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ARTICLE

Evaluation of drought tolerance in various petunia genotypes based on their morpho-physiological and biochemical responses

Avaliação da tolerância à seca em vários genótipos de petúnia com base em suas respostas morfofisiológicas e bioquímicas

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Abstract
Due to water resource constraints, studying and selecting drought-tolerant ornamental plants, along with a better understanding of their physiological and biochemical mechanisms, can help enhance their productivity in arid and semi-arid environments. A factorial experiment based on a completely randomized design was conducted with three water regimes (no stress – 90% container capacity (CC), moderate stress – 60% CC, and severe stress – 30% CC) in eight petunia genotypes, namely: Heirloom petunia (P1), *Petunia* × *hybrida* Supercascade White (P2), *Petunia* × *hybrida* grandiflora Frost Blue (P3), *Petunia* × *hybrida* grandiflora crimson star (P4) *Petunia milliflora* Picobella Rose Morn (P5), *Petunia grandiflora* Tritunia White (P6), *Petunia grandiflora* Success 360 Blue (P7) and *Petunia Supercascade* Red (P8). After the application period of the water regimes, morpho-physiological, biochemical, and growth variables were evaluated over a total experimental duration of 120 days. Photosynthetic pigments and growth parameters were reduced under stress conditions in all Petunia genotypes. Under drought conditions, P1and P5 genotypes exhibited higher performance, production of osmotically active solutes and activities of antioxidant enzymes, lower electrolyte leakage, and smaller reduction of relative water content than the other genotypes. Catalase and superoxide dismutase had no major antioxidative functions in P8 genotype and was recognized as the most vulnerable to severe water stress. These findings highlight the superior drought tolerance of genotype P1, followed by P5, indicating their potential for cultivation in water-limited environments. This provides valuable insights for breeding programs and landscaping applications in drought-prone areas.
Keywords: antioxidant, growth, *Petunia* × *hybrida*, tolerant, water scarcity.

Resumo
Devido às restrições dos recursos hídricos, o estudo e a seleção de plantas ornamentais tolerantes à seca, juntamente com uma melhor compreensão de seus mecanismos fisiológicos e bioquímicos, podem contribuir para aumentar sua produtividade em ambientes áridos e semiáridos. Um experimento fatorial baseado no delineamento inteiramente casualizado foi conduzido com três regimes hídricos (sem estresse – 90% da capacidade do vaso, estresse moderado – 60% da capacidade do vaso e estresse severo – 30% da capacidade do vaso) em oito genótipos de petúnia, a saber: Heirloom petunia (P1), *Petunia* × *hybrida* Supercascade White (P2), *Petunia* × *hybrida* grandiflora Frost Blue (P3), *Petunia* × *hybrida* grandiflora Crimson Star (P4), *Petunia milliflora* Picobella Rose Morn (P5), *Petunia grandiflora* Tritunia White (P6), *Petunia grandiflora* Success 360 Blue (P7) e *Petunia Supercascade* Red (P8). Após o período de aplicação dos regimes hídricos, foram avaliadas variáveis morfofisiológicas, bioquímicas e de crescimento, com duração total do experimento de 120 dias. Os pigmentos fotossintéticos e os parâmetros de crescimento foram reduzidos sob condições de estresse em todos os genótipos de petúnia. Sob condições de seca, os genótipos P1 e P5 apresentaram melhor desempenho, maior produção de solutos osmoticamente ativos e maior atividade de enzimas antioxidantes, menor extravasamento de eletrólitos e menor redução do teor relativo de água em comparação com os demais genótipos. A catalase e a superóxido dismutase não desempenharam funções antioxidativas significativas no genótipo P8, que foi identificado como o mais vulnerável ao estresse hídrico severo. Esses resultados destacam o potencial do genótipo P1, seguido por P5, para o cultivo em ambientes com limitação de água, oferecendo informações valiosas para o melhoramento genético e aplicações em paisagismo em áreas propensas à seca.
Palavras-chave: antioxidante, crescimento, escassez de água, *Petunia* × *hybrida*, tolerante.

Introduction

Water scarcity is one of the most critical constraints in agricultural and horticultural production, especially in arid and semi-arid regions (Goldani et al., 2021). It severely limits plant vegetative and reproductive growth and affects the visual quality and attractiveness of ornamental plants. Drought stress disrupts plant morphology, physiology, water relations, and metabolism, with its impact influenced by plant species, developmental stage, soil conditions, and environmental factors (Tafaghodi et al., 2018). Given the increasing unpredictability of rainfall patterns and global warming, improving drought tolerance in plants has become a pressing concern, especially for plants cultivated for aesthetic purposes. Thus, screening for resistant genotypes is indispensable with respect to the restricted water resources.

Plants exhibit varying degrees of drought stress tolerance, which largely depends on genotypic differences. These differences offer opportunities to select genotypes with superior adaptive traits, such as enhanced photosynthesis and yield under limited water availability (Gholami et al., 2012). To survive drought conditions, plants have evolved a range of morphological, physiological, and biochemical mechanisms, including delaying or accelerating flowering, reducing growth, and initiating osmotic adjustment via the accumulation of compatible solutes like proline and soluble sugars (Chirivì and Betti, 2023; Khosravi and Haghighi, 2021). Additionally, drought triggers the overproduction of reactive oxygen species (ROS), which can harm cellular components. To mitigate these effects, plants activate antioxidant defense systems composed of enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Tafaghodi et al., 2018). Drought-tolerant plants with low water requirements during the hot season should be assessed to determine further water requirements (Wang et al., 2021). Recently, there has been increasing attention on ornamental plants with low water demand. Therefore, it is essential to detect drought-tolerant plants (Rebi et al., 2024).

Petunia (*Petunia* sp.), belonging to the family Solanaceae, is an annual flowering plant that originated in South America (Mexico and Argentina). It is one of the most economically fabulous ornamental plants with genotypes appropriate for summer planting in the landscape because of its diversity in shape, size, odor, and flower color (Goldani et al., 2021;

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Rezaei et al., 2023; Tran et al., 2024). Moreover, it is extensively grown in gardens due to the extended flowering period and is recognized as the most significant and noteworthy seasonal plant (Tafaghodi et al., 2018). Nevertheless, Petunia is vulnerable to water limitations (Tran et al., 2024). Moreover, petunia is a tropical plant and is highly likely to experience prolonged water deficit. Therefore, comparing heirloom petunia and commercially significant genotypes under the same environmental conditions can identify the best-performing genotypes in dry climate.

Therefore, this study aims to evaluate the morpho-physiological, biochemical, and growth responses of eight Petunia genotypes – including both heirloom and commercially important varieties – under controlled drought stress conditions. To the best of our knowledge, no comprehensive study has yet reported the comparative drought tolerance of these genotypes. We hypothesize that genotypic variability plays a key role in drought response, and that specific genotypes will exhibit superior adaptive traits. By encompassing a broad spectrum of genetic diversity, this research seeks to identify key indicators and mechanisms of drought tolerance, providing valuable insights for future breeding programs and ornamental plant cultivation in water-limited environments.

Material and Methods

This experiment was conducted in 2023 in the greenhouse of the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran (36°17′ N, 59°36′ E). Greenhouse conditions were maintained at an average temperature of 24 ± 2 °C, midday photosynthetic photon flux density (PPFD) ranging from 300 to 1500 µmol m^{-2} s^{-1}, and relative humidity of 60 ± 1%.

