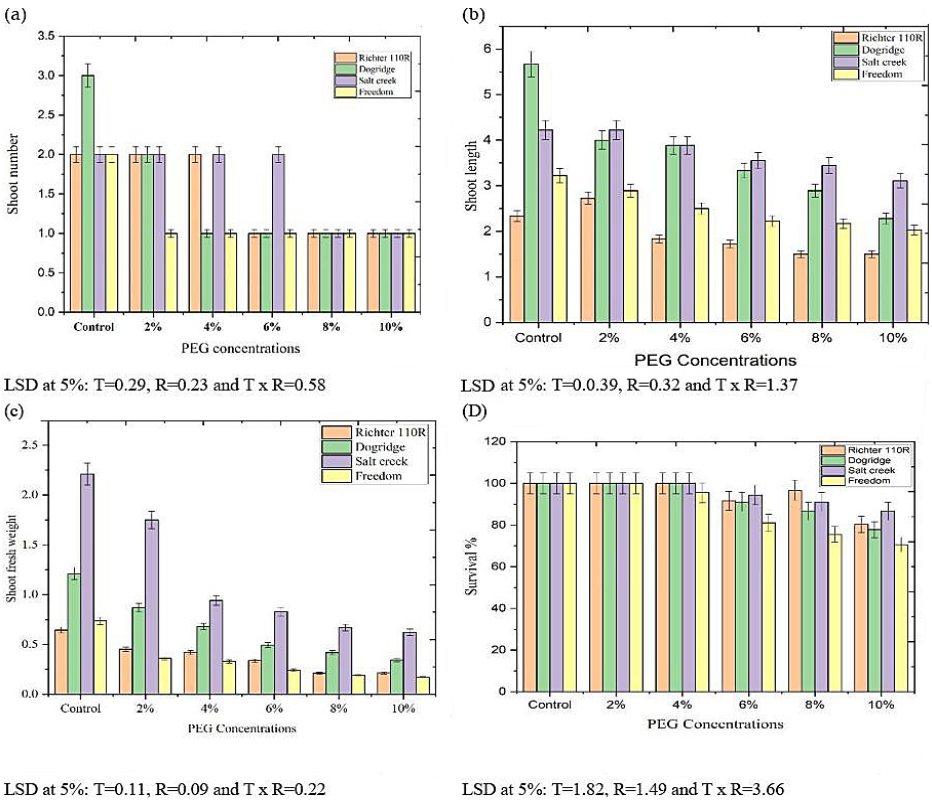
The present examine followed a completely randomized block layout. Analysis of variance was executed using manner ANOVA within the Co-stat software program, and way were as compared using the Duncan check with an importance level of $p \leq 0.05$.

RESULTS

Evaluation morphological characteristics

The micro-shoots of all evaluated grape rootstocks were transferred to MS media enriched with 1.0 mg/L BA and 0.01 mg/L NAA, alongside different concentrations of PEG (0%, 2%, 4%, 6%, 8%, and 10%), to perceive drought-tolerant grape rootstocks. The interaction between grape rootstocks, PEG levels, and their impact on shoot range, shoot duration, shoot sparkling weight, and shoot survival percent of the four examined grape rootstocks became located to be large. Figure 1 suggests that growing the PEG level from 2% to 10% within the medium decreased the morphological characteristics of all tested grape rootstocks. However, the volume of the decline various most of the rootstocks. Some rootstocks exhibited much less loss in morphological tendencies at lower PEG degrees in comparison to higher ranges. Richter and Dogridge were observed to be the least drought-tolerant, followed with the aid of Freedom and Salt Creek rootstocks in ascending order.

Figure 2 affords facts on how the 4 tested grape rootstocks replied to drought pressure triggered by different doses of PEG (0%, 6%, 8%, and 10%) the use of the sluggish version method. The 4 tested grape rootstocks confirmed full-size differences in shoot variety, shoot period, shoot clean weight, and shoot survival percentage depending at the rootstock, the diploma of drought, and their interactions. Adding 6% PEG to the growth medium improved the quantity of shoots for Dogridge, Salt Creek, and Freedom rootstocks, at the same time as Richter rootstock did no longer display an increase compared to the manage. When the MS medium became supplemented with 8% PEG, all tested grape rootstocks experienced an growth in shoot quantity as compared to the manage. Transferring micro-shoots to MS media with 10% PEG concentration led to an accelerated shoot wide variety in comparison to the manage, as the sluggish remedy with PEG allowed the callus to shape genes that produce surprise proteins, which might be considered an effective anti-drought system. However, those proteins have been now not produced when the calli were uncovered to PEG surprise remedies (Al-Taha, 2013). Similar outcomes had been suggested by way of Szopkó (2017) in wheat-barley suggesting that sluggish treatments are more effective than shock treatments. In conclusion, gradual variation of micro-shoots to PEG advanced the drought tolerance of all tested rootstocks, specifically at excessive PEG levels of 8% to 10%. These findings align with Mahmood et al. (2012), who suggested that the boom of bananas became inhibited by using PEG concentrations ranging from 10% to 30%. High doses of PEG (30%) resulted in the minimum shoot fresh weight, shoot length, and shoot quantity as compared to the manipulate in numerous banana cultivars. Cui et al., (2020) additionally supported these findings and stated that the very best awareness of PEG in MS media negatively affected vegetative boom and decreased all growth parameters compared to the lowest PEG awareness. PEG triggered a discount in growth, elevated overall soluble protein and unfastened proline, and brought about cellular membrane damage, mobile demise, and accumulation of O_2 and H_2O_2 within the growth medium. Akbarpour et al. (2017) located a decrease in growth indices, consisting of shoot sparkling weight, shoot dry weight, and shoot range, when Almond micro-cultures had been grown on MS media supplemented with a high awareness of PEG (7.0%). Growth factors can function selection criteria for drought tolerance (Asfere et al., 2020). Abiotic strain, such as osmotic and drought pressure, inhibits protein synthesis by affecting amino acid incorporation and proteolysis (Sharma et al., 2021). Higher concentrations of PEG within the media can purpose osmotic stress, ensuing in cellular department inhibition, shrinkage imbalance, nutritional imbalance, multiplied electrolyte leakage, and decreased



