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Effects of Removing Seed Hardness and Salinity Stress on Seed Germination Traits and Antioxidant Enzymes Activity in Two Populations of *Alhagi camelorum*

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ABSTRACT

Using saline water for cultivating halophyte forage is a practical approach when freshwater resources are scarce. This study aimed to examine the effects of seed dormancy-breaking treatments and salt stress on germination characteristics and antioxidant enzyme activity in *Alhagi camelorum* seeds. A factorial experiment was conducted using a completely randomized design with four replicates. The factors included two Iranian populations (Gorgan and Mashhad), three salinity levels (control, 100 mM, and 200 mM NaCl), and seed dormancy-breaking treatments (exposure to water at 100 °C for 30 s and concentrated sulfuric acid for 35 min). The results indicated that salinity stress significantly influenced germination percentage and rate, seedling length, and seedling dry weight. Both populations demonstrated the ability to germinate under saline conditions up to 200 mM NaCl, although germination indices decreased with increasing salinity. Between the two populations, the Gorgan population exhibited greater tolerance. Antioxidant enzyme activity analysis revealed that catalase (CAT) levels increased by 77.9% in the Mashhad population and 75.1% in the Gorgan population when treated with 200 mM salinity and hot water at 100 °C compared to the control. Similarly, superoxide dismutase (SOD) activity increased under the same conditions, with Mashhad and Gorgan populations showing enhancements of 56.9% and 45.4%, respectively, compared to the control. Notably, the Mashhad population treated with sulfuric acid demonstrated greater salinity tolerance.

Introduction

A significant portion of global water resources is affected by varying degrees of salinity, and soil salinity is recognized as a progressive issue. Addressing or mitigating this phenomenon necessitates the adoption of all available scientific and practical solutions. One practical approach is to replace salt-sensitive crops with salt-tolerant plants in agricultural systems. This strategy not only enhances income and living conditions for communities in affected areas but also provides substantial fodder for ruminants in saline and arid regions (Ranjbar et al., 2018). With the depletion of freshwater reserves and their prioritization for agricultural and horticultural crops, the cultivation of salt-tolerant plants offers a sustainable solution. By utilizing saline water,

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these plants can serve as a reliable source of fodder. For instance, the cultivation of salt-resistant plants like *Alhagi camelorum* is a viable option under freshwater-scarce conditions. These plants can produce significant amounts of fodder, catering to the dietary needs of ruminants in water-deficient regions (Pirasteh-Anosheh et al., 2017). *Alhagi camelorum* belongs to the Fabaceae (Leguminosae) family, which comprises 550 genera and 13,000 species. Many species in this family have applications in traditional medicine and pharmaceuticals (Urabee et al., 2020).

Members of the *Alhagi* genus are widely utilized in weed management, animal feed production, erosion control, and pharmaceutical industries, and several scientific studies have been conducted to explore these uses (Ibrahim, 2015; Muhammad et al., 2015). Bioactive compounds such as fatty acids, sterols, flavonoids, coumarins, and alkaloids have been identified in *Alhagi* species (Ibrahim, 2015). The protein content of *Alhagi* varies, ranging from 12.8% during the early vegetative stage to 10.8% during seed maturation (Temel et al., 2015). *Alhagi* is a valuable genus due to its resilience to drought and salinity, its ability to remain green throughout the year, and its presence in erosion-prone areas. It also has substantial potential for medicinal applications and is suitable for evaluating saline, arid, and degraded rangelands (Keskin et al., 2022). Successful cultivation of *Alhagi* requires overcoming seed hardness to achieve uniform germination, optimal seedling establishment, and efficient resource use (Baskin and Baskin, 2014). Significant improvements in total germination rates of *Alhagi pseudalhagi* seeds have been observed when seeds were soaked in boiling water for varying durations. Soaking seeds in boiling water for 2 min was found sufficient to achieve high germination rates. This treatment enhances seed coat permeability, leading to increased germination rates (Keskin et al., 2022). Chemical scarification using sulfuric acid has also proven effective in breaking seed dormancy in various *Alhagi* species, including *A. pseudalhagi*, *A. cancencens*, *A. kirghisorum*, *A. sparsifolia*, and *A. maurorum* (Karshibaev, 2014; Anosheh, 2020).

