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ORIGINAL PAPER

Effects of salt stress on germination, seedling growth, and macro element content of barley (*Hordeum vulgare* L.) varieties*

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Abstract

Barley (*Hordeum vulgare* L.) is an important cereal crop grown worldwide, but it is highly susceptible to salinity stress during early developmental stages. This study aimed to investigate the effects of salinity stress on four barley cultivars commonly grown in Turkey (Aydanhanım, Baronesse) and Bulgaria (Zemela, Bojin) at critical stages of germination and seedling growth. The experiment was conducted in a randomized block design with four replications, and salt treatments (NaCI) were applied as a solution. Two different concentrations of sodium chloride (NaCl) were used: 0 (control),100, and 200 mM. Germination, seedling characteristics, and the content of macroelements (P, K, Mg, Na, and Ca) were assessed. Salinity stress significantly reduced key growth parameters such as the root length, shoot length, plant height (root+shoot), fresh weight, dry weight, germination capacity (GC), initial vigor (IV), and germination index (GI). Genotypic differences were observed among the cultivars for all the evaluated features. As the salinity stress increased, the content of P, K, Mg, and Ca decreased in all the cultivars, while the Na content increased. Aydanhanım and Baronesse were the least affected, whereas Zemela and Bojin were the most affected by the increasing salt doses (100, 200 mM). At the largest salinity level (200 mM NaCl), the germination capacity of Zemela and Bojin was significantly reduced, while Aydanhanım and Baronesse showed improved germination capacity compared to the control and 100 mM salt dose treatments.

Keywords: two-row barley, germination index, initial vigor, mineral composition, stress index, toxic effect, salinity

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INTRODUCTION

Soil salinity affects more than 20% of the world's arable land. Marginal land continues to increase because of water scarcity and increased use of saline water, unsustainable irrigation and the incorporation of saline soils into agricultural cropping systems. Therefore, salinity will be a serious problem for world agriculture in the future. Munns, Tester (2008) reported that salinity affects 1.5 million hectares. Thus, 50% of arable land will have been lost by 2050 (Alzahrani et al. 2021). Cereal crops are exposed to many environmental factors that limit their yield. Soil salinity is one of the constraints that effect plant growth.

Seed germination is the first stage of the plant's cycle (Patade et al. 2011), which largely determines crop yield (Rajjou et al. 2012). This stage is known to be sensitive to salt stress in diverse plant species. Soil salinity is responsible for reducing, delaying and sometimes preventing the seed germination stage and early seedling formation. Like other crops, the seed germination and seedling growth of barley are adversely affected by salinity stress (Pastuszak et al. 2022). Poor germination and reduced seedling emergence result in poor crop establishment, which in turn leads to losses in plant growth and yield. Previous studies by Doruk Kahraman et al. (2024), and Bouzidi et al. (2021) showed that salinity affected the wheat seed germination process, resulting in a reduced germination rate and prolonged germination time that depended on a NaCl concentration. In the vegetative stage, the detrimental effect of salinity is explained by the disruption of enzymes, respiration, photosynthesis, protein synthesis and damage to the cell membrane (Parida, Das 2005, Daszkowska-Golec 2011). Different solutions have been proposed to reduce risks to agricultural production. However, these solutions are expensive and difficult to implement. Instead, the negative effects of salinity on crop yields can be minimized by screening for species, genotypes and cultivars better suited to salinity conditions.

Barley is frequently exposed to soil and water salinity, especially during the germination stage, and this stage is important for the tolerance of plants. Mrani Alaoui et al. (2013) demonstrated that the germination phase, which determines the subsequent stages of plant growth and development, is of critical importance, especially in arid and semi-arid regions. Similarly, Mbarki et al. (2020) emphasized that a plant's tolerance to salt stress during the germination and early seedling formation phases is vital. Investigating this characteristic is a rapid and safe approach for screening cultivars with good germination and seedling emergence under salinity stress. Thus, correct initiation of the plant's cycle under saline conditions can guarantee its subsequent success. In plants, specific translocation and absorption of potassium (K) and calcium (Ca) compared to sodium (Na) have been identified as another mechanism of salt tolerance (Shakeri et al. 2020). However, high sodium concentrations lead to toxic accumulation in sorghum leaves, impairing the absorption and translocation of K, Ca, and magnesium (Mg), and thereby affecting plant development and photosynthetic activity (Netondo et al. 2004*a*,*b*, Bavei et al. 2011 Joardar et al. 2018). Specifically, high NaCl concentrations, such as 250 mM, have been reported to cause toxic effects in sorghum, adversely affecting plant development and photosynthetic activity. In this context, identifying salt-tolerant varieties not only reveals plants' inherent adaptive capabilities, reducing the need for chemical interventions, but also lays the foundation for implementing eco-friendly and sustainable agricultural practices in saline environments. Plants have different responses to salt stress, including photosynthetic processes, increasing accumulation of Na+ macroelements on shoots and roots and induction of oxidative stress (Ahmad et al. 2021). Sodium (Na^+) is excluded by plants when present at high levels in the soil solution to avoid toxic effects (Trapp et al. 2008). Several transporter systems interacting with sodium have been identified and the existence of a sodium/hydrogen pump in the root membrane has been proposed (Balasubramaniam et al. 2023). The Na^+/H^+ antiporter and other macroelement channels in plant cell membranes have been identified to play important roles in both reducing the toxic effects of high salt concentrations and in salt tolerance mechanisms (Trapp et al. 2008, Balasubramaniam et al. 2023).

