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Research Article

Investigation of Drought Stress Effects on Physiological, Biochemical, and Morphological Characteristics of Wild Almond (Amygdalus scoparia) Populations in Isfahan Province

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Abstract: Drought stress is one of the most significant environmental challenges, directly impacting the growth and survival of plants, particularly in arid and semi-arid regions. This stress disrupts physiological processes such as photosynthesis, osmotic balance, and cellular metabolism, leading to reduced performance and plant viability. Considering Iran's arid and semi-arid climate and the importance of native species in conserving natural ecosystems, this study examines the effects of drought stress on the morphological, physiological, and biochemical traits of various wild almond (Amygdalus scoparia) populations in Isfahan province. The study was conducted as a factorial experiment in a randomized complete block design. Drought treatments included four levels of field capacity (25%, 50%, 75%, and 100%), applied to 600 seedlings from ten different populations. Parameters studied included chlorophyll a and b levels, relative water content, ion leakage, fresh and dry weight of shoots and roots, proline content, and antioxidant enzyme activities such as peroxidase, ascorbate peroxidase, and catalase. Data were analyzed using SPSS software, and mean comparisons were performed using the Least Significant Difference (LSD) method at a 5% probability level. The results indicated that drought stress significantly decreased the relative water content of leaves, fresh and dry weights of shoots and roots, and chlorophyll a levels, while increasing ion leakage and proline accumulation in green shoots. Antioxidant enzyme activities were influenced by drought stress, with an increase in ascorbate peroxidase, while changes in catalase and peroxidase activities were not significant at the population level. The populations of Fereydunshahr and Khur-Biabanak exhibited the highest drought resistance, maintaining water content and reducing ion leakage under stress conditions. This research demonstrated that Amygdalus scoparia can effectively cope with the adverse effects of drought stress through diverse mechanisms such as osmotic adjustment, proline accumulation, and enhanced antioxidant enzyme activities. These features make this species a suitable candidate for afforestation in arid and semi-arid regions. The findings can play a significant role in water resource management programs and the development of strategies for ecosystem restoration.

Keywords: Antioxidant enzymes, Wild almond (Amygdalus scoparia), Proline, Drought stress, Relative water content.

Introduction

Drought stress is one of the major environmental challenges, particularly affecting plant growth and survival in arid and semi-arid regions. By reducing water availability, drought disrupts vital plant processes such as photosynthesis, osmotic balance, and cellular metabolism, ultimately leading to reduced growth, yield, and plant viability [32]. Due to its predominantly arid and semi-arid climate, Iran faces this challenge acutely, emphasizing the importance of studying the effects of drought stress on native plant species [10]. One such species of significant interest in this context is the wild almond (Amygdalus scoparia).

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The wild almond plays a vital role as a valuable forest species in the mountainous and arid regions of Iran. Besides stabilizing soil and preventing erosion, it provides critical habitats for various wildlife species. Its tolerance to harsh environmental conditions, such as drought, makes it a suitable candidate for afforestation in arid regions [15,27]. However, reduced rainfall and climate change exert significant pressure on its survival, highlighting the need to study its physiological and biochemical responses to drought stress [40].

Numerous studies, both domestic and international, have investigated the effects of drought stress on the characteristics of wild almond. [10] reported that drought stress reduces leaf water potential and increases proline accumulation in this species, showcasing its defensive mechanisms for maintaining osmotic balance. Similarly, [38] found that antioxidant enzymes such as catalase and peroxidase exhibit increased activity under drought conditions, helping to mitigate oxidative damage. Furthermore, [2] demonstrated that drought reduces photosynthesis and increases phenolic compound accumulation in wild almond leaves, where these compounds serve as antioxidants to protect against oxidative stress.

In terms of soil improvement, [27] reported that afforestation with wild almond improves physical and chemical soil properties while reducing erosion in arid areas. [36] identified morphological markers to introduce drought-resistant genotypes of wild almond. [29] found that reduced leaf area and increased leaf thickness under drought conditions are effective mechanisms for reducing transpiration. [22] reported the positive effect of nitric oxide in mitigating drought stress in this species, demonstrating the role of chemical regulators in enhancing plant resistance.

[32] emphasized reduced transpiration and increased water-use efficiency as key responses of wild almond to drought stress. [16] showed that drought increases phenolic compounds while reducing photosynthetic activity in almonds, suggesting these changes serve as protective adaptations. [15] found that changes in the root system, including increased root depth, are among the critical mechanisms for wild almond to adapt to drought. [5] highlighted the accumulation of secondary metabolites such as flavonoids and phenols under drought conditions, acting as antioxidants to reduce oxidative damage. [40] further emphasized the importance of genes involved in osmolyte regulation, such as proline and soluble sugars, in enhancing drought tolerance.