Eight petunia genotypes were evaluated in this study: Heirloom petunia (P1), *Petunia* × *hybrida* Supercascade White (P2), *Petunia* × *hybrida* grandiflora Frost Blue (P3), *Petunia* × *hybrida* grandiflora Crimson Star (P4), *Petunia milliflora* Picobella Rose Morn (P5), *Petunia grandiflora* Tritunia White (P6), *Petunia grandiflora* Success 360 Blue (P7), and *Petunia Supercascade* Red (P8). These genotypes represent a diverse array of petunia cultivars with distinct ornamental traits:

P1: Heirloom Petunia refers to a variety of petunias that have been passed down through generations, typically through traditional gardening practices, and these petunias are non-hybrid. Characteristics such as flower color, size, and growth habits may vary depending on the specific variety. P2: Known for large, white flowers and trailing habit, ideal for hanging baskets and containers. P3: Features pale blue to lavender blooms with a frosted appearance, appreciated for their striking color. P4: Distinguished by vibrant red flowers with a star-shaped white center, visually appealing for gardens. P5: Characterized by smaller, rose-pink flowers with a lighter center, providing a delicate look. P6: Known for large, pure white flowers, versatile for various settings. P7: Features deep blue or purple-blue flowers, noted for uniform growth and continuous blooming. P8: Similar to Supercascade White but with striking red flowers, ideal for adding a splash of color to containers. These genotypes represent a diverse array of petunia varieties with unique characteristics that contribute to their ornamental value in horticulture (Fig. 1).

Seeds were sown in trays filled with a substrate of cocopeat, peatmoss, and perlite (2:2:1, v/v/v). After approximately 50 days, when the seedlings had developed four true leaves, they were transplanted into 5-L containers filled with a 1:1:1 (v/v/v) mix of leaf mold, sand, and loamy soil, with a gravel layer at the bottom for drainage. The physicochemical properties of the soil were as follows: pH = 6.5, EC = 1.1 dS m^{-1}, and a loamy texture. This study was conducted as a factorial experiment based on a completely randomized design (CRD) with four replicates.

Seedlings were irrigated daily with tap water to 100% field capacity (FC) for four months (April 20th to August 20th) up to be established. The soil field capacity was determined according to the method described by Cambell and Mulla (1990). Three levels of watering were then imposed: no stress (90% FC), moderate stress (60% FC), and severe stress (30% FC). Preservation of the water treatments was achieved by daily weighing of the pots, substituting the water lost by transpiration using a precision scale. These regimes were applied for 120 days. The following parameters were determined at the end of the experiment.

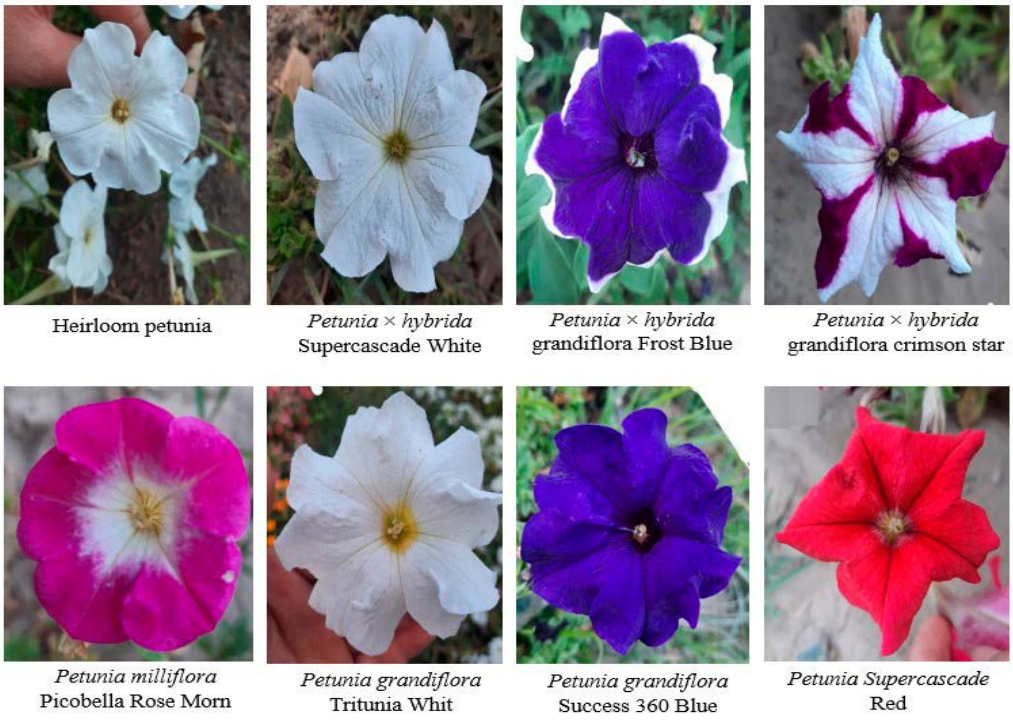

Fig. 1. Identification and characterization of the petunia genotypes under investigation.

Fresh and dry weight of roots and aerial part

At the end of the experimental drought treatments, shoots (stems, leaves, and flowers) and roots were weighed using a digital balance, placed in separate envelopes, and oven-dried at 65 - 70 ˚C for 48 h.

Stem length

Stem length was measured from the crown to the top of the stem with a ruler and expressed in centimeters.

Root diameter

Root diameter was measured at the crown of the plant using a digital caliper and expressed in millimetres.

Lateral branch number

The number of lateral branches was counted at the end of the experiment.

Flower diameter and number

Five fully open flowers in each replicate were randomly selected to measure flower diameter. Flower diameter was measured from one side of the flower petals to the opposite side using a digital caliper. The flower numbers were counted for each replication.

Electrolyte leakage

Leaf electrolyte leakage was measured using the method described by Lutts et al., (1996). Six discs with a diameter of 1 cm were prepared from fully developed young leaves and washed twice with distilled water and once with deionized water. Then, 10 ml deionized water was added to the leaf disk and placed at room temperature for 12 h. Electrolytic conductivity (Lt1) of the samples was measured using a digital conductivity meter. Samples were then autoclaved at 120 ˚C for an hour and following cooled, and the electrical conductivity of the samples was measured (Lt2). The electrolyte leakage (EC%) was calculated using the following equation:

$(EC\% = Lt_1/Lt_2 \times 100)$.

Relative water content

The relative water content (RWC) of the leaf samples was measured using the method described by Ghoulam et al. (2002). Five uniform disk of fully developed young leaves were used, and RWC (%) was calculated using the following equation:

$$RWC = \left[\frac{(FW - DW)}{(TW - DW)}\right] \times 100$$

where FW is the fresh weight, DW is the dry weight of the leaf discs after drying in the oven at 70˚C for 24 h, and TW is the turgid weight when rehydration of the leaf discs occurred by soaking for 4 h in the dark.

Total carbohydrate concentration

To determine the total carbohydrate content, 0.1 g of leaf powder was extracted with 25 mL ethanol (80%) and shaken for a few seconds. After centrifuging at 5,000 rpm, the supernatant was separated, decolorized with activated carbon, and filtered. Then, the extract volume was made up to 100 ml with distilled water. Approximately 1 ml of supernatant was added to 10 ml anthrone solution (0.15%) and then heated at 95 ˚C for 8 min. After transferring to an ice bath, the absorbance of the samples was recorded at 625 nm. The results were expressed as mg g^{-1} DW (McCready et al., 1950).

Proline content

Free proline was extracted from 0.5 g samples of fully expanded leaves with 10 mL of sulfuric acid 3% and estimated using ninhydrin reagent, according to the protocol described by Bates et al. (1973). The absorbance of the fraction with toluene was read spectrophotometrically at 520 nm. Proline concentration was calculated using a calibration curve and expressed as µmol g^{-1} FW.

Chlorophyll determination

Chlorophyll (a and b) content was measured according to the method described by Lichtenthaler (1987). Then, 4 mL of 80% acetone was added to 0.1 g fresh leaves. Following centrifugation at 3000 rpm for 10 min, the UV absorbance of the samples was read at 647 nm and 664 nm against acetone as a blank. The result was expressed as milligrams per fresh weight of leaf, using following equations.

$$\text{Chlorophyll a (mg g}^{-1}\text{ FW)} = \frac{12.25(A_{664}) - 2.79(A_{647}) \times \text{Volume made}}{\text{Wt of the sample}}$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ FW)} = \frac{21.21(A_{647}) - 5.10(A_{664}) \times \text{Volume made}}{\text{Wt of the sample}}$$

Where Wt is the weight of the sample, and Al is the absorption at wavelength l (nm).

