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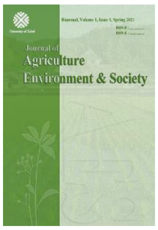

Agrophysiological of barley genotypes responses to zinc fertilization and water saline irrigation

Mehrdad Mahlooji*[a], Abas Nasiri Dehsorkhi [b]

[a] Assistant Professor, Horticulture Crops Research Department, Isfahan Agricultural and Natural Resources Research and Education Center, AREEO, Isfahan, Iran

[b] M.Sc Graduate of Agroecology, Department of Agronomy and Plant Breeding, Faculty of Agriculture, Shahrood University of Technology, Shahrood, Iran

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ABSTRACT

Salinity is one of the most important abiotic stresses because it causes zinc to precipitate in an unusable form for plants and is influenced by saline-calcareous soils. This experiment was carried out in a strip split block design with three replications at the Esfahan Rodasht Drainage and Salinity Research Station to investigate the effects of agrophysiological responses of barley genotypes to zinc fertilization and water saline irrigation. As vertical factors, water irrigation quality at three levels, 2, 10, and 18, dS/m, were used. Fertilizer application included Nano zinc-oxide, zinc-chelate, a mixture of Nano zinc-oxide and zinc-chelate, and water as a control. Within vertical factors, three different barley genotypes are arranged, including Morocco (moderate semi-sensitive), Nosrat (moderate tolerant), and Khatam (tolerant). The results showed that the application of Zn-chelate fertilizer resulted in the highest grain yield, K^+ concentration, and K^+/Na^+ ratio in shoots. In Khatam, stomatal conductance (gs), the maximum quantum efficiency of PSII (Fv/Fm), K^+ and Zn^{2+} concentrations, and the K^+/Na^+ ratio were all higher than in Morocco. In comparison to Morocco, Khatam had lower Na^+ and $Ca^{2+}+Na^+$ contents. Furthermore, as salinity stress increased, all barley genotypes showed a decreasing trend in K^+ content and the K^+/Na^+ ratio in shoots.

1. Introduction

Population rise and salinity in the world have increased water requirements. The use of gray water (drainage and recycled water) for production has been suggested as a solution to save high-quality water. However, the application of gray water causes salinity tension (Zhuo and Hoekstra, 2017).

Salinity is a serious threat to agricultural sustainability because over 800 million hectares of land throughout the world are salt-affected (Munns, 2005). Salinity can hamper plant growth and yield in two ways (Arzani and Ashraf, 2017): firstly, high concentrations of salts in the soil disrupt the capacity of roots to extract water (Mahlooji et al., 2018) and decrease the root-zone osmotic potential (Ashraf et al., 2008), and secondly, high concentrations of salts within the plant itself can be toxic (Munns, 2002; Munns and Tester, 2008) and later cause ion toxicity (Tabatabaei and Ehsanzadeh, 2016), particularly through the accumulation of Na^+ ions (James et al., 2008).

Understanding salt-tolerant responses and mechanisms is imperative for crop improvement in salt regions. Plants' responses to salt stress are an expression of the physiological changes that occur in the plant to overcome the environmental stress imposed by salinity (Venkateswarlu et al., 2012). The initial and most dramatic response of plants, when exposed to salt conditions, is a decrease in stomatal conductivity. Stomatal conductance (gs) and resistance are reduced by salinity in the root zone. The stomatal conductance is initially affected by salinity due to water relations and subsequently due to the synthesis of ABA (Fricke et al., 2004; Fricke et al., 2006; Mahlooji et al., 2014). There is a signal from root to stomata when the plants are exposed to water stress (Davies et al., 2005) or when the plants are exposed to saline soil with a high concentration of salts (Termaat et al., 1985).

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* Corresponding author.
E-mail address: mmahlooji2000@yahoo.com (M. Mahlooji)

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Some reports of salinity effects on the photochemical efficiency of Photosystem II (PSII) of different plant organs, tissues, and cell preparations are limited and conflicting. Some researchers have demonstrated that salt stress inhibits PSII activity (Hichem et al., 2009), whereas others have indicated that salt stress has no effect on PSII (Lu et al., 2003a, b; Demiral and Türkan, 2006). It has been reported that mild-salinity levels do not induce sustained photodamage to PSII as revealed by unvaried Fv/Fm (photosynthetic activity or maximal PSII photochemistry) in plants (Baker and Rosenqvist, 2004; Naumann et al., 2007), even if leaf gas exchanges are reduced. In the first stages of salinity stress, stomatal conductance decreases and, consequently, CO_2 fixation leaves PSII unaffected (Baker and Rosenqvist, 2004). However, some studies have shown changes in the Fv/Fm ratio after dark-adaptation of the leaf as a result of salinity (Lee et al., 2004).

