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Application of Salicylic Acid and Ascorbic Acid on the Vegetative and Physiological Characteristics of *Pistacia vera* cv. Badami-riz-Zarand under Water Deficit

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ABSTRACT

This study examined the effects of foliar application of plant growth regulators—salicylic acid (SA) and ascorbic acid (AA)—on the growth and qualitative traits of *Pistacia vera* cv. Badami-riz-Zarand under drought stress. The experiment was conducted over two consecutive years in a greenhouse at the Research and Education Center for Agriculture and Natural Resources in Kerman Province, Iran. Using a factorial design within a randomized complete block framework, the experiment included four replications. Drought stress was evaluated at three levels (100%, 60%, and 30% field capacity [FC]), with SA and AA applied at three concentrations (0, 0.5, and 1.0 mM). Results indicated that SA and AA applications significantly influenced plant traits under stress conditions ($P < 0.05$). Under severe drought stress (30% FC), applying 1.0 mM of SA and AA resulted in increases of 15%, 14%, and 6% in relative water content, total chlorophyll, and biomass, respectively, as well as 51%, 49%, and 11% in guaiacol peroxidase, polyphenol peroxidase, and phenylalanine ammonia-lyase activities, respectively. Simultaneously, reductions of 36%, 4%, and 4% were observed in catalase activity, electrolyte leakage, and soluble sugars, respectively. Under severe drought conditions, SA demonstrated a greater ability than AA to maintain yield and quality characteristics of the plant. Overall, the application of 0.5 to 1.0 mM SA was effective in mitigating the adverse effects of severe drought stress, helping to preserve plant performance.

Abbreviation: Salicylic acid (SA), Ascorbic acid (AA), Field capacity (FC), Drought stress (DS), Glycine betaine (GB), Proline (Pro), Protein (Prt), Guaiacol peroxidase (GP), Superoxide dismutase (SOD), Catalase (CAT), Polyphenol peroxidase (POD), Soluble sugars (SSs), Phenylalanine ammonylase (Phe), Electrolyte leakage (EL)

Introduction

Water scarcity is the primary factor limiting crop production worldwide (Hassani Asl et al., 2024). By 2025, it is projected that plant production will decrease by 30% due to water shortages

(Schwalm et al., 2017). This issue is exacerbated by global warming and severe climatic conditions, which intensify water scarcity (Loutfy et al., 2020). Plants subjected to water scarcity exhibit various physiological and biochemical responses, such as suppressed cell growth, stomatal closure, impaired photosynthesis, and respiration issues (Hussain et al., 2023).

In arid and semi-arid regions, water scarcity and drought stress are the most critical factors limiting plant growth and performance. These stresses affect all physiological and biochemical processes, leading to reduced yields, lower product quality, and, ultimately, plant mortality (Shahrivar et al., 2020). Pistachio (*Pistacia vera* L.), a key medicinal, edible, and commercial plant, has experienced significant performance declines in recent decades due to limited water resources. A deciduous species from the Anacardiaceae family, pistachio is well-adapted to high summer temperatures and thrives in dry, warm regions such as Iran, Iraq, Syria, and parts of Turkey (Mohammed and Arkwazee, 2024; Nezami and Gallego, 2023). Pistachio nuts are nutritionally rich, containing essential minerals and non-mineral elements such as potassium, magnesium, calcium, protein, dietary fibers, carbohydrates, folic acid, fat, vitamin K, gamma-tocopherols, phytochemicals, and polyphenols (Dreher, 2012). These nuts offer medicinal benefits, including improved blood pressure, vascular stiffness, cholesterol levels, weight management, endothelial and gastrointestinal function, glucose metabolism, kidney function, and allergy management (Nadimi et al., 2019).

In Iran, pistachios are a vital export crop with a long history of cultivation. However, production has decreased by 50% since 2017 due to water scarcity (Heydari et al., 2023). Iran is home to approximately 90 known varieties of pistachio, several of which are commercially cultivated. Among them, *P. vera* var. Badami-Riz-Zarand is considered an optimal rootstock for grafting, offering enhanced water and nutrient uptake, especially under environmental stress conditions (Mohammed and Arkwazee, 2024). This early-flowering cultivar produces small, almond-shaped, dark-colored fruit and originates from the Zarand region in Kerman Province. In recent years, researchers have focused on developing effective and cost-efficient methods to enhance drought resistance and sustain plant performance under challenging conditions. Using plant growth regulators (PGRs), such as ascorbic acid (AA) and salicylic acid (SA), is an emerging method for preserving plant performance under drought stress. Studies have identified SA and AA as cost-effective and efficient solutions for mitigating stress in various crops (Ayuba et al., 2020; Miladinov et al., 2020; Nawaz et al., 2021).

SA is a phenolic compound naturally produced in small quantities in plant roots. It plays a vital role in regulating physiological processes, such as photosynthesis, stomatal closure, transpiration, chlorophyll and protein synthesis, inhibition of ethylene biosynthesis, and the absorption and transport of nutrients (Hassani Asl et al., 2024). Additionally, SA is recognized as a key molecular signal in plants' responses to environmental stresses (Zahid et al., 2023). Its effects on photosynthesis are mediated through stomatal regulation, pigment stabilization, chloroplast structure, and enzyme activity (Souri and Tohidloo, 2019). SA has been shown to significantly enhance leaf area, biomass, and total chlorophyll content in maize (Moghaddam et al., 2011) and improve germination and biomass accumulation in *Mentha piperita* (Abdi and Karami, 2020; Kunpratum et al., 2024). AA, a small water-soluble antioxidant, plays a crucial role in detoxifying reactive oxygen species (ROS), particularly hydrogen peroxide (Loutfy et al., 2020). It also directly neutralizes superoxide and singlet oxygen radicals while acting as a secondary antioxidant in the production of alpha-tocopherol and other lipophilic antioxidants (Maghsoudi et al., 2019). AA enhances resistance to abiotic stresses and mitigates oxidative damage (Hassani Asl et al., 2024). The application of AA and SA has been demonstrated to improve fruit characteristics under stress, particularly in tree crops (Raees et al., 2023; El Refaey et al., 2022).

Based on this background, the present study aims to investigate the effects of foliar application of AA and SA on the morphological (e.g., plant height, root length) and physiological (e.g., chlorophyll content, enzyme activity) characteristics of *P. vera* var. *Badami-Riz-Zarand* seedlings under water deficit conditions. Water deficit stress poses a significant challenge to the growth and productivity of pistachio, especially in arid and semi-arid regions where water resources are limited. The selected *Badami-Riz-Zarand* variety, known for its adaptability and commercial importance, is an ideal candidate for exploring strategies to enhance drought tolerance. The findings from this research could provide valuable insights into cost-effective and sustainable approaches for improving the tolerance and productivity of pistachio in water-limited environments.

Material and methods

The experiment

The experiment was conducted in the greenhouse of the Center for Research and Education of Agriculture and Natural Resources, Kerman Province, during 2021-2022. The study followed a factorial design arranged in a randomized complete block layout with four replications. The greenhouse soil was classified as sandy loam. Field capacity (FC) was determined by measuring soil water content and weight. The nitrogen, phosphorus, potassium levels, pH, and organic matter content of the soil used in this experiment appear in Table 1.

Table1. Physicochemical properties of the soil used for the experiment.