Given the importance of wild almond in arid and semi-arid ecosystems and the adverse effects of drought stress on its physiological and biochemical traits, this study aims to examine the impacts of drought stress on the morphological, physiological, and biochemical characteristics of wild almond in Isfahan Province. This research includes evaluating plant responses to different drought levels, the effects of growth regulators and bio-fertilizers on mitigating drought stress, and assessing water-use efficiency in this species. The findings can provide valuable insights for developing management strategies and restoring this valuable forest species.

Materials and Methods

Application of Drought Stress and Measurement of Morphological Traits in Seedlings

In late summer 2021, an experiment was conducted to investigate drought stress in a factorial arrangement within a completely randomized block design. Two factors were considered: drought at four levels (25% as severe, 50% as moderate, 75% as mild, and 100% as control of field capacity) and almond genotypes at ten levels, with three replications (600 seedlings) over five weeks in the Jabal Amalian nursery of Najafabad. To determine the field capacity, soil samples were transferred to the soil science laboratory. After determining the soil's field capacity, drought treatments were applied based on field capacity using the gravimetric method. Based on calculations, the weight of each pot for the four irrigation treatments field capacity as the control treatment and irrigation after depletion to 75%, 50%, and 20% of available water for mild, moderate, and severe stress treatments was determined.

At the end of the experimental period, parts of chlorophyll-containing almond stems with several leaves were cut with scissors from seedlings under different drought stress treatments. These were immediately placed in labeled aluminum sheets, frozen with liquid nitrogen, and transported to the laboratory, where they were stored at -80°C until analysis. Physiological and biochemical parameters, including photosynthetic pigments, relative water content (RWC) of leaves, ionic leakage of chlorophyll-containing stems, proline content, activities of peroxidase, ascorbate peroxidase, and catalase enzymes, as well as growth indices such as leaf dimensions, fresh and dry weight of roots and stems, were measured under different drought treatments.

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For measuring fresh weight, the plant was divided into root and stem sections, and their fresh weights were measured with a digital scale accurate to 0.01 g. After placing the fresh roots and stems in an oven at 105°C for 48 hours, the dry weights were measured using the same scale. To determine the relative water content (RWC) of leaves, fresh leaf weight was measured to the nearest milligram. The leaves were then placed in sealed containers filled with distilled water. After 24 hours in darkness at 4°C, excess water was removed from the leaf surface using filter paper, and the turgid weight of the leaves was recorded. The leaves were then placed in an oven at 70°C for 24 hours to measure the dry weight. Finally, RWC was estimated using the following formula (30):

$$RWC\% = [(FW-DW)/ (TW-DW)] \times 100$$

In this formula, TW, DW, and FW represent the turgid weight, dry weight, and fresh weight of the leaf samples, respectively.

The measurement of ionic leakage was performed using the method described by [33]. First, a 3 cm segment of healthy, chlorophyll-containing almond stems was excised and placed in a test tube. Subsequently, 20 mL of double-distilled water was added, and the sample was left at room temperature for 24 hours. The initial electrical conductivity (EC_0) of the solution was measured using an electrical conductivity meter. The samples were then frozen at 0°C in a freezer for 24 hours. After removal from the freezer, the samples were kept at room temperature for another 24 hours, and the final electrical conductivity (EC_1) was measured. The percentage of ionic leakage in the chlorophyll-containing almond stems was calculated using the following formula:

Ionic Leakage (%)=(EC_0/EC_1)×100 Where:
EC_0: Electrical conductivity of the solution before freezing
EC_1: Electrical conductivity of the solution after freezing
The measurement of photosynthetic pigments (chlorophyll and carotenoids) was carried out based on the method described by [14]. According to this method, 0.5 grams of fresh leaf tissue was weighed and finely ground in a porcelain mortar using liquid nitrogen. Subsequently, 5 mL of 80% acetone was added to the sample, which was then centrifuged at 6000 rpm for 10 minutes. Three milliliters of the supernatant, containing chlorophyll a, chlorophyll b, and carotenoids (xanthophylls and beta-carotene), were transferred into a cuvette. The absorbance was measured using a spectrophotometer at wavelengths of 663 nm for chlorophyll a, 645 nm for chlorophyll b, and 470 nm for carotenoids. Using the equations below, the concentrations of chlorophyll a, chlorophyll b, and carotenoids were calculated in milligrams per gram of fresh weight (mg/g FW) of the sample.