Antioxidant enzymes activity

Leaf tissue (0.5 g) was ground with 1 mL extraction buffer and centrifuged at 15,000 rpm for 20 min at 4 ˚C. The supernatant was then used to measure the antioxidant enzyme activity. To measure the antioxidant activity of catalase, phosphate buffer containing dipotassium phosphate, monopotassium phosphate, and hydrogen peroxide was added to the enzyme extract, and the absorbance was read at 240 nm using a spectrophotometer. The results were expressed as U g^{-1} FW (Aebi, 1984). Peroxidase enzyme activity was measured using the method described by Ferreira et al. (2010) and expressed as U g^{-1} FW. The activity of superoxide dismutase was measured according to the method described by Giannopolitis and Ries (1977). Superoxide dismutase enzyme activity was determined by its ability to prevent the photochemical reduction of nitroblue tetrazolium (NBT), and the absorbance was recorded at 560 nm using a spectrophotometer. Superoxide dismutase activity was expressed as U mg^{-1} protein.

Statistical Analysis

Data were analysed using SAS software (ver. 9.4), and the significance of the differences was compared using the least significant difference (LSD) test at $p \leq 5\%$. Principal component analysis (PCA) was performed using MINITAB software (version 16). Pearson correlation analysis was performed using SAS software.

Results

Effect of drought stress on growth parameters

Growth parameters significantly decreased in eight petunia genotypes under different water regimes (60% FC and 30% FC) after 120 days. In this case, the average fresh and dry weights of stems and roots were significantly reduced by increasing the drought levels from 90 to 30% FC. (Fig. 2A). The fresh shoot weight of eight genotypes was at its peak after under 90% FC. The shoot's fresh weight declined as levels of drought stress increased. P1 genotype produced the highest shoot fresh weight (52.03 g), followed by the same genotype following the 60% FC. P1 genotype usually outperformed for all treatments compared with other genotypes.

The shoot's dry weight rapidly declined as the drought stress increased. The minimum dry shoot weight (1.89 g) values were recorded in the genotype P8 at 30% FC. When the plants were subjected to 30% FC, there was a reduction in the dry shoot weight in all genotypes which reveals the sensitivity of eight genotypes to water scarcity (Fig. 2B).

Drought stress significantly reduced the fresh weight of the roots in eight petunia genotypes. Severe stress (30% FC) significantly reduced the fresh weight of roots compared to the control (90% FC) and moderate (60% FC) stress. P1 genotype showed the lowest reduction in fresh weight of root throughout the stress conditions. In control treatment (90% FC), the highest root fresh weight was related to P1 genotype, while P5 genotype had the lowest root fresh weight. Nonetheless, there was no significant difference among P3, P4, P5, and P7 genotypes in 90% FC. Drought stress caused a significant decrease in root fresh weight among studied genotypes. In moderate stress (60% FC), P1 and P6 genotypes with 16.91 g and 9.19 g had the highest root fresh weight and after that, P2, P5, P3, P4, and P8 genotypes were in the next orders, respectively. Under severe stress (30% FC), the highest root fresh weight was observed in P1 genotype with the value of 14.75 g compared to other genotypes (Fig. 2C).

When the plants were subjected to 90% FC, the dry weight of the roots of eight genotypes was at its highest. As the drought stress level increased, the root dry weight decreased. The P1 genotype revealed a significant decrease in root dry weight, from 60% FC to 30% FC, whilst, other genotypes did not show substantial drop as the drought stress level decreased (Fig. 2D).

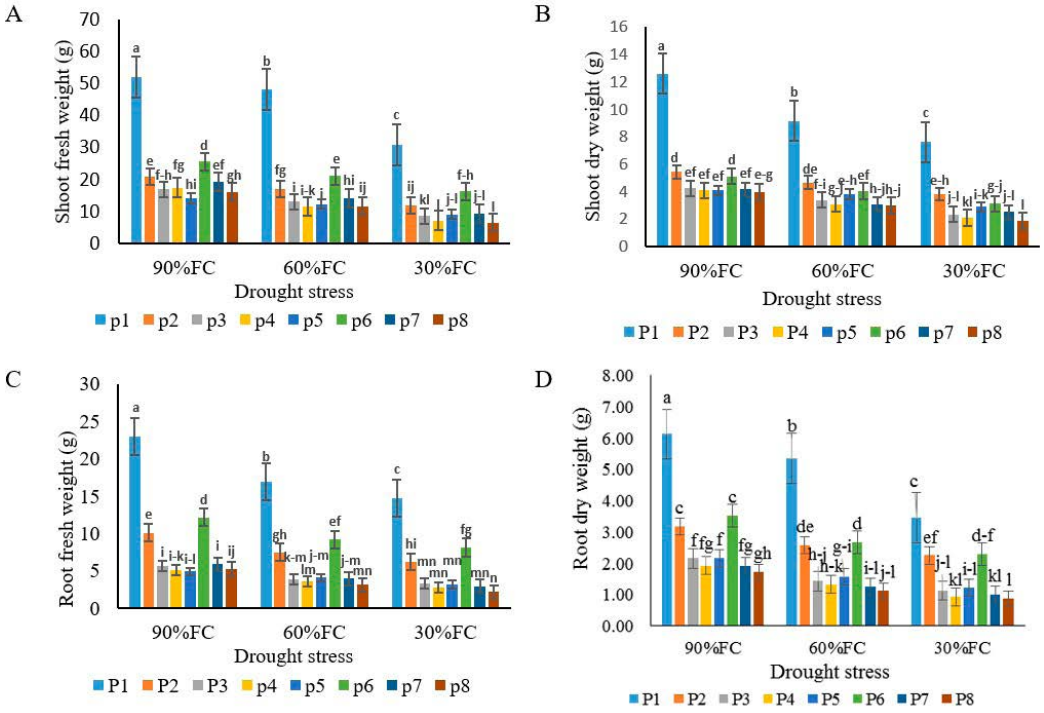

Fig. 2. Interaction effect of petunia genotypes and drought stress levels on shoot and root dry and fresh weight of eight genotypes of petunia plants. The graph's bars represent the average value over three replicates, while the error bars denote the standard deviation. Different lowercase letters indicate significant differences among genotype × drought stress combinations according to LSD test at $p \leq 0.05$.

Under drought conditions (60% and 30% FC), no significant changes in stem height were recorded for the P4, P5, and P6 genotypes. Stem height decreased significantly in all genotypes under moderate stress (60% FC) and severe stress (30% FC) compared to the control plants, reaching a minimum value of 38.24 cm, 18.22 cm, 13.1 cm, 10.31 cm, 14.09 cm, 15.82 cm, 11.89 cm, and 11.12 cm in P1, P2, P3, P4, P5, P6, P7, and P8 genotypes, respectively, at 30% FC (Fig. 3A).

The comparison of means in Fig. 3 revealed that flower number showed variations in eight genotypes against all water stress conditions. From the LSD contrasting results, the maximum flower number (30.75) was observed in the genotype P1, followed by the genotype p6 (25.5) following treatment 90% FC. Following subjected to 60% FC, all genotypes reduced the flower number, reaching the minimum value in genotype P8 (5). Eight genotypes showed poor performance following subjecting to sever stress (Fig. 3). Poor performance for flower size was exhibited against 30 % FC. So, it can be showed that with declining water levels, flower size decreases (Fig. 3B).

Water stress treatments significantly influenced branch number in all eight genotypes compared with non-stress conditions. The branch number increased in P1, P3 and P7 up to moderate stress (from 90% to 60% FC), but decreased sharply under severe stress (30%) compared to control plants. In the rest of the genotypes, the branch number showed a non-significant differences with increasing water shortage from 90% FC to 60% FC. (Fig. 3C).