Differences in ion partitioning and the maintenance of higher nutrients such as nitrogen, phosphorus, potassium, and calcium to Na^+ ratios, especially in young growing and recently expanded tissues of barley, would appear to be important mechanisms contributing to the improved salt tolerance (Wei et al., 2003). Among glycophytic plants, barley is one of the most salinity-stress tolerant species, with an 8 dS/m degree of salt tolerance (Pessarakli, 1995). Salt stress causes a nutritional imbalance through lowering phosphorus (Evelin et al., 2009), potassium (Khosh Kholgh Sima et al., 2009), nitrate (Aslam et al., 1984), calcium (Hu and Schmidhalter, 2005), and zinc (Karimian and Moafpouryan, 1999; Khoshgoftarmanesh et al., 2004) absorption.

Plant mineral element nutrition is affected by salinity. Pearson and Rengel (1994) investigated Zn^{2+} and Fe^{2+} transport into remobilization conditions from leaves and found that Zn^{2+} showed good remobilization. Soil salinity is also associated with zinc efficiency in alkaline conditions (Khoshgoftar et al., 2006). By reducing the amount of soil moisture in saline soil, Zn^{2+} and Fe^{2+} in the soil solution are reduced in mobility. Application of Zn fertilizers is a common practice to correct Zn^{2+} deficiency. Zn^{2+} is an essential micronutrient that is deficient in many regions worldwide, such as in the calcareous and salt-affected soils of central Iran (Khoshgoftarmanesh et al., 2004). Zinc application increased corn, wheat , and soybean yields (Hemantaranjan and Gray, 1988). However, soil application of Zn^{2+} has not been very successful under furrow irrigation. Zinc deficiency in plants grown in calcareous soils can be fairly corrected by the application of inorganic zinc salts such as $ZnSO_4$ (Sielsepour, 2006). El-Fouly et al. (2010) suggest that in saline soils, some elements such as magnesium, calcium, and sodium have antagonistic effects on micronutrients uptake by the roots. The high pH and $CaCO_3$ content of these soils are usually considered the reasons for the low availability of Zn (Karimian and Moafpouryan, 1999; Havlin et al., 2005; Mahlooji and Pessarakli, 2017). Most Zn^{2+} deficiencies can be corrected with foliar zinc application (Christensen and Peacock, 2000). The deficient elements in plants can be sprayed with a solution to compensate for their deficiencies (Cakmak, 2008). Applying the micronutrients could restore the negative effect of salinity on dry weight and nutrient uptake (El-Fouly et al., 2010). Many studies suggest that micronutrient fertilizers could increase plant resistance to environmental stresses such as drought and salinity (Shahlaby et al., 1993). Khoshgoftar et al. (2006) found that the application of Zn^{2+} had a positive effect on the salt tolerance of wheat. In Iran, the increasing importance of Zn fertilizers and the use of water in saline soil to suppress regular salinity have been demonstrated as a requirement for studies of their combined effects on crop growth. Limited research has been conducted on the effects of zinc foliar application and salt resistance. Salinizing with NaCl has been shown to decrease free Zn^{2+} concentrations in soil solutions (Khoshgoftar et al., 2004). Improving the salt resistance of crop plants is a major focus of agricultural research. There are a few studies where links have been established between concentrations of Zn^{2+} in different barley genotypes under saline soils.

The purpose of this study was to investigate the effects of salinity and zinc fertilization on the physiological responses of barley genotypes. Furthermore, Zn^{2+} fertilizations (in the forms of EDTA and Nano-ZnO) were tested as a means of reducing salinity stress.