Chlorophyll a)= 12.25A663-2.79A645(V/100

Chlorophyll b = (1.50A645 -5.10 A663) V/100W

Chlorophyll total = (7.15 A663 – 18.71 A645) V/100W

Carotenoides = 1000 A470 -1.82 (mg chl. a) -85.02(mg chl. b)/198

V: Volume of the clarified solution (the supernatant obtained after centrifugation).

A: Absorbance of light at wavelengths 663 nm, 645 nm, and 470 nm.

W: Fresh weight of the sample in grams.

The content of free proline was measured using a spectrophotometer based on the method of [4] and reported in milligrams per gram of fresh weight of the chlorophyll-containing stem. For this purpose, 0.5 g of fresh tissue from the chlorophyll-containing part of the almond stem was ground in a mortar. Subsequently, 10 mL of 3% sulfosalicylic acid was added, and the mortar contents were mixed thoroughly. The mixture was then filtered, and 2 mL of the filtrate was mixed with 2 mL of ninhydrin acid and 2 mL of acetic acid in a test tube. The test tubes were capped and placed in a boiling water bath at 100°C for 1 hour. After the tubes reached room temperature equilibrium, 4 mL of toluene was added to each tube and vortexed for 30 minutes. The tubes were then allowed to rest at room temperature, forming two distinct layers. Finally, the absorbance of the colored upper layer was measured at a wavelength of 520 nm using toluene as a blank, and the proline content was determined using a standard curve.

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The second part involved measuring the activities of the enzymes peroxidase, catalase, and ascorbate peroxidase in the chlorophyll-containing stems of almond seedlings from various populations under drought stress. For enzyme extraction, the chlorophyll-containing parts of the almond stems were ground in a sterilized mortar using liquid nitrogen. Subsequently, 1 g of the sample was weighed with a balance accurate to 0.01 g and placed into a test tube. Three milliliters of extraction buffer (as shown in Table 1) were added to the sample, and the tube was sealed with sterilized parafilm. The samples were stored in a refrigerator at 4°C for 26 hours and then centrifuged at 3000 rpm for 20 minutes. The clear supernatant was separated and stored in a freezer for quantitative enzyme analysis [1].

Table 1: Materials Required for Preparing Enzyme Extraction Solution in a Final Volume of 1 Liter [1]

Required Amount (gr)	Material Name
1.2	Tris base
2	Ascorbic acide
3.8	Borax
3.6	Nacl
2	EDTA-Na_2
50	Polyethylonglycol

To measure peroxidase activity, 2 mL of 0.01 M acetate buffer (pH = 7), 0.4 mL of 3% hydrogen peroxide, and 0.2 mL of 0.01 M benzidine were mixed, and 50 µL of the extract was added to the mixture. After mixing, the absorbance was recorded at a wavelength of 530 nm at intervals of 10, 20, and 120 seconds using a Unico 2100 UV spectrophotometer, and the enzyme activity was calculated in units of time [12]. The activity of ascorbate peroxidase was measured using the method of [24]. The reaction mixture contained 600 µL of 0.1 M EDTA, 1500 µL of 0.05 M phosphate buffer (pH = 7), 400 µL of 0.5 mM ascorbic acid, 0.3 µL of 30% hydrogen peroxide, and 50 µL of the enzyme extract. The decrease in absorbance at 290 nm was recorded with a 15-second delay over 60 and 120 seconds, and the average of these readings was recorded as activity per unit time. Catalase activity was determined using the method of [7]. To 2 mL of phosphate buffer (pH = 7), 50 µL of enzyme extract and 2 µL of 3% hydrogen peroxide were added. The enzymatic reaction began upon the addition of hydrogen peroxide, and the decrease in absorbance at 260 nm was recorded with a 10-second delay over 60 seconds, and the activity was expressed as units per unit time. To study the effect of drought stress on seed germination in different almond populations, treatments were initiated in mid-February 2022. To apply cold and moisture treatments (almond seeds exhibit physiological and mechanical dormancy, as described by [26], seeds from different populations were placed in labeled plastic containers. Twice the seed weight in water was added to the containers, and they were subjected to cold and moist conditions at ambient outdoor temperatures (average 4°C) for 10 days. During this period, the water in the containers was replaced three times. In early March, the soaked seed coats were carefully removed without damaging the kernels using a hammer. The de-coated seeds were sterilized with 5% sodium hypochlorite for three minutes [10], thoroughly rinsed with distilled water, and placed in plastic containers containing washed and sterilized sand. Distilled water was used to create an osmotic potential of zero (control), and three different drought stress levels (-0.05 MPa, -0.1 MPa, and -0.5 MPa) were prepared by adding various concentrations of PEG 6000, following the method described by [18], using the calculated formula for osmotic potential.

$$s = (1.18 \times 10^{-2})C(1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T$$

Where:
S: Osmotic potential in megapascals (MPa).
C: Concentration in grams per gram of water.
T: Temperature in degrees Celsius (°C).

