

Physiological and antioxidant response of three cultivars of cucumber (*Cucumis sativus* L.) to salinity

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Abstract: The effects of salinity on the growth, relative water content (RWC), chlorophyll content, inorganic ions, proline accumulation, lipid peroxidation and antioxidant enzymes, superoxide dismutase (SOD), ascorbate peroxidase (APOX), and catalase (CAT) of 3 cucumber (*Cucumis sativus* L.) cultivars (Çengelköy, Anadolu F1, and Beith Alpha) were investigated. Cucumber cultivars were grown in perlite culture and irrigated with half-strength Hoagland nutrient solution for a period of 35 days under controlled conditions. After this period, seedlings were treated with 150 mM NaCl solution and the plants were harvested on day 7 and day 14 of salt treatment. The fresh and dry weights, RWC, inorganic ions, total chlorophyll, proline and malondialdehyde (MDA) content, and SOD, CAT, and APOX activities were determined. In general, the applied salinity affected all of the considered parameters depending on the duration of salt treatment and the cultivars analyzed. Proline content also increased with salinity, but accumulation of proline was lower in Çengelköy than in the other cultivars. The results suggest that the protection mechanism against oxidative damage due to induced activity of antioxidant enzymes may be better in Çengelköy than in Beith Alpha or Anadolu F1. The higher K⁺ concentrations, RWC, total chlorophyll content, and SOD, CAT, and APOX activities, together with the lower MDA, proline content, and Na⁺ and Cl⁻ concentrations detected in Çengelköy might make this cultivar more salt-tolerant than Beith Alpha or Anadolu F1.

Key words: Salinity, cucumber, proline, chlorophyll, malondialdehyde, antioxidant enzymes

Üç salatalık (*Cucumis sativus* L.) çeşidinin tuzluluğa karşı fizyolojik ve antioksidant tepkileri

Özet: Bu çalışmada salatalık (*Cucumis sativus* L.) çeşitlerinde (Çengelköy, Anadolu F1, Beith Alpha) tuzluluğun, büyüme, oransal su kapsamı, klorofil miktarı, inorganik iyonlar, prolin birikimi, lipid peroksidasyonu ile süperoksit dismutaz (SOD), askorbat peroksidaz (APOX) ve katalaz (CAT) antioksidant enzimlerine etkisi araştırılmıştır. Salatalık çeşitleri kontrollü koşullarda, perlit ortamında, ½ Hoagland besin çözeltisi ile sulanarak 35 gün büyütülmüştür. Bu periyot sonunda; fidelere 150 mM NaCl uygulanmıştır ve tuz uygulamasının 7. ve 14. gününde bitkiler hasat edilmiştir. Taze ve kuru ağırlıkları, oransal su kapsamı (OSK), inorganik iyonlar, toplam klorofil, prolin ve malondialdehid (MDA) miktarları ile süperoksit dismutaz (SOD), katalaz (CAT), askorbat peroksidaz (AP) enzim aktiviteleri belirlenmiştir. Genel olarak, uygulanan tuzluluk tuz muamele süreci ve çeşitli farklılıklara bağlı olarak etkilenmiştir. Ayrıca tuzlulukla birlikte prolin içeriği de artmış, fakat Çengelköy çeşidinde prolin birikimi diğer çeşitlere oranla daha az olmuştur. Sonuçlar, Çengelköy çeşidinin artan antioksidant enzim aktivitesine bağlı olarak oksidatif hasara karşı koruma mekanizmasının Beith Alpha ve Anadolu F1 çeşitlerinden daha iyi olabileceğini göstermiştir. Muhtemelen, Çengelköy'ün içerdiği daha düşük MDA ve prolin içeriği ile Na⁺ ve Cl⁻ konsantrasyonu ve daha yüksek K⁺ konsantrasyonu ile oransal su kapsamı, toplam klorofil içeriği, süperoksit dismutaz, katalaz ve askorbat peroksidaz aktivitesi bu çeşidin tuza Beith Alpha ve Anadolu F1'e oranla daha dayanıklı olmasını sağlamaktadır.