2. Materials and methods

2.1. Plant materials and treatments

In this experiment, the effects of irrigation water salinity stress and zinc fertilizer applications on barley genotypes were evaluated. In order to find out the changes in agrophysiological properties, such as yield, stomatal conductance, chlorophyll fluorescence, and nutrient content of three barley genotypes, Morocco (salt-sensitive), Nosrat (semi-salt tolerant), and Khatam (salt-tolerant). This experiment was conducted in a strip-split-plot design with three replications at Esfahan Rodasht Drainage and Salinity Research Station (32º 30′ N, 52º 09′ E) in two cropping seasons (2012-14). Three irrigation water qualities, including W1=2 dS/m (low salinity as a check), W2=10 dS/m (common salinity in the region), and W3=18 dS/m (high salinity), were evaluated as vertical strip factors. The horizontal factors were four levels of foliar application, including Nano zinc-oxide, Zn-chelate, a mixture of Nano zinc-oxide and Zn-chelate , and water application as a check. The application rates of Nano-ZnO and Zn-chelate were 100 and 1000 grams per hectare, respectively. Soil characteristics and chemical analysis of irrigation water quality have been shown in Table 1.

Barley genotypes were planted in 1.2×4 m plots in November. The seeding rate was 450 seeds per square meter in June. Samples for grain yield are harvested and weighed at physiological maturity stage. Phenological stages were determined according to the method suggested by Zadoks et al. (1974).

2.2. Nutrient concentration

Digestion apparatus methods determined the nutrient concentration in the shoots. The rates of N, P, and K are determined by the Auto Analyzer (Quikchem IC+FIA 8000

Series) and the rate of Na+ is determined by the Atomic Absorption Spectrometer (Perkin Elmer Model 3110 USA) (Bauder et al., 2014).

2.3. Physiological parameters

Stomatal conductance: Direct measurements of photosynthesis through gas exchange rate were performed by an infrared gas analyzer (IRGA), which measures the carbon dioxide flux within a sealed chamber containing a leaf sample.

At grain filling, stomatal conductance was determined by using a portable photosynthetic system IRGA (Model:LCA-4, USA) on intact plants (abaxial surface of the mid portion of flag leaf) between 10:00 and 14:00 hours, and light intensity was set at 1200-1400 µmol/m^{2}s with an infra-red/blue light source (Fischer et al., 1998).

Table 1. Selected physico-chemical properties of the soil before planting and three levels of water irrigation quality

Soil characteristic	Amount	Water characteristics	Saline water		
			W_1=2 (dS/m)	W_2=10 (dS/m)	W_3=18 (dS/m)
pH	7.7	pH	7.7	8.1	7.6
Electrical conductivity (dS/m)	13	Electrical conductivity(dS/m)	1.4	9.7	17.8
Available K (mg kg^{-1})	340	So4 $^{2-}$ (meq/lit)	0.8	26.9	172.3
Available Zn (mg kg^{-1})	0.72	HCo^{3-} (meq/lit)	2.0	5.7	6.4
Available Fe (mg kg^{-1})	5.54	Cl$^-$ (meq/lit)	1.4	60	111
Available Na (meq/lit)	79.1	Na (meq/lit)	1.5	47.8	99.3
Available Ca+Mg (meq/lit)	60	Ca+Mg (meq/lit)	2.6	44	72

Chlorophyll Fluorescence: Chlorophyll fluorescence was measured with a leaf prometer (Handy OS1-FL, USA) on intact plants (abaxial surface of the mid portion of the flag leaf) between 10:00 and 14:00 hours at the heading stage. The leaves were maintained in darkness by clips for 20 min before taking the data on chlorophyll fluorescence (Bake and Rosengvist, 2004; Li et al., 2009).

The chlorophyll fluorescence characters included the minimal fluorescence level from dark-adapted leaves (Fo), maximal fluorescence level from dark-adapted leaves (Fm), variable fluorescence level (Fv), the maximum quantum efficiency of PSII photochemistry (Fv/Fm= (Fm-F0)/Fm). For each treatment, the chlorophyll fluorescence of 3 individual leaves was measured.

2.4. Data analysis

Data were subjected to analysis of variance by SAS (SAS Institute 2007). Means of treatments were compared by the least significant differences (LSD) test at ($P \leq 0.05$).