In the control treatment, 10 mL of distilled water was added, while in the other treatments, 10 mL of the prepared solutions was added, ensuring the seeds were in contact with the solution. The seeds were kept at a constant temperature of 20°C for a 30-day period. Counting of germinated seeds began on the third day and continued daily until the end of the experiment.

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The emergence of a radicle at least 2 mm in length was considered the criterion for germination [26]. Germinated seeds were counted and removed from the germination container. After the experiment, ungerminated seeds were examined, and those lacking an embryo were excluded from the analysis. The final germination percentage was calculated by dividing the number of germinated seeds by the number of viable seeds.

At the end of the experiment, germination percentage, mean germination time, germination rate coefficient, germination rate index, germination index, and germination value were calculated based on the formulas presented in Table 2 [11]. Due to the non-normal distribution of data for some germination indices, angular transformation (arcsine) was applied before statistical analysis. The experiment was conducted in a factorial arrangement within a completely randomized design with four replications.

Table 2: Germination Indices Studied in Seeds of Wild Almond (Amygdalus scoparia) Populations

Calculation Formula	Studied Indices
GP= n /N1 ×100	Germination Percentage
$CVG=N_1+N_2+...+N_x/100\times N_1T_1+...+N_xT_x$	Coefficient of Velocity of Germination
$GRI= G_1/1a+G_2/2+G_x/x$	Germination Rate Index
$GI= (30\times n_1)+(29\times n_2)+...+ (1\times n_{30})$	Germination Index
MGT =Σ (ni×ti) /Σn	Mean Germination Time
GV= PV × MDG	Kazabator Germination Value
GV= (ΣDGS/N) ×GP×10	Javanshir Germination Value - GV

In this study, drought stress was investigated in the nursery and laboratory through a factorial experiment within a completely randomized block design. The following definitions apply: n represents the total number of germinated seeds during the period, N is the total number of seeds sown, ni is the number of seeds germinated in a specific time interval, ti is the number of days after the start of germination, N1and N2 are the number of seeds germinated on the first and second days, respectively, G1and G2are the germination percentages multiplied by 100 on the first and second days after sowing, n1n_1, n2n_2, and n30n_{30} are the number of seeds germinated on the first, second, and thirtieth days, respectively, PVPV denotes the maximum germination rate value, MDGMDG indicates the mean daily germination, and DGSDGS refers to the daily germination speed. When the interaction effect was significant, slicing was performed, and mean comparisons were carried out using the least significant difference (LSD) method at a 5% probability level. For statistical analysis, data on leaf, seed, and seedling traits were recorded in Excel, and to test for normality, assess variance homogeneity, calculate statistical indices, perform simple Pearson correlation coefficients, conduct analysis of variance (ANOVA), compare means, perform slicing, cluster analysis, principal component analysis, and generate statistical charts, SPSS 26 software was used.

The results related to the effects of drought stress on seedlings from different populations of wild almond

The effect of drought stress on the levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in wild almond leaves

The analysis of variance showed that the effect of population on the levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in wild almond leaves was not significant. The effect of drought stress was significant only for chlorophyll levels in wild almond leaves at the 5% probability level. The interaction effect of population and drought stress on the levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in wild almond leaves was significant at the 1% error probability level (Table 3). The significance of the interaction effect indicates different responses of populations to various levels of drought stress. The breakdown of the interaction effect by population treatment revealed significant differences in the levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in the wild almond populations of Isfahan and Fereydunshahr (Table 4). The mean comparison of the interaction effect using the Least Significant Difference (LSD) method in the populations of Isfahan and Fereydunshahr showed that drought stress levels of 50% and 20% of field capacity caused significant differences in the levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in wild almond leaves. However, in other populations, no significant differences were observed between the different levels of drought stress.