Root diameter was affected by water scarcity. Severe water shortage (30% FC) considerably reduced diameter in all eight genotypes. The result showed that root diameter was changed over time. The root diameter peaked when eight genotypes were at non-stress condition. The P1 genotype observed a maximum root diameter of 7.65 mm, followed by the same genotype for treatment 60% FC. Root diameter declined rapidly as the water shortage increased, reaching a minimum value of 4.74 mm, 3.42mm, 2.55mm, 2.61 mm, 2.01 mm, 3.12 mm, 2.68 mm, and 2.65 mm in P1, P2, P3, P4, P5, P6, P7, and P8, respectively, in 30% FC (Fig. 3D).

The comparison of means in Fig. 3E revealed that flower diameter varied among the eight genotypes under drought stress treatments. From the LSD contrasting results, the maximum flower diameter (71.78 mm) was observed in the genotype P6, following 90% FC treatment. The behaviour of all genotypes was the same as the reduction trend for the 60% FC treatment, except for P7. All genotypes showed low flower diameter under severe water stress, (30% FC), that the lowest flower diameter was recorded in genotype P1 (43.57 mm).

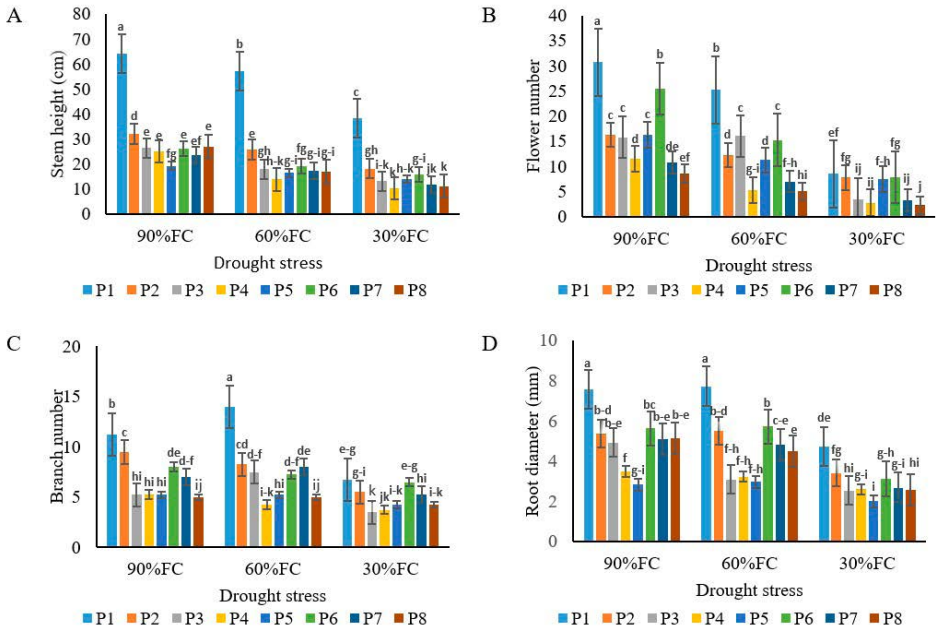

Fig. 3. Interaction effect of petunia genotypes and drought stress levels on stem height, flower and branch number, root and flower diameter of eight genotypes of petunia plants. The graph's bars represent the average value over three replicates, while the error bars denote the standard deviation. Different lowercase letters indicate significant differences among genotype × drought stress combinations according to LSD test at $p \leq 0.05$.

Electrolyte leakage

After the experiment, the percentage of electrolyte leakage was assayed and was found to differ for all water stress treatments. The electrolyte leakage of all eight genotypes was not significantly differences in 90% FC condition. Subsequently, it increased as the water requirement decreased. P4 genotype produced the highest electrolyte leakage (41.26 %) at 60% FC. The maximum percentage of electrolyte leakage was recorded in P8 genotype at 30% FC (Table 1).

Table 1. Effect of drought stress on electrolyte leakage, relative water content, proline and total carbohydrate content of eight genotypes of petunia plants.

Rose genotype	Drought stress	Electrolyte leakage (%)	Relative water content (%)	Proline (µmol g^{-1} FW)	Total carbohydrates (mg g^{-1} DW)
P1	90%FC	20.65^{i}	80.35^{a}	1.69^{de}	65.07^{ef}
	60%FC	22.28^{hi}	82.01^{a}	1.87^{cd}	63.28^{cd}
	30%FC	25.23^{gh}	67.67^{b-c}	2.24^{ab}	68.17^{a}
P2	90%FC	19.53^{i}	79.22^{a}	1.49^{e-g}	56.17^{ef}
	60%FC	27.18^{fg}	82.11^{a}	1.56^{ef}	65.18^{a-c}
	30%FC	27.91^{fg}	69.16^{b}	2.04^{a-c}	67.12^{ab}
P3	90%FC	20.11^{i}	82.01^{a}	1.12^{ij}	42.83^{k}
	60%FC	29.01^{ef}	71.87^{b}	1.36^{f-j}	48.11^{ij}
	30%FC	35.81^{cd}	64.19^{c-e}	1.34^{f-j}	46.77^{j}
P4	90%FC	19.44^{i}	80.42^{a}	1.11^{j}	51.39^{hi}
	60%FC	41.26^{b}	72.16^{b}	1.40^{f-h}	54.77^{f-h}
	30%FC	36.09^{c}	60.82^{e}	1.28^{g-j}	53.32^{f-h}
P5	90%FC	19.63^{i}	80.17^{a}	1.38^{f-j}	55.17^{fg}
	60%FC	25.68^{f-h}	79.18^{a}	1.53^{e-g}	64.28^{bc}
	30%FC	27.98^{fg}	70.12^{b}	2.31^{a}	68.19^{a}
P6	90%FC	19.47^{i}	81.30^{a}	1.27^{g-j}	54.19^{f-h}
	60%FC	28.17^{fg}	70.14^{b}	1.39^{f-i}	59.71^{de}
	30%FC	32.14^{de}	63.29^{de}	1.98^{bc}	63.17^{cd}
P7	90%FC	21.05^{i}	80.72^{a}	1.21^{h-j}	51.24^{hi}
	60%FC	32.19^{de}	70.19^{b}	1.37^{f-j}	55.22^{fg}
	30%FC	42.18^{b}	61.13^{e}	1.52^{e-g}	54.19^{f-h}
P8	90%FC	20.19^{i}	81.88^{a}	1.22^{h-j}	52.18^{gh}
	60%FC	35.12^{cd}	69.12^{bc}	1.42^{f-h}	54.68^{f-h}
	30%FC	47.37^{a}	60.53^{e}	1.30^{f-j}	53.27^{f-h}

Means followed by the same letter within a column are not significantly different according to LSD test at $p < 0.05$

Relative water content

The relative water content was not influenced in all eight genotypes under non-stress conditions. While, the relative water content was affected as the drought stress level increased. Severe water shortage (30% FC) considerably reduced the relative water content in all eight genotypes, reaching a minimum value of 67.67%, 69.16%, 64.19%, 60.82%, 70.12%, 63.29%, 61.13%, and 60.53% in P1, P2, P3, P4, P5, P6, P7, and P8, respectively, in 30% FC.

Proline content

Although there was an increasing pattern during the experiment, proline content in P3, and P8 genotypes did not show a significant change compared to that ones in the well-watered condition. The highest proline content was recorded in the P5 genotype with a value of 2.31 µmol g^{-1} FW compared to other genotypes under 30% FC (Table 1). Nonetheless, there were no significant difference with P1 and P2 genotypes.

Total carbohydrate content

Under drought conditions (60% FC and 30% FC), no significant changes were recorded in the total carbohydrate concentrations for the P3, P4, P6, P7, and P8 genotypes. Total carbohydrate content increased significantly in P1, P2, P3, P5, P6, and P7genotypes under moderate stress (60% FC) compared to control plants. The highest total carbohydrate content was recorded in the P5 genotype with a value of 68.19 mg g^{-1} DW compared to other genotypes under 30% FC. Nonetheless, there were no significant difference with P1 and P2 genotypes (Table 1).