This study aimed to examine the effects of salt stress on germination and early vegetative development of barley seeds. In particular, it was aimed to select salt tolerant genotypes and reduce the effects of salt stress by identifying genotypic differences against this abiotic constraint. In addition, macroelement contents (P, K, Na, Mg and Ca) in shoots of these genotypes were evaluated during germination and early vegetative stage, the most sensitive stages of plant development.

MATERIALS AND METHODS

This study was carried out on barley, one of the most widely produced cereals in Turkey. Two barley varieties obtained from the Institute of Agriculture in Karnobat, Bulgaria (Zemela, Bojin) and two barley varieties selected from Central Anatolia (Baronesse, Aydanhanim) were used in this study. The cultivars were subjected to increasing salinity stress during germination and early plant formation. The study was conducted in a 2-factor (genotype × NaCl concentration) randomized experimental design with four replications, by using four genotypes and three germination media. 25 seeds were placed in each Petri dish lined with filter paper and containing 5 ml of NaCl in a concentration chosen for a given treatment (saline solution), and then covered with another filter paper, after which another 5 ml of saline solution was added (24±1°C and 75% relative humidity in a climate room at Selcuk University, Faculty of Agriculture). Seeds with a rootlet length exceeding

2 mm were considered germinated and counted (Fuller et al. 2012). Shoot and root lengths of the varieties that did not germinate at high salt doses were defined as '0'.

Hundred seeds for each genotype were tested in batches of twenty-five seeds per Petri dish (four dishes per treatment and genotype) containing filter paper embedded with distilled water (control-0 salt) and saline solution containing 100 mM, 200 mM sodium chloride (NaCl). Germination percentage (GP) was recorded daily and the experiment ended after three consecutive constant GPs, which were considered as germination capacity (GC). The results presented are an average for 100 seeds. The recovery of seed germination allows us to distinguish between toxic and osmotic effect. For this purpose, non-germinated seeds were transferred to pure water for another three days and the percentage of germination was recorded.

Germinated seeds from each treatment were transferred to 1 kg plastic pots containing 2/3 peat and 1/3 perlite (Hamrouni et al. 2011). Four pots containing three germinated seeds for each genotype and each treatment were watered once a week with 50 ml of Hoagland nutrient solution per pot at 24±1°C and 75% relative humidity 16 h light/8 h dark photoperiod and 200 µmol m^{-2} s^{-1} light intensity, in a climate room at Selcuk University, Faculty of Agriculture. In addition, 50 ml of 0, 100 and 200 mM NaCl were given to the plants every two days. Seedling growth was evaluated after four weeks of treatment. The study lasted approximately 2 months. Before harvesting, plant height was measured, followed by shoot, root and total biomass quantification.

The study was designed with 2 factors (genotype × NaCl concentration) and was set up in a completely randomized design with 4 replications of 25 seeds at the germination stage (results are presented as the mean of 100 seeds) and 4 replications at the seedling stage (4 pots each containing three seedlings). The following traits were determined based on various formulas:

The morphological traits were examined by measuring and counting on days 4 and 8 in accordance with ISTA rules. Seeds with rootlets exceeding 2 mm were considered germinated and counted (Fuller et al. 2012). Seedlings in pots were measured after 4 weeks and root length, shoot length, root length/shoot length, plant height (root length+shoot length), fresh mass and dry weight were determined by counting and measuring (in cm with ruler). Dry weight was determined by drying the fresh shoots at 70°C for 24 h (Atak et al. 2006, Saboora et al. 2006).

Germination capacity (GC, %): This parameter allows the determination of the salt concentration that constitutes the physiological limit of durum wheat seed germination.

$$GC= b/a \times 100 \quad (1)$$

where a = total number of seeds, b = maximum number of germinated seeds.