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Table 3: Analysis of Variance for the Effect of Drought Stress and Population on Levels of Chlorophyll a, Chlorophyll b, Chlorophyll a+b, Carotenoids, and Leaves of Wild Almonds

Mean Squares					Source of Variation
Carotenoids	Total Chlorophyll	Chlorophyll b	Chlorophyll a	Degrees of Freedom (df)	
12.28	0.014	0.002	0.00582	9	Population
5.62	0.016	0.001	0.00938*	3	Drought Stress
19.22**	0.018**	0.002**	0.00811**	27	Population × Drought Stress
768.58	0.792	0.109	0.354	112	Error
25.93	27.62	35.6	25.88	-	Coefficient of Variation (CV%)

**Significant difference at the 0.01 error **Significant difference at the 0.05 error level

Table 4: Mean Squares Resulting from the Split-Plot Interaction of Different Levels of Drought Stress for Physiological Traits of Wild Almond Populations

Mean Squares							Population
Water Potential (MPa)	Relative Water Content (RWC)	Carotenoids	Total Chlorophyll	Chlorophyll b	Chlorophyll a	Degrees of Freedom (df)	
32.98	166.35	2.430	0.0026	0.0002	0.0050	9	Natanz
991.84**	204.53	8.393	0.0113	0.0009	0.0178	9	Semirom
123.31	1373.24**	19.60*	0.0286**	0.0027*	0.0440 **	9	Isfahan
170.94	633.12**	52.28**	0.0529**	0.0045**	0.0811 **	9	Fereydunshahr
741.86**	76.36	25.04*	0.0123	0.0021	0.0128	9	Khoor
256.05	670.98**	5.46	0.0055	0.0007	0.0081	9	Naein
21.60	943.92**	7.69	0.0066	0.0018	0.0048	9	Borkhar
281.74	203.51	7.492	0.0111	0.0008	0.0175	9	Kashan
255.99	669.98**	5.35	0.0054	0.0006	0.0080	9	Ardestan
239.85	75.96	24.99*	0.0121	0.0020	0.0125	9	Shahinshahr

*Significant difference at the 0.05 error level **Significant difference at the 0.01 error

The Effect of Drought Stress on the Ion Leakage of Chlorophyll-Containing Stems in Wild Almond Populations

The analysis of variance indicated a statistically significant effect of drought stress, as well as a significant interaction effect of population and drought stress, on the ion leakage of chlorophyll-containing stems in wild almond populations at the 1% error probability level (Table 3). The significance of the interaction effect between population and drought stress suggested that the ion leakage of chlorophyll-containing stems varies among populations in response to drought stress. The breakdown of the interaction effect by population revealed significant differences in ion leakage levels in the chlorophyll-containing stems of wild almond populations in Semirom and Khur and Biabanak (Table 4).

The mean comparison of the interaction effect using the Least Significant Difference (LSD) method in the populations of Semirom and Khur and Biabanak demonstrated that drought stress levels of 50% and 25% of field capacity significantly increased ion leakage in the chlorophyll-containing stems of the Khur and Biabanak population. In the Semirom population, drought stress levels of 75%, 50%, and 25% of field capacity led to a significant increase in ion leakage. Therefore, regarding ion leakage, the Semirom population exhibited greater sensitivity to drought stress compared to the other studied populations.

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The Effect of Drought Stress on the Relative Water Content of Wild Almond Populations

The analysis of variance indicated statistically significant effects of population, drought stress, and the interaction between population and drought stress on the relative water content (RWC) of wild almond leaves at the 1% probability level (Table 4). The significance of the interaction effect between population and drought stress suggests that the RWC levels of wild almond leaves vary significantly among populations in response to drought stress.

The analysis of variance, segmented by population treatment, showed that different levels of drought stress caused significant differences in the RWC levels of wild almond leaves in the populations of Isfahan, Fereydunshahr, Naeen, Borkhar, and Ardestan (Table 4).

The Effect of Drought Stress on Fresh Weight, Dry Weight, and Relative Water Content of Stems in Wild Almond Populations

The effect of drought stress on the fresh weight, dry weight, and relative water content of stems in wild almond populations was not statistically significant. However, the effects of population and the interaction between population and drought stress on stem fresh weight, dry weight, and relative water content were statistically significant at the 1% probability level (Table 4). The significance of the interaction effect indicates that the fresh weight, dry weight, and relative water content of stems differed among populations in response to drought stress (Table 6).

The breakdown of the interaction effect by population treatment revealed that in the populations of Fereydunshahr and Khur and Biabanak, different levels of drought stress caused significant differences in stem fresh weight, dry weight, and relative water content, whereas no such significant differences were observed in other populations. A mean comparison of the interaction effect using the Least Significant Difference (LSD) method for the populations of Fereydunshahr and Khur and Biabanak showed that drought stress levels of 50% and 20% of field capacity significantly reduced stem fresh weight, dry weight, and relative water content in these populations.