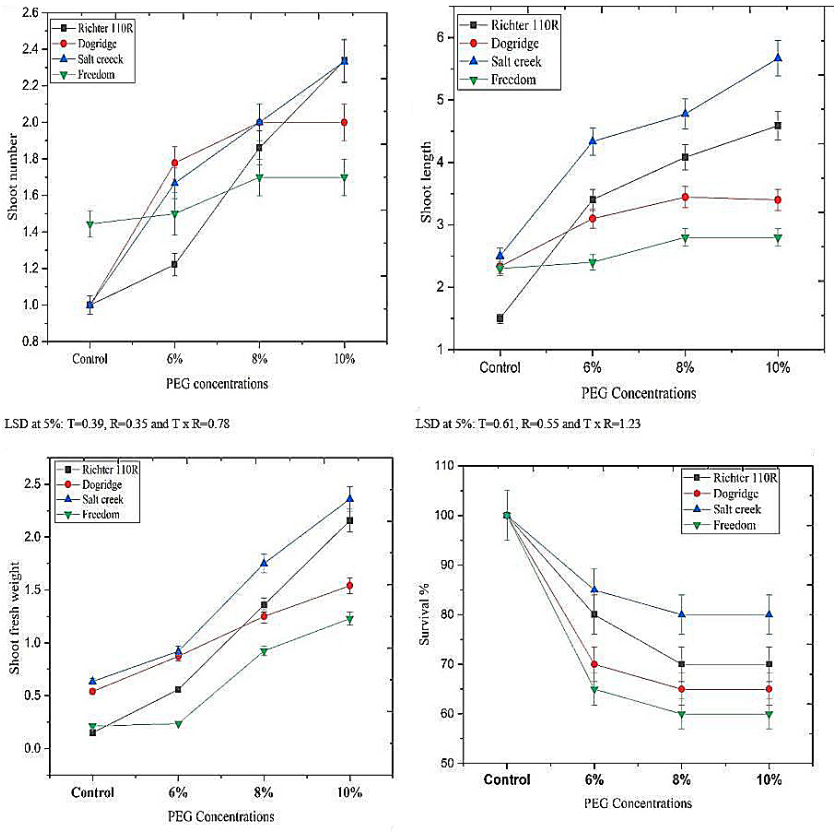
LSD at 5%: T=0.29, R=0.23 and T x R=0.58

LSD at 5%: T=0.0.39, R=0.32 and T x R=1.37

LSD at 5%: T=0.11, R=0.09 and T x R=0.22

LSD at 5%: T=1.82, R=1.49 and T x R=3.66

Figure 1. Effect of drought stress using PEG on morphological characteristics of grape rootstocks grown in vitro by using shock treatment



LSD at 5%: T=0.39, R=0.35 and T x R=0.78

LSD at 5%: T=0.61, R=0.55 and T x R=1.23

LSD at 5%: T=0.28, R=0.25 and T x R=0.57

LSD at 5%: T=0.92, R=0.82 and T x R= 1.84

Figure 2. Effect of drought stress using PEG on morphological characteristics of grape rootstocks grown in vitro by using gradual treatment

cell water content (Khodabin et al., 2020). PEG 6000, being an osmotic agent, does now not reason plasmolysis and is harmless to plant life. The dying of the callus in PEG-supplemented media is attributed to a lower inside the medium's water capacity in place of PEG absorption into the cells (Luo et al., 2019). Decreased turgor strain, which restricts cellular elongation, is the number one reason of boom dilemma (Medina-Villar et al., 2020). The inhibitory effects of dryness and osmotic stress on vegetative growth had been more stated in grapevine leaf roll-inflamed shoots as compared to healthful ones (Chacón-Vozmediano et al., 2020).

Evaluation of photosynthesis pigments

The statistics presented in Figure 3 exhibit the effect of MS media supplemented with PEG at concentrations of 0%, 2%, 4%, 6%, 8%, and 10% (the use of the surprise treatment approach) on photosynthetic pigments along with chlorophyll a, chlorophyll b, overall chlorophyll, and carotenoids of the 4 tested grape rootstocks. Grape rootstocks, PEG degrees, and their interactions had a full-size effect at the photosynthetic pigment content of the four tested grape rootstocks. Gradually increasing the PEG level in the boom medium resulted in a sluggish discount of photosynthetic pigments in all of the studied grape rootstocks. However, the photosynthetic pigments of all the studied grape rootstocks showed a decline in an ascending order from low to high PEG degrees in comparison to the control. In end, Salt Creek and Freedom have been located to be the most drought-tolerant grape rootstocks, observed by using Dogridge and Richter in descending order. On the other hand, the statistics supplied in Figure four displays the effect of drought strain at the photosynthetic pigments of the 4 studied grape rootstocks with the aid of adding PEG concentrations of 0.0%, 6%, 8%, and 10% to the increase medium the usage of the gradual adaptation approach. The photosynthetic pigments of the four tested grape rootstocks were substantially influenced by the grape

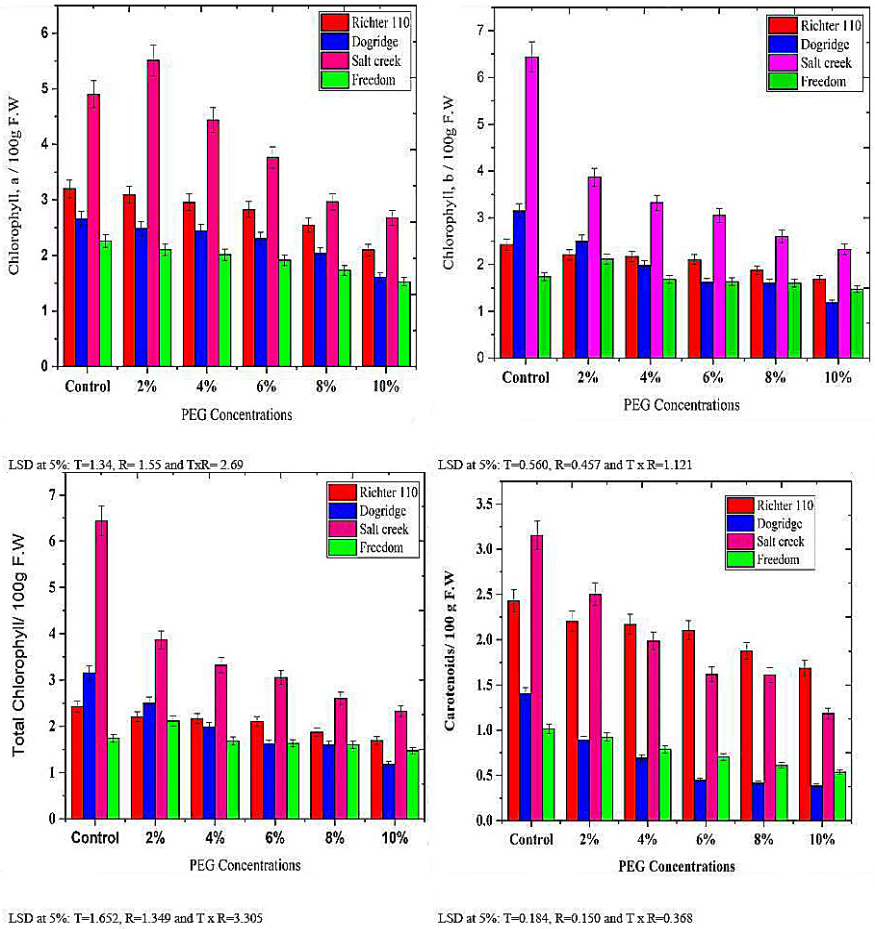


Figure 3. Effect of drought stress using PEG on photosynthetic pigments of grape rootstocks grown in vitro by using shock treatment