Initial vigor (IV): This parameter relates germination capacity to plant growth. The results were calculated as follows:

$$IV= GC \times DW \quad (2)$$

where GC – germination capacity, DW – plant dry weight.

Germination index (GI): It is expressed as the ratio of GC under salt stress (GCS) to GC in the control (0 NaCl, GCC). The results were calculated as follows:

$$GI = GCs / GCc \quad (3)$$

Mineral content

Total sodium, magnesium, calcium and potassium content: 0.5 g of the dried (70°C at 48 h in oven) shoot samples was taken and 3 ml of HCl (37%), 9 ml of HNO_3 (65%) were added. Fresh combustion was performed in a DigiBlock (Labtech ED 36S) combustion unit (U.S. EPA 2007) The total content of Na, P Mg, Ca and K was determined by ICP-OES (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA).

Statistical analysis

All data measured and observed in the experiment were subjected to analysis of variance (ANOVA) in JMP 13.2.0 program in accordance with the factorial experimental design in coincidence plots and the differences between the means were determined using the Duncan multiple comparison test (Snedecor, Cochran 1967). PCA analysis was performed in the R statistical program.

RESULTS AND DISCUSSION

Salinity stress decreased root length and shoot length in barley, depending on a NaCl concentration and genotypes (Tables 1, 2). In the study, statistically significant differences were found between varieties and salt doses at 1% level in terms of root length and shoot length. In addition, cultivar x salt interaction was also found significant at 1% level.

Table 1

Root lengths (cm) of barley genotypes at different sodium chloride (NaCl) concentrations

Genotypes	NaCl (mM)		
	0	100	200
Zemela	12.20 *d**	21.50 *a*	no germination
Baronesse	15.00 *abcd*	18.90 *abc*	13.00 *cd*
Bojin	15.20 *abcd*	16.50 *abcd*	no germination
Aydanhanim	14.00 *bcd*	14.40 *bcd*	20.10 *ab*

* Differences between values shown with different letters are statistically significant ($p<0.01$)

Table 2

Shoot lengths (cm) of barley genotypes at different sodium chloride (NaCl) concentrations

Genotypes	NaCl (mM)		
	0	100	200
Zemela	35.80 *abcd**	37.70 *ab*	no germination
Baronesse	37.60 *ab*	39.00 *a*	33.10 *bcd*
Bojin	37.20 *abc*	35.10 *abcd*	no germination
Aydanhanim	34.20 *bcd*	31.10 *d*	32.70 *cd*

* Differences between values shown with different letters are statistically significant (p<0.01).

The biggest root length was achieved at 100 mM salt dose (17.80) and the genotype with the biggest root length was Aydanhanım (16.10). Considering the cultivar x salt interaction, the biggest root length was determined in Zemela cultivar at 100 mM NaCI dose (21.50). The smallest root length was determined at 200 mM salt dose and Bojin genotypes (Table 1). Regarding the cultivar x salt interaction, the smallest root length was determined in Zemela and Bojin cultivars at 200 mM NaCl dose. Regarding the shoot length of the varieties, the largest shoot length was determined in the control object without NaCl (36.20) and Baronesse genotypes (36.60), while the largest shoot length was determined in Baronesse genotypes at 100 mM salt dose (Table 2). The smallest shoot length was determined in Zemela and Bojin cultivars at 200 mM salt dose.

Salinity stress decreased seedling length of barley depending on a NaCl concentration and genotypes (Table 3). In the study, statistically significant differences were found between cultivars and salt doses at 1% level in terms of the seedling length. In addition, the cultivar x salt interaction was also significant at 1% level. The biggest plant length was determined at 100 mM salt dose and the biggest plant length was determined in Baronesse genotypes (52.10), while the biggest plant length was determined in Zemela genotypes (59.10) at 100 mM NaCI dose (Table 3). The smallest seedling height was determined in Bojin genotypes at 200 mM salt dose. In the interaction, the smallest plant height was determined in Zemela and Bojin cultivars at 200 mM NaCI dose (no germination).

Table 3

Plant length (Root lengths+Shoot lengths cm) of barley genotypes at different sodium chloride (NaCl) concentrations

Genotypes	NaCl (mM)		
	0	100	200
Zemela	48.10 *bc**	59.10 *a*	no germination
Baronesse	52.40 *abc*	57.90 *ab*	46.00 *c*
Bojin	52.40 *abc*	51.70 *abc*	no germination
Aydanhanim	48.20 *bc*	45.50 *c*	50.80 *abc*

* Differences between values shown with different letters are statistically significant (p<0.01).

In the study, statistically significant differences were found between salt doses and cultivar x salt interaction in terms of fresh mass, between salt doses in terms of dry weight, and between salt doses, cultivars and cultivar x salt interaction in terms of root/shoot ratio at 1% level (Tables 4,5,6).