Photosynthetic pigments

When the plants were watered with 90% FC, the chlorophyll a content was the highest in all eight genotypes. The genotype P3 produced the most chlorophyll a (1.51 mg g^{-1} FW) under 90% FC condition, having no significant difference with genotypes P7 (Fig. 4A). The chlorophyll a concentration declined substantially as the water shortage increased, reaching a minimum of 0.68 mg g^{-1} FW under 30% FC in P4 genotype.

Under non-stressed conditions (90% FC), the highest chlorophyll b content was observed in P3 genotype (0.76 mg g^{-1} FW). As water stress increased, the chlorophyll b concentration of the plants decreased. However, the chlorophyll b content was not significantly influenced in P2 and P3 at 60% FC. There was a sharp decline in chlorophyll b content with increasing levels of water stress up to 30% FC. The minimum chlorophyll b content was detected in P8 genotype with the value of 0.51 mg g^{-1} FW under 30% FC. Nonetheless, there were no significant difference with P4 genotype (Fig. 4B).

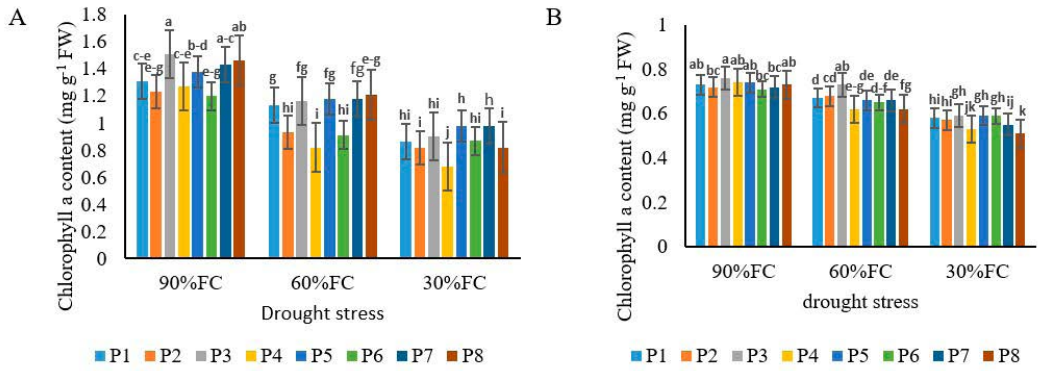

Fig. 4. Interaction effect of petunia genotypes and drought stress levels on chlorophyll a and b content of eight genotypes of petunia plants. The graph's bars represent the average value over three replicates, while the error bars denote the standard deviation. Different lowercase letters indicate significant differences among genotype × drought stress combinations according to LSD test at $p \leq 0.05$.

Antioxidant enzyme activities

CAT activity in leaves increased with drought stress in P1, P2, P3, P7, and P8 genotypes compared to those ones in the control plants. CAT activity significantly increased in P1 genotype from 60% FC to 30% FC (1.01 U g^{-1} FW) compared with other genotypes (Fig. 5A).

The SOD activity increased in all eight genotypes up to moderate stress (from 90% to 60% FC). P2 and P1 genotypes recorded the highest SOD enzyme activity under 60%FC (22.3 U mg^{-1} protein, 21.65 U mg^{-1} protein, respectively). As water shortage increased to 30% FC, the highest SOD enzyme activity was observed in P5 genotype with the value of 23.86 U mg^{-1} protein, having no significant difference with genotypes P1, and P2 (Fig. 5B).

Under non-stressed conditions (90% FC), POD activity was the highest content in P5 genotype (5.71 U g^{-1} FW). Nonetheless, there were no significant difference with P1 genotype. As water stress increased up to 60% FC, the POD activity of the plants increased. P5 and P1 genotypes recorded the highest POD enzyme activity under 60%FC (6.12 U g^{-1} FW, 6.05 U g^{-1} FW, respectively). As water shortage increased up to 30% FC, the lowest POD enzyme activity was observed in P8 genotype with the value of 3.63 U g^{-1} FW, having no significant difference with genotypes P3, P4, and P7 (Fig. 5C).

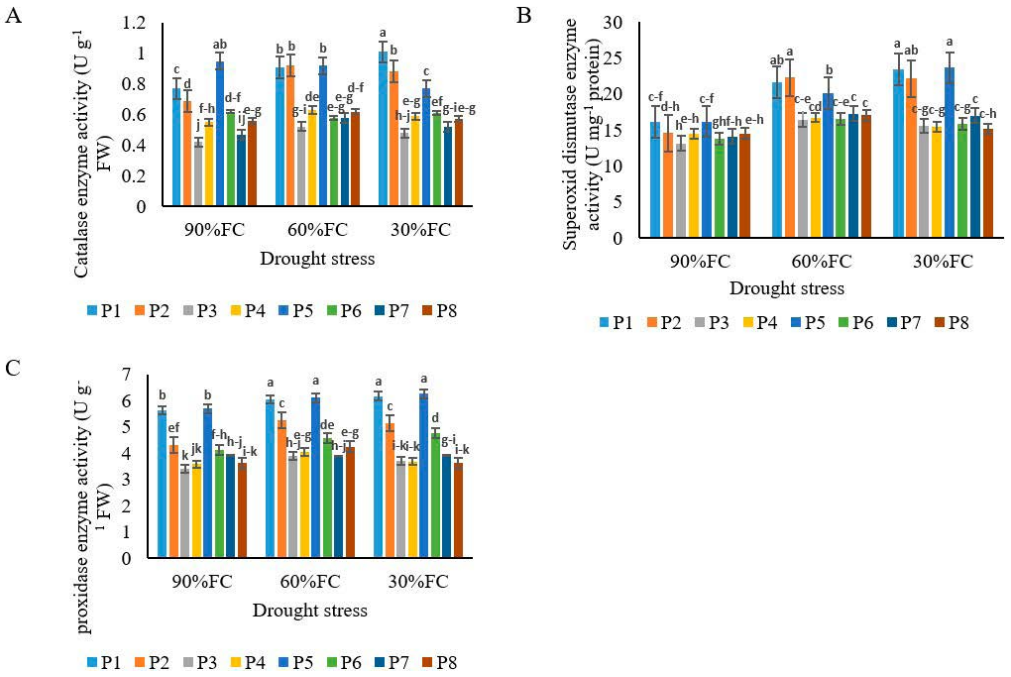

Fig. 5. Interaction effect of petunia genotypes and drought stress levels on antioxidant enzyme activity of eight genotypes of petunia plants. The graph's bars represent the average value over three replicates, while the error bars denote the standard deviation. Different lowercase letters indicate significant differences among genotype × drought stress combinations according to LSD test at $p \leq 0.05$.

Pearson's correlation coefficients between some of the studied chemical indices

Proline and total carbohydrate contents were positively associated with antioxidant enzyme activity (Table 2). Chlorophyll a was closely correlated with chlorophyll b content, and chlorophyll a and b showed a negative correlation with electrolyte leakage. Total carbohydrate showed a positive correlation with proline content (r = 0.73). The highest correlation was recorded between POD and CAT enzyme activities (r = 0.86).

Table 2. Pearson's correlation coefficients (r) of the measured factors in various petunia genotypes under drought stress

	Electrolyte leakage	RWC	Chla	Chlb	Proline	Total carbohydrate	SOD	CAT	POD
Electrolyte leakage	1								
RWC	-0.74**	1							
Chla	-0.63**	0.63**	1						
Chlb	-0.71**	0.79**	0.78**	1					
Proline	-0.01ns	-0.21*	-0.34*	-0.35**	1				
Total carbohydrate	-0.02ns	-0.06ns	-0.39**	-0.32**	0.73**	1			
SOD	0.05ns	-0.07ns	-0.37**	-0.29**	0.67**	0.76**	1		
CAT	-0.18ns	0.14ns	-0.20*	-0.10ns	0.66**	0.81**	0.78**	1	
POD	-0.26**	0.17 ns	-0.08 ns	-0.03ns	0.69**	0.76**	0.71**	0.86**	1

ns: not significant. *$p < 0.05$.** $p < 0.01$

PCA analysis

Principal components analysis (PCA) was performed to visualize the grouping of genotypes-drought treatments (Fig. 6A and 6B) as well as the relation of the measured factors with principal components. Eigenvalues greater than 1 was selected to extract the main principal components.