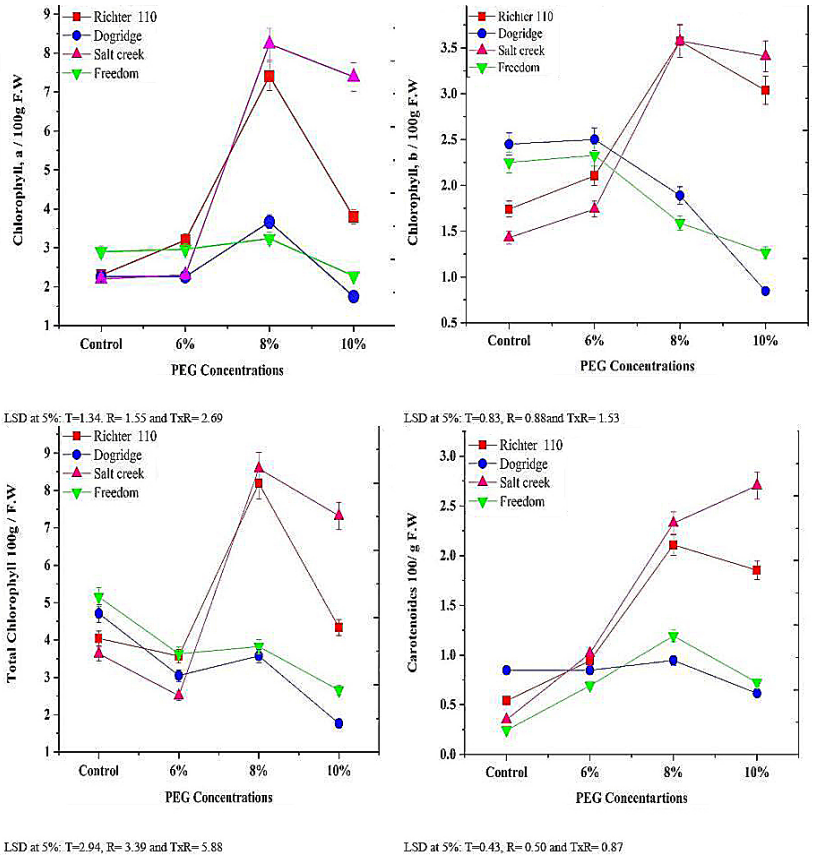


Figure 4. Effect of drought stress using PEG on photosynthetic pigments of grape rootstocks grown in vitro by using gradual treatment

rootstocks' PEG tiers and their interactions. Transferring cultures step by step from a medium containing 6% PEG to a medium containing eight% PEG elevated the photosynthetic pigments, while moving from 8% to 10% decreased the photosynthetic pigments in all of the tested grape rootstocks. In end, Salt Creek rootstock exhibited the best drought tolerance a number of the grape rootstocks, observed by means of Richter, Dogridge, and Freedom in descending order. These findings are steady with the ones of Sivritepe et al. (2008), who tested that drought stress due to PEG had a deleterious effect at the chlorophyll a, chlorophyll b, and general chlorophyll of cherry rootstocks. Furthermore, Hong et al. (2013) found that MS media supplemented with PEG at exceptional concentrations (10%, 15%, 20%, 25%, MW 6,000) affected chlorophyll content. The reduction in general chlorophyll content became found in a few pear rootstocks when shoots had been cultured on a excessive stage of PEG from 10% to twenty-five% as compared to the manipulate. Su et al. (2017) additionally found that drought pressure generated by way of PEG at distinctive doses ranging from 2.0% to 10% reduced the whole chlorophyll content as compared to the control. When shoots of positive grape rootstocks have been grown on MS media supplemented with excessive ranges of PEG (8% and 10%) compared to the bottom level of PEG (6% to 2.0%) and the manage, the bottom price of chlorophyll content material become acquired. Chlorophyll attention has been used as a source evaluation index, and a decrease in this will be taken into consideration a non-stomatal limiting aspect in drought pressure situations (Gholamin and Khayatnezhad, 2020). Water deficiency stress has varied physiological outcomes on vegetation, and the type and quantity of damage are determined by means of the severity of the stress and the plant's resistance. If the chloroplasts of leaves are broken, photosynthesis inside the plant cannot arise and will be compromised (Blum, 2011). Abiotic stressors, in fashionable, lead to chloroplast damage and a decrease in green pigmentation. The lower in chlorophyll content will be attributed to chlorophyll breakdown or the activation of chlorophyllase, which converts chlorophyll b to chlorophyll a. It could also be because of a

lack of chlorophyll synthesis along with adjustments in thylakoid membrane shape (Nxele et al., 2017). The increasing leaf chlorophyll concentration in osmotic pressure-tolerant species under excessive osmotic pressure levels suggests that chlorophyll attention may be used as an initial selection criterion for osmotic strain tolerance (Mirbehbahani et al., 2023; Prgomet et al., 2020).

Effect of PEG on proline content

The records offered in Figure five show the influence of MS media supplemented with PEG at concentrations of 0%, 2%, 4%, 6%, 8%, and 10% (the usage of the surprise treatment technique) on the buildup of proline within the 4 studied grape rootstocks. The accumulation of proline within the 4 tested grape rootstocks is particularly encouraged by the grape rootstocks, PEG levels, and their interaction. Increasing the PEG level in the increase medium regularly enhances proline accumulation in all tested grape rootstocks. However, the significance of the boom varies from low to excessive PEG stages and from rootstock to rootstock. All tested grape rootstocks show a smaller increase in proline accumulation at decrease PEG concentrations in comparison to better concentrations. When PEG is introduced at concentrations ranging from 2% to ten%, all investigated grape rootstocks display an augmentation of proline accumulation. The extent of augmentation varies among rootstocks, with Salt Creek exhibiting the highest proline accumulation, observed by using Richter, Freedom, and Dogridge in descending order in comparison to the manage. According to this take a look at, the maximum drought-resistant grape rootstock is Salt Creek, observed by means of Richter, Freedom, and Dogridge. On the other hand, the consequences offered in Figure 5 exhibit the impact of drought strain caused by including PEG at concentrations of 0%, 6%, 8%, and 10% in the increase medium (the usage of the sluggish edition method) on the proline accumulation of the 4 studied grape rootstocks. The proline accumulation in the four studied grape rootstocks is substantially influenced by way of their interactions, PEG levels, and the grape rootstocks themselves. Gradually transferring cultures from PEG-unfastened media to media containing 6% PEG increases proline accumulation in all investigated rootstocks compared to the controls, with Richter rootstock showing the highest proline content, followed by means of Salt Creek, Dogridge, and Freedom in descending order. Transferring micro-shoots to MS media supplemented with 8% PEG will increase proline accumulation in all tested rootstocks, with Richter rootstock displaying the best accumulation, accompanied by using Dogridge, Salt Creek, and Freedom. Further switch to MS media supplemented with 10% PEG considerably increases proline accumulation in all examined rootstocks, with Richter rootstock demonstrating the highest proline content material and being extra drought tolerant than Salt Creek, Dogridge, and Freedom rootstocks, which showcase lower proline accumulation. The most drought-resistant grape rootstock, consistent with these findings, is Richter, accompanied with the aid of Salt Creek,