Plants are often most sensitive to environmental stresses, such as salinity and drought, during seedling growth and establishment (Kafi et al., 2005). Salinity stress adversely affects plant growth and development by disrupting ion balance, water status, nutrient uptake, stomatal function, and photosynthetic efficiency. These disruptions impair critical processes such as germination and seedling growth, ultimately reducing yield (Munns, 2002; Zamani et al., 2018). Among the primary factors influencing plant growth under salinity are osmotic effects, which reduce growth rates, limit leaf surface area, hinder lateral stem development, and decrease the size, as well as the dry and wet weights, of various plant organs (Weisany et al., 2012; Postini and Sioce Mardedeh, 2001).

Seed germination, the most crucial stage for seedling establishment, determines the success of plant production and is influenced by environmental and genetic factors (Kabiri et al., 2018). Seeds that exhibit higher stress tolerance during germination often result in crops with a stronger root system and higher root index. Salinity stress reduces environmental water potential, induces osmotic stress, and causes ion accumulation, which disrupts the metabolism of the growing embryo and impairs germination (Ahmadi et al., 2018). For example, Broumand Reza Zadeh and Kocheki (2005) found that germination percentage and rate in medicinal plants such as fennel (*Foeniculum vulgare*), ajowan caraway (*Trachyspermum ammi*), and dill (*Anethum graveolens*) decreased under drought and salt stress conditions.

Numerous studies have reported reduced seed vigor, shorter root and shoot lengths, and lower dry weights in species such as *Alhagi* and *Salicornia* when exposed to drought and salinity stress (Amiri et al., 2012; Farkhah et al., 2002; Zhao et al., 2005; Arndt et al., 2004; Jie et al., 2008; Zhang et al., 2010; Zobayed et al., 2006). Antioxidant enzymes play a critical role in mitigating the harmful effects of reactive oxygen species (ROS) generated under stress. These enzymes, including glutathione peroxidase, catalase, superoxide dismutase, and ascorbate peroxidase, constitute the first line of defense against oxidative damage. They protect cellular membranes from ROS-induced destruction under abiotic stress conditions, thereby enhancing plant resilience (Tan et al., 2006; Mohammadkhani and Heidari, 2007).

The effects of salinity on antioxidant enzyme activity vary depending on plant genotype, growth stage, salt type and concentration, and environmental conditions (Rezaei et al., 2006). Germination and the early stages of seedling growth are particularly sensitive to salinity stress. However, little is known about the early growth responses of *Alhagi camelorum* populations in Iran under saline conditions. This study aimed to evaluate the tolerance of two Iranian *Alhagi camelorum* populations to salinity stress during germination and early growth stages. Furthermore, the effects of different salinity levels on antioxidant enzyme activity

were assessed to identify the population with greater resistance to salinity.

Material and methods

This study was conducted in 2023 at the Seed Analysis Laboratory of the Seed and Plant Registration and Certification Research Institute. Seeds from two Iranian populations of *Alhagi camelorum*, Mashhad and Gorgan, were provided by Pakan Kavir Agricultural Support Services. A factorial experiment was designed using a completely randomized design with four replications to evaluate methods for overcoming seed hardness. Two treatments were tested to address seed hardness. In the first treatment, seeds were subjected to concentrated sulfuric acid (98%) (Merck, Germany) for 35 min to scarify the hard seed coat. The seeds were then rinsed with sterile distilled water and dried. The second treatment involved immersing the seeds in boiling water (100 °C) for 30 s. A control group, with no scarification treatment, was also included. After these treatments, all seeds were washed with sterile distilled water and dried (Haghanifar et al., 2024).

Petri dishes and the seed beds, composed of Whatman No. 1 filter paper (90 mm diameter), were sterilized in an autoclave at 120 °C for 120 min. Twenty-five seeds of uniform size were sterilized in a 10% sodium hypochlorite solution for 30 s, followed by rinsing three times with distilled water. These seeds were then placed in the prepared petri dishes. Salinity stress was applied using laboratory-grade sodium chloride solutions at concentrations of 100 and 200 mM. Distilled water was used as the control (zero-stress level) in both experiments. Ten ml of the respective solutions were added to each petri dish. The initial weight of each dish was recorded, and the dishes were placed in an air-conditioned germinator set to 30 °C, with a photoperiod of 16 h of light and 8 h of darkness (Haghanifar et al., 2024).