PCA score plot (Fig. 6A) and biplot (Fig. 6B) of genotypes- drought treatments generated the first four component using 18 variables where the first, second, third and fourth component explained, 49.5%, 26.8%, 9.2%, and 6% of the total variance (91.6%), respectively. 'P1' genotype at 90% FC had higher positive correlation with the first component and P1 genotype at the drought level of 30% FC had a higher positive correlation with the first component (Fig. 6A). On the other hand, the first component was positively associated with root and shoot fresh and dry weight, flower number, lateral branch number, root diameter, and RWC (Fig. 6B), which is indicating the higher performance of 'P1' and 'P5' under drought condition than the other six genotypes and 'P8' genotype was recognized as the most vulnerable to drought stress.

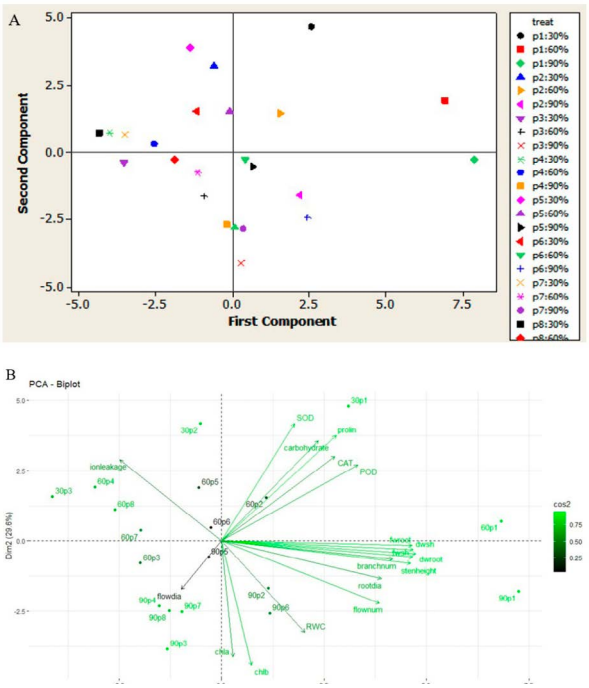

Fig. 6. Principal components analysis (PCA) of genotype -drought treatments (Fig. (A) [score plot] and B [biplot]) in eight studied petunia genotypes.

Discussion

Water scarcity influences plant growth, although the particular impact shows various patterns that depend on the severity of the water stress (Ilyas et al., 2021). Plants respond to water limitation via changes in morphological, physiological, and biochemical processes.

The production ratio of fresh and dry materials in the aerial parts is affected by water stress, leading to a decline in the fresh and dry weights. Water scarcity has adverse effects on numerous growth indices, including stem length and areal fresh and dry weights (Riaz et al., 2013; Khosravi and Haghighi, 2021; Babaei et al., 2021), which is in accordance with this research. The reduction of photosynthesis, plant growth and development, and fresh and dry biomass under drought stress resulted in decreased shoot fresh and dry weights of plants (Hatamifar and Samani, 2017; Kour et al., 2020; Khosravi and Haghighi, 2021). Moreover, the synthesis of dry mass is positively correlated with transpired water content (Riaz et al., 2013). In previous studies, the reduced biomass production under water stress has been reported in *Tagetes minuta L.* (Babaei et al., 2021), and *Capsicum annuum L.* (Khosravi and Haghighi, 2021).

Plant growth and production under water stress are strongly associated with the dry matter partitioning process and spectral and progressive root allocation and biomass distribution to the roots (Riaz et al., 2013). Since greater root growth under water scarcity conditions can enhance drought tolerance in plants (Ilyas et al., 2021), the P1 genotype can increase drought tolerance more than other genotypes. The reduction in the dry weight of roots under water shortage has been previously reported (Hatamifar and Samani, 2017). Shoot fresh and dry weights were particularly higher in the P1 genotype, possibly because it was a tall genotype that created greener biomass.

The decline in stem height in Petunia genotypes under severe drought stress can be attributed to the prevention of cell expansion and cell growth due to low turgor (Rebi et al., 2024). In previous studies, a reduction in stem height under drought stress was reported in *Petunia* (Hatamifar and Samani, 2017) and *Rosa hybrid* (Dolatkhahi et al., 2020). The shortest stem length observed in the P4 genotype was probably due to the decrease in the draining fragment as a result of the depletion of available water around the active root zone. Previous studies have shown that water stress led to the growth reduction of the root attributes (root length, root density, and root diameter) owing to low water supply (Riaz et al., 2013). The reason for the better performance of the P1 genotype could be that its root system may have established a substantial mechanism to tolerate drought stress. A root system that improves the ability of plants to attain water is an important adaptation mechanism to drought (Riaz et al., 2013).

The reduced branch number in Petunia genotypes under severe water stress resulted in more living during these conditions. A reduction in branch number under water scarcity has been previously reported (Shams et al., 2015). The decrease in branch number due to drought stress can be attributed to the fact that plants apply decisive mechanisms to survive under conditions of water limitation.

Flower size (diameter) is substantial in seasonal ornamental plants cultivated in greenspace. The P1 genotype had the lowest flower diameter, although it had the highest number of flowers. Previously, water scarcity during flowering caused a remarkable decrease in flower diameter and quality indices (Rebi et al., 2024).

Poor performance for flower number was exhibited against 30% FC. Therefore, it can be detected that with decreasing water levels, flower number decreases. Moreover, moderate water stress can exert a positive effect on flower number in P1 genotype can be through the influence flowering by preventing vegetative growth. The effect of water deficit on decreasing the flower number of petunia has been observed (Goldani et al., 2021) due to the reduction of stomatal conductance and the subsequent rate of net photosynthesis under water stress, which is consistent with the results of this study.

The degree of cell membrane integrity and stability during drought periods is associated with drought stress tolerance. On the other hand, the reduction of electrolyte leakage under water stress is a key indicator of drought tolerance in plants (Giordano et al., 2021). The results of this study showed that there was a significant increase in electrolyte leakage, membrane destruction, and generation of reactive oxygen species when the petunia genotypes were exposed to severe drought stress, with the highest value in the P8 genotype under severe water stress. Therefore, this genotype is more susceptible to water stress than other genotypes, and other strategies should be applied, including osmotic adjustment, maintaining cell turgor, and reactive oxygen species scavengers to reduce damage to biological membranes (Oraee and Tehranifar, 2020).

Relative water content is widely used as a meaningful index for assessing the water status and drought tolerance of plants (Tran et al., 2024). The reduction in relative water content in P8 genotype might have been due to the inaccessibility of water in the soil and/or root systems, which are not capable of making up for water. Stomatal closure, elevated leaf temperature, and decreased plasma membrane integrity result from a decrease in the relative water content (Seleiman et al., 2021). In addition, during severe drought stress, ABA initiates the biosynthesis

and accumulation of osmolytes to protect the cellular structural stability (Takahashi et al., 2020). However, our study revealed that in the P4 genotype with the lowest relative water content and highest electrolyte leakage under severe stress, osmoprotectants did not accumulate to sufficient levels for effective osmotic adjustment to drought stress (Tran et al., 2024). Therefore, this genotype was introduced as the most sensitive genotype compared with other genotypes.

The accumulation of non-protein amino acids such as proline with increasing water limitation can contribute to the process of osmoregulation inside the cell (Shams et al., 2015; Goldani et al., 2021; Khosravi et al., 2023). Because proline accumulation is considered a common marker of drought tolerance, the highest content in the P5 genotype was probably due to its tolerance to drought conditions. The highest concentrations of proline in drought-stressed plants resulted in osmotic adjustment and prevention of cell dehydration (Khosravi et al., 2023; Khan et al., 2025), and as a non-enzymatic antioxidant, it induces antioxidant enzyme activity in plants (Giordano et al., 2021).