The largest fresh mass value was determined at 100 mM NaCI dose and the smallest at 200 mM NaCI. Considering the cultivar x salt interaction, the largest fresh mass was determined in Zemela cultivar at 100 mM dose (1.880). Similarly, the largest dry weight value was observed at 100 mM NaCI dose (0.390). In a study conducted on maize plants, salt stress caused a significant reduction by 33-45% in fresh and dry weight, and this decrease occurred independently of external phosphate concentrations (Sacala et al. 2016).

Table 4

Fresh mass (g) of barley genotypes at different sodium chloride (NaCl) concentrations

Genotypes	NaCl (mM)		
	0	100	200
Zemela	0.780 *b**	1.880 *a*	no germination
Baronesse	1.220 *b*	1.260 *ab*	0.770 *b*
Bojin	1.080 *b*	1.250 *ab*	no germination
Aydanhanim	0.610 *bc*	1.140 *b*	0.780 *b*

* Differences between values shown with different letters are statistically significant ($p<0.01$).

Table 5

Dry weight (g) of barley genotypes at different sodium chloride (NaCl) concentrations

Genotypes	NaCl (mM)		
	0	100	200
Zemela	0.230	0.490	no germination
Baronesse	0.450	0.380	0.220
Bojin	0.310	0.380	no germination
Aydanhanim	0.170	0.330	0.200

* Differences between values shown with different letters are statistically significant ($p<0.01$).

Table 6

Root/shoot (cm) of barley genotypes at different sodium chloride (NaCl) concentrations

Genotypes	NaCl (mM)		
	0	100	200
Zemela	0.340 *c*	0.570 *ab*	no germination
Baronesse	0.390 *c*	0.480 *abc*	0.390 *c*
Bojin	0.400 *bc*	0.470 *abc*	no germination
Aydanhanim	0.400 *bc*	0.450	0.630 *a*

* Differences between values shown with different letters are statistically significant ($p<0.01$).

The root length/shoot length ratio is the root length divided by shoot length, and it indicates the balance between root and shoot development. This ratio reflects the water and nutrient uptake capacity of the plant, its adaptation to environmental conditions and its general physiological state. For example, it has been observed that under saline conditions this ratio increases, meaning that roots grow longer than shoots. In terms of the root/shoot ratio, the largest value was observed at 100 mM NaCI dose. The genotype with the largest root/shoot ratio was Aydanhanim. In terms of the cultivar x interaction, the largest value was observed at 200 mM NaCI dose and for Aydanhanim cultivar (0.630).

Salinity stress affected the germination capacity (GC) of barley differently depending on the NaCl concentration and genotype (Table 7). Statistically

Table 7

Germination capacity (GC; %) of barley genotypes at different sodium chloride (NaCl) concentrations

Genotypes	NaCl (mM)		
	0	100	200
Zemela	51.00 *bc**	93.00 *a*	no germination
Baronesse	27.00 *de*	63.00 *b*	98.00 *a*
Bojin	95.00 *a*	44.00 *cd*	no germination
Aydanhanim	97.00 *a*	26.00 *e*	83.00 *a*

* Differences between values shown with different letters are statistically significant (p<0.01).

significant differences at 1% level were found between salt doses, cultivars and cultivar x salt interaction in terms of germination capacity.

The largest germination capacity was realized in the control and the genotypes with the largest germination capacity was Aydanhanim (68.20). In the cultivar x salt interaction, Baronesse cultivar showed the largest value at 200 mM NaCI dose. In the study, Zemela and Bojin varieties did not germinate at 200 mM NaCI dose, and their germination capacity could not be calculated (Table 7).

To determine genotypic variability in plant establishment in response to salinity stress, initial vigor (IV), which correlates germination capacity with plant development, was calculated (Table 3). Increasing NaCl concentration decreased IV in all cultivars. However, this parameter shows a clear discrimination between cultivars and clarifies the sensitivity of Zemela and Bojin, especially compared to Baronesse and Aydanhanim. In the study, statistically significant differences were found between salt doses and cultivar x salt interaction at 1% level in terms of the initial vigor (Table 8). The largest initial viability was determined at 100 mM salt dose, and the largest value in the cultivar x salt interaction was detected for Zemela cultivar at 100 mM NaCI dose.

In the study, statistically significant differences were found between cultivars, salt doses and cultivar x salt interaction at 1% level in terms of the germination index (Table 9). Germination index (GI) was calculated to

Table 8

Initial vigour (IV) of barley genotypes at different sodium chloride (NaCl) concentrations

Genotypes	NaCl (mM)		
	0	100	200
Zemela	11.30 *cde**	45.10 *a*	no germination
Baronesse	12.00 *cde*	23.10 *bc*	21.20 *bc*
Bojin	30.20 *b*	17.10 *cd*	no germination
Aydanhanim	16.30 *cd*	8.40 *de*	17.000 *cd*

* Differences between values shown with different letters are statistically significant (p<0.01).