Table 5: Analysis of Variance (ANOVA) for Relative Water Content, Ionic Leakage, Fresh and Dry Weights, Stem Water Content, Fresh and Dry Weights of Roots in Almond Populations Under Drought Stress

Mean Squares									Source of Variation
Root Water Content	Dry Root Weight	Fresh Root Weight	Stem Water Content	Dry Stem Weight	Fresh Stem Weight	Ionic Leakage	Relative Water Content	Degrees of Freedom	
0.151**	0.884**	0.566**	0.453**	0.47**	1.790**	128.34	747.78**	9	Population
0.094	0.063	0.196	0.0634	0.0823	0.289	716.62**	1368.62**	3	Drought Stress
0.021	0.44	0.070	0.133**	0.148**	0.559**	270.34**	449.98**	27	Population× Drought Stress
3.02	3.10	10.69	7.17	7.44	28.53	12390.800	16507.80	112	Error
34.9	30.13	30.16	51.2	50.15	50.5	23.50	19.42		Coefficient of Variation (CV%)

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Table 6: Mean Squares from Analysis of Variance (ANOVA) for the Effect of Drought Stress at Different Levels of Almond Populations for Traits of Fresh and Dry Weights and Stem Water Content

Mean Squares							Population
Water Potential (MPa)	Relative Water Content (RWC)	Carotenoids	Total Chlorophyll	Chlorophyll b	Chlorophyll a	Degrees of Freedom (df)	
32.98	166.35	2.430	0.0026	0.0002	0.0050	9	Natanz
991.84**	204.53	8.393	0.0113	0.0009	0.0178	9	Semirom
123.31	1373.24**	19.60*	0.0286**	0.0027*	0.0440 **	9	Isfahan
170.94	633.12**	52.28**	0.0529**	0.0045**	0.0811 **	9	Fereydunshahr
741.86**	76.36	25.04*	0.0123	0.0021	0.0128	9	Khoor
256.05	670.98**	5.46	0.0055	0.0007	0.0081	9	Naein
21.60	943.92**	7.69	0.0066	0.0018	0.0048	9	Borkhar
281.74	203.51	7.492	0.0111	0.0008	0.0175	9	Kashan
255.99	669.98**	5.35	0.0054	0.0006	0.0080	9	Ardestan
239.85	75.96	24.99*	0.0121	0.0020	0.0125	9	Shahinshahr

*Significant difference at the 0.05 error level **Significant difference at the 0.01 error level

The Effect of Drought Stress on Proline Content in Chlorophyll-Containing Stems of Wild Almond Populations

The analysis of variance showed that the effect of population on the proline content of chlorophyll-containing stems in wild almond was not statistically significant. However, the effects of drought stress levels and the interaction between population and drought stress level on proline content were statistically significant (Table 6). The breakdown of the interaction effect indicated that different levels of drought stress had varying impacts on the proline content of chlorophyll-containing stems in wild almond populations. Further analysis by population treatment revealed that in the populations of Fereydunshahr and Khur and Biabanak, different levels of drought stress caused significant differences in proline content in chlorophyll-containing stems, whereas in other populations, no significant differences were observed (Table 7). The mean comparison of the interaction effect using the Least Significant Difference (LSD) method for the populations of Fereydunshahr and Khur and Biabanak showed a significant increase in the proline content of chlorophyll-containing stems at drought stress levels of 25% and 50% of field capacity (Table 7).

The Effect of Drought Stress on Ascorbate Peroxidase Enzyme Activity in Chlorophyll-Containing Stems of Wild Almond Populations

The analysis of variance indicated that the effects of population and the interaction between drought stress and population on ascorbate peroxidase enzyme activity in chlorophyll-containing stems were not statistically significant (Table 7). However, the enzyme activity was influenced by different levels of drought stress.

The mean comparison results showed the highest ascorbate peroxidase enzyme activity at the drought stress level of 25% of field capacity.

Table 7: Analysis of Variance (ANOVA) for the Effect of Population and Drought Stress on Peroxidase Activity, Ascorbate Peroxidase, Catalase, and Proline Content in Green Stem Populations of Almond

Mean Squares					Source of Variation
Proline	Catalase	Ascorbate Peroxidase	Peroxidase	Degrees of Freedom	
12.28	0.014	0.002	0.00582	9	Population
5.62	0.016	0.001	0.00938*	3	Drought Stress
19.22**	0.018**	0.002**	0.00811**	27	Population × Drought Stress
768.58	0.792	0.109	0.354	112	Error
25.93	27.62	35.6	25.88	-	Coefficient of Variation (CV%)

*Significant difference at the 0.05 error level **Significant difference at the 0.01 error level

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The Results of Drought Stress Induced by Polyethylene Glycol on Germination

The analysis of variance revealed statistically significant differences in germination indices among the studied populations. The effect of population was significant for traits such as germination rate coefficient, mean germination time, and germination rate index at the 1% level, and for traits such as germination index and germination value at the 5% level.

