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Abstract: The effects of salinity on the growth, chemical composition and superoxide dismutase (SOD) and peroxidase (POX) activities of 2 malting barley (*Hordeum vulgare* L.) cultivars (Kaya and Scarlet) were studied. In 10-day-old seedlings, salinity stress was initiated by applying an appropriate amount of NaCl to water, and it lasted for 30 days. Salinity decreased growth of the cultivars significantly. Scarlet restricted entry of Na^+ and Cl^- into root and translocation to leaves more efficiently than did Kaya. As a whole Scarlet produced a higher amount total chlorophyll under salinity than did Kaya. POX activity of the cultivars decreased with salinity up to 20 dS m^{-1}, and then increased. In contrast to POX, salinity increased the SOD activity of leaves. Compared to the control application, 5.31% and 16.34% increases were determined in SOD activity, in 10 dS m^{-1} salinity, for Kaya and Scarlet, respectively.

Key Words: *Hordeum vulgare* L., superoxide dismutase, peroxidase, salinity stress, chemical content, growth

Tuzluluğun İki Maltlık Arpa (*Hordeum vulgare* L.) Çeşidinde Gelişim Kimyasal Bileşim ve Antioksidatif Enzim Aktivitesi Üzerine Etkisi

Özet: Tuzluluğun iki farklı arpa (*Hordeum vulgare* L.) çeşidinde (Kaya ve Scarlet) gelişim, kimyasal içerik, süperokside dismutase (SOD) ve peroksidase (POX) aktiviteleri üzerine olan etkileri araştırıldı. Tuz stresi, fidelerin 10 günlük olduğu dönemde uygun miktarlarda NaCl karıştırılmış su verilmesiyle başlatıldı. Tuz uygulaması çeşitlerin gelişimlerini önemli ölçüde geriletti. Scarlet çeşidi Kaya çeşidine göre Na^+ ve Cl^- elementlerinin köke girişini ve yapraklara taşınmasını daha iyi sınırlandırdı. Tuz stresi altında genel olarak Scarlet çeşidi Kaya'ya göre daha yüksek miktarlarda klorofil üretti. Çeşitlerin (POX) aktiviteleri, 20 dS m^{-1} tuz düzeyine kadar azaldı ve daha sonra arttı. POX aktivitesinin aksine, tuzluluk yaprakların SOD içeriğini arttırdı. Kontrole göre 10 dS m^{-1} tuz düzeyinde SOD aktivitesi Kaya çeşidinde %5,31 ve Scarlet çeşidinde %16,34 artış gösterdi.

Anahtar Sözcükler: *Hordeum vulgare* L., superoxide dismutase, peroxidase, tuzluluk stresi, kimyasal bileşim, gelişim

Introduction

Excessive soluble salts in the soil are harmful to most plants. In fact, no toxic substance restricts plant growth more than does salt on a world scale. It is estimated that salinity affects at least 20% of the world's arable land and more than 40% of irrigated land to various degrees (1), (2). Sodium toxicity represents the major ionic stress associated with high salinity. Additionally some plant species are also sensitive to Cl^-, the major anion found in saline soils (2). It is well known that there are significant differences, with respect to salt tolerance, between and within plant species (3). Among agricultural crops, barley (*Hordeum vulgare* L.) is considered a highly salt-tolerant plant (4). The objectives of this study were to evaluate the differential response of 2 barley cultivars to NaCl-induced salinity, especially with respect to antioxidative enzyme activity, uptake and translocation of ions and growth parameters. The practical implication is to provide data enabling the expansion of barley growing in areas possessing high salinity.

Materials and Methods

Plant Material

In the research 2 malting barley (*Hordeum vulgare* L.) cultivars, Kaya and Scarlet, were used as plant material. Kaya is a Turkish malting barley cultivar grown in Turkey. Scarlet is of German origin and, due to its higher quality properties, it might be a better choice to grow in the region in order to use in malt production. Seedlings of the

cultivars were planted on February 12, 2004, in 5-l buckets filled with coarse sand of 0.6-0.8 mm particle size. Plants were irrigated with an amount of water accounting for a leaching factor of 20-25% and fertilized with concentrated Hoagland solution (5). After germination 40 plants, which are equal to 450 plant m^2, were left in each pot.