Table 9

Germination index (GI) of barley genotypes at different sodium chloride (NaCl) concentrations

Genotypes	100	200
Zemela	0.690 *b*	no ermination
Baronesse	0.070 *c*	0.090 *c*
Bojin	0.960 *a*	no ermination
Aydanhanim	0.650 *b*	0.850 *ab*

* Differences between values shown with different letters are statistically significant (p<0.01).

characterize differences between the genotypes. The results show that there is a significant genotypic difference in response to salinity stress in barley (Table 9). Since Zemela and Bojin did not germinate at 200 mM NaCI, their GI values could not be calculated. The largest GI value was determined for Aydanhanim genotypes. When the cultivar x salt interaction was analyzed, Bojin cultivar had the largest GI value at 100 mM NaCI dose.

Macronutrient content

The results of variance analysis of the mineral content are given. According to the results, the differences were found significant at 0.01 level for all macroelements. We identified P, Na, K, Ca, and Mg concentrations in shoot samples at three NaCl levels: 0, 100 and 200 mM NaCl. All P, Na, K, Ca, and Mg parameters were influenced by the salinity level (Table 10).

Table 10

Variance analyses (*F* values) of the investigated macroelements

Source of variance	Salt stress	Genotypes	Salt Stress x Genotypes
P	184.8269**	24.0896**	1.5780
K	125.4979**	100.233**	2.1885
Mg	574.474*	19.6458**	3.0365*
Na	1336.648**	38.1428**	11.2824**
Ca	2419.77**	25.6341**	12.2177**

** p<0.01, * p<0.5

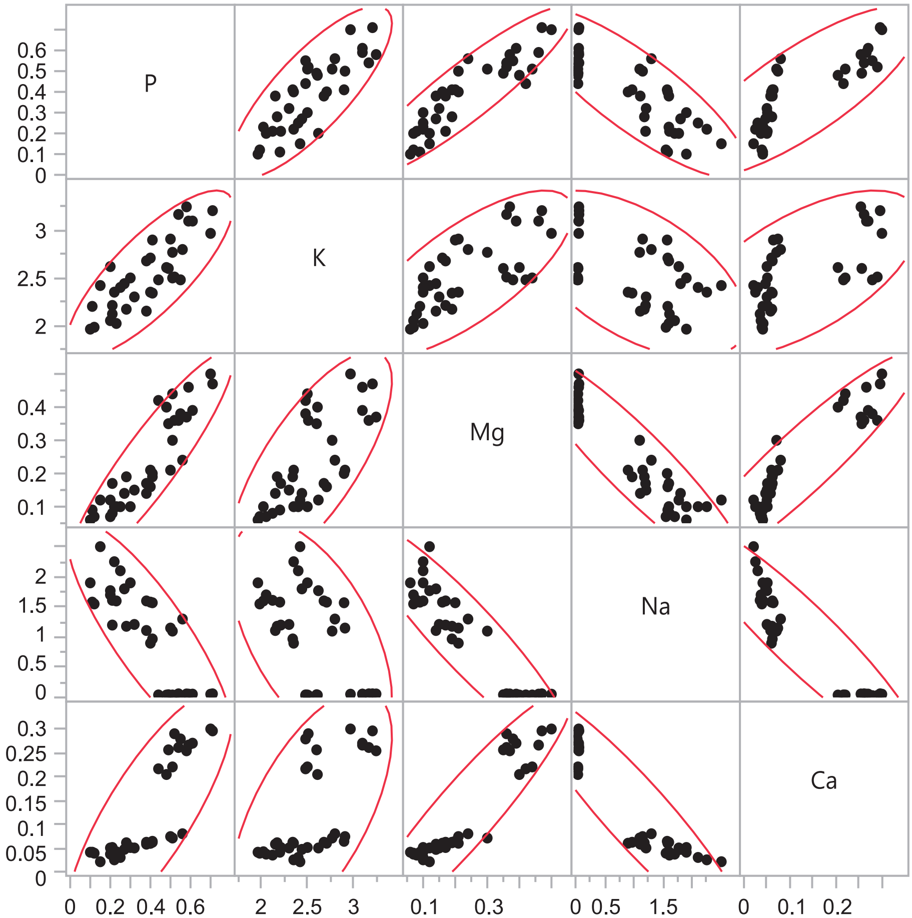

Fig. 1. Scatterplot Matrix

The correlation values between elements are given in Table 11 and Figure 1. According to the results, a positive correlation of 0.9292 was observed between Ca and Mg, while a negative correlation of 0.9253 was observed between Na and Ca.