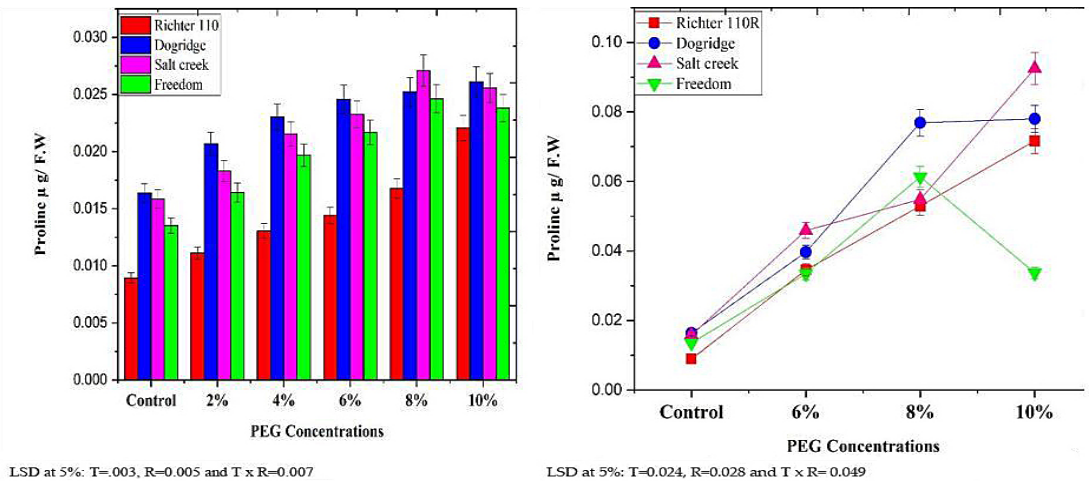


Figure 5. Effect of drought stress using PEG on proline content of grape rootstocks grown in vitro by using shock and gradual treatment

Dogridge, and Freedom in descending order. These findings are consistent with previous research. Sivritepe et al. (2008) verified that culturing cherry rootstock micro-shoots on MS media supplemented with PEG resulted in proline accumulation. Hong et al. (2021) reported that the proline content material of pear rootstocks become undoubtedly laid low with drought strain caused by PEG at various concentrations. Additionally, Cui et al.,(2020) found that growing PEG awareness had a superb impact at the proline content of grape cultivars. Proline accumulation is taken into consideration an adaptive response to strain and performs a role in preserving cell membrane integrity and shielding subcellular structures in drought-harassed flowers. It acts as an osmoregulator, osmoprotector, and stabilizes membranes and cell structures. Proline also enables in detoxifying free radicals and can act as an organic nitrogen reserve for amino acid and protein synthesis. In conclusion, the buildup of Proline is prompted by using the grape rootstocks, PEG ranges, and their interactions. Salt Creek and Richter rootstocks showcase the very best Proline accumulation and drought tolerance among the grape rootstocks studied.

Effect of PEG on RWC

The relative water content material (RWC) of the four studied grape rootstocks become affected by the MS medium combined with PEG at 0%, 2%, 4%, 6%, 8%, and 10% (shock remedy approach), as proven by means of the statistics in Figure 6. The RWC of the four tested grape rootstocks appeared to be extensively encouraged by way of the PEG degree, the grape rootstocks, and their interactions. Increasing the PEG level inside the increase medium gradually reduced the RWC content material of every examined grape rootstock. The extent of decrease, however, various from low to excessive PEG levels and amongst special rootstocks. All examined grape rootstocks showed a decrease when PEG become brought to the growth medium at concentrations starting from 2% to 10%, as compared to the control. However, the degree of reduction differed among rootstocks, with Salt Creek showing the lowest percentage of discount, accompanied by way of Richter in descending order, and Freedom and Dogridge exhibiting the highest percentage of reduction compared to the control. It can be inferred that Salt Creek and Richter are the most drought-tolerant grape rootstocks, followed by using Freedom and Dogridge in descending order. On the other hand, the statistics in Figure 6 demonstrated the impact of drought stress at the RWC of the four studied grape rootstocks caused by way of adding PEG at concentrations of 0%, 6%, 8%, and 10% to the boom medium the use of the gradual edition technique. Gradually transferring cultures from PEG-free media to media containing up to 6%, 8%, and 10% led to a reduction within the RWC of all studied grape rootstocks. The level of reduction, however, varied amongst rootstocks, with Richter displaying the lowest percent of reduction, accompanied by Salt Creek, Freedom, and Dogridge in ascending order as compared to the manage. These findings are steady with the ones of Sivritepe et al. (2008), who observed that polyethylene glycol (PEG) had a terrible impact on RWC while shoots of

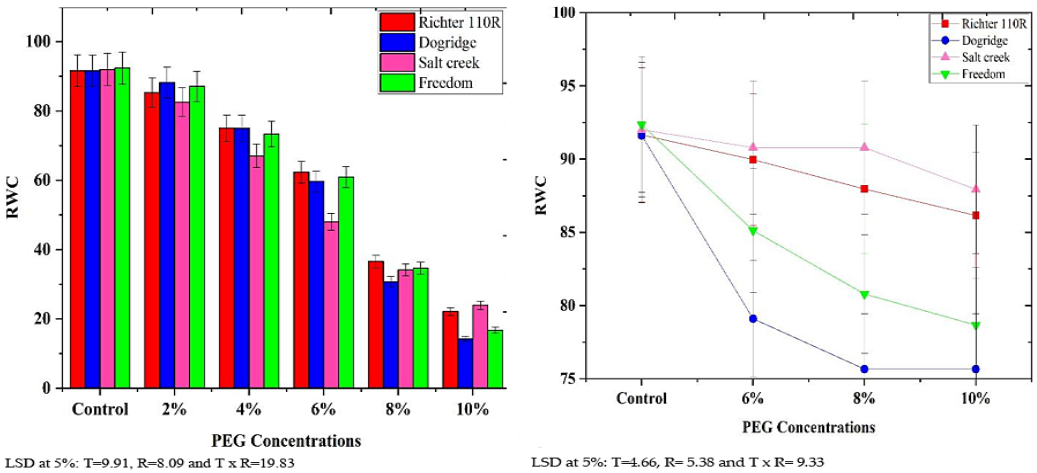


Figure 6. Effect of drought stress using PEG on RWC content of grape rootstocks grown in vitro by using shock and gradual treatment

cherry rootstock had been cultured on MS media containing an excessive level of PEG compared to the control and other remedies. Additionally, Akbarpour et al. (2017) proven that including PEG from 2% to 15% to the growing medium decreased the RWC of fig 'Sabz' CV as compared to the control. When shoots of fig 'Sabz' CV have been cultivated on MS media supplemented with a high quantity of PEG as compared to the manipulate and the bottom concentrations of PEG, the lowest fee of RWC turned into received. According to RWC statistics, the boom of fig explants might also have been slowed due to a lack of water availability beneath the drought situations of the apple rootstock MM 106 (Molassiotis and Fotopoulos, 2011). Furthermore, Su et al. (2017) pronounced that reducing the RWC percent became the final results of including PEG to the boom medium at diverse concentrations of 2%, 4%, 6%, and 8%. When shoots from several grape rootstocks had been cultivated on MS media enriched with an excessive dose of PEG from 6% to 8%, the bottom percent of RWC turned into observed as compared to the manipulate and different treatments. Relative water content (RWC) is taken into consideration an essential physiological characteristic associated with drought tolerance. It displays the ability of leaves to preserve water and is influenced