Factor	Amount
pH	7.6
OM (%)	1.40
K (ppm)	287
P (ppm),	28.00
N (%)	1.50
Silt (%)	7.7
Clay (%)	18.5
Sand (%)	73.8

Pistachio seeds were sourced from Zarand gardens in Kerman and disinfected before sowing. In 2022, four seeds were planted in each of 81 pots. Irrigation was uniformly maintained at soil field capacity (FC), and stress was introduced when the seedlings reached the four-leaf stage. The soil's FC was measured using the method described by Jahantab et al. (2022). In summary, soil in the pots was saturated with water, and after 24 h of drainage, the soil was weighed. It was then oven-dried at 100 °C for 24 h to determine the FC. Stress treatments were applied at three levels: 100% FC (control), 60% FC (moderate stress), and 30% FC (severe stress). Foliar applications of salicylic acid (SA) and ascorbic acid (AA) at concentrations of 0, 0.5, and 1.0 mM were carried out on the seedlings' leaves between 9:00 and 10:00 am, one week after the onset of drought stress. For enhanced effectiveness, the foliar spray was repeated after two weeks, following the protocol of El Refaey et al. (2022). Plant traits were assessed at the end of the experiment in the winter of 2022.

Measuring plant traits

The morphophysiological and biochemical traits of the seedlings were evaluated, including the fresh and dry weights of the stem, root, and leaves; number of leaves; plant height; root length; leaf thickness; chlorophyll index; relative water content (RWC); electrolyte leakage (EL); and levels of proline (Pro), glycine betaine (GB), chlorophyll a, chlorophyll b, total chlorophyll, soluble sugars (SSs), leaf protein (Prt), phenylalanine ammonia-lyase (Phe), catalase (CAT), guaiacol peroxidase (GP), and polyphenol peroxidase (POD).

Plant height and root length were measured using straightforward methods described by Tavili et al. (2019). Biomass and RWC were calculated based on fresh and dry weight measurements, following a procedure outlined by Jahantab et al. (2022).

Enzyme activity measurements

To measure enzyme activities, protein (Prt) extracts were prepared as follows. A total of 0.5 g of plant tissue was ground in a mortar containing 5 mL of 0.05 M Tris-HCl buffer (pH 7.5) until a homogeneous solution was obtained. The solution was transferred to a centrifuge tube, allowed to rest for 10 min, and then centrifuged for 25 min at 10,000 rpm and 4 °C using a refrigerated centrifuge. After centrifugation, the supernatant was collected into test tubes as the protein extract.

To measure Prt content, 5 mL of biuret reagent was added to test tubes containing 100 µL of the protein extract. The mixture was vortexed rapidly and incubated for 25 minutes. Absorbance was measured at 529 nm using a spectrophotometer, and protein content was calculated using a standard curve. Polyphenol peroxidase (POD) activity was measured using a mixture of 100 mM Tris buffer, 5 mM hydrogen peroxide, and 10 mM pyrogallol. The reaction was performed in an ice bath, and absorbance was read at 425 nm using a spectrophotometer (2802 UV/VIS, China), following the method of Kochba et al. (1977).

Catalase (CAT) activity was assessed using the method described by Aebi (1984). Approximately 0.1 g of leaf tissue was ground in liquid nitrogen, and 1 mL of sodium phosphate extraction buffer was added. The buffer contained monosodium phosphate and 15 mM hydrogen peroxide. Phenylalanine ammonia-lyase (Phe) activity was measured according to the method of Amrhein et al. (1976). Guaiacol peroxidase (GP) activity was determined using the method outlined by Panda and Patra (2007). These methods ensured precise and reliable measurements of enzyme activities, which are critical for understanding plant physiological responses.

Proline (Pro)

The Pro content was measured according to a method outlined by Bates et al. (1973). Briefly, the procedure involved preparing 0.5 g of the plant sample, keeping it in liquid nitrogen, adding

sulfosalicylic acid, and centrifuging the solution for 20 min at 10,000 rpm at 4 °C. Pro was measured using a spectrophotometer (UV-1800 Shimadzu, Japan) at a wavelength of 520 nm.

Statistical analysis

The data were checked for normality. Then, they were statistically analyzed using the SPSS software (version 22). Duncan's statistical test was used for grouping the treatments at 0.05 significance. All graphs were drawn using Microsoft Excel.

Results

Stress significantly altered seedling traits, including chlorophyll content, biomass, and stem/leaf characteristics. However, the application of AA and SA significantly mitigated the negative effects of water stress and enhanced the properties of pistachio seedlings ($P < 0.05$). Furthermore, the interaction between drought stress and acid application had notable effects on certain evaluated traits, such as leaf and stem thickness, root length, and leaf chlorophyll content (Table 2).

Table 2. Effects of salicylic acid (SA), ascorbic acid (AA) and drought stress (DS) on the morpho-physiologichal characteristics of elmond pistachio (n = 4) ($P < 0.05$).

Treatments	DS	SA	SA*DS	AA	AA*DS	SA*AA	SA*AA*DS	CV%
SL (cm)	11.151^{ns}	63.111^{*}	21.688	19.445^{ns}	22.703^{ns}	2.088^{ns}	8.225^{ns}	17.0
SD (mm)	0.002^{ns}	0.003^{*}	0.009^{**}	0.005^{**}	0.002^{*}	0.0005^{ns}	0.001^{*}	14.0
SFW (g)	0.185^{ns}	0.02^{ns}	0.035^{ns}	0.026^{ns}	0.029^{ns}	0.088^{ns}	0.064^{ns}	26.0
SDW (g)	0.034^{ns}	0.01^{ns}	0.017^{ns}	0.013^{ns}	0.004^{ns}	0.022^{ns}	0.012^{ns}	29.0
RL (cm)	372^{*}	122.445^{ns}	37.6^{ns}	32.118^{ns}	90.847^{ns}	317.281^{*}	89.337^{ns}	37.0
RFW (g)	0.285^{**}	0.146^{*}	0.032^{ns}	0.009^{ns}	0.017^{ns}	0.141^{*}	0.027^{ns}	50.0
RDW (g)	0.006^{ns}	0.066^{**}	0.007^{ns}	0.044^{*}	0.004^{ns}	0.006^{ns}	0.015^{ns}	56.0
Bio (g)	0.318^{**}	0.245^{**}	0.154^{*}	0.111^{*}	0.88^{*}	0.081^{ns}	0.072^{*}	39.0
NLs	65.398^{*}	65.176^{*}	51.843^{**}	2.787^{ns}	7.162^{ns}	4.856^{ns}	17.481^{ns}	31.0
LT (cm)	0.002^{ns}	0.003^{*}	0.009^{**}	0.005^{**}	0.002^{*}	0.0005^{ns}	0.001^{*}	14.0
LFW (g)	0.758^{*}	0.222^{ns}	0.15^{ns}	0.08^{ns}	0.029^{ns}	0.16^{ns}	0.08^{ns}	29.0
LDW (g)	0.057^{*}	0.005^{ns}	0.013^{ns}	0.005^{ns}	0.004^{ns}	0.02^{ns}	0.007^{ns}	32.0
Clo a	0.011^{**}	0.012^{**}	0.011^{**}	0.0001^{ns}	0.0003^{ns}	0.001^{*}	0.00038^{ns}	24.0
Clo b	0.027^{**}	0.007^{**}	0.005^{**}	0.0005^{ns}	0.0002^{ns}	0.00075^{ns}	0.00038^{ns}	38.0
TClo	0.074^{**}	0.037^{**}	0.03^{**}	0.001^{ns}	0.00025^{ns}	0.003^{*}	0.001^{ns}	27.0
RWC	301.97^{*}	1075.31^{**}	298.33^{*}	3.629^{ns}	46.879^{ns}	184.569^{ns}	131.176^{ns}	13.9
EL	0.034^{**}	0.018^{*}	0.012^{ns}	0.009^{ns}	0.002^{ns}	0.008^{ns}	0.005^{ns}	9.25
Pro	74838.56^{**}	1293.813^{ns}	1627.792^{ns}	553.068^{ns}	212.944^{ns}	829.957^{ns}	927.54^{ns}	47
GB	66692.6^{**}	3330.857^{ns}	6581.255^{ns}	757.347^{ns}	1280.14^{ns}	3236.89^{ns}	1590.16^{ns}	21

DS: Drought stress, SA: Salicylic acid, AA: Ascorbic acid, SL: Stem length, SD: Stem diameter, SFW: Stem fresh weight, SDW: Stem dry weight, RL: Root length, RFW: Root fresh weight, RDW: Root dry weight, Bio: Biomass, NLs: Number of leaves, LT: Leaf thickness, LFW: Leaf fresh weight, LDW: Leaf dry weight, Clo: Chlorophyll, TClo: Total Chlorophyll, RWC: Relative water content, EL: Electrolyte Leakage, Pro: Proline content, GB: Glycine betaine, **: Significant at 1%, *: Significant at 5%, ^{ns}: Not significant.