3. Results

Rainfall in the first year (2012-13), second year (2013-14), and the average (2002-2014) was 149.8, 97.2, and 93.5 mm, respectively. Rainfall in the first year of the study was 60.2% (56.3 mm) greater than the average mid-term rainfall. In May of the first year (the time of heading and grain filling stages), the rainfall was three times that of normal years, and the temperature was 7 °C lower than normal years.

Table 2. Effects of water quality, fertilizer application and genotypes on grain yield, stomatal conductance (gs), Fv/Fm (maximum quantum efficiency) on shoot nutrient contents (Fe, Zn)

Treatments	Grain yield		Stomatal conductance		Fv/Fm		Fe		Zn	
	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14
Quality(dS/m)										
2	6006.30a	3123.26a	0.505a	0.211a	0.799a	0.76a	204.83a	171.39c	26.83 a	23.50 a
10	4592.20b	1737.85ab	0.392b	0.114b	0.792a	0.76a	175.67b	190.42b	20.39 b	13.00 c
18	2054.40c	1524.03c	0.329b	0.096b	0.795a	0.76a	165.67c	193.75a	26.50 a	13.33b
Fertilizer										
Nano-ZnO	4163.30a	2069.81b	0.447a	0.120c	0.795ab	0.756a	219.71a	161.59d	32.00 a	9.33 d
Zn-Chelate	4365.10a	2365.46a	0.383b	0.126bc	0.794ab	0.754a	129.11d	173.33c	25.56 b	14.89 c
Mix	4209.80a	2259.72ab	0.397ab	0.140b	0.801a	0.771a	178.07c	179.93b	18.44 d	23.33 a
Check	4132.40a	1818.52c	0.407ab	0.175a	0.790b	0.754a	201.33b	226.48a	22.30 c	18.89 b
Genotype										
Morocco	3843.59b	1381.94c	0.415a	0.124b	0.795ab	0.751b	175.72b	196.78a	25.17 a	14.67 b
Nosrat	4402.67a	2232.57b	0.406a	0.133b	0.789b	0.765a	168.06c	197.58a	22.50 b	17.83 a
Khatam	4406.68a	2770.63a	0.406a	0.164a	0.801a	0.761ab	202.39a	161.19b	26.06a	17.33 a

Means within each column with similar letters are not significantly different (Duncan's 5%).

Due to significant differences in climatic conditions in the two growing seasons, and according to Bartlett's test, combined ANOVA was not advisable. Therefore ANOVA was carried out for each year separately.

3.1. Grain Yield

The effects of irrigation water quality (salinity) and genotypes on grain yield were highly significant in both years. The effects of fertilizer application on grain yield were not significant in the first year (2012-13), but they were significant in the second year (2013–14).

The results of this study showed that salinity decreased grain yield. These adverse effects can be significantly reduced by Zn foliar application. The highest grain yield was produced in the Zn-chelate application treatments. Khatam had the highest grain yield due to a higher grain number per spike (Table 2). Consistent with these results, many researchers showed that the growth of plants declined under saline conditions, but it’s degree depended on the level of salt, environmental conditions, type of plant, and stage of growth of barley (Shafaqat et al., 2012) and wheat (Pessarakli 1995).

3.2. Photosynthetic parameters

There are a lot of reports on photosynthetic characteristics under salt stress and, generally, photosynthesis is inhibited by salt stress (Venkateswarlu et al., 2012; Qiu et al., 2003; Munns, 2005; Chaves et al., 2009).

3.2.1. Stomatal conductance (gs)

The results showed that salinity stress reduced stomatal conductance (gs). The effects of irrigation water quality (salinity) and genotype on gs were highly significant in both years. The effect of fertilizer on gs was not significant in the first year (2012-13), but it was significant in the second year (2013-14) (Table 2). The highest gs was obtained in the lowest salinity treatment (W1=2 dS/m) and the Nano-ZnO fertilizer application treatment in the first year and also in the check fertilizer application treatment in the second year. However, the Khatam genotype had the lowest gs in Nano-ZnO fertilizer application in the second year.

In addition, James et al. (2002) reported that salinity decreased by more than half in tolerant barley and wheat varieties, indicating that the sensitivity of the root system to NaCl could also be detected in leaves (Katsuhara et al., 2011).