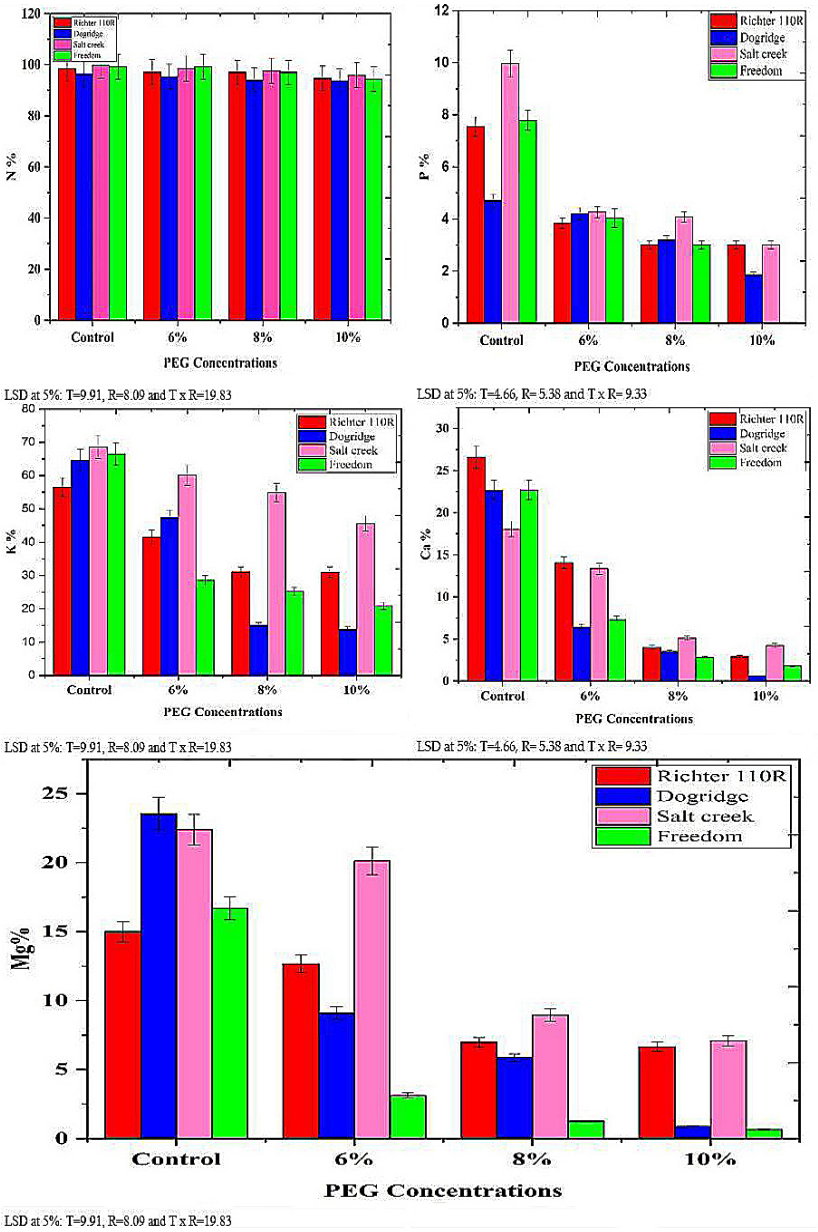


Figure 7. Effect of drought stress using PEG on ion content of grape rootstocks grown in vitro by using shock treatment

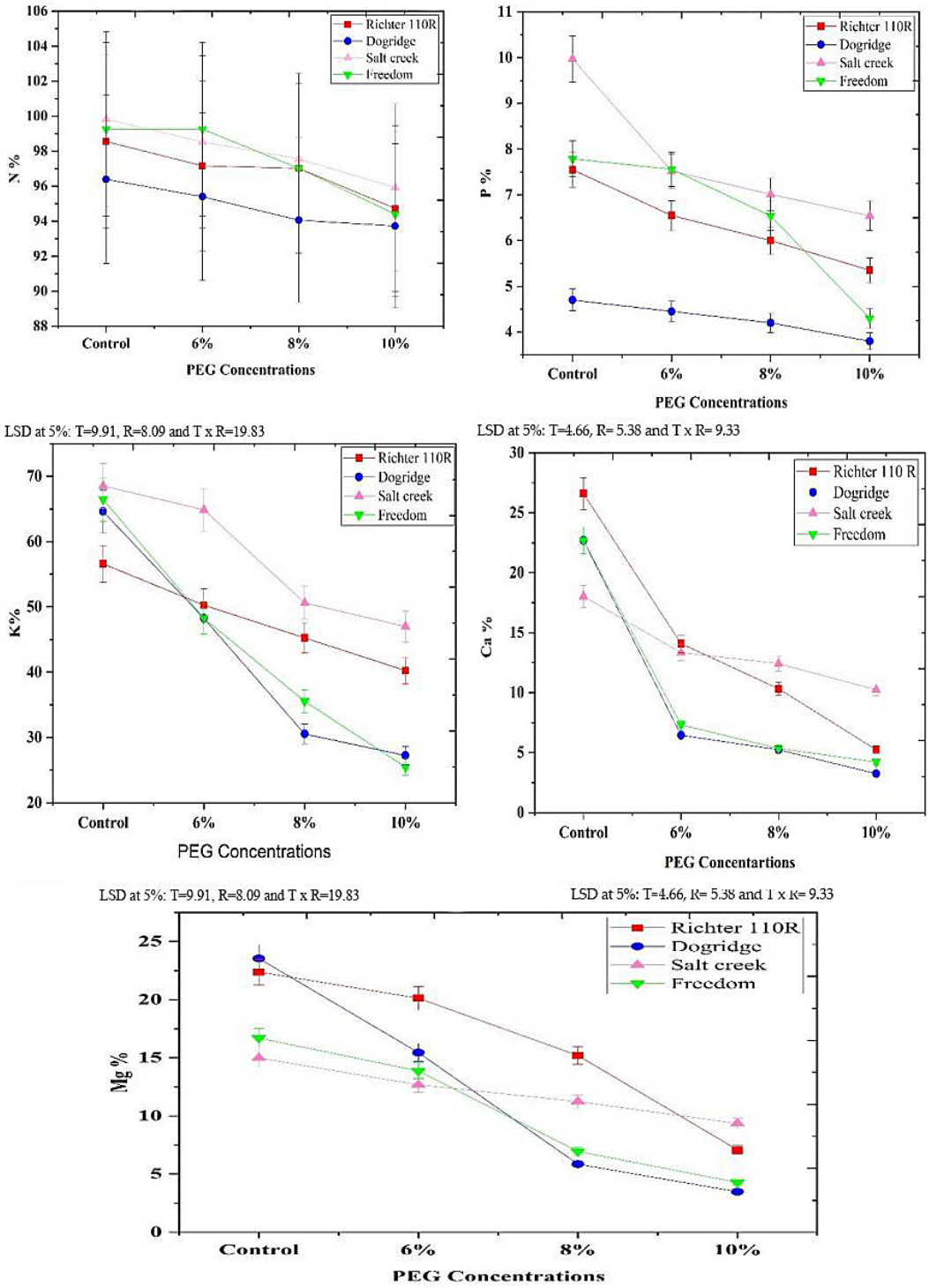


Figure 8. Effect of drought stress using PEG on ion content of grape rootstocks grown in vitro by using gradual treatment