The results indicated that all characteristics of pistachio seedlings were significantly influenced by drought stress and the application of AA or SA ($P < 0.05$). Traits such as stem and root length, number of leaves, stem diameter, leaf thickness, fresh weights of the stem, root, and leaves, root and leaf biomass, RWC, EL, chlorophyll content, Pro, and glycine betaine were notably affected by AA and SA application under drought stress conditions, as shown in Table 2. In most cases, drought stress had detrimental effects, reducing vegetative traits. However, the application of 0.5 mM and 1.0 mM AA or SA mitigated these effects and improved seedling vegetative traits. For example, 30% FC resulted in a 19% reduction in biomass, whereas the application of 0.5 mM and 1.0 mM SA increased biomass by 5% and 33%, respectively. Physiological traits such as Pro and glycine betaine showed significant increases under drought stress. Specifically, Pro content increased by 18% and 95% under stress at 60% and 30% FC, respectively (Table 3).

Chlorophyll content

The effect of drought stress and SA on chlorophyll *a* content was significant, as were the interactions of drought stress, SA, and the combined application of SA × AA ($P < 0.05$). Stress levels of 60% and 30% FC led to reductions of 31% and 37% in chlorophyll *a* content, respectively. However, the application of 1.0 mM SA resulted in a 12% increase in chlorophyll *a* compared to the control. The triple interaction effects revealed that the highest chlorophyll *a* content was observed under 100% FC with the combined application of 0.5 mM SA and 0.5 mM AA (Fig. 1A).

Table 3. Grouping the mean values of the pistachio traits affected by salicylic acid (SA: 0.0, 0.5, 1.0 mM) and ascorbic acid (AA: 0.0, 0.5, 1.0 mM) under drought stress (100, 30% FC) (n = 4) ($P < 0.05$).

Treatments	Ctl	30 FC	SA (0 mM)	SA (0.5 mM)	SA (1 Mm)	AA (0 Mm)	AA(0.5 Mm)	AA (1 Mm)
SL (cm)	24.96 ^{a}	24.98^{a}	23.81 ^{b}	26.36^{a}	25.69^{ab}	24.51^{a}	25.38^{a}	25.97^{a}
SD (mm)	3.206^{ab}	2.967^{b}	3.367^{a}	2.981^{b}	3.186^{ab}	3.193^{a}	3.174^{a}	3.167^{a}
SFW (g)	1.05^{a}	0.93^{b}	0.95^{a}	0.96^{a}	0.99^{a}	0.95^{a}	0.96^{a}	1.0^{a}
SDW (g)	0.43^{a}	0.37^{b}	0.41^{ab}	0.39^{b}	0.42^{a}	0.40^{ab}	0.39^{b}	0.43^{a}
RL (cm)	31.25^{a}	25.80^{b}	26.93^{b}	30.08^{a}	30.17^{a}	28.06^{ab}	29.19^{a}	29.93^{a}
RFW (g)	0.48^{a}	0.33^{b}	0.37^{b}	0.45^{a}	0.47^{a}	0.41^{a}	0.44^{a}	0.44^{a}
RDW (g)	0.22^{a}	0.20^{a}	0.18^{b}	0.19^{ab}	0.24^{a}	0.18^{b}	0.22^{a}	0.23^{a}
NLs	13.22^{a}	10.75^{b}	11.22^{b}	11.86^{b}	13.81^{a}	12.03^{a}	12.28^{a}	12.58^{a}
LT (cm)	0.183^{a}	0.169^{ab}	0.166^{b}	0.181^{a}	0.182^{a}	0.168^{ab}	0.172^{a}	0.189^{a}
LFW (g)	1.09^{a}	0.81^{b}	0.88^{b}	1.00^{a}	1.03^{a}	0.92^{a}	0.98^{a}	1.01^{a}
LDW (g)	0.37^{a}	0.30^{b}	0.33^{a}	0.34^{a}	0.35^{a}	0.33^{a}	0.34^{a}	0.35^{a}
Clo a	0.09^{a}	0.06^{b}	0.05^{c}	0.08^{a}	0.09^{a}	0.07^{b}	0.07^{b}	0.07^{b}
Clo a b	0.09^{a}	0.04^{b}	0.04^{b}	0.06^{a}	0.07^{a}	0.05^{a}	0.05^{a}	0.05^{a}
TClo	0.18^{a}	0.10^{b}	0.09^{b}	0.14^{ab}	0.16^{a}	0.12^{b}	0.12^{b}	0.12^{b}
RWC	68.38^{a}	64.00^{b}	62.67^{b}	71.55^{a}	72.63^{a}	69.15^{a}	68.58^{a}	69.11^{a}
EL	0.75^{b}	0.80^{a}	0.77^{b}	0.79 ^{ab}	0.82^{a}	0.80^{a}	0.80^{a}	0.82^{a}
Pro	70.2^{c}	137.1^{a}	83.3^{b}	81.4^{b}	79.2^{b}	82.3^{b}	85.0^{b}	90.0^{ab}

Ctl = Control (100 FC/non-stress; SA/AA = 0.0), SA = Salicylic acid, AA = Ascorbic acid, SL = Stem length, SD = Stem diameter, SFW = Stem fresh weight, SDW = Stem dry weight, RL = Root length, RFW = Root fresh weight, RDW = Root dry weight, NLs = Number of leaves, LT = Leaf thickness, LFW = Leaf fresh weight, LDW = Leaf dry weight, Clo = Chlorophyll, TClo = Total Chlorophyll, RWC = Relative water content, EL = Electrolyte Leakage, Pro = Proline content. Means followed by common letters are not significantly different using Dunkan 0.05.

The interaction between drought stress and SA had a significant effect on chlorophyll b content. Stress levels of 60% and 30% FC resulted in reductions of 57% and 77% in chlorophyll b, respectively. However, the application of 1.0 mM SA increased chlorophyll b by 16%. Triple interaction effects revealed that the highest chlorophyll b content was observed under 100% FC with the combined application of 1.0 mM SA and ascorbic acid (AA) (Fig. 1B).

For total chlorophyll, stress levels of 60% and 30% FC led to reductions of 43% and 46%, respectively. In contrast, the application of 1.0 mM SA resulted in a 14% increase in total chlorophyll compared to the control. Triple interaction effects showed that the highest total chlorophyll content was achieved under 100% FC with the combined application of 0.5 mM SA and 0.5 mM AA (Fig. 1C).

Biomass and relative water content (RWC)

Water stress significantly reduced the biomass of pistachio seedlings. However, the application of SA and AA significantly improved biomass under drought stress ($P < 0.005$). The use of 0.5 mM and 1.0 mM AA increased biomass by 8% and 6%, respectively. Conversely, applying 0.5 mM and 1.0 mM SA resulted in decreases of 6% and 2% in root length compared to the control (Fig. 2A).

The effects of drought stress, SA, and their interactions on the RWC of pistachio leaves were also significant ($P < 0.05$). Under 30% FC, the application of 0.5 mM and 1.0 mM SA increased RWC by 14% and 15%, respectively, compared to the control. Triple interaction effects revealed that the highest RWC content occurred under 100% FC with the combined application of 1.0 mM SA and 1.0 mM AA (Fig. 2B).