3.2.2. Chlorophyll fluorescence (Fv/Fm)

The effects of irrigation water quality (salinity) and fertilizer on Fv/Fm were not significant in both years, but the effect of genotype was significant. Salt-tolerant genotype (Khatam) had a higher maximum quantum efficiency (Fv/Fm) of PSII than a salt-sensitive genotype (Morocco) (Table 2). According to James et al. (2002), the Fv/Fm ratio of a salt-tolerant wheat genotype remained unchanged after salt treatment, indicating that there was no salt-induced decrease in intrinsic or actual quantum efficiency of PSII. In contrast, sensitive wheat genotypes showed a small but significant decline in Fv/Fm after salt treatment. Also, unstressed plants had higher Fv/Fm values than salt-treated plants (James et al., 2008). This may be due to irreversible photoinhibition resulting from a sustained, high PPFD over the course of the experiment (Bilger et al., 1995).

3.2.3. Shoot nutrient element contents

The effects of irrigation water quality (salinity), fertilizer and genotype on shoot nutrient element content (K^+, Na^+, K^+/Na^+, Fe^{2+}, Zn^{2+} and Na^++Ca^{2+}) were significant in both years.

3.2.3.1. Potassium (K^+) and K^+/Na^+ ratio

There was the highest K^+ and K^+/Na^+ratio in the lowest salinity treatment. (W1=2 dS/m), Zn-chelate fertilizer application, and Khatam (salt-tolerant) genotype (Table 3). Salinity reduces K^+ and K^+/Na^+ content in shoots. $K^+$and K^+/Na^+ contents in the shoots increased in Zn-chelate (foliar) application treatments and decreased in Nano-ZnO application treatments in comparison with the check (fertilizer without Zn).

The results of this study revealed that uptake of K^+ and K^+/Na^+ content was greater in tolerant and semi-tolerant genotypes compared with the sensitive genotype. Under saline conditions, a low K^+/Na^+ ratio may indicate that Na impaired Ca^{2+}, K^+, and Mg transport, which could disrupt plant metabolism and reduce plant growth.

Therefore, investigations dealing with the development of salt-tolerant varieties have concentrated on the uptake, transport, and accumulation of K^+, Na^{2+}, and Ca^{2+} in plants (Morshedi and Farahbakhash, 2012; Munns, 2005; James et al., 2008).

The concentrations of these nutrients and their ratios (e.g., $K^+/Na^+$and Ca^{2+} /Na) are reliable, useful, and widely used as screening parameters in ranking varieties for their tolerance to salt toxicity. Torabi (2010) confirmed the increasing K^+/Na^+ ratio under salt stress. Tavakoli et al. (2010) reported that salt tolerant barley genotype "Afzal" produced higher dry mass compared to salt sensitive genotype under salt stress conditions (200 mM NaCl) and that higher tolerance in genotype Afzal was associated with a higher K^+/Na^+ ratio in the shoots.

Table 3. Effects of water quality, fertilizer and genotypes on shoot nutrient contents (K, Na, K/Na and Na+Ca)

Treatments	K		Na		K/Na		Na+Ca	
	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14
Water quality (dS/m)								
2	1.57 a	1.73 a	0.61 b	0.94 c	2.64 a	1.87 a	0.76 b	1.18 b
10	1.54 b	1.67 b	0.63 b	0.96 b	2.54 b	1.84 a	0.77 b	1.17 b
18	1.47 c	1.55 c	0.70 a	1.08 a	2.22 c	1.52 b	0.83 a	1.33 a
Fertilizer								
Nano-ZnO	1.49 c	1.48 d	0.63 b	0.97 b	2.44b	1.55 c	0.76 c	1.24 a
Zn-Chelate	1.57 a	1.83 a	0.65 a	0.94 c	2.53 a	2.02 a	0.82 a	1.24 a
Mix	1.52 b	1.57 c	0.66 a	0.97 b	2.39 b	1.69 b	0.80 ab	1.20 b
Check	1.54 b	1.73 b	0.64 a	1.08 a	2.38 b	1.71 b	0.79 b	1.24 a
Genotype								
Morocco	1.34 c	1.59 b	0.74 a	1.08 a	1.82 c	1.54 c	0.85 a	1.33 a
Nosrat	1.52 b	1.58 b	0.67 b	0.97 b	2.30 b	1.66 b	0.78 b	1.21 b
Khatam	1.73 a	1.79 a	0.55 c	0.93 c	3.18 a	2.03 a	0.74 c	1.14 c