by means of the balance between water supply and transpiration price suffering from drought pressure. The use of drought-tolerant rootstocks in grapevines has been proposed to mitigate the impact of water regulations by enhancing water uptake and delivery, as well as managing plant transpiration via chemical and hydraulic signaling (Lavoie-Lamoureux et al., 2017).

Effect of PEG on ion content.

The data presented in Figures 7 and 8 exhibits the impact of adding PEG to MS media at concentrations of 0%, 6%, 8%, and 10% the use of both a shock and stepwise remedy method on the buildup of N, P, K, Ca, and Mg in the leaves of the 4 examined grape rootstocks. The levels of PEG inside

the grape rootstocks and their interactions notably stimulated the accumulation of these elements. Adding PEG from 6% to 10% to the increase medium resulted in a lower inside the accumulation of those factors compared to the control for all tested grape rootstocks. When the micro-shoots have been transferred to the increase medium supplemented with 10% PEG, all rootstocks skilled a tremendous decline in element accumulation, with Salt Creek displaying the finest decline, followed with the aid of Richter, Dogridge, and Freedom in ascending order. In end, Salt Creek verified the highest resilience to drought some of the grape rootstocks, mainly at the high stage of PEG, while Richter, Dogridge, and Freedom exhibited decrease drought tolerance. These findings align with preceding research by using Abdel-Aziz et al. (2023), who determined extended Proline content in Musa spp. Leaves when PEG became introduced to MS media at concentrations of 2% to 6%, and Mohsen et al. (2006) who investigated the fundamental composition of diverse banana cultivars below water deficiency strain caused via special degrees of PEG in MS media. Sivritepe et al. (2008) additionally suggested a lower in sure detail stages in cherry rootstocks underneath PEG-caused water stress. Drought pressure influences nutrient uptake and growth in flora, leading to reduced output and lengthy-time period plant fitness. Nitrogen, phosphorus, and potassium are in particular impacted by using drought strain (Singh et al., 2015). Potassium, mainly, performs a critical position in maintaining photosynthetic CO_2 fixation and is needed in higher quantities in the course of environmental strain (Kathpalia and Bhatla, 2018). Phosphorus supplementation has been shown to relieve the results of drought strain on flowers by using improving stomatal conductance, photosynthesis, and cellular wall membrane integrity. Calcium, even though to a lesser extent than potassium, phosphorus, and nitrogen, is likewise constrained below drought pressure but is crucial for mobile wall development and signaling (Gelaw et al., 2023).

Effect of PEG on stomata behavior

The data presented in Figure 9 reveal the effect of including PEG to MS medium at concentrations of 0%, 6%, 8%, and 10% the use of both a shock or stepwise remedy approach at the density of open stomata, inside period and width of stomata (μm), out of doors duration and width of mobile shield (μm) within the four tested grape rootstocks. The stomata status of the 4 studied grape rootstocks became appreciably inspired by way of the rootstocks, PEG degrees, and their interactions. Increasing the PEG level within the boom medium step by step reduced the stomata status of every rootstock, with varying degrees of decrease determined among specific PEG tiers and rootstocks. All studied grape rootstocks confirmed a decrease in open stomata density while 8% PEG changed into introduced to the increase medium. While the density of open stomata in Freedom leaves become now not negatively impacted by means of including 8% PEG, Richter rootstock exhibited the very best discount percentage, observed via Salt Creek and Dogridge, which had the bottom discount percentage compared to the control. When the MS medium was supplemented with 10% PEG, the density of open stomata considerably reduced in all tested rootstocks, with Richter displaying the best reduction, observed via Salt Creek, Dogridge, and Freedom in descending order. Research shows that Richter rootstock is the most drought-resistant grape rootstock, while Salt Creek, Dogridge, and Freedom are the least tolerant. In temperate deciduous bushes, there may be a sturdy terrible affiliation between sensitivity to drought and stomata frequency, and an immediate correlation among low stomata density and tolerance to drought pressure. Beyene et al. (2015) suggested similar findings, pointing out that the addition of PEG (0%, 10%, 20%, 30%) to MS media decreased the mesophyll's substomatal chambers and intercellular spaces, multiplied cuticle thickness, epicuticular wax depositions, and plastid density, improving the capacity of stomata to close in Date palm Sewi cv plantlets. They additionally located a lower in stomata aperture duration. Similarly, Wang et al., (Wang et al., 2015) observed that stomata density decreased and stomata length and width accelerated in a few grape cultivars below water deficit conditions in comparison to the manage. The presence of small guard cells in leaf stomata may additionally contribute to balancing CO_2 uptake and water loss whilst keeping favorable stages. Levin et al. (2018) found out that water deficit caused a lower in all stomata conduct parameters, along with stomata density, period, and width, in a few grape cultivars compared to the manage. They located huge variations between all examined grape cultivars, with Syrah recording the lowest stomata density and the longest stomata period and width, followed by Grenache, Xinomavro, and Agiorgitiko. Stomata