Electrolyte leakage, proline, and glycine betaine

The effects of drought stress and SA application on EL were significant. Stress levels of 60% and 30% FC resulted in increases of 6% and 7% in EL compared to the control. However, the application of 0.5 mM and 1.0 mM SA reduced EL by 6% and 4%, respectively. Triple interaction effects revealed that the highest EL value occurred under moisture stress conditions without the application of SA or AA (Fig. 3A).

Changes in Pro content were significant under drought stress with SA application. Stress levels of 60% and 30% FC led to increases of 18% and 95% in Pro content, respectively. Triple interaction effects showed that the highest Pro value was observed with the combined application of 1.0 mM SA and 0.5 mM AA under 30% FC (Fig. 3B). In addition, glycine betaine content increased significantly by 18% under 30% FC. However, the application of SA and AA did not have a significant effect on glycine betaine levels under stress conditions (Fig. 3C).

Soluble sugars and protein

The effects of SA and AA on soluble sugars were significant under drought stress ($P < 0.05$). The application of 0.5 mM and 1.0 mM SA resulted in decreases of 8% and 2% in soluble sugars, respectively, while the application of 0.5 mM and 1.0 mM AA led to increases of 3% and 4% in soluble sugars under 30% FC. Triple interaction effects revealed that the highest soluble sugars content was observed with the combined application of 1.0 mM SA and 0.5 mM AA under 100% FC (Fig. 4A). However, the effects of SA and AA application under drought stress on leaf protein content were not significant (Fig. 4B).

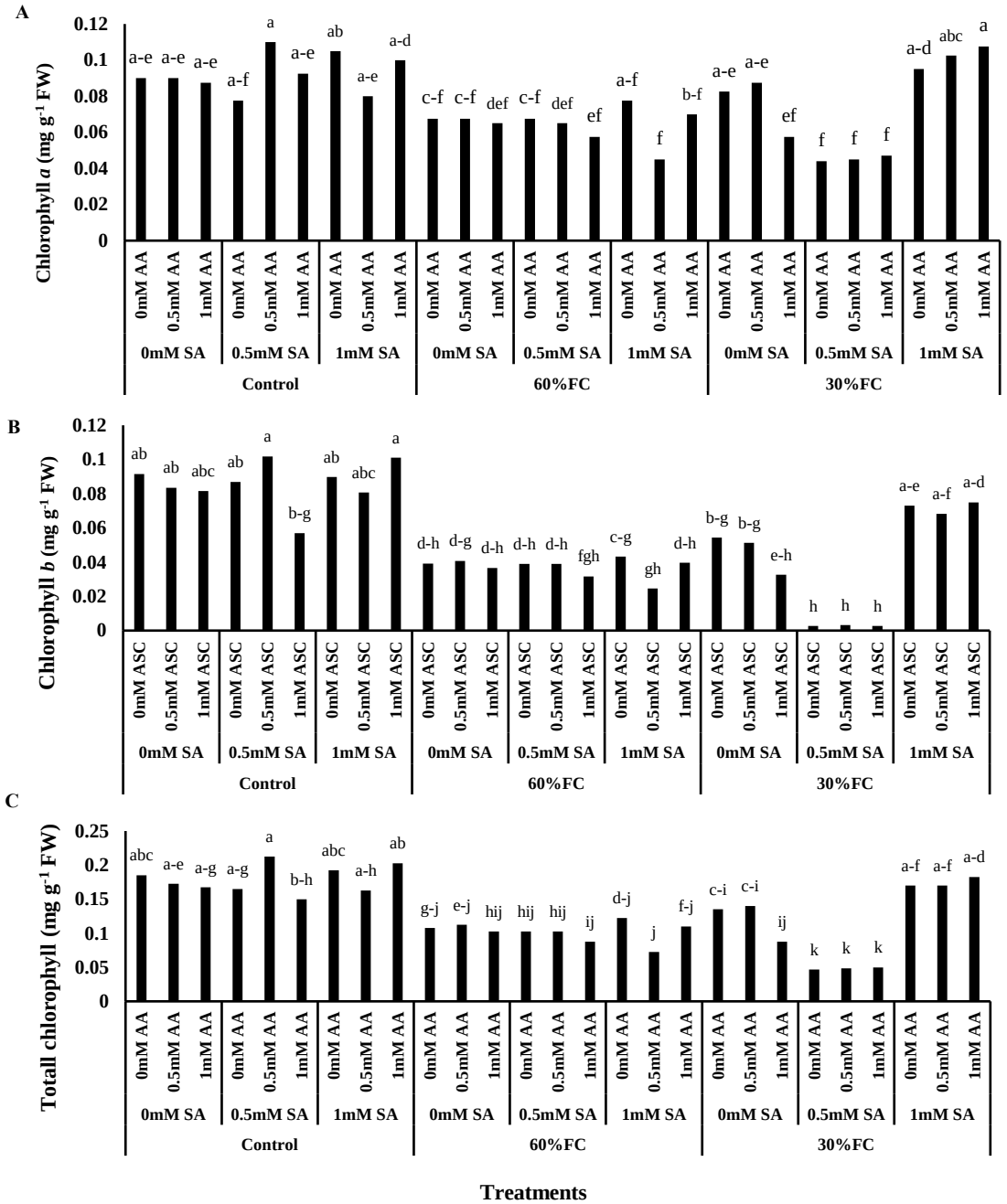


Fig. 1. Effect of salicylic acid (SA: 0.0, 0.5, 1.0 mM) and ascorbic acid (AA: 0.0, 0.5, 1.0 mM) on the chlorophyll content of pistachio seedlings under drought stress (100, 60, 30% FC) (n = 4) ($P < 0.05$).