To prevent changes in solution potential due to water evaporation, the dishes were weighed daily, and distilled water was added to compensate for any weight loss compared to their initial weight. Germination was monitored daily from the second day, with seeds considered germinated if their plumule length was at least 2 mm (Miller and Chapman, 1978). Counting continued until the number of germinated seeds remained constant for three consecutive days. After 28 d, ten seedlings were randomly selected from each petri dish. Root length, shoot length, and seedling length were measured using a ruler. The dry weights of roots and shoots were determined using a precision scale ($\pm$ 0.001 mg) after drying the samples at 75 °C for 24 h in an oven (Turan et al., 2010). The germination percentage was calculated using Equation 1 (Maguire, 1962):

$$G = (\frac{n}{N}) \times 100\% \quad (1)$$

Where G is the percentage of germination, n is the final number of germinated seeds, and N is the number of cultivated seeds. The germination rate of seeds was calculated using Equation 2 (Maguire, 1962):

$$GR = \sum_{i=1}^{n} \frac{Ni}{Ti} \quad (2)$$

Where GR is the germination rate, Ni is the number of germinated seeds in each count and Ti is the time from the beginning of planting to the n*th* count in days. The average time of germination, which is an indicator of the rate and acceleration of germination, was calculated using equation 3 (Ranal and De Santana, 2006):

$$MGT = \Sigma \frac{Ni\ Di}{N} \quad (3)$$

Where Ni is the number of germinated seeds on the i*th* day, Di is the number of days from the beginning of the test (during cultivation) to the i*th* count (the end of the experiment period), and N is the total number of germinated seeds. To record dry weight of seedlings, 25 normal seedlings were randomly selected from each replication after germination experiments. The seedlings were dried in an oven at 75 °C for 24 h, and the stems' weight was determined. Dry weights of seedlings (shoots + roots) were determined using a precision scale with an accuracy of ± 0.001. The effects of salinity stress on the activity of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) enzymes of the seeds were measured in the early stages of germination. The enzymatic activity of SOD was assayed following Ullah et al. (2022). Pulverized samples of *Alhagi camelorum* leaves were homogenized in a chilled mortar along with 0.1 M phosphate buffer (pH = 7.3) and 0.5 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was then centrifuged at 8000 × g and 4 °C for 10 min. The activity of SOD was determined in terms of the reduction rate of nitroblue tetrazolium (NBT) at 560 nm, where each unit of SOD was defined as the amount of enzyme required for 50% inhibition of NBT reduction at 560 nm. The

enzymatic activity of POD was determined according to the method described by Ullah et al. (2022) with some modifications. To prepare the reaction mixture, 2 mL of buffer substrate (8 mM guaiacol and 100 mM Na_3PO_4, pH 6.4) and 24 mM H_2O_2 in 0.5 mL were added to the *Alhagi camelorum* leaf extract. Absorbance values were measured at 460 nm twice at 1 min intervals. The activity of POD was recorded as U g min^{-1} calculated in terms of increasing the absorbance of the reaction system by 0.01 up to a maximum of 1 U min^{-1}.

The enzymatic activity of CAT was determined by monitoring the disappearance of H_2O_2 (Ullah et al., 2022). First, 50 mL of *Alhagi camelorum* leaf extract was added to 1.5 mL of reaction mixture prepared by mixing 50 mM K-phosphate buffer (Ph = 7.0) and 15 mM H_2O_2. The absorbance was recorded at 240 nm for 1 min, and each unit of CAT was defined as degradation of one mole of H_2O_2 min^{-1}. Analysis of variance the collected data was done using SAS (v9.4) software and mean comparisons were made based on the least significant difference (LSD) test ($P \leq 0.01$). Graphs were drawn using Microsoft Excel software.

Results

Germination percentage

The analysis of variance showed significant effects of different levels of salinity stress on the germination percentage ($p \leq 0.01$) (Table 1). The control plants were not significantly different from the 100 mM salinity stress treatment group, while showing a significant difference from the plants treated with 200 mM salinity. There was a decreasing trend in the germination percentage of *Alhagi camelorum* seeds with an increase in salinity level (Fig. 1). The highest level of salinity (200 mM NaCl) recorded a decrease of 15.9% in germination, compared to the control.