Irrigation water

The experimental treatments consisted of 3 salinity levels of the irrigation water (10, 20 and 30 dS m^{-1}), besides the control. Tap water (1 dS m^{-1}) served as the non-saline control. The salinity levels were obtained by the addition of appropriate amounts of NaCl to that water. Each treatment was in 3 replicates, 40 plants per pot, making a total of 120 plants per replicate. The data obtained are presented with the least significant difference ($LSD_{0.05}$) between treatments, derived from analyses of variance (6).

Chemical and Physiological Analyses

Electrical conductivity (EC) and volume of leach were measured over the entire irrigation period. Although the leaching factor was high some fluctuations in EC were observed. Average water applied per pot was approximately 1940 ml during the experiment.

Plant height was expressed as a percentage, in order to eliminate differences in vigor of the cultivars. Dry matter (DM) accumulation was also determined as percentage. Salinization continued for 30 days; then the plants were gently removed from the substrate, the roots were washed with deionized water and the plants partitioned into roots and leaves. The roots were selected by hand mechanically from the growing media. For nutrient analyses, plant organs were placed in paper bags and dried in a forced-air oven at 70 °C for 72 h. The samples were then ground in a stainless steel Wiley mill with 0.5 mm particle size (7). The ground samples were wet digested in a mixture of nitric acid:perchloric acid (HNO_3:$HClO_4$) (4:1). Sodium (Na^+), potassium (K^+) and calcium (Ca^{+2}) contents were measured by flamephotometer (Jenway PFP7), magnesium (Mg^{+2}) contents were measured by atomic absorption spectrophotometer (Varian SpectrAA 220FS) and Cl^- contents were measured by chloridemeter (Jenway PCLM 3) in the samples (7). Concentrations of chlorophyll were determined in 80% acetone extract of leaf using the method described by Lichtenthaler and Wellburn (8).

Enzyme Assays

The extracts used in the determination of superoxide dismutase (SOD), peroxidase (POX) and total protein analyses were prepared by homogenizing 0.5 g of frozen leaf material in 3 ml of cold solution containing 50 x 10^{-3} M Na phosphate buffer (pH 7.8), 1 x 10^{-3} M EDTA and 2% (w/v) PVPP. The homogenate was centrifuged at 0 °C for 40 min at 13,000 rpm. Spectrophotometric analyses were conducted on a Thermo MultiScanSpectrum 1500 spectrophotometer. The SOD and POX activity assays were based on the methods described by Beauchamp and Fridovich (9) and Herzog and Fahimi (10), respectively. Total soluble protein content in the enzyme extracts was determined (11) using bovine serum albumin (BSA) as standard.

Results and Discussion

Growth of the cultivars was inhibited by salinity to different extents. Both cultivars easily tolerated the first salinity application, 10 dS m^{-1}. Salinity decreased plant height significantly (Table 1). Another important approach to assess the effects of salinity on plant growth is to measure accumulation of DM in plant organs. Salinity increased the leaf and root DM content of the cultivars significantly (Table 1). Probably because of its higher plant height reduction, Kaya produced more DM content in all treatments except for leaf DM in the highest treatment (30 dS m^{-1}).

Salinity reduced the tillering ratio (%) of the cultivars significantly. In the control application, Kaya and Scarlet had almost the same tillering ratio (343.5% and 345.3%, respectively). However increasing salinity reduced the tillering ratios of the cultivars to different extents and in this respect Scarlet tolerated the salinity better. Compared to the control, increasing salinity rates (10, 20 and 30 dS m^{-1}) reduced the tillering ratio 27.3%, 32.9% and 65% in Kaya, and 11.2%, 27% and 30% in Scarlet, respectively (Table 1). The results clearly showed that increases in irrigation water salinity decreased plant growth parameters significantly. According to Mer et al. (12), increasing salinity reduced the growth and seed germination ratio of barley. Similar results were reported by Bagcı et al. (3), Hussain et al. (13) and Isla et al. (14) for growth parameters such as plant height, root length, tillering, green matter and DM production.