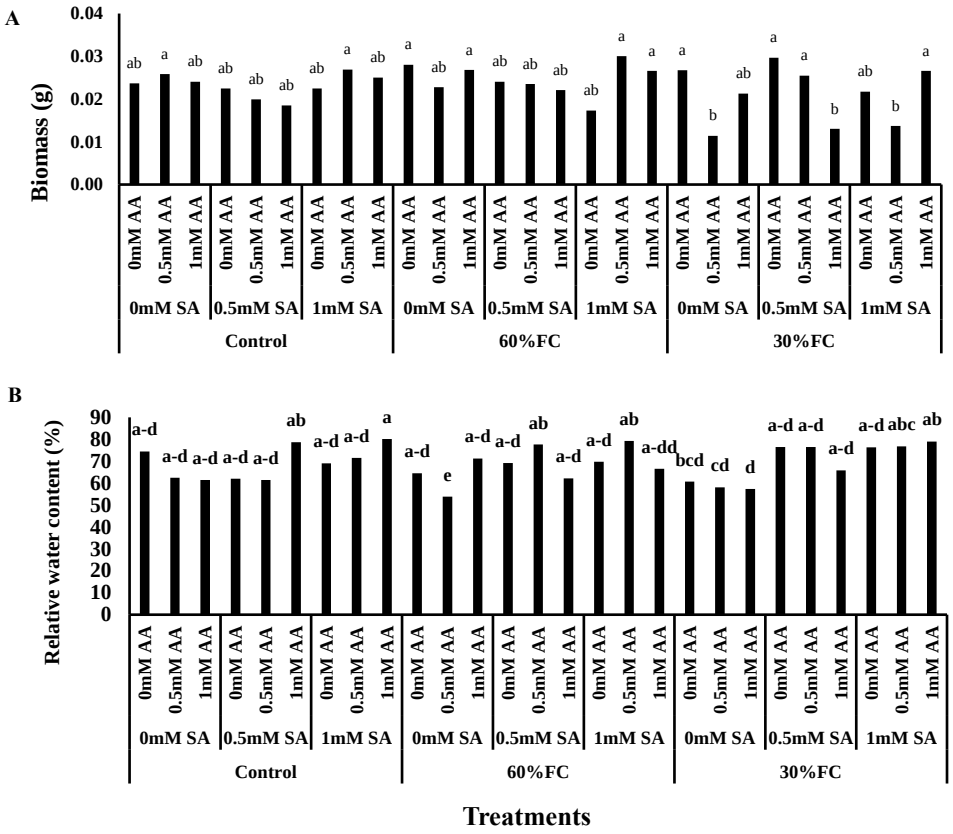
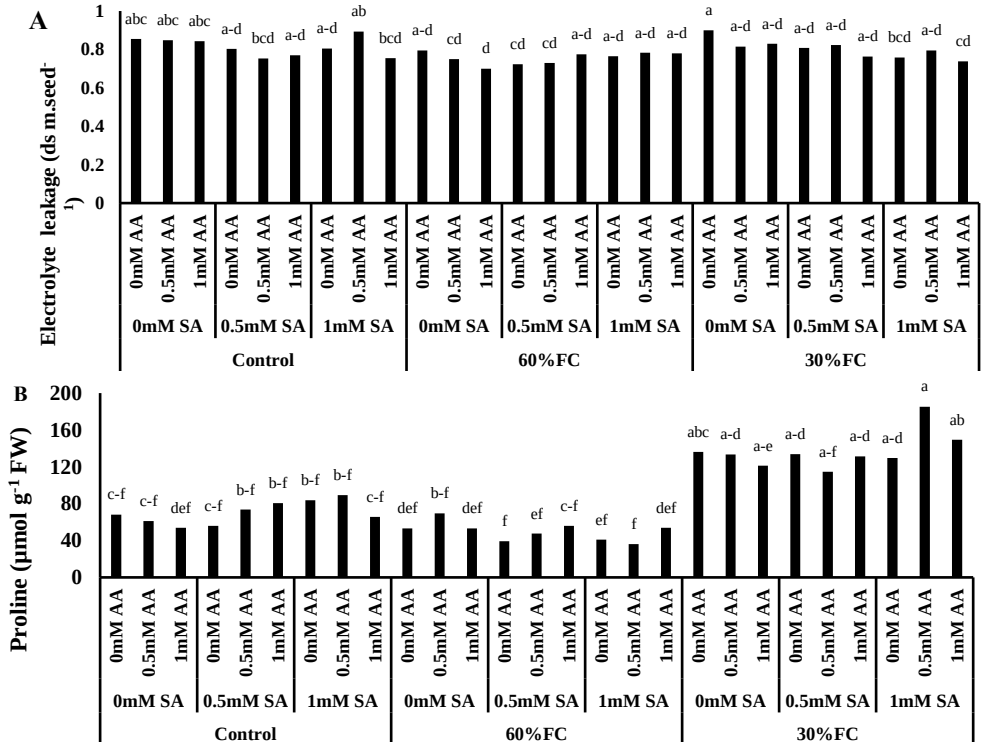


Fig. 2. Effect of salicylic acid (SA: 0.0, 0.5, 1.0 mM) and ascorbic acid (AA: 0.0, 0.5, 1.0 mM) on the biomass and relative water content (RWC) of pistachio seedlings under drought stress (100, 60, 30% FC) (n = 4) ($P < 0.05$).



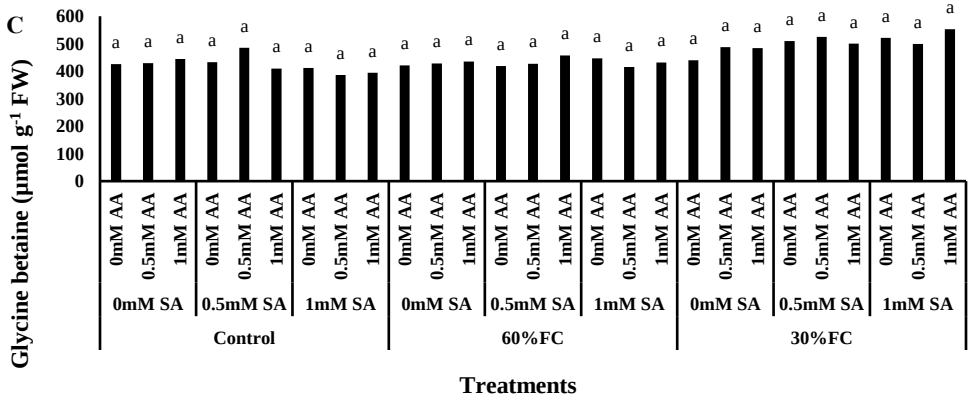


Fig. 3. Effect of salicylic acid (SA: 0.0, 0.5, 1 mM) and ascorbic acid (AA: 0.0, 0.5, 1.0 mM) on the proline (Pro), electrolyte leakage (EL) and glycine betaine (GB) under drought stress (100, 60, 30% FC) (n = 4) ($P < 0.05$).

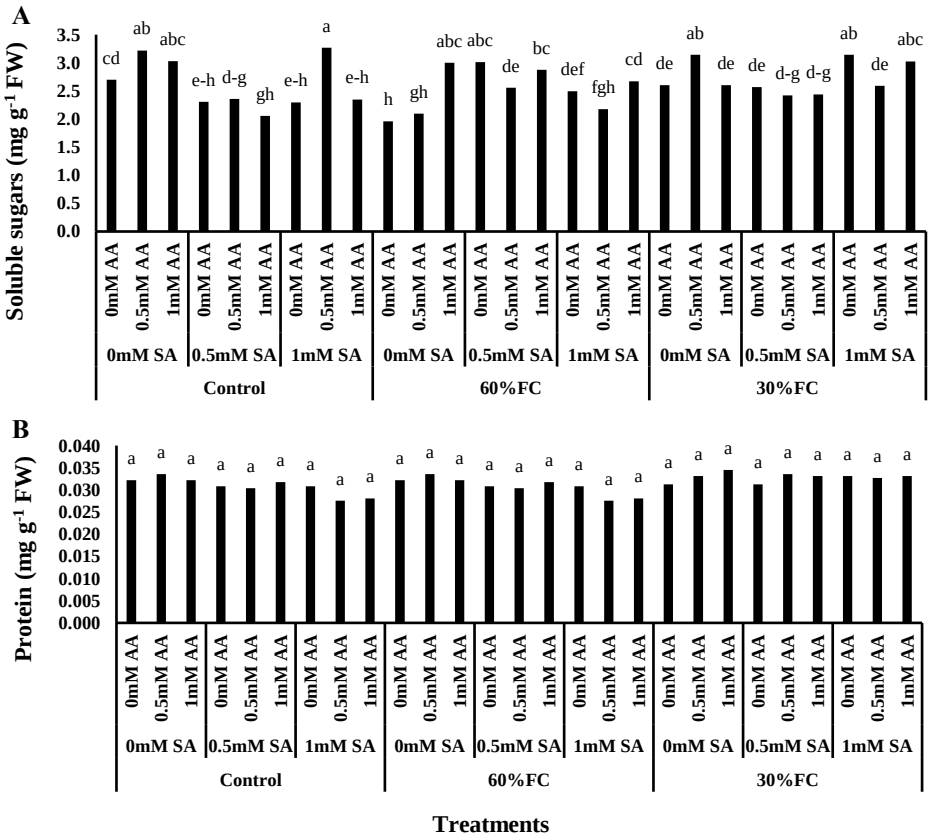


Fig. 4. Effect of salicylic acid (SA: 0.0, 0.5, 1.0 mM) and ascorbic acid (AA: 0.0, 0.5, 1.0 mM) on the soluble sugars (SS) and protein (Prt) under drought stress (100, 60, 30% FC) (n = 4) ($P < 0.05$).

Enzyme activities of Phe, CAT, GP, POD

The SA and AA application significantly changed Phe under drought stress. Homogeneous interaction effects of DS×SA, DS×AA, SA×AA and triple interaction effects were also significant on the Phe. Stresses of 60 and 30% FC led to a 3 and 6% decrease in the Phe, but the application of 1.0 mM SA treatment led to a 12% increase in the Phe and the treatment 0.5 and 1.0 mM of AA led to 2 and 11% increases in Phe compared to the control. The triple interaction effects showed that the highest value of Phe was assigned to the 1 mM SA and 1 mM AA application under 30% FC (Fig. 5A).