Table 11

Correlation between investigated macroelements

Macro-elements	P	K	Mg	Na	Ca
P	*				
K	0.8013	*			
Mg	0.9127	0.7386	*		
Na	-0.8415	-0.5519	-0.9190	*	
Ca	0.8040	0.6206	0.9292	-0.9253	*

Table 12

Mean P and K content (mg) in shoots at two NaCl levels for four barley genotypes

Genotypes	NaCl					
	0		100		200	
	P	K	P	K	P	K
Zemela	0.476 *bc**	2.530 *cd*	0.270 *de*	2.228 *ef*	0.153 *e*	2.066 *f*
Baronesse	0.576 *ab*	3.173 *a*	0.397 *cd*	2.763 *bc*	0.207 *e*	2.390 *de*
Bojin	0.667 *a*	3.090 *a*	0.523 *bc*	2.827 *b*	0.256 *e*	2.520 *cd*
Aydanhanim	0.520 *bc*	2.530 *cd*	0.365 *cd*	2.280 *def*	0.170 *e*	2.043 *f*

* Differences between values shown with different letters are statistically significant (p<0.01).

Phosphorus and potassium contents are given in Table 12. According to the results, the largest phosphorus value was 0.67 mg in Bojin cultivar in the control object without NaCl, and the smallest value was 0.17 mg in Aydanhanim cultivar in 200 mM salt stress. The largest potassium value of 3.17 mg was obtained in the control treatment of Baronesse genotypes, and the smallest value of 2.04 mg was obtained in the 200 mM salt stress of Aydanhanim genotypes.

Magnesium and sodium contents are given in Table 13. According to the results obtained, the largest magnesium value was 0.48 mg in Bojin genotypes in control object without NaCl and the smallest value was 0.07 mg

Table 13

Mean Mg and Na content (mg in shoots at two NaCl levels for four barley genotypes

Genotypes	NaCl					
	0		100		200	
	Mg	Na	Mg	Na	Mg	Na
Zemela	0.420 *ab*	0.041 *d*	0.170 *de*	1.197 *c*	0.087 *f*	1.576 *b*
Baronesse	0.373 *b*	0.057 *d*	0.177 *d*	1.583 *b*	0.106 *ef*	2.282 *a*
Bojin	0.477 *a*	0.056 *d*	0.250 *c*	1.183 *c*	0.120 *def*	1.824 *b*
Aydanhanim	0.383 *b*	0.035 *d*	0.180 *d*	0.993 *c*	0.070 *f*	1.736 *b*

* Differences between values shown with different letters are statistically significant (p<0.01).

in Aydanhanim genotypes in 200 mM salt stress. The largest sodium value of 2.28 mg was obtained from the 200 mM salt application of Baronesse genotypes and the smallest value of 0.03 mg was obtained from the control application of Aydanhanim genotypes.

Calcium contents are given in Table 14. According to the results, the largest calcium content was 0.28 mg in Bojin cultivar and the smallest 0.023 mg in Baronesse cultivar under 200 mM salt stress.

PCA values of mineral results because of germination are given in Figure 2. When the graph is analyzed, 2 principal components with eigenvalues higher than one and 6 independent principal components were obtained.