Table 1. Effect of salinity on growth parameters of barley cultivars.

Treatment NaCl (dS m^{-1})	cv. Kaya		cv. Scarlet	
	Leaf	Root	Leaf	Root
		Dry Matter (%)		
Control	13.423 b*	7.247 bc	12.540 d	6.803 b
10	13.410 b	7.137 c	13.363 c	6.947 b
20	16.637 ab	8.177 ab	15.460 b	7.273 ab
30	19.960 a	9.187 a	20.237 a	9.130 a
		Tillering (%)		
Control	343.533 a		345.333 a	
10	249.633 b		306.633 a	
20	230.333 b		251.933 ab	
30	120.233 c		159.133 b	
		Plant Height (cm)		
Control	38.390 a		31.110 a	
10	36. 277 a		28.833 a	
20	29.223 b		24.383 b	
30	22.503 c		19.053 c	

* Values are means of 3 replications. Means separations by least significant difference (LSD) at $P \leq 0.05$. ns: nonsignificant

The effect of salinity on the Na^+ content of plant organs was statistically significant (Table 2). In the control treatment, Kaya and Scarlet had similar Na^+ contents in the roots (0.173% and 0.160%, respectively). However, in the first salinity level, 10 dS m^{-1}, the root Na^+ content of Kaya was almost 25% lower than that of Scarlet. Then, root Na^+ content of Kaya increased sharply in the second (20 dS m^{-1}) and third salinity levels (30 dS m^{-1}). The root Na^+ content of Scarlet increased steadily up to the second salinity level, 20 dS m^{-1}, and then decreased (Table 2). Probably both cultivars inhibited the translocation of Na^+ from root to leaf in the control treatment. However, increasing salinity enhanced the Na^+ transportation to the leaves. In the first salinity level, 10 dS m^{-1}, Kaya translocated higher amounts of Na^+ from root to leaf than did Scarlet. Hence, the root Na^+ content of Kaya was lower in this salinity level. Presumably, Scarlet managed increasing salinity better, and inhibited translocation of Na^+ from root to leaf up to the second salinity level, 20 dS m^{-1}, and then could not prevent translocation of Na^+ in the third salinity level, 30 dS m^{-1} (Table 2). It is well known that ability to restrict Na^+ transport from the roots to the aerial parts is an important determinant of salt tolerance. According to Lazof and Bernstein (15), growth inhibition is mainly restricted to shoots under moderate salt stress. One related process in the aerial parts is to retain Na^+ in older leaves, which reduces transport to young organs (16).

The amount of Cl^- translocated from root to leaves was higher than that of the other elements in leaves (Table 2). Seedlings take up Cl^- very rapidly and in considerable amounts. According to Mengel and Kirkby (17), the uptake rate of Cl^- depends primarily on the concentration in the nutrient or soil solution.

The cultivars differed in their uptake and translocation of Cl^-. With salinity, Kaya uptook and translocated Cl^-. Scarlet uptook a very large amount of Cl^- in the first application rate and then tried to decrease it into the roots and translocate it to the leaves. Probably Scarlet governed uptake and/or translocation of Cl^- in the tissues better and managed to reduce the root Cl^- content in the

Table 2. Effect of salinity on mineral content (%/g DW) in leaf and root of barley cultivars.