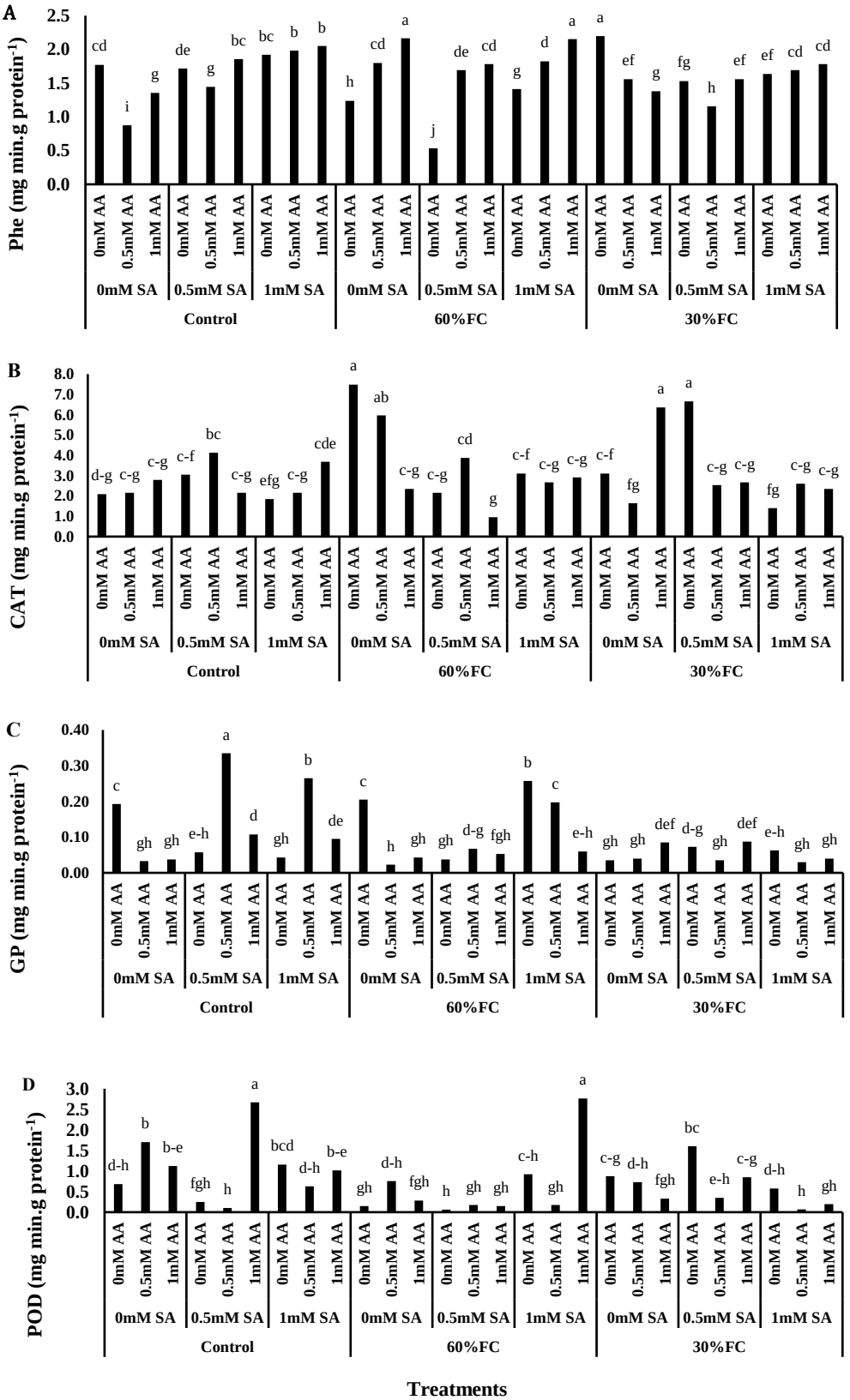


Fig. 5. Effect of salicylic acid (SA: 0.0, 0.5, 1.0 mM) and ascorbic acid (AA: 0.0, 0.5, 1.0 mM) on the phenylalanine ammonylase (Phe), catalase (CAT), guaiacol peroxidase (GP), and polyphenol peroxidase (POD) under drought under drought stress (100, 60, 30% FC).

Moreover, stresses of 60 and 30% FC led to an increase of 30 and 22% in CAT, respectively, but the application of 0.5 and 1.0 mM SA led to a decrease of 18 and 36% in CAT. Application of 0.5 and 1.0 mM AA also resulted in 11 and 16% decrease in CAT, respectively (Fig. 5B). The effect of DS and SA and the mutual effects of DS×SA, DS×AA and SA×AA were significant on GP ($P < 0.05$). The 60 and 30% FC led to a 20 and 60% decrease in GP, but the application of 0.5 and 1.0 mM SA led to a 23 and 51% increase in GP. However, triple interaction effects showed that the highest value of GP was observed using 1.0 mM SA and 1.0 mM AA under 100% FC. It was found that the stresses of 60 and 30% FC led to a 42% and 41% decrease in POD, but the application of 1.0 mM SA treatment resulted in a 13% increase in POD and 1.0 mM AA application resulted in a 49% increase in POD compared to the control, and the triple interactions showed that the highest amount of POD was assigned to the 1.0 mM SA/AA application under 60% FC (Fig. 5).

Discussion

Drought stress is one of the most significant environmental factors that limit plant growth and performance. At the cellular level, plants mitigate the harmful effects of stress by enhancing metabolic activity and regulating osmotic potential. This is achieved through the accumulation of organic compounds (such as amines) and mineral elements, which help maintain turgor pressure and stabilize water potential during stress (Ayuba et al., 2020).

The application of SA and AA to pistachio seedlings under drought stress conditions decreased phenylalanine ammonylase (Phe) activity. However, the application of 1.0 mM SA and both 0.5 mM and 1.0 mM AA increased Phe activity compared to the control. The highest Phe activity was observed with the combined application of 1.0 mM SA and 1.0 mM AA under 30% FC.

Generally, environmental stress reduces plant growth as energy is redirected to counteract stress effects. Acid foliar sprays enhance plant tolerance to drought stress by reducing biochemical biomarkers associated with oxidative damage caused by reactive oxygen species (ROS). This reduction allows the plant to conserve energy that would otherwise be spent on antioxidant enzyme production (Akhter et al., 2021). The application of SA significantly mitigated the negative effects of drought stress on pistachio seedlings, primarily by enhancing the activity of antioxidant enzymes such as superoxide dismutase (SOD), which counteracts ROS like hydrogen peroxide (H_2O_2) (Loutfy et al., 2020). Plants with increased antioxidant enzyme activity, such as peroxidase (POD), catalase (CAT), and SOD, are more effective at suppressing ROS and minimizing stress-induced damage (Zare et al., 2019).

Under drought stress, osmotic pressure changes are critical physiological responses. Regulating osmotic pressure helps maintain cell turgor, which supports metabolic activity. Even small changes in turgor can significantly impact a plant's metabolism under stress (Bagheri et al., 2018). Drought stress often increases the concentration of dissolved salts in the root environment, raising soil osmotic potential and reducing nutrient absorption (Moghaddam et al., 2011; Souri and Tohidloo, 2019). Additionally, higher soil pH exacerbates the limited absorption of certain micronutrients.

Drought stress also negatively impacts photosynthesis by reducing leaf area, closing stomata, decreasing protoplasmic activity, stabilizing carbon dioxide, and inhibiting protein and chlorophyll synthesis. Stomatal closure conserves water but limits photosynthetic efficiency (Zeeshan et al., 2020). In response, stress activates specific genes that aid adaptation by increasing total Prt, which supports growth under stress and stabilizes cellular membranes (Akhter et al., 2021).