Table 1. ANOVA results of germination characteristics of two populations of *Alhagi camelorum* under salinity stress.

Sources of Variations	df	Final Germination Percentage	Germination Rate	Mean Germination Time	Seedling Length	Seedling Dry Weight
P	1	196.00**	0.00130^{ns}	0.1340^{ns}	0.0215^{ns}	3.3611111E-8^{ns}
SHRT	1	1995.11**	1.01484**	30.7448**	8.8804**	3.8027778E-7*
SS	2	517.33**	0.13757**	5.9700**	16.6191**	9.7861111E-7**
P × SHRT	1	100.00*	0.01369*	0.4082*	0.0642^{ns}	1.7336111E-6**
P × SS	2	16.00^{ns}	0.00475^{ns}	0.0760^{ns}	1.7453**	5.6194444E-7**
SHRT × SS	2	49.78^{ns}	0.04174**	4.7257**	0.1101^{ns}	2.8527778E-7*
P × SHRT× SS	2	5.33^{ns}	0.00461^{ns}	0.0444^{ns}	0.5987**	2.1944444E-8^{ns}
Error	24	18.666667	0.00242124	0.08185121	0.05001111	8.4166667E-8
CV (%)	-	4.96	9.63	11.81	6.73	7.50

P: population, SHRT: seed hardness removal treatment, SS: salinity stress, CV: coefficient of variation, df: degree of freedom.

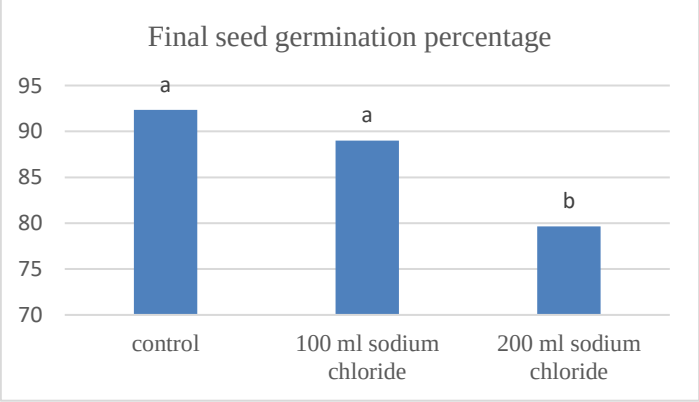

Fig. 1. Changes in the germination percentage of *Alhagi camelorum* seeds under different levels of sodium chloride salinity.

The interaction effects of population type and seed-hardness removal treatment were significant ($p \leq 0.05$) (Table 1). The results suggested that under the same abiotic conditions, the seed-hardness removal treatment with sulfuric acid for 35 min in both populations caused a significant increase in their germination percentage in comparison with the 1-min immersion of seeds in 100 °C water. Merku et al. (2017) showed that using sulfuric acid stimulates the germination of hard-shelled *Alhagi sparsifolia* seeds, and the immersion of seeds in concentrated sulfuric acid for 30-50 min led to an increase in seed germination and production of seedlings, whereas untreated seeds did not germinate.

Table 2. Mean comparison of germination characteristics of *Alhagi camelorum* under the interaction effect of population and seed hardness removal treatments.

Population	Seed-hardness removal treatment	Final germination percentage	Germination rate (seed d^{-1})	Mean germination time (d)	Seedling dry weight (g)
Mashhad	control (without treatment)	30.54^{e}	0.186130^{d}	5.29122^{e}	0.0036170^{b}
	100 °C water for 1 min	75.56^{c}	0.306231^{c}	3.17744^{b}	0.0039333^{ab}
	sulfuric acid for 30 min	93.78^{a}	0.653034^{b}	1.54214^{c}	0.0033667^{c}
Gorgan	control (without treatment)	42.66^{d}	0.164021^{e}	6.16401^{f}	0.0039220^{ab}
	100 °C water for 1 min	83.56^{b}	0.329251^{c}	3.51244^{a}	0.0042000^{a}
	sulfuric acid for 30 min	95.11^{a}	0.704044^{a}	1.15120^{d}	0.0036167^{b}
	LSD	4.20	4.20	0.27	0.0001

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	sulfuric acid for 30 min	95.11^{a}	0.704044^{a}	1.15120^{d}	0.0036167^{b}
	LSD	4.20	4.20	0.27	0.0001

Table 3. Mean comparison of germination characteristics of two populations of *Alhagi camelorum* under the interaction effects of seed-hardness removal and salinity stress.