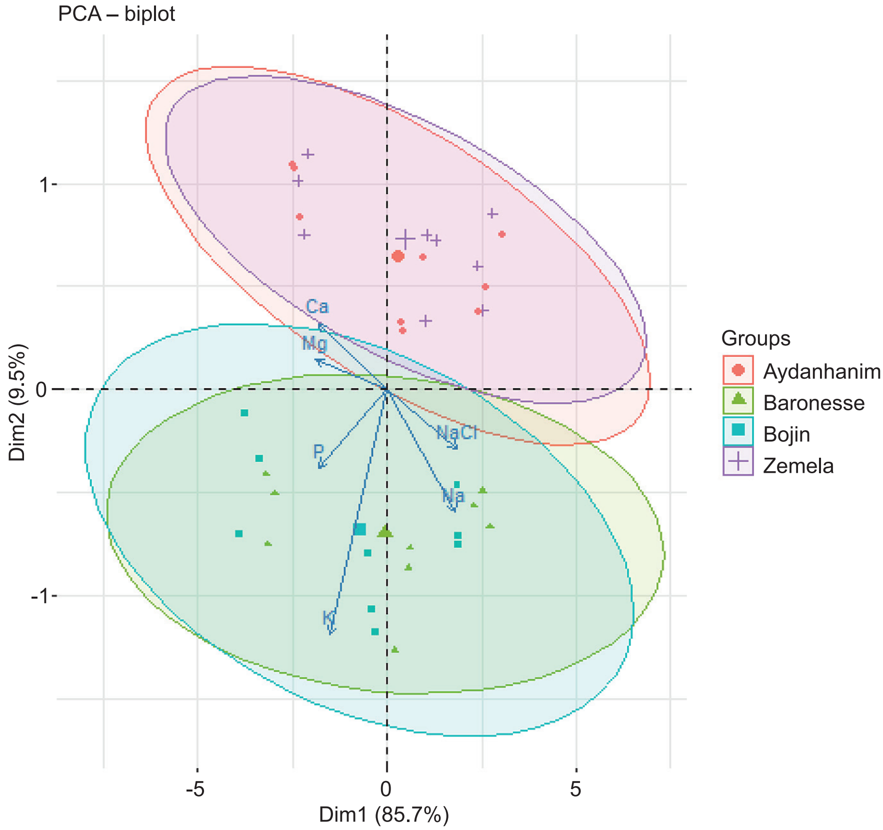

Fig. 2. PCA analysis

Table 14

Mean Ca content (mg in shoots at two NaCl levels for four barley genotypes

Genotypes	NaCl (mM)		
	0	100	200
Zemela	0.214 *c*	0.054 *de*	0.041 *ef*
Baronesse	0.263 *b*	0.062 *de*	0.023 *f*
Bojin	0.288 *a*	0.074 *d*	0.049 *ef*
Aydanhanim	0.276 *ab*	0.060 *de*	0.097 *ef*

* Differences between values shown with different letters are statistically significant ($p<0.01$).

PC1 is +0.4245 with NaCl. These values indicate that the principal component analysis can be interpreted as successful.

Field crops are known to be affected by different abiotic stresses during their growth stages. The early stages of plant development determine the response to stress. In this study, four barley cultivars, two of which were Bulgarian barley cultivars, were exposed to increasing salinity stress at the germination and early plant formation stages to determine their physiological responses and differences in the responses of the cultivars. Our idea was that a cultivar that is tolerant at the early stages of germination and development will be tolerant at later stages of plant development. We also tried to identify the most critical stage (germination or seedling formation). The findings of this study may provide useful physiological indicators for screening cultivars for salt tolerance in related studies. Depending on the cultivar, salinity stress significantly reduced all traits. Aydanhanim and Baronesse were the most tolerant cultivars, while Zemela and Bojin were the most sensitive ones.

Many authors have reported that an increase in salt concentration decreases germination percentage and delays germination (Patade et al. 2011, Ansari, Hussain 2012, Thiam et al. 2013). In the present study, these effects were more pronounced in sensitive varieties (Zemela, Bojin). This is confirmed by the absence of germination in Zemela and Bojin cultivars at 200 mM NaCl. Germination index also decreased with increasing NaCl concentration (Table 9). The values recorded in Aydanhanim and Baronesse were the highest, while those recorded in Aydanhanim and Bojin were the lowest, indicating some genotypic differences in the response of barley to salinity at the germination stage. Sima et al. (2013) suggested that the reason for the high sensitivity of germination to salinity is related to damaged enzyme activity. On the other hand, Bouzidi et al. (2021) stated that low ambient salt concentration causes a state of dormancy. In contrast, high salt concentration inhibited seed germination and reduced germination capacity due to the toxic effect of sodium macroelements.

Under salt stress, plants are severely damaged during their first development period, and their growth and development periods are significantly reduced (Okumus, Uzun 2022). Salt stress inhibits seed germination and causes a decrease in germination capacity in wheat (Doruk Kahraman, Topal 2024). The reduction in germination observed in this study due to severe stress (200 mM NaCl) may be caused by osmotic or toxic effects (Neamatollahi, Bannayan 2009) that would inhibit the developmental activities of the seed (Yildirim, Guvenc 2006). The results we obtained are in agreement with the existing literature. The decrease in germination percentage and germination observed in this study is due to the high rate of uptake of Na^+ and Cl^- during germination, thus may suspected to have be caused by the occurrence of toxicity.