The percentage of germination and germination value were not significant at the population level. However, the effects of drought treatment and the interaction between genotype and drought were significant for all studied traits at the 1% probability level (Table 8).

Table 8: Analysis of Variance (ANOVA) Table for Germination Traits of Almond Populations at Different Levels of Drought Stress

Germination Rate Index	Germination Value	Germination Value	Germination Index	Mean Germination Time	Germination Rate Coefficient	Germination Percentage	df	Sources of Variation
0.59**	0.06**	0.12	5027.5*	32.85**	21.53**	187.9	9	Population
10.43**	1.44**	4.84**	268348**	95.02**	72.2**	14064.3**	3	Drought Treatment
1.12**	0.18**	0.49**	20158.6**	26.04**	18.53**	870.6**	27	Population × Drought
0.069	0.02	0.060	1880	0.324	0.24	112.2	84	Treatment
29.98	49.9	47.3	25.07	4.5	21.12	23.63	-	Coefficient of Variation

Drought Stress and Studied Parameters

Under drought stress, among the studied parameters, populations of Amygdalus scoparia showed significant differences in relative water content, ion leakage, stem fresh weight, stem dry weight, stem water content, root fresh weight, root dry weight, and stem water content at the population level. Other parameters, such as chlorophyll content, relative water content, ion leakage, ascorbate peroxidase enzyme activity, and proline, showed significant differences under drought stress levels at the 5% and 1% probability levels. The chlorophyll content of living plants is one of the critical factors in maintaining photosynthetic capacity. Under drought stress, the chlorophyll concentration of leaves decreases due to increased chlorophyllase and peroxidase enzyme activity, accumulation of phenolic compounds, and reduced nitrogen uptake [25]. Changes in chlorophyll concentration are considered a short-term response to stress and a measure of the ability to maintain source capacity under drought conditions. It appears that reactive oxygen species lead to peroxidation and, consequently, the degradation of these pigments [35]. Although chlorophyll a has a higher concentration in the photosynthetic apparatus compared to chlorophyll b, it is more sensitive to environmental stresses, including drought [8].

[38] found no significant differences in carotenoid content under drought stress in several almond species, consistent with this study. Drought stress reduced relative water content compared to the control treatment. The highest and lowest relative water content values were observed at 75% (99%) and 25% (50%) field capacity, respectively. Generally, drought-tolerant populations maintain higher relative water content under drought stress. In this study, the highest relative water content was found in the Fereydunshahr population, while the lowest was in the Natanz population. It seems that the higher accumulation of free proline in the seedlings of the Fereydunshahr population somewhat mitigated water absorption difficulties, resulting in less reduction in leaf relative water content. Semi-tolerant and drought-tolerant cultivars mainly rely on drought-tolerance mechanisms. One of the serious reported damages under drought stress is membrane damage and ion leakage from cells into the intercellular space [28]. The first cellular component affected by drought stress is the cell membrane, and the loss of its integrity leads to electrolyte leakage. This study showed that Amygdalus scoparia partially loses membrane stability under drought stress, with ion leakage increasing in its populations as drought stress intensifies. Drought stress reduced membrane stability in the studied populations.

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[13] noted that physiological responses of tree and shrub species change with increasing altitude. Populations located at higher altitudes may experience less drought stress due to reduced temperature and evapotranspiration, but they exhibit greater sensitivity to drought stress compared to lower-altitude populations. As drought stress progressed, proline levels increased across different populations, with a more pronounced increase in Borkhar and Fereydunshahr populations as stress levels rose. [23] reported greater proline accumulation under drought stress in one chickpea variety compared to two others. This study showed that ascorbate peroxidase activity in the chlorophyll-containing stems of Amygdalus scoparia was affected by drought stress, increasing under these conditions. However, catalase and peroxidase enzymes in chlorophyll-containing stems showed no significant increase under various drought stress levels. Thus, Amygdalus scoparia can enhance proline levels in chlorophyll-containing stems to counteract drought stress, while the role of antioxidant enzymes like catalase and peroxidase in drought resistance appears limited. [39] reported that ascorbate peroxidase activity in turmeric leaves was more affected by drought stress than other plant organs. Various antioxidant enzymes coordinate to regulate high concentrations of H_2O_2 effectively.