Seed-hardness removal treatment	**Salinity-stress mM NaCl**	**Germination-rate (seed d^{-1})**	**Mean germination time (d)**	**Seedling dry weight (g)**
100 °C water for 1 min	0 (control)	0.501727^{f}	2.80525^{c}	0.0042333^{a}
	100	0.521318^{d}	2.52525^{b}	0.0035167^{e}
	200	0.285179^{e}	4.18253^{b}	0.0035333^{e}
Sulfuric acid for 35 min	0 (control)	0.716387^{b}	3.80194^{b}	0.0041333^{b}
	100	0.747130^{a}	3.57150^{c}	0.0040333^{c}
	200	0.602100^{c}	4.95232^{a}	0.0037333^{d}
	LSD	0.053	0.34	0.0001

Mean germination time

The analysis of variance showed that the interaction effect of seed-hardness removal treatment and salinity stress was significant on the mean germination time (Table 1). Increasing the salinity stress increased the mean germination time (Table 2). The number of germinated seeds decreased at high levels of stress, although the treatment groups with the same seed-hardness removal method were placed in the same group, despite different salinity levels, thus showing no significant difference (Table 3). The longest mean germination time pertained to the control group, which indicated the slowest germination compared to the other treatments. Also, the shortest mean germination time occurred in the treatment of soaking the seeds in acid for 35 min. Variance analysis (Table 1) showed that the mean germination time of *Alhagi camelorum* seeds was influenced by the interaction effects of population and seed-hardness removal treatments ($p \leq 0.05$). The comparison of mean values showed that the two populations of *Alhagi camelorum* had different responses to the seed-hardness removal treatments (Table 3). Immersion of the Gorgan population of *Alhagi camelorum* seeds in sulfuric acid for 35 min reduced the mean germination time compared to the treatment of 1 min soaking of the seeds in 100 °C water.

Seedling length

The results of ANOVA showed that the interaction effect of population × seed-hardness removal treatment × salinity level was significant on seedling length ($p \leq 0.01$) (Table 1). In analyzing the effects of different salinity levels on seedling length, it was revealed that seedling length decreased with the application of salinity (Table 4). In comparing seed-hardness removal treatments, it was observed that placing seeds in concentrated sulfuric acid for 35 min resulted in longer seedling lengths than in other treatment groups. Also, the interaction effect among population, seed-hardness removal treatment, and different salinity levels led to diverse results. The longest and shortest seedling lengths were observed in the control group and the 100 °C water treatment under 200 mM salinity, respectively (Table 4).

Table 4. Mean comparison of seedling length of *Alhagi camelorum* under the interaction effect of population, seed-hardness removal, and salinity stress.

Seed-hardness removal treatment	Salinity stress mM NaCl	Seedling length (mm)	
		Gorgan population	Mashhad population
100 °C water for 1 min	control	3.71333^{bc}	3.72667^{bc}
	100	3.30667^{c}	3.58000^{c}
	200	1.70667^{e}	1.36000^{e}
Sulfuric acid for 35 min	control	4.04667^{b}	5.36000^{a}
	100	4.64000^{a}	4.05333^{b}
	200	2.81333^{d}	1.98000^{d}
LSD		0.378	0.416

Seedling dry weight

Breaking seed hardness was significantly ($p \leq 0.01$) affected by interaction of population and seed hardness treatment (Table 1). The highest dry weight of the seedlings (0.0042000 g) was related to the population of Gorgan in seeds soaked in 100 °C water for one min. The lowest dry weight of the seedlings (0.0033667 g) occurred in response to 200 mM NaCl. Dry weights of the seedlings of the two *Alhagi camelorum* populations (Table 1) showed significant effects of salinity stress ($p \leq 0.01$). Moreover, the seed-hardness removal and different salinity levels had a significant effect on plant dry weight (Table 1). The comparison of mean values suggested a significant difference between the population type and the seed-hardness removal treatments, as well as between the population type and different salinity levels (Table 1). The maximum and minimum dry weights of seedlings (0.0042000 g and 0.0033667 g, respectively) were observed in the control and the salinity treatment of 200 mM sodium chloride, respectively, in both populations (Table 5).