Means within each column with similar letters are not significantly different (Duncan’s 5%)

3.2.3.2. Sodium (Na^+) and (Na^++Ca^{2+})

The highest Na^+ and Na^++Ca^{2+} rates in shoots were in the highest salinity (W3=18 dS/m) and in check fertilizer application treatments, as well as in the Morocco genotype. Increasing salt stress causes an imbalance of the nutrient elements due to competition in uptake and toxicity in plants under salt stress (Pessarakli, 1999).

With increasing salinity, the ion concentration of Na^+ and Na^++Ca^{2+} increased in the shoot tissues. This is confirmed by many authors (Khorshidi et al., 2009). There are some reports of antagonism between the absorption of K^+ and Na^+ at the root surface (Ahmadi et al., 2009).

3.2.3.3. Fe^{2+} and Zn^{2+}

The highest Fe^{2+} content was in the lowest and highest salinity treatments in the first and second years, respectively (Table 2). The highest Fe^{2+} content was in Nano-ZnO fertilizer application and in check fertilizer application in the first and second year, respectively.

The Khatam genotype in the first year and the Morroco and Nosrat genotypes in the second year had the highest Fe content. The highest rate of Zn^{2+} was in the lowest salinity treatment (W1=2 dS/m), and in Nano-ZnO fertilizer application treatments in the first year and in fertilizer mixtures in the second year, and for the Khatam genotype. Salinity decreased the total amount of Zn^{2+} in the shoots (about 36% in the second year) and Zn-chelate fertilizer application had the highest grain yield.

However, Khoshgoftar et al. (2004), Pahlavan-Rad and Pessarakli (2009) reported that salinity in irrigation water had no effect on Zn^{2+} concentration in the shoots of wheat. Shoot concentrations of Zn^{2+} varied among barley genotypes.

This may indicate that barley genotypes were different in their ability to accumulate Zn^{2+} both with and without Zn^{2+} fertilization. In the second year, the Khatam and Nosrat genotypes accumulated much more Zn^{2+} in their shoots than Morocco.

4. Discussion

The effects of irrigation water quality (salinity) on grain yield, stomatal conductance (gs) and shoot nutrient element content were highly significant in both years. With increasing salinity, grain yield, stomatal conductance, K^+ ion, and K^+/Na^+ ratio decreased, but Na^+ ion and Ca^{2+}+Na^+ions increased. The effects of fertilizer application on grain yield, gs (only in the second year) and shoot nutrient element content were significant. Application of Zn-chelate fertilizer provided the highest grain yield, K^+, K^+/Na^+ ratio and Ca^{2+}+Na^+. The Nano-ZnO fertilizer had the lowest K^+ ion.

The effects of genotypes on grain yield, gs, Fv/Fm, and shoot nutrient element content were highly significant in both years. Sodium content in shoots increased due to salinity in all barley genotypes. However, the Morocco genotype maintained the highest leaf Na^+ concentration. The Khatam genotype had the highest grain yield, gs, Fv/Fm, K^+, K^+/Na^+, and Zn^{2+} contents, and higher maximum quantum efficiency (Fv/Fm) than PSII. Based on the results of this report, it can be concluded that Khatam and Nosrat genotypes maintained a lower level of Na^+ in their shoots, and hence these genotypes can be considered as saline tolerant genotypes. Genotype Khatam (salt-tolerant) is comparatively higher in gs, Fv/Fm, K^+, K^+/Na^+, and Zn^{2+} content than Morocco (sensitive ones). The results showed that the tolerant genotype had fewer Na^+ and Ca^{2+}+Na^+contents insensitive ones compared to sensitive ones. Moreover, all barley genotypes showed a decreasing trend in K^+ content and the K^+/Na^+ ratio due to salinity stress.

From the results of this study, it can be concluded that agrophysiological characteristics such as gs and leaf K^+/Na^+ ratio may be used as potential traits for selecting barley genotypes with superior performance under salinity conditions. Furthermore, the genotypes that maintained a higher K^+/Na^+ ratio can be considered salt tolerant.

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