This study also showed that different drought levels had no significant effect on the fresh and dry weights of stems and roots in Amygdalus scoparia. [31] observed that roots of different almond ecotypes were less affected by water stress levels compared to genotypes. [20] in their study on pistachio seedlings, found no significant effect of drought stress on root length, root-to-shoot ratio, and leaf surface temperature. Since Amygdalus scoparia is a drought-adapted species, it appears that five weeks of drought stress did not cause significant changes in stem and root biomass. As drought stress activates mechanisms such as proline accumulation and increased antioxidant enzyme activity, Amygdalus scoparia may preserve cell turgor, preventing significant changes in cell growth and biomass reduction. [9] suggested that drought stress reduces plant growth by affecting leaf photochemical processes and indirectly by stomatal closure and reduced leaf area. Thus, in Amygdalus scoparia, photosynthesis by chlorophyll-containing stems may compensate for reduced leaf photosynthesis, explaining the lack of significant changes in stem and root biomass during the studied drought period.

General Conclusion

This study demonstrated that Amygdalus scoparia employs various mechanisms, such as osmotic adjustment via proline accumulation, increased antioxidant enzyme activity, and maintenance of cell structure under drought conditions, to effectively combat drought stress. Moreover, changes in ion leakage and relative water content in leaves and stems reflect complex cellular responses to drought stress that directly affect plant growth and performance.

Certain populations, such as Fereydunshahr and Khur and Biabanak, showed greater drought tolerance, maintaining water content and reducing drought-induced damage more effectively. These characteristics were particularly evident in the germination process, a critical stage in the plant's life cycle. Given the ecological importance of Amygdalus scoparia in dry and semi-dry ecosystems and its drought-resistance traits, this research provides a scientific basis for better water resource management in these areas and developing effective strategies for cultivation and afforestation with this species.

Drought and Germination

The transition from seed to seedling and initial plant establishment is one of the most challenging phases in a plant's life. During this stage, plants need to exhibit their highest adaptability traits to survive environmental stresses, especially drought. This stage is not only critical for the plant's life cycle but often determines a population's success or failure under various stresses, particularly drought. Water potential in the environment is one of the most fundamental parameters influencing water uptake and seed swelling. Each seed requires a minimum level of water absorption and swelling to germinate, which necessitates that the environmental water potential does not fall below a critical threshold [17]. Numerous studies have shown that reduced water potential decreases seed water absorption and germination ability. Successful germination requires an environment with sufficient water potential to enable proper swelling and germination [19].

This study showed that increasing drought stress led to a decrease in mean germination percentage, germination rate coefficient, germination index, and germination value, while mean germination time increased. These changes clearly indicate the negative effects of drought on germination processes, with significant differences observed between various osmotic potentials. The increase in mean germination time suggests that plants require more water demand for optimal germination.

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This increased moisture demand reflects the limited capacity of Amygdalus scoparia populations to cope with drought stress. In Mediterranean regions studied, rainfall occurs almost exclusively from mid-spring to mid-autumn, and there is no precipitation during this period. Thus, germination is restricted to a short window with adequate moisture and temperature. Rapid water absorption and germination during this period facilitate better seedling establishment [3]. One mechanism to counter osmotic stress involves the accumulation of organic osmolytes such as proline and soluble sugars, which help the plant maintain water potential under drought conditions. Gradual proline accumulation as drought stress increases indicates that Amygdalus scoparia seedlings can increase proline levels to create lower osmotic potential and maintain cell structure under drought stress. This may explain why Amygdalus scoparia seeds in some populations can withstand mild stresses. Proline acts as an osmolyte, improving water absorption and playing a critical role in countering drought-induced osmotic changes [6]. The increased proline concentration with heightened drought stress helps reduce cellular osmotic potential and enhance the water potential gradient between plant cells and soil, improving water uptake by seeds [34].

This study also highlighted that early and late germination in some populations may serve as an adaptive trait for greater ecological compatibility. Some populations naturally tend to germinate earlier or later, aiding them in adapting to specific climates. This study demonstrated that drought stress significantly affects germination processes, particularly by increasing germination time and reducing germination percentages. Plants effectively counter water potential reduction and maintain growth capacity under drought through mechanisms such as proline accumulation and other organic osmolytes. These adaptive mechanisms are valuable for selecting drought-tolerant populations and improving water resource management strategies in dry and semi-dry conditions.

Conclusion

This study showed that drought stress has extensive effects on the physiological, biochemical, and morphological traits of Amygdalus scoparia. The populations studied demonstrated significant differences in response to various levels of drought stress. Using mechanisms like osmotic adjustment through proline and osmolyte accumulation, increased antioxidant enzyme activity, and cellular structure maintenance under drought conditions, Amygdalus scoparia effectively combats drought stress. Furthermore, changes in ion leakage and relative water content in leaves and stems reflect cellular responses to drought stress, directly impacting plant growth and performance.

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