As reported (Patade et al. 2011), seedling formation is an important stage of plant development. Our results showed that salinity stress significantly reduced plant growth in the observed cultivars according to the observed genotypic differences (Aydanhanim and Baronesse less affected, Zemela and Bojin highly affected). Liu et al. (2023) explained that seedling growth under salinity is adversely affected by the restriction of water and nutrient uptake in highly saline soils, which slows metabolism and inhibits growth. The initial vigor values (DW × GC, Table 8) supported this idea and confirmed genotypic differences. Indeed, the increase in the initial vigor value of Aydanhanim cultivar at 200 mM NaCI dose is an example of this. This trait showed the tolerance of Aydanhanim and Baronesse, especially when compared with Zemela and Bojin. Doruk Kahraman and Topal (2024) reported some genotypic differences among 10 cultivars. This suggests that plants can develop genetically diverse responses to salt stress, and that these differences are important in assessing tolerance to salinity. In particular, the cultivar Altintas-95 While Svevo variety was assessed as tolerant to salinity in the cited study, while Svevo variety was determined to be sensitive. In the research, initial vigor (IC) and stress index (SI) were used to differentiate the studied varieties from each other, and to identify traits suitable for identification of salinity tolerant cultivars.

The element content in plant seeds and the elemental requirements of agricultural crops vary widely. The functions of essential elements for plant growth and development cannot be fulfilled by other elements (Kahraman, Onder 2018). In this study, the genotypic differences observed were explained by differences in Na^+ uptake at the germination stage, which affects seedling growth. Thus, the relative tolerance of Aydanhanim and Baronesse can be explained by their lower macroelement toxicity, while the sensitivity of Zemela and Bojin is explained by the high accumulation of Na^+ and Cl^-, which creates a toxicity situation. The existence of a wide, intraspecific genetic diversity for salt tolerance has been previously observed in wheat genotypes (Krishnamurthy et al. 2007). Salinity decreased germination parameters as the salt level increased. Kandil et al. (2012) stated that the toxic effects of Na^+ and Cl^- macroelements on germinating seeds affect germination percentage and germination rate.

Salt stress in seeds is an important environmental stress factor that negatively affects the growth and development processes of plants. Significant changes were observed in mineral contents of plant under salt stress. It was reported that the accumulation of the macroelements sodium (Na^+) and chlorine (Cl^-) increased, while the concentrations of important nutrients such as potassium (K^+) and calcium (Ca^{2+}) decreased in wheat (*Triticum aestivum*) seeds exposed to salt stress (Parida, Das 2005). These changes cause imbalance of macroelements and an increase in cellular osmotic pressure, leading to water loss and reduced growth rate (Munns, Tester 2008). It has also been reported that salt stress inhibits the uptake of micronutrients, such as magnesium (Mg) in seeds. Deficiency of these micronutrients may cause physiological problems, such as decreased enzymatic activities and impaired chlorophyll synthesis (Marschner 2012). Adaptation mechanisms developed by plants against salt stress include the maintenance of macroelemental homeostasis and the compartmentalization of toxic macroelements within the cell. For example, plants with high salt tolerance can limit the accumulation of Na^+ and Cl^- in the roots and prevent the transport of these macroelements to the leaves (Ashraf 2004). Furthermore, these plants accumulate intracellular osmotic regulators, such as proline, betaine and sorbitol to maintain osmotic balance (Bohnert, Jensen 1996). These compounds reduce the water loss of cells by balancing the intracellular water potential and stabilize enzymatic activities (Szabados, Savouré 2010). Other studies on the mineral changes of salt stress in seeds show similar trends in different plant species. For example, one study found that Na^+ and Cl^- accumulation increased and K^+, $Ca2^+$, and Mg^{2+} concentrations decreased in tomato (*Solanum lycopersicum*) seeds exposed to salt stress (Kaya, Higgs 2003). It has been reported that these changes may differ depending on the plant species and the severity of salt stress applied. Similar mineral changes were also reported in rice (*Oryza sativa*) seeds under salt stress (Zeng, Shannon 2000).

CONCLUSIONS

According to the results of this study, it can be said that increasing salinity caused a decrease in germination and seedling development in all the determined traits of barley varieties. Genotypic differences were found among the four barley cultivars. Aydanhanim and Baronesse were identified as tolerant, while Bulgarian genotypes Zemela and Bojin were identified as sensitive to salnity. The barley varieties showed better tolerance to salinity at germination than at the seedling stage. GC and IV differed among the genotypes. In conclusion, salt stress altering the macroelements (P, K, Mg, Na, Ca) is a complex mechanism that negatively affects the growth and development processes of plants. Understanding the adaptation strategies developed by plants against this stress factor is very important for sustainable food production. It can be said that Aydanhanim genotype, which was determined in the study to be salt tolerant, can be used as breeding material in the development of plant genotypes with high salt tolerance. As a matter of fact, it is thought that the traits in the study may be effective traits in determining salinity tolerant genotypes.

Author contributions

Conceptualization – N.D.K, methodology – N.D.K and O.O., investigation – N.D.K, writing – original draft preparation – N.D.K and O.O., writing – review and editing – N.D.K and O.O., visualization – N.D.K and O.O. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

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