The interaction effect of salinity × seed-hardness removal treatment on the dry weight of *Alhagi camelorum* seedlings under laboratory

conditions was significant ($p \leq 0.05$) (Table 1). Comparisons of mean values pertaining to the reciprocal effects showed that the seedlings grown in the absence of salinity stress (control) had the highest dry weight.

Antioxidant enzyme activities

CAT activity

Analysis of variance of the data showed that the interaction effects of population × seed-hardness removal treatment × different levels of salinity had a significant effect ($p \leq 0.05$) on the activity of catalase enzyme in the two Iranian populations of *Alhagi camelorum* (Table 6).

Comparisons of mean catalase (CAT) enzyme activity in the two populations under varying salinity levels revealed that CAT activity increased with higher salinity and seed hardness removal treatments (Table 7). A significant difference was observed between the control (no salt stress) and the salinity treatments, with notable increases in CAT activity at higher salinity levels. Specifically, CAT activity increased by 77.9% and 75.1% in the Mashhad and Gorgan populations, respectively, when exposed to 200 mM salinity and boiling water treatment (100 °C) compared to the control. Furthermore, the combined treatment of concentrated sulfuric acid and 200 mM salinity led to increases in CAT activity of 74.3% and 84.9% in the Mashhad and Gorgan populations, respectively, compared to the control.

Table 5. Mean comparison of dry weight of *Alhagi camelorum* seedlings under the interaction effects of population and salinity stress.

Population	Salinity stress mM NaCl	Seedling dry weight (g)
Mashhad	Control	0.0042000[a]
	100 mL sodium chloride	0.0039333[ab]
	200 mL sodium chloride	0.0033667[c]
Gorgan	Control	0.0041667[a]
	100 mL sodium chloride	0.0036167[b]
	200 mL sodium chloride	0.0034000[c]
LSD		0.0001

Table 6. ANOVA results of antioxidant enzymes activities (catalase, peroxidase, superoxide dismutase) of two populations of *Alhagi camelorum* under salinity stress.

Source of Variation	Df	Catalase Activity	Peroxidase Activity	Superoxide Activity
Population	1	0.309079**	1578.45**	46.087^{ns}
Seed Hardness Removal Treatment	1	0.000107^{ns}	1254.68**	66.008^{ns}
Salinity Stress	2	0.338826**	185.93^{ns}	58.301^{ns}
Population × Seed Hardness Removal Treatment	1	0.145748**	38.48^{ns}	338.686*
Population × Salinity stress	2	0.088182**	569.37*	117.514^{ns}
Seed Hardness Removal Treatment × Salinity Stress	2	0.016949^{ns}	27.30 ns	913.846**
Population × Seed Hardness Removal Treatment × Salinity Stress	2	0.055224*	122.05^{ns}	932.982**
Error	24	0.01307105	119.590006	64.003479
(%)CV	-	41.20	17.83	15.72

SOD activity

The interaction between seed hardness treatment and salinity stress in the Gorgan and Mashhad populations of *Alhagi camelorum* had a significant effect ($p \leq 0.01$) on the activity of the superoxide dismutase (SOD) enzyme (Table 6). Additionally, SOD activity significantly increased with rising salinity concentrations, although no significant difference was observed between the

control and the 100 mM salinity treatment (Table 7). The combined effect of seed dormancy breaking treatment with boiling water (100 °C) and 200 mM salinity stress led to increases in SOD activity by 56.9% and 45.4% in the Mashhad and Gorgan populations, respectively, compared to the control. Similarly, under the combined treatment of concentrated sulfuric acid and 200 mM salinity stress, SOD activity increased by 25.4% in the Mashhad population and 0.13% in the Gorgan population relative to the control.