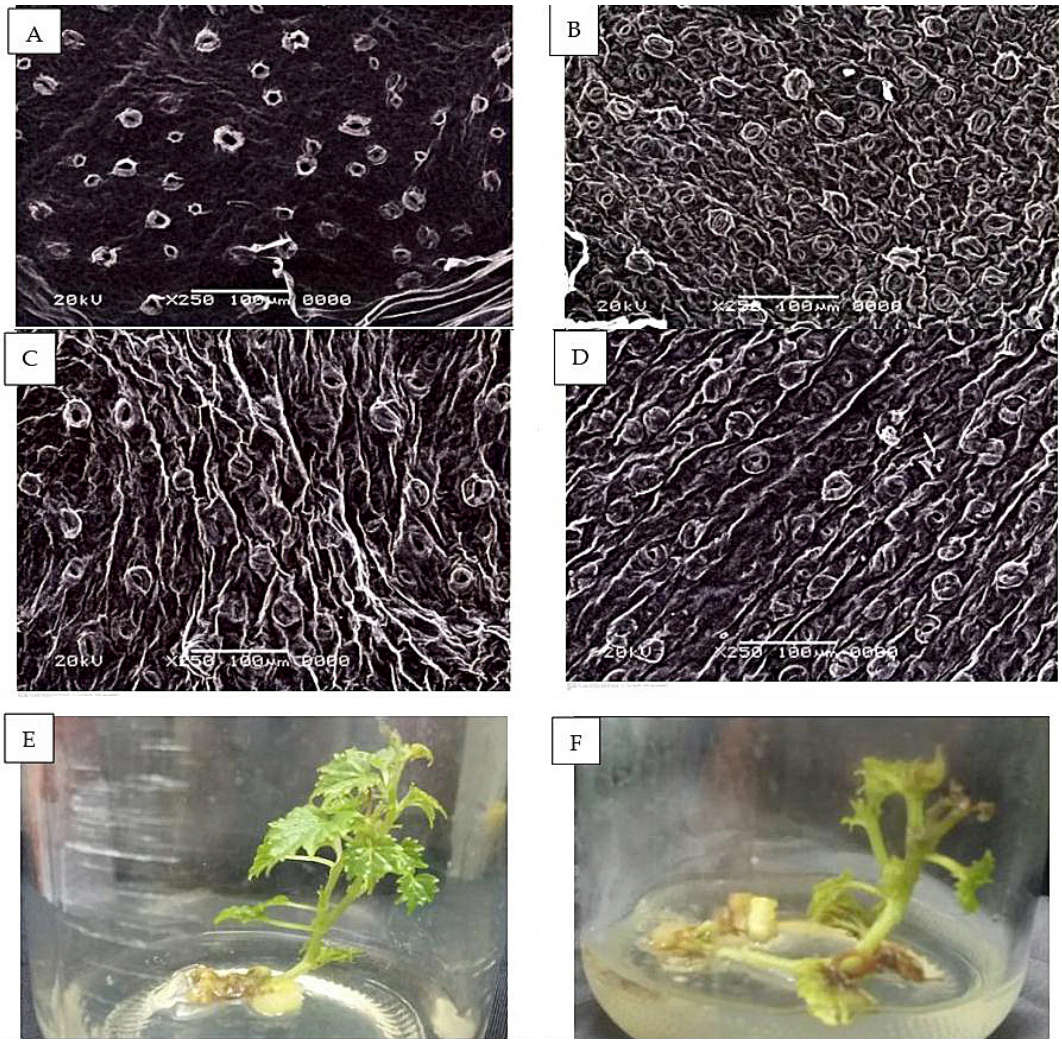


Figure 9. Effect of MS media supplemented PEG at 0%, 6% , 8% and 10% on stomata behavior of some tested grape rootstocks by SEM: (A) control, (B) PEG at 6%, (C) PEG at 8% and (D) PEG at 10%

density became notably stimulated by using genotypes, as stomata play a position in transpiration, photosynthesis, water loss, water use performance, and plant yield. In temperate deciduous timber, there is a right away correlation among low stomata density and resistance to drought stress, as well as a fantastic correlation between sensitivity to drought and stomata duration. Larger stomata tend to open and near more slowly than smaller ones, making them greater touchy to drought deficit. P. Webbii well-knownshows decrease sensitivity to water stress compared to cultivated almonds, in part because of its decrease stomata density and size. Lower stomata length may be related to drought resistance in cultivated almonds. Additionally, a lower in stomata density and cellular size under water deficit shows ability drought edition. Drought induces senescence, decreases increase and plant water potential, reasons stomata closure, and lowers transpiration and photosynthetic prices. Stomata play a crucial position in gas alternate and photosynthesis. In grapevines, water deficit ends in a reduction in stomata conductance and photosynthesis. Stomata manipulate water loss and had been recognized as an early event in plant reaction to water deficit, main to carbon uptake hindrance via the leaves. Drought-tolerant species modify stomata function to improve water use efficiency and permit a few carbon fixation below stress, even as stomata may close in extreme pressure, with photosynthesis being controlled by means of the chloroplast's capacity to restoration CO_2 as opposed to multiplied diffusiveresistance.

Antioxidant enzymes

Data in Figure 10 show that adding PEG at concentrations starting from 2% to 10% (shock treatment method) caused a boom within the activity of antioxidant enzymes, such as PPO, CAT,

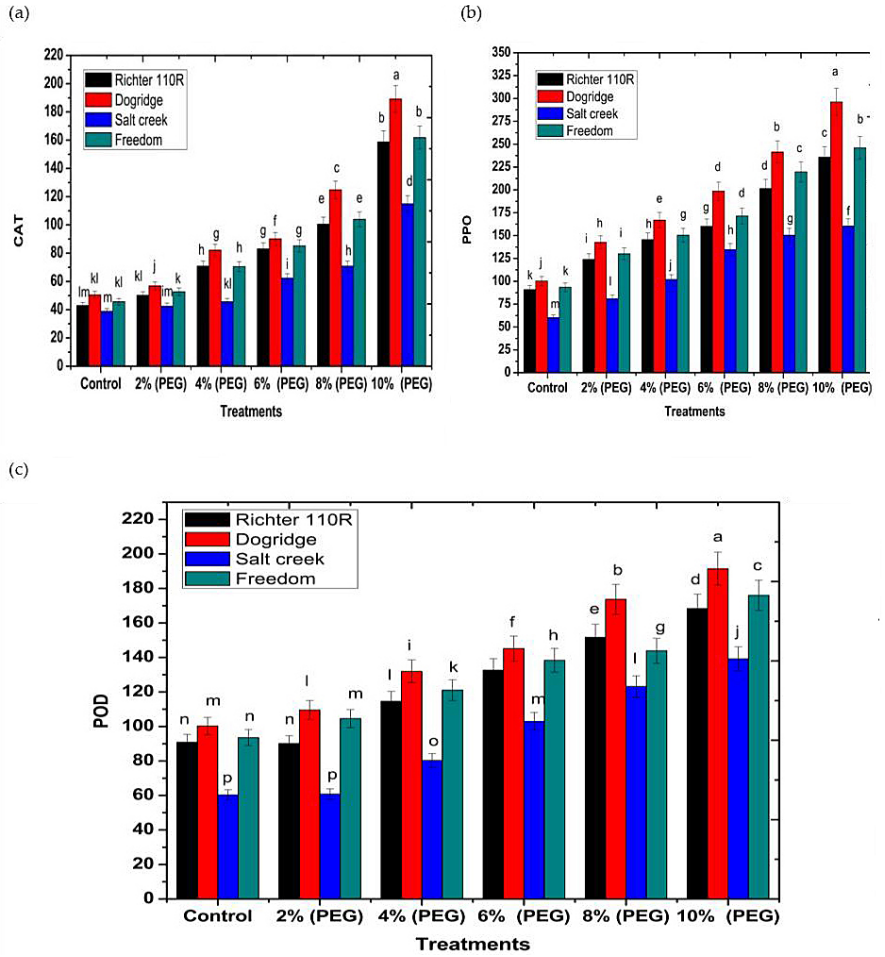


Figure 10. Effect of drought stress using PEG on antioxidant enzymes activity of grape rootstocks grown *in vitro* by using shock treatment