The increase of total carbohydrate content during stress can be associated with the inhibition of growth or synthesis of this compound from non-photosynthesis process (Jang et al., 2020). Moreover, a higher accumulation of compatible solutes (soluble sugars) may help in drought stress tolerance in Petunia genotypes by enhancing osmotic adjustment, reactive oxygen species detoxification, and cell membrane maintenance (Oraee and Tehranifar, 2020).

Photosynthetic pigments (chlorophyll *a* and *b*) are required for photosynthesis, and water scarcity results in a chain of molecular, biochemical, and physiological modifications, including loss of chlorophyll in plants (Shams et al., 2015). One key enzymatic factor contributing to this process is chlorophyllase, which catalyzes the removal of the phytol chain from chlorophyll a, leading to its breakdown and reduction in concentration. Under drought stress, the activity of this enzyme has been reported to increase, thereby accelerating chlorophyll degradation and reducing photosynthetic efficiency (Hatamifar and Samani, 2017). In addition to enhanced catabolism, other causes such as chloroplast damage, lipid peroxidation, disintegration of pigment-protein complexes, and inhibition of pigment biosynthesis further contribute to chlorophyll loss, as previously reported (Shams et al., 2015; Goldani et al., 2021; Rebi et al., 2024) and have been detected in drought-stressed plants of eight genotypes. The reduction in chlorophyll concentration under drought stress has very significant effects on photosynthesis by changing stomata operation and reducing CO_2 absorption, which has been defined by differences in chlorophyll concentration in drought-stressed plants (Rebi et al., 2024). Previously, the adverse effect of drought stress on photosynthetic pigments of different leaves of plants has been reported (Gholami et al., 2012).

Abiotic stress, such as drought, often enhances reactive oxygen species generation in various plant tissues (Naing and Kim, 2021). Antioxidant enzymes (SOD, CAT, and POD) play an important role in defense mechanisms by scavenging the toxic effects of excess reactive oxygen species. O^{2-} removal is conducted by the SOD enzyme by catalyzing its dismutation, while CAT and POD remove H_2O_2 (Naing et al., 2022). The ability of antioxidant enzymes to detoxify reactive oxygen species and decrease their destructive effects may be associated with drought tolerance in plants (Giordano et al., 2021). Thus, the finding that the specific CAT and SOD activities did not change significantly with drought in P3, P4, P6, P7, and P8 suggests that these enzymes have no major antioxidative function in these genotypes. In contrast, a greater magnitude of increase in the concentrations of CAT, POD, and SOD in P1, P2, and P5 genotypes under 60% FC reflects the activity of these enzyme systems in these plants, which increases their tolerance to water stress. The presence of antioxidant enzymes has been reported to determine the stress tolerance of plants (Oraee and Tehranifar, 2020; Giordano et al., 2021).

Our results demonstrated that the effects of drought stress is genotype dependent and is different among studied genotypes. Correlation analysis revealed significant positive relationships among traits related to drought tolerance, including shoot and root biomass, RWC, flower number, and lateral branches. These correlations suggest that genotypes maintaining higher biomass and water status under drought stress also exhibited better reproductive performance. Genotype P1 at 90% FC and P5 at 30% FC showed high positive loadings on the first component, indicating superior performance under well-watered and stress conditions, respectively. In contrast, P8 was clearly separated and negatively correlated with major growth and physiological traits, highlighting its sensitivity to drought stress. This pattern is consistent with the correlation matrix, where traits such as proline and electrolyte leakage (markers of stress) showed negative associations with growth-related parameters. Together, the PCA and correlation analysis reinforce that drought-tolerant genotypes maintain physiological integrity (e.g., RWC, chlorophyll content) and biomass under stress, making them suitable candidates for breeding programs targeting drought resilience.

Conclusions

Petunia genotypes revealed, different morphological and physiochemical traits in terms of growth, antioxidant enzyme activity, and generation of osmotically active solutes under different levels of drought stress conditions. In the current study, it can be concluded that among the eight assessed genotypes, P1 genotype showed good tolerance to water scarcity. The higher tolerance induced by the P1 genotype was correlated with higher performance and more effective osmotic adjustment, which was reflected by minor declines in relative water content and electrolyte leakage and better values of photosynthetic pigments and antioxidant capacity. These physiological and biochemical indicators are noteworthy because they can be rapidly assessed and may serve as useful tools in breeding programs aimed at selecting drought-tolerant petunia genotypes. However, it is important to note that this conclusion is based on phenotypic and biochemical observations, and more specific studies (e.g., gene expression profiling, molecular marker analysis, or multi-environment trials) are needed to confirm the drought tolerance of P1. Based on the data obtained, it is clear that the most tolerant genotype was P1, followed by P5, P2, P3, P4, P7, P6, and P8 was the most susceptible genotype studied.

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Author Contribution

LC: Investigation, Data Curation, Writing - Original Draft. **SK**: Investigation, Data Curation, Formal Analysis. **MS:** Conceptualization, Methodology, Validation. **AT:** Validation, Writing – Review & Editing. **HN:** Conceptualization, Methodology, Writing – Review & Editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data will be made available upon request to the authors.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that the use of AI and AI-assisted technologies was not applied in the writing process.

References

AEBI, H. Catalase in vitro. **Methods in Enzymology**, v.105, p.121-126, 1984. https://doi.org/10.1016/S0076-6879(84)05016-3

BABAEI, K.; MOGHADDAM, M.; FARHADI, N.; PIRBALOUTI, A.G. Morphological, physiological and phytochemical responses of Mexican marigold (*Tagetes minuta* L.) to drought stress. **Scientia Horticulturae**, v.284, p.110116, 2021. https://doi.org/10.1016/j.scienta.2021.110116

BATES, L.S.; WALDREN, R.P.; TEARE, I.D. Rapid determination of free proline for water-stress studies. **Plant and Soil**, v.39, p.205-207, 1973. https://doi.org/10.1007/BF00018060

CAMBELL, G.S.; MULLA, D.J. Measurement of soil water content and potential. In: STEWART, B.A.; NIELSEN, D.R. **Irrigation of Agricultural Crops**. Madison: American Society of Agronomy, 1990. p.127-142.

CHIRIVÌ, D.; BETTI, C. Molecular links between flowering and abiotic stress response: A focus on Poaceae. **Plants**, v.12, n.2, p.331, 2023. https://doi.org/10.3390/plants12020331

DOLATKHAHI, A.; SHOOR, M.; BANNAYAN, M.; TEHRANIFAR, A.; ALIZADEH, A. Water deficit decreases gas exchange parameters and marketable quality of *Rosa hybrida* 'Club-Nika' irrespective of training systems**. Journal of Agricultural Science and Technology**, v.22, n.3, p.837-849, 2020. http://jast.modares.ac.ir/article-23-18994-en.html

FERREIRA, L.C.; CATANEO, A.C.; REMAEH, L.M.R.; CORNIANI, N.; DE FATIMA FUMIS, T.; DE SOUZA, Y.A.; SCAVRONI, J.; SOARES, B.J. Nitric oxide reduces oxidative stress generated by lactofen in soybean plants. **Pesticide Biochemistry and Physiology**, v.97, n.1, p.47-54, 2010. https://doi.org/10.1016/j.pestbp.2009.12.003

GHOLAMI, M.; RAHEMI, M.; KHOLDEBARIN, B.; RASTEGAR, S. Biochemical responses in leaves of four fig cultivars subjected to water stress and recovery. **Scientia Horticulturae**, v.148, p.109-117, 2012. https://doi.org/10.1016/j.scienta.2012.09.005

GIANNOPOLITIS, C.N.; RIES, S.K. Superoxide dismutases: I. Occurrence in higher plants. **Plant Physiology**, v.59, n.2, p.309-314, 1977. https://doi.org/10.1104/pp.59.2.309