POD activity

Analysis of the data revealed that the interaction effects of salinity treatment and population on peroxidase (POX) enzyme activity were significant at the 5% probability level (Table 6). A comparison of means indicated that, among the different salinity levels, the highest POX activity was observed in the Mashhad population treated with 200 mM sodium chloride. In contrast, the lowest POX activity across all salinity stress levels was recorded in the Gorgan population under the same 200 mM sodium chloride treatment (Table 8).

Table 7. Mean comparison of catalase and superoxide dismutase activity under the interaction effects of seed hardness removal and salinity stress treatments.

Seed Hardness Removal Treatment	Salinity Stress mM NaCl	Superoxide Activity (unit mg^{-1})		Catalase Activity (unit mg^{-1})	
		Gorgan Population	Mashhad Population	Gorgan Population	Mashhad Population
100 °C Water for 1 min	Control	29.8690^{c}	27.0599^{c}	0.103702^{cd}	0.162218^{cd}
	100	54.6274^{a}	54.2030^{ab}	0.189217^{bc}	0.424363^{bc}
	200	54.7912^{a}	62.8319^{a}	0.416825^{a}	0.730709^{a}
Sulfuric Acid for 35 min	Control	48.0938^{ab}	46.6292^{b}	0.065308^{d}	0.142485^{d}
	100	56.9892^{a}	68.2228^{a}	0.255851^{b}	0.252804^{b}
	200	55.3145^{a}	62.5512^{a}	0.434825^{a}	0.554875^{ab}
LSD		12.22	15.98	0.090	0.278

Table 8. Mean comparison of peroxidase enzyme activity under the interaction effects of population and salinity stress.

Population	Salinity Stress mM NaCl	Peroxidase (units mg^{-1})
Mashhad	Control	46.1805^{d}
	100	58.9180^{bcd}
	200	74.4785^{a}
Gorgan	Control	47.2218^{d}
	100	61.0805^{bc}
	200	67.9892^{ab}
LSD		13.03

Discussion

The results of this study demonstrate that sodium chloride (NaCl) salinity significantly affects the germination indices of *Alhagi camelorum*. Specifically, increasing salinity levels created an unsuitable environment for germination, leading to a decline in the germination index with higher sodium chloride concentrations. This finding aligns with the work of Pirasteh-Anosheh (2020), who reported that increasing salinity from 12 dS m^{-1} to 15 dS m^{-1} and above reduced the germination percentage of *Alhagi camelorum* seeds. Most agricultural species are sensitive to drought and salinity stress during the early stages of growth, including germination and seedling establishment. The germination and establishment phases are considered critical

indicators of plant tolerance to stress. Uniform seedling emergence under drought and salinity conditions is essential for achieving maximum yield and, ultimately, optimal annual crop profitability (Feghhenabi et al., 2020).

Osmotic stress, caused by reduced water absorption, disrupts seed metabolic and physiological processes, limiting the availability of materials necessary for growth (Kaya and Day, 2008). Consequently, this disruption negatively impacts seedling growth indices. Consistent with the present findings, Ghorbani et al. (2013) reported that increasing salinity stress decreased both the germination percentage and rate in *Alhagi camelorum* seeds. Germination rate, a critical stress tolerance indicator, also declined with increasing salinity levels in this experiment. Khammari et al. (2007) similarly observed decreased germination percentage and speed in several medicinal plant species under salinity stress. Germination speed is a vital measure of tolerance, as faster germination improves the likelihood of sprouting under stress conditions (Kaya and Day, 2008). Osmotic stress delays metabolic activities, slowing radicle emergence and thereby reducing germination rates (Kaya and Day, 2008).

The present study also found that increasing salinity stress levels extended the average germination time. High salinity levels negatively impacted membrane permeability, cell division, protein synthesis, and enzyme activity, which increased mean germination time while reducing germination rate and root growth (Hardegree and Emmerich, 1990; Bal and Chattopadhyay, 1985). Additionally, higher salt concentrations intensified adverse effects, as reflected in increased mean germination time and decreased seedling dry weight. Severe salinity stress disrupts seedling water potential homeostasis and ion distribution, resulting in more pronounced reductions in growth (Kafi et al., 2009). The study also showed that treating *Alhagi camelorum* seeds with sulfuric acid significantly reduced mean germination time by dissolving the seed coat. This treatment accelerated germination speed, consistent with findings by Fahimi et al. (2017). Overall, sulfuric acid treatment proved more effective than 100 °C water in breaking seed hardness and reducing mean germination time.