Treatment NaCl (dS m^{-1})	cv. Kaya		cv. Scarlet	
	Root	Leaf	Root	Leaf
		Na (%)		
Control	0.173 d*	0.114 d	0.160 c	0.126 d
10	0.740 c	1.207 c	1.007 b	1.127 c
20	1.400 b	1.973 b	1.370 a	1.713 b
30	1.427 a	2.450 a	1.220 ab	2.493 a
		Cl (%)		
Control	0.875 ns	2.588 c	0.729 c	2.837 ns
10	1.723 ns	4.680 bc	2.412 a	4.307 ns
20	1.730 ns	6.297 b	2.157 ab	6.320 ns
30	2.075 ns	9.730 a	1.675 b	6.430 ns
		K (%)		
Control	2.997 a	5.447 a	2.860 a	5.357 a
10	1.223 b	4.360 b	1.633 b	4.247 b
20	0.910 c	3.270 c	0.656 c	3.107 c
30	0.480 d	2.727 d	0.540 c	2.633 c
		Ca (%)		
Control	0.200 ns	0.257 ns	0.173 ns	0.197 ns
10	0.163 ns	0.203 ns	0.170 ns	0.187 ns
20	0.120 ns	0.197 ns	0.097 ns	0.177 ns
30	0.190 ns	0.210 ns	0.160 ns	0.200 ns
		Mg (%)		
Control	0.184 ns	0.198 ns	0.164 ns	0.225 ns
10	0.187 ns	0.228 ns	0.241 ns	0.209 ns
20	0.226 ns	0.213 ns	0.198 ns	0.210 ns
30	0.205 ns	0.223 ns	0.172 ns	0.243 ns

* Values are means of 3 replications. Means separations by least significant difference (LSD) at $P \leq 0.05$. ns: nonsignificant

highest salinity level, 30 dS m^{-1} (Table 2). On the other hand, the leaf Cl^- content of Kaya was significantly higher than that of Scarlet in the highest salinity level, 30 dS m^{-1}.

The effects of salinity on the K^+, Ca^{+2} and Mg^{+2} contents of different plant organs are given in Table 2. While increasing salinity negatively affected the K^+ and Ca^{+2} contents of plant tissues, it positively affected Mg^{+2} contents. However, only the salinity&K interaction was statistically significant. In the control, the cultivars had similar amounts of K^+ in their tissues. With increasing salinity Scarlet accumulated K^+ in the root tissues and limited its translocation. Kaya translocated more K^+ to the leaves (Table 2). Probably the presence of Ca^{+2} and K^+ enhances Na^+ exclusion by controlling channel selectivity.

A high K^+ concentration in the growing medium also ensures its adequate supply to the plant in the presence of excess Na^+ (18). As reported by Ashraf (19), there is an inverse relationship between Na^+ and K^+, Ca^{+2} and Mg^{+2} contents of cotton leaves. Low accumulations of Na^+ and K^+ were found in the shoots of cotton subjected to saline conditions. The presence of K^+ and in particular Ca^{+2} ions has been shown to decrease Na^+ influx to plant cells (20) and consequently to decrease Na^+ damage (21) and yield reduction (22). Bağcı et al. (3) stated that there is an inverse relation between K^+ and Na^+ contents of 8 different barley cultivars grown under increasing salinity conditions.

The effects of salinity on total chlorophyll, protein, POX and SOD activity are given in Table 3. As a whole, the total chlorophyll content of Scarlet was higher than that of Kaya. Although salinity affected the total chlorophyll contents of the cultivars, these interactions were not statistically significant. Chlorophyll content was increased by salinity up to the second salinity level, 20 dS m^{-1}, in Kaya, and up to the first salinity level, 10 dS m^{-1}, in Scarlet. Probably the reduction in the total chlorophyll content of the cultivars is related to the effect of salinity on stomatal closure (23). Another possible factor contributing to decreased photosynthesis is the inhibitory effect of salt stress on the efficiency of translocation and assimilation of photosynthetic products.

Total protein content of the cultivars increased up to the second salinity level, 20 dS m^{-1}, and then decreased. However, this interaction was only statistically significant in Scarlet, probably because of the low protein content of this cultivar in the control application. However, with increasing salinity, the protein contents of the cultivars were equalized. This result, in combination with the reduction in plant growth, suggests lower rates of protein synthesis, possibly accompanied by synthesis of stress-induced proteins (24). Declines in protein content in the highest salinity level, 30 dS m^{-1}, may be features of salinity stress in plants in which salt reduces photosynthesis (25).