The leaf water potential plays a critical role in photosynthesis. As drought stress increases, photosynthesis declines, directly affecting the biochemical processes of photosynthesis and indirectly influencing the entry of carbon dioxide (CO_2) into the stomata, which close during drought stress. Under extreme stress, respiration, CO_2 absorption, photosynthate transport, and raw material transfer within xylem vessels drastically decrease. Concurrently, the activity of hydrolyzing enzymes rises, while reduced photosynthate availability leads to leaf saturation (Zeeshan et al., 2020), ultimately decreasing photosynthetic efficiency.

The reduction in dry matter observed during stress is largely due to decreased cell turgor caused by a reduction in leaf area and a decline in photosynthetic rates. These reductions are often linked to biochemical constraints, such as the loss of photosynthetic pigments, especially chlorophylls. In this study, drought stress reduced chlorophyll content and biomass, whereas acid applications (e.g., SA and AA) increased both, indicating their positive role in mitigating stress effects.

Plants employ intricate resistance mechanisms under stress, relying on a coordinated and

complex response system. Stress conditions disrupt the balance between energy absorption and consumption in the photosynthetic apparatus, leading to the overproduction of reactive oxygen species (ROS). The inability to regulate ROS causes oxidative stress in cellular membranes and triggers oxidative damage symptoms (Blokhina and Fagerstedt, 2010). Oxidation by ROS, including oxygen radicals, damages lipids, proteins, and DNA. Lipid peroxidation and protein degradation are common consequences of ROS activity (Jiang et al., 2022).

To counteract such damage, plants are equipped with antioxidant defense systems, including enzymes such as superoxide dismutase (SOD) and catalase (CAT), which neutralize ROS. The production of ROS plays a dual role, both contributing to damage and stimulating the plant's antioxidant defense, particularly through enzymes like CAT, peroxidase (POD), and SOD, which mitigate ROS effects (Van Dat and Viet, 2019). SOD is a key factor in the survival and activity of organisms, as it catalyzes the dismutation of superoxide radicals into hydrogen peroxide (H_2O_2) and oxygen. Increased POD activity under drought stress reflects the simultaneous production of H_2O_2 and the electron transfer mediated by ferredoxin and NADP. As POD activity rises, the production of SOD may decrease (Jiang et al., 2022; Smirnoff et al., 1998). Enzymes such as CAT, POD, and guaiacol peroxidase are essential for breaking down excess H_2O_2, although their activity may be insufficient under severe stress. Moreover, microelements are vital under stress conditions for the synthesis and activity of growth hormones, chloroplast formation, material regeneration, nucleotide synthesis, and the regulation of plant water relations (Zeng et al., 2019). These elements play an indispensable role in supporting plant adaptation and recovery during and after stress events. SA, an integral part of signaling pathways activated by various biotic and abiotic stresses, plays a significant role in mitigating the adverse effects of drought stress (Loutfy et al., 2020). Recognized as a crucial internal regulatory signal, SA enhances the plant's ability to cope with unfavorable environmental conditions. Alongside other phenolic compounds, SA modulates plant resistance to a wide range of stressors (Shahrivar et al., 2020). SA promotes the development of a defense system in all plant organs, helping to reduce excessive water loss by minimizing sub-branching and dehydration. Additionally, it reduces evaporation and transpiration, thereby maintaining moisture levels within the plant (Hussain et al., 2023).

In this study, SA application proved more effective than AA in preserving the yield and quality traits of pistachio seedlings under severe drought stress. SA significantly improved growth indices and increased proline content, consistent with findings in lentil seeds subjected to stress (Mardani et al., 2014). As a growth regulator, SA enhances flowering induction, nutrient translocation, photosynthesis, stomatal closure, and chlorophyll content. Furthermore, it inhibits ethylene biosynthesis and promotes glycolysis, fruit production, and the regulation of various physiological processes (Maghsoudi et al., 2019; Shahrivar et al., 2020). By balancing cell expansion, division, and senescence, SA fosters a harmonious relationship between plant growth and aging. This study's results align with previous research, such as Abdi and Karami (2020) and Lewis and McFarlane (1986), which also reported the positive impact of SA in enhancing plant resistance to drought stress. While both AA and SA applications demonstrated the ability to improve pistachio seedling performance under drought stress, further research is recommended. Specifically, future studies should focus on the economic evaluation of acid applications for pistachio cultivation under natural conditions across larger areas to ensure practical and cost-effective applications.

Conclusions

This research investigated the effects of foliar application of SA and AA on the growth properties and qualitative traits of *Pistacia vera* cv. Badami-riz-Zarand under drought stress over two consecutive years. The findings revealed that the application of SA and AA significantly mitigated the adverse effects of DS and improved the morphophysiological characteristics of pistachio seedlings. Specifically, the application of 0.5–1.0 mM SA and AA increased relative water content, total chlorophyll, biomass, guaiacol peroxidase, polyphenol oxidase, and phenylalanine ammonylase, while decreasing catalase, electrolyte leakage, and soluble sugars under drought stress conditions. Among the treatments, SA demonstrated greater efficiency than AA in maintaining seedling performance and quality traits under severe drought stress. The most pronounced improvements in morphophysiological traits were observed with the application of 1.0 mM SA and AA under 30% field capacity. In conclusion, SA showed a superior effect on enhancing physiological responses of pistachio seedlings compared to AA under drought conditions. The application of 0.5–1.0 mM SA proved effective in alleviating the

detrimental impacts of severe drought stress, supporting plant growth and maintaining performance. These results highlight the potential of SA as an agent for improving drought tolerance in pistachio seedlings.

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Author contributions
BP and AT designed the research. EE and IT collected the materials and plant data. AT and EE analysed the data. All the authors wrote the manuscript and approved the fnal version of the manuscript.

Conflict of Interest
The authors indicate no conflict of interest in this work.

References

Abdi G, Karami L. 2020. Salicylic acid effects on some physiochemical properties and secondary metabolite accumulation in *Mentha piperita* L. under water deficit stress. Advances in Horticultural Science 34, 81-91.

Aebi H. 1984. Catalase in vitro, in: Methods in Enzymology. Elsevier, pp. 121-126.

Akhter MS, Noreen S, Mahmood S, Ashraf M, Alsahli AA, Ahmad P. 2021. Influence of salinity stress on PSII in barley (*Hordeum vulgare* L.) genotypes, probed by chlorophyll-a fluorescence. Journal of King Saud University 33, 101239.

Amrhein N, Gödeke KH, Gerhardt J. 1976. The estimation of phenylalanine ammonia-lyase (PAL)-activity in intact cells of higher plant tissue: I. Parameters of the assay. Planta, 131: 33-40.

Ayuba Q, Khana SM, Mehmoodb A, Haqc NU, Alia S, Ahmadd T, Ayuba MU, Hassaana M, Hayata U, Shoukata MF. 2020. Enhancement of Physiological and Biochemical Attributes of Okra by Application of Salicylic Acid under Drought Stress. International Journal of Horticultural Science and Technology 3, 113-119.

Bagheri V, Shamshiri MH, Shirani H, Roosta HR. 2018. Nutrient uptake and distribution in mycorrhizal pistachio seedlings under drought stress. Journal of Agricultural Science and Technology 14(7),1591-1604.

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. Plant and Soil 39, 205-207.

Blokhina O, Fagerstedt KV. 2010. Reactive oxygen species and nitric oxide in plant mitochondria: origin and redundant regulatory systems. Journal of Plant Physiology 138, 447-462.

Dreher ML. 2012. Pistachio nuts: composition and potential health benefits. Nutrition Reviews 70, 234-240.

El Refaey A, Mohamed YI, El-Shazly SM, Abd El, Salam AA. 2022. Effect of salicylic and ascorbic acids foliar application on Picual olive trees growth under water stress condition. Egyptian Journal of Soil Science 62, 1-17.