GIORDANO, M.; PETROPOULOS, S.A.; CIRILLO, C.; ROUPHAEL, Y. Biochemical, physiological, and molecular aspects of ornamental plants adaptation to deficit irrigation. **Horticulturae**, v.7, n.5, p.107, 2021. https://doi.org/10.3390/horticulturae7050107

GHOULAM, C.; FOURSY, A.; FARES, K. Effects of salt stress on growth, inorganic ions, and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. **Environmental and Experimental Botany**, v.47, p.39-50, 2002. https://doi.org/10.1016/S0098-8472(01)00109-5

GOLDANI, M.; DOLATKHAHI, A.; PARSA, M.; VAHDATI, N.; RASOULI, Z. Investigation of improving the drought tolerance in Persian petunia (*Petunia* sp.) by exogenous application of salicylic acid and gibberellic acid. **Acta Scientiarum Polonorum Horticulturae Cultus**, v.20, n.1, p.37-48, 2021. https://doi.org/10.24326/asphc.2021.1.4

HATAMIFAR, N.; SAMANI, R. B. Effect of paclobutrazol on some morphological and physiological characteristics of petunia under drought stress. **Journal of Ornamental Plants**, v.7, n.2, p.125-136, 2017. Available at: https://journals.iau.ir/article_531555.html

ILYAS, M.; NISAR, M.; KHAN, N.; HAZRAT, A.; KHAN, A.H.; HAYAT, K.; FAHAD, S.; KHAN, A.; ULLAH, A. Drought tolerance strategies in plants: a mechanistic approach. **Journal of Plant Growth Regulation**, v.40, p.926-944, 2021. https://doi.org/10.1007/s00344-020-10174-5

JANG, I.; DO, G.; SUH, S.; YU, J.; JANG, I.; MOON, J.; CHUN, C. Physiological responses and ginsenoside production of *Panax ginseng* seedlings grown under various ratios of red to blue light-emitting diodes. **Horticulture, Environment, and Biotechnology**, v.61, p.663-672, 2020. https://doi.org/10.1007/s13580-020-00255-5

KHAN, P.; ABDELBACKI, A.M.M.; ALBAQAMI, M.; JAN, R.; KIM, K.-M. Proline promotes drought tolerance in maize. **Biology**, v.14, n.1, p.41, 2025. https://doi.org/10.3390/biology14010041

KHOSRAVI, S.; HAGHIGHI, M. The effect of foliar spray of brassinosteroid on sweet pepper (*Capsicum annuum* L.) seedling under drought stress. **Journal of Horticultural Science**, v.35, n.3, p.367-381, 2021. https://doi.org/10.22067/jhs.2021.61838.0

KHOSRAVI, S.; HAGHIGHI, M.; MOTTAGHIPISHEH, J. Effects of melatonin foliar application on hot pepper growth and stress tolerance. **Plant Stress**, v.9, p.100192, 2023. https://doi.org/10.1016/j.stress.2023.100192

KOUR, D.; RANA, K.L.; YADAV, A.N.; SHEIKH, I.; KUMAR, V.; DHALIWAL, H.S.; SAXENA, A.K. Amelioration of drought stress in foxtail millet (*Setaria italica* L.) by P-solubilizing drought-tolerant microbes with multifarious plant growth-promoting attributes. **Environmental Sustainability**, v.3, p.23-34, 2020. https://doi.org/10.1007/s42398-020-00094-1

LICHTENTHALER, H.K. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. **Methods in Enzymology**, v.148, p.350-382, 1987. https://doi.org/10.1016/0076-6879(87)48036-1

LUTTS, S.; KINET, J.M.; BOUHARMONT, J. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. **Annals of Botany**, v.78, p.389-398, 1996. https://doi.org/10.1006/anbo.1996.0134

McCREADY, M.R.; GUGGOLZ, J.; SILVIERA, V.; OWENS, S.H. Determination of starch and amylose in vegetables. **Analytical Chemistry**, v.22, p.1156-1158, 1950. https://doi.org/10.1021/ac60045a016

NAING, A.H.; CAMPOL, J.R.; KANG, H.; XU, J.; CHUNG, M.Y.; KIM, C.K. Role of ethylene biosynthesis genes in the regulation of salt stress and drought stress tolerance in petunia. **Frontiers in Plant Science**, v.13, p.844449, 2022. https://doi.org/10.3389/fpls.2022.844449

NAING, A.H.; KIM, C.K. Abiotic stress-induced anthocyanins in plants: Their role in tolerance to abiotic stresses. **Physiologia Plantarum**, v.172, n.3, p.1711-1723, 2021. https://doi.org/10.1111/ppl.13373

ORAEE, A.; TEHRANIFAR, A. Evaluating the potential drought tolerance of pansy through its physiological and biochemical responses to drought and recovery periods. **Scientia Horticulturae**, v.265, p.109225, 2020. https://doi.org/10.1016/j.scienta.2020.109225

REBI, A.; EJAZ, I.; KHATANA, M.A.; ALVI, A.B.A.; IRFAN, M.; WANG, G.; GANG, Y. Y.; WANG, L.; MENG, Y.; GHAZANFAR, S.; ZHOU, J. Effect of irrigation levels on the physiological responses of petunia cultivars for selection. **Ecology Frontiers**, v.44, n.1, p.206-216, 2024. https://doi.org/10.1016/j.chnaes.2023.12.001

REZAEI, H.; MIRZAIE-ASL, A.; ABDOLLAHI, M.R.; TOHIDFAR, M. Comparative analysis of different artificial neural networks for predicting and optimizing *in vitro* seed germination and sterilization of petunia. **PLoS One**, v.18, n.5, e0285416, 2023. https://doi.org/10.1371/journal.pone.0285657

RIAZ, A.T.I.F.; YOUNIS, A.; TAJ, A.R.; KARIM, A.; TARIQ, U.; MUNIR, S.; RIAZ, S.I.T.W\.A.T. Effect of drought stress on growth and flowering of marigold (*Tagetes erecta* L.). **Pakistan Journal of Botany**, v.45, n.1, p.123-131, 2013.

SELEIMAN, M.F.; AL-SUHAIBANI, N.; ALI, N.; AKMAL, M.; ALOTAIBI, M.; REFAY, Y.; DINDAROGLU, T.; ABDUL-WAJID, H.H.; BATTAGLIA, M.L. Drought stress impacts on plants and different approaches to alleviate its adverse effects. **Plants**, v.10, n.2, p.259, 2021. https://doi.org/10.3390/plants10020259

SHAMS, J.; NAJAFI, P.; ETEMADI, N.A. Effect of water deficiency on growth indices of *Petunia hybrida* cultivars and *Petunia violacea* grown in Isfahan region of Iran. **Crop Research**, v.49, n.1, p.55-61, 2015.

TAFAGHODI, R.; MARASHI, H.; MOSHTAGHI, N.; ZARGHAMI, M.M. Expression patterns of catalase and superoxide dismutase (Cu/Zn-SOD) genes under drought stress in *Petunia hybrida*. **Journal of Advanced Plant Science**, v.1, p.209, 2018.

TAKAHASHI, F.; KUROMORI, T.; URANO, K.; YAMAGUCHI-SHINOZAKI, K.; SHINOZAKI, K. Drought stress responses and resistance in plants: From cellular responses to long-distance intercellular communication. **Frontiers in Plant Science**, v.11, p.556972, 2020. https://doi.org/10.3389/fpls.2020.556972

TRAN, N.H.T.; VAN HOANG, D.; PHAN, L.T. Drought stress induces early flowering and the stress tolerance of offspring in *Petunia hybrida*. **Plant Biotechnology**, v.41, n.1, p.53-63, 2024. https://doi.org/10.5511/plantbiotechnology.23.1220a

WANG, C.; TURNER, V.K.; WENTZ, E.A.; ZHAO, Q.; MYINT, S.W. Optimization of residential green space for environmental sustainability and property appreciation in metropolitan Phoenix, Arizona. **Science of the Total Environment**, v.763, p.144605, 2021. https://doi.org/10.1016/j.scitotenv.2020.144605