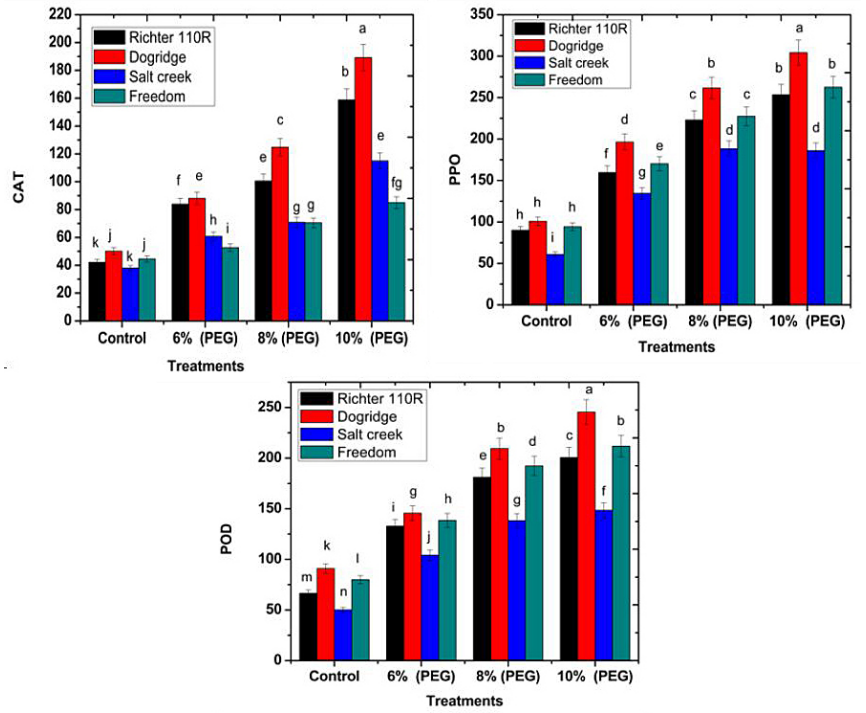


Figure 11. Effect of drought stress using PEG on antioxidant enzymes activity of grape rootstocks grown *in vitro* by using stepwise treatment

and POD, inside the leaves of some examined grape rootstocks in comparison with the control. The maximum stage of antioxidant enzyme pastime changed into executed while micro shoots of all examined grape rootstocks were cultured on MS media supplemented with 10% PEG, in comparison to each the control and other treatments. Similarly, statistics in Figure 11 display that regularly growing the PEG concentration in the MS media from 6% to ten% resulted inside the most level of antioxidant enzyme hobby inside the leaves of all examined grape rootstocks in comparison with the control. There had been top notch variations between all examined grape rootstocks concerning the effect of each shock and stepwise PEG remedy strategies on antioxidant enzyme pastime. Richter emerged as the most drought-resistant grape rootstock, accompanied by using Salt Creek, Freedom, and Dogridge in descending order of resistance. These outcomes are in settlement with Abdel-Aziz et al. (2023), who discovered that MS media supplemented with exclusive concentrations (from 2% to 6%) of PEG triggered water strain, main to a massive growth in the interest of antioxidant enzymes (peroxidase, catalase, and polyphenol oxidase) in *Musa acuminata* L. Grandinin cv. in comparison with the manage. PEG concentrations growing from 2% to 6% resulted in a great growth in CAT, PPO, and POD activity ($p \leq 0.05$) as compared to the manipulate. Similarly, Patade et al. (2012) confirmed that sugarcane (*Saccharum officinarum* L cv. Co 86032) calli's antioxidant enzyme activity turned into higher in MS media supplemented with four% PEG than in MS media without PEG.

Antioxidant enzymes are crucial for resistance to abiotic stresses like drought. Drought pressure induces lipid peroxidation, which in flip produces reactive oxygen species (ROS) including hydrogen peroxide, unfastened radicals, and hydroxyl radicals. These ROS motive membrane harm, protein degradation, and enzyme inactivation (Davies, 1993). Similarly, CAT, POX, and PPO enzyme sports were recognized as key enzymes for the defense towards ROS below various stress situations and play an important role inside the resistance of Naomi mango bushes to cold strain (Abdel-Aziz et al. 2023). According to Xia et al. (2020), an accumulation of enzymatic and non-enzymatic compounds protects mobile membranes and different cell additives. Plants utilize enzymes like CAT, GPX, SOD, GR, and APX to mitigate oxidative harm (Mishra et al., 2021). This defense gadget closely relies on enzymatic additives such as superoxide peroxidase (POD), catalase (CAT), and SOD. Adhikari et al. (2021) describe an internal protection machine in flora that shields mobile and subcellular structures from doubtlessly dangerous free radicals via the movement of antioxidant enzymes like SOD, CAT, APX, and POX. Catalase is taken into consideration the maximum normal enzyme for scavenging hydrogen peroxide, which accumulates whilst vegetation are subjected to abiotic strain and can harm the photosynthetic equipment, leading to oxidative harm to proteins, lipids, and nucleic acids, ultimately impairing ordinary metabolism. Additionally, polyphenol oxidase (PPO) and peroxidase (POD) are two antioxidant enzymes that play a first-rate role in preventing mobile destruction (Zhan et al., 2013).

CONCLUSIONS

This observe supplied proof that positive morphological characteristics and photosynthetic pigments, such as chlorophyll a and b, RWC percentage, and Proline content, in the leaves of all examined grape rootstocks had been affected by applying PEG treatments as drought stress beneath tissue way of life method, the use of either shock or stepwise strategies, as compared with the control. MS media supplemented with PEG, mainly at high concentrations, brought on a decrease in both morphological and biochemical characteristics besides for proline content, which improved with growing PEG concentrations as compared with the manipulate. Additionally, including PEG to the MS media caused an increase in leaf content material of antioxidants, indicated by way of higher CAT, POX, and PPO enzyme interest compared with the manipulate. Interestingly, this examine found that step by step growing PEG from 6% to 8% led to an boom in all morphological and biochemical characteristics in comparison with the manipulate. However, in addition growing PEG within the increase medium from 8% to 10% regularly prompted a lower in all formerly mentioned biochemical traits, RWC, and all expected elements of all tested rootstocks. Despite this lower, a corresponding growth turned into determined in proline and antioxidant enzyme interest (PPO, CAT, and POD) in comparison with the control. As the effects of PEG by means of shock and stepwise treatment approaches on the morphological, biochemical, and

antioxidant enzyme interest inside the leaves of all tested grape rootstocks numerous, Richter and Salt Creek emerged because the most drought-resistant grape rootstocks, accompanied in descending order of resistance by Freedom and Dogridge. Further research is needed at the genetic level to identify the genes responsible for drought tolerance in European grape varieties. Moving forward, these genes can then be used in breeding and hybridization programs to improve the resistance of varieties to water stress.

Acknowledgments

This research was funded by Researchers Supporting Project number (RSP2024R334), King Saud University, Riyadh, Saudi Arabia.

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