Hassani Asl, NH, Vash FF, Roshdi M, Shekari BM, Gaffari M. 2024. The effect of exogenous application of salicylic acid and ascorbic acid on forage quality and yield of maize (Zea mays L.) under water deficit conditions. Plant, Soil and Environment 70 (3), 142-153.

Heydari A, Daghighi S, Azarmi-Atajan F. 2023. The effect of application of some nitrogen fertilizers along with different levels of potassium sulfate and humic acid on growth rate, yield and photosynthetic pigments of Pistachio Badami Sefid-e-Mahvalat Variety. Journal of Horticultural Science 38 (1), 52-65.

Hussain I, Ashraf MY, Saleem MH, Ashraf MA, Ali B, Shereen A, Farid G, Ali M, Shieazi MU, Saleem A. 2023. Alleviating effects of salicylic acid spray on stage-based growth and antioxidative defense system in two drought-stressed rice (*Oryza sativa* L.) cultivars. Turkish Journal of Agriculture and Forestry 47, 79-99.

Jahantab E, Farzadmehr J, Matinkhah S, Mohammad Abadi NT, Shafeiyan E, Yazdanshenas H. 2022. Effect of metal oxide nanoparticles on the activity of glutathione reductase, catalase, peroxidase and superoxide dismutase in plants under drought. Irrigation and Drainage 71, 1351-1362.

Jiang H, Wang J, Zhang W, Hu L, Mao L. 2022. Oxidation and reduction reactions of (Al/FexCr1-x) $2O_3$ caused by CaO during thermal treatment of solid waste containing Cr. Environmental Research 204, 112356.

Kochba J, Lavee S, Spiegel-Roy P. 1977. Differences in peroxidase activity and isoenzymes in embryogenic ane non-embryogenic 'Shamouti'orange ovular callus lines. Plant and Cell Physiology 18, 463-467.

Kunpratum N, Phalawat C, Thoradit T, Kamoltheptawin K, Thongyoo K, Khiaokhoen P,

Tunprasert L, Jiajitsawat S, Pooam M. 2024. Effect of salicylic acid on germination and seedling growth of KhaoRai Leum Pua Petchabun (*Oryza sativa* L.) under combined drought stress. Plant Physiology Reports 29, 51-64.

Lewis DC, McFarlane JD. 1986. Effect of foliar applied manganese on the growth of safflower (*Carthamus tinctorius* L.) and the diagnosis of manganese deficiency by plant tissue and seed analysis. Australian Journal of Agricultural Research 37, 567-572.

Loutfy N, Azooz M, Abou Alhamd MF. 2020. Exogenously-applied salicylic acid and ascorbic acid modulate some physiological traits and antioxidative defense system in Zea mays L. seedlings under drought stress. Egyptian Journal of Botany 60, 313-324.

Maghsoudi K, Emam Y, Ashraf M, Arvin MJ. 2019. Alleviation of field water stress in wheat cultivars by using silicon and salicylic acid applied separately or in combination. Crop & Pasture Science 70, 36-43.

Mardani Z, Rabiei B, Sabouri H, Sabouri A. 2014. Identification of molecular markers linked to salt-tolerant genes at germination stage of rice. Plant breeding 133, 196-202.

Miladinov Z, Balešević-Tubić S, Crnobarac J, Miladinović J, Čanak P, \DJukić V, Petrović K. 2020. Effects of foliar application of solutions of ascorbic acid, glycine betaine, salicylic acid on the yield and seed germination of soybean in South Eastern Europe conditions. Zemdirbyste-Agriculture 107, 337-344.

Moghaddam NM, Arvin MJ, Nezhad GRK, Maghsoudi K. 2011. Effect of salicylic acid on growth and forage and grain yield of maize under drought stress in field conditions. Seed and Plant Production Journal 27, 41-55.

Mohammed AA, Arkwazee HA. 2024. Micrografting of *Pistacia vera* L.: A review. SVU-International Journal of Agricultural Science 6, 61-72.

Nadimi AE, Ahmadi Z, Falahati-Pour SK, Mohamadi M, Nazari A, Hassanshahi G, Ekramzadeh M. 2019. Physicochemical properties and health benefits of pistachio nuts. International journal for vitamin and nutrition research 90(5-6), 564-574.

Nawaz M, Ashraf MY, Khan A, Nawaz F. 2021. Salicylic acid--and ascorbic acid--induced salt tolerance in mung bean (*Vigna radiata* (L.) Wilczek) accompanied by oxidative defense mechanisms. Journal of Soil Science and Plant Nutrition 21, 2057-2071.

Nezami E, Gallego PP. 2023. History, phylogeny, biodiversity, and new computer-based tools for efficient micropropagation and conservation of pistachio (Pistacia spp.) germplasm. Plants 12(2), 323.

Panda SK, Patra HK. 2007. Effect of salicylic acid potentiates cadmium-induced oxidative damage in *Oryza sativa* L. leaves. Acta Physiologiae Plantarum 29, 567-575.

Raees N, Ullah S, Nafees M. 2023. Interactive effect of tocopherol, salicylic acid and ascorbic acid on agronomic characters of two genotypes of *brassica napus* L. under induced drought and salinity stresses. Gesunde Pflanz 75, 1905-1923.

Schwalm CR, Anderegg WRL, Michalak AM, Fisher JB, Biondi F, Koch G, Litvak M, Ogle K, Shaw JD, Wolf A. 2017. Global patterns of drought recovery. Nature 548, 202-205.

Shahrivar Z, Abtahi F, Hatami M. 2020. Effect of growth regulator salicylate on some physiological and biochemical parameters of peppermint (*Mentha piperita* L.) under drought stress. Journal of Plant Research (Iranian Journal of Biology) 32, 815-830.

Smirnoff M, Patt C, Seckler B, Adler JJ. 1998. Tuberculin and anergy skin testing of patients receiving long-term hemodialysis. Chest 113, 25-27.

Souri, MK, Tohidloo G. 2019. Effectiveness of different methods of salicylic acid application on growth characteristics of tomato seedlings under salinity. Chemical and Biological Technologies in Agriculture 6, 1-7.

Tavili A, Bashari H, Yazdanshenas H, Jafari M, Arzani H, Azarnivand H. 2019. Morphophysiological changes of wild Stachys multicaulis species under physical conditions during the cultivation process. Heliyon 5. https://doi.org/10.1016/j.heliyon.2019.e02093

Torun AA, Bozgeyik S, Duymu\cs E. 2021. The Effect of Boron application in increasing doses on shoot boron concentration and uptake of other nutrients of Pistachio (*Pistacia vera* L.). Turkish Journal of Agriculture - Food Science and Technology 9, 855-862.

Van Dat P, Viet NX. 2019. Facile synthesis of novel areca flower like Cu2O nanowire on copper foil for a highly sensitive enzyme-free glucose sensor. Materials Science and Engineering 103, 109758.

Zahid A, Razzaq AA, Munawar M, Ramzan M, Almutairi BO, Almutairi MH. 2023. Foliar spray of

salicylic acid and ascorbic acid ameliorates the biochemical compounds in hybrid chillies. Journal of King Saud University 35, 102660.

Zare S, Nazarian-Firouzabadi F, Ismaili A, Pakniyat H. 2019. Identification of miRNAs and evaluation of candidate genes expression profile associated with drought stress in barley. Plant Gene 20, 100205.

Zeeshan M, Lu M, Sehar S, Holford P, Wu F. 2020. Comparison of biochemical, anatomical, morphological, and physiological responses to salinity stress in wheat and barley genotypes deferring in salinity tolerance. Agronomy 10, 127.

Zeng Y, Zhang X, Qin R, Liu X, Fang P, Zheng D, Tong Y, Lu X. 2019. Dendrite-free zinc deposition induced by multifunctional CNT frameworks for stable flexible Zn-ion batteries. Advanced Materials 31, 1903675.