In respect to POX activity, the cultivars were similar. However, POX activity was not accompanied by an increase in SOD activity (26). Leaf POX activities of the cultivars decreased with the salinity up to the second treatment, 20 dS m^{-1}, and then increased. Presumably this tendency was related to decreasing salinity tolerance of the cultivars under increasing salinity. Increasing protein accumulation in leaves caused decreasing POX activities in the cultivars.

In contrast to POX, salinity increased the SOD activity in leaves. In the control treatment, the SOD activity of Kaya was lower than that of Scarlet. While the SOD activity of Kaya was highest in the second treatment, 20 dS m^{-1}, the SOD activity of Scarlet was highest in the first

Table 3. Effect of salinity on total chlorophyll, protein, peroxidase (POX) and superoxide dismutase (SOD) activity of barley cultivars.

Treatment NaCl (dS m^{-1})	Total Chlorophyll (mg g^{-1})	Protein (mg l^{-1})	POX (IU)	SOD (IU)
	cv. Kaya			
Control	3.604*	121.5 ns	0.180 a	1.96 ab
10	3.934ns	125.1 ns	0.142 b	2.07 ab
20	4.105 ns	131.6 ns	0.131 b	2.27 a
30	3.303 ns	124.0 ns	0.141 b	1.65 b
	cv. Scarlet			
Control	4.084 ns	112 b	0.188 a	2.20 ab
10	4.832 ns	127.4 a	0.147 bc	2.63 a
20	4.563 ns	133.6 a	0.116 c	1.65 ab
30	4.253 ns	123.7 a	0.154 b	1.24 b

* Values are means of 3 replications. Means separations by least significant difference (LSD) at $P \leq 0.05$. ns: nonsignificant

treatment, 10 dS m^{-1}. In the control treatment, the SOD activity of Scarlet was higher than that of Kaya. Actually, this value was higher even than the SOD activity of Kaya in the first salinity level, 10 dS m^{-1}. According to Acar et al. (26), the SOD activity of drought-resistant barley cultivars is higher than that of drought-sensitive barley cultivars. As a whole, the highest SOD activity was determined in Scarlet in the first salinity level, 10 dS m^{-1}. These findings suggested that the plant's defense system against reactive O_2 species (ROS) is active in unstressed conditions as well. According to Alscher and Hess (27), ROS are produced in both unstressed and stressed cells. However, the defense system, when presented with increased ROS formation under stress conditions, can be overwhelmed.

It is clear that there is a similarity between the increase&decrease trend in SOD and total chlorophyll content of the cultivars (Table 3). Total chlorophyll contents and SOD activity were increased by salinity up to the second salinity level, 20 dS m^{-1}, in Kaya, and up to the first salinity level, 10 dS m^{-1}, in Scarlet. Probably, SODs are present in some subcellular locations, including chloroplasts (27). According to Fridovich (28), while all compartments of the cell are possible sites for O_2 formation chloroplasts, mitochondria and peroxisomes are thought to be the most important generators of ROS.

Conclusion

The results clearly showed that increases in irrigation water salinity decreased crop yield significantly. Both cultivars easily tolerated the first salinity level, 10 dS m^{-1}. However, increasing salinity applications were tolerated better by Scarlet. This cultivar more efficiently restricted entry of Na^+ and Cl^- into the roots and translocation to the leaves than Kaya. As a whole, Scarlet produced higher amounts of total chlorophyll under salinity than Kaya. SOD activity was affected differently under salinity stress, indicating a variety dependent difference in SOD content. The overproduction of SOD in Scarlet may be a mechanism of salinity resistance. However, changes in POX activity showed no clear pattern following salinity stress. The results clearly showed that there is a relation among SOD activity, protein and total chlorophyll content of the cultivars.

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