As shown in Table 4, the reduction in seedling length with increasing salinity levels was evident across all treatments. In this study, seedling growth decreased under sodium chloride stress, likely due to limited turgor pressure or the accumulation of dry matter in root storage tissues. Shamsodin-Saied et al. (2007) similarly reported that increased salinity reduces plant length by raising the osmotic pressure of the soil solution, which decreases water absorption (cell swelling) and subsequently inhibits cell division, elongation, and differentiation.

The findings indicated that treating seeds with 100 °C water effectively removed seed shell hardness, broke dormancy in both *Alhagi camelorum* populations, and significantly increased seedling dry weight compared to other treatments (Table 2). Additionally, *Alhagi camelorum* plants appeared to tolerate salinity up to 100 mM without significant reductions in seedling dry weight (Table 5). However, at 200 mM salinity, a notable decrease in seedling dry weight was observed in both populations (Table 5). These results align with those of Cicek and Cakilar (2002), who reported yield reductions in corn under sodium chloride salinity stress. A decreasing trend in seedling dry weight was observed in both populations as salinity stress increased, with significant reductions recorded at both 100 and 200 mM NaCl compared to the control (Table 3).

Under salinity stress, reduced seed access to moisture impairs food hydrolysis, which is essential for seedling tissue production, ultimately leading to lower seedling dry weight (Soltani et al., 2006). Biomass reduction in plants under saline conditions—such as black cumin (*Nigella sativa*), Moldavian balm (*Dracocephalum moldavica*), *Salicornia herbacea*, *Alhagi persarum*, *Alyssum homalocarpum*, and camelthorn (*Alhagi maurorum*)—has been attributed to decreased photosynthetic levels and the high energy demands associated with maintaining osmotic balance and cell mass (Kabiri et al., 2015, 2018; Amiri et al., 2012; Ganjali et al., 2017; Pirasteh-Anosheh, 2020).

Stress-induced scavenging of ROS is primarily mediated by increased activity of antioxidant enzymes, which protect plants from severe oxidative damage. In this study, activities of SOD, POD, and CAT were progressively enhanced with rising salinity levels compared to the unstressed control. Plants rely on a highly specialized enzymatic antioxidant defense system to mitigate ROS effects at the cellular level under salt stress (Munns, 2008). Koca et al. (2007) and Athar et al. (2008) found that SOD activity increased significantly under saline conditions in salt-resistant sesame and wheat cultivars, respectively. Plant resistance to stress depends on the efficacy of this antioxidant system (Bor et al., 2003). Similarly, studies by Ghorbanli et al. (2012) on cumin and Bor et al. (2003) on sugar beet reported increased catalase and peroxidase activities with rising salinity, consistent with the

findings of this study. Enhanced activity of these enzymes mitigates ROS production and activity, thereby preventing cellular damage and death, which ultimately strengthens plant resistance to abiotic stresses, including salinity stress (Motohashi et al., 2010).

Conclusions

Treatment of Iranian populations of *Alhagi camelorum* seeds with concentrated sulfuric acid (98%) and hot water (100 °C) effectively broke seed dormancy and resolved the issue of seed hardness in this study. The hardness of these seeds may be attributed to their mechanical resistance to sprouting. The results demonstrated that treatment with concentrated sulfuric acid significantly improved the germination index compared to the 100 °C hot-water treatment in both populations. Salinity stress was found to negatively affect the entire seedling growth period, resulting in decreased values for all studied traits. However, the activities of antioxidant enzymes, CAT, SOD, and POD, increased under cumulative levels of salinity stress, indicating their roles in enhancing plant resistance to salinity. These enzymes are crucial in mitigating oxidative stress caused by saline conditions. Since the cultivation of *Alhagi camelorum* is commonly recommended for animal forage production and achieving remedial purposes in folk medicine, further studies are required to establish camelthorn's resistance thresholds in seed germination and early seedling growth. Among the treatments, seeds from the Mashhad population treated with concentrated sulfuric acid for 35 min exhibited superior resistance to salinity compared to the Gorgan population. Both populations can be suitable for planting in saline soils.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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