Anahtar sözcükler: Tuzluluk, salatalık, prolin, klorofil, malondialdehit, antioksidant enzimler

Introduction

A better understanding of the physiological and biochemical mechanisms conferring salinity tolerance is key for developing selection and breeding strategies (1). Plant breeding methodologies have been frequently used to develop most of the salt-tolerant crop genotypes in different parts of the world. Unfortunately, no single physiological process can explain why a plant is tolerant or sensitive; rather, it is an interplay of physiological processes controlled by a group of genes. This clearly explains the complexity of salt tolerance (2). Salt tolerance mechanisms are complex, including osmotic adjustment, compartmentation of toxic ions, metabolic accumulation, ion homeostasis, redox control, and scavenging of activated oxygen species (3).

Large amounts of salt in the cytoplasm are not tolerable for plants, whether glycophyte or halophyte. Therefore, as a defensive mechanism to facilitate their metabolic functions under saline conditions, they limit the excess salts in the vacuole or separate the ions into isolated compartments in different tissues (4-6). Glycophytes restrict the absorption of sodium or remove extra sodium into older tissues that act as storage and are eventually sacrificed (7). Other mechanisms of salt regulation are salt secretion and selective salt accumulation or exclusion. Selective accumulation of ions or solutes enables the plants to make osmotic adjustments, which occur through mass action and result in increased water retention and/or sodium exclusion (5).

To accommodate the ionic balance in the vacuoles, cytoplasm accumulates low molecular mass compounds, known as compatible solutes, because they do not interfere with normal biochemical reactions; rather, they replace water in biochemical reactions. These compatible solutes mainly include proline, glycine betaine, sugars, and polyols (3,6).

Salt stress is complex and imposes a water deficit because of osmotic effects on a wide variety of metabolic activities (4,7). This water deficit leads to the formation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide (H_2O_2), hydroxyl radical (OH^\cdot), and singlet oxygen (O_2^\cdot). These cytotoxic activated oxygen species can seriously disrupt normal metabolism through oxidative damage of lipids, proteins, and nucleic acids (8).

When plants are subjected to environmental stress, the balance between the production of reactive oxygen species and the quenching activity of the antioxidants is upset, often resulting in oxidative damage. Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage. The activities of the antioxidative enzymes, such as catalase (CAT), ascorbate peroxidase (APOX), guaiacol peroxidase (POD), glutathione reductase (GR), and superoxide dismutase (SOD), increase under salt stress in plants, and a correlation exists between these enzyme levels and salt tolerance (9,10).

It has been known for many years that there are large differences in salt tolerance among crop species (11). Cucumber (*Cucumis sativus* L.), an important crop produced in Turkey, is moderately susceptible to salinity stress, especially at the germination and seedling stages. There is not much information about the effects of salinity on cucumber species in the current literature, with the exception of a few studies (12-15). The aim of this study was to determine the relative salt tolerance of 3 different cucumber cultivars on the basis of their growth, chlorophyll and relative water content, proline accumulation, lipid peroxidation, and antioxidant changes.

Materials and method

Anadolu F1, Çengelköy, and Beith Alpha cucumber cultivars were provided by Betaseed, Inc. Seeds of each cultivar were placed in plastic pans (20 × 14 × 6 cm) on filter paper and irrigated with half-strength Hoagland nutrient solution for a period of 7 days. Seedlings at the cotyledon stage were transferred to flowerpots and grown under controlled conditions (25 ± 2 °C, 50%-60% relative humidity, a photoperiod of 16 h light and 8 h dark, and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity) in perlite culture. The seedlings were irrigated with half-strength Hoagland nutrient solution for a period of 28 days. After this period, the cucumber seedlings were harvested and samples were taken for analyses as control; then they were treated with 150 mM of NaCl solution. To avoid osmotic shock, the NaCl concentrations were increased gradually by 50 mM each day until the desired concentration was reached. After reaching the

optimum concentration points on days 7 and 14 of salt treatment, the plants were harvested and sampled for analyses.

The fresh weight (FW) and dry weight (DW) (after drying at 70 °C for 48 h) of the leaves were measured. Leaves' relative water content (RWC) was measured according to the method of Smart and Bingham (16). Total chlorophyll content was determined using the method of Lichtenthaler (17). The mineral ions were determined from extracts prepared according to the methods of Prakash and Prathapasenan (18). Na^+ , K^+ , and Ca^{2+} were measured using an Eppendorf flame photometer. Cl^- was measured using a Cotlove chloridometer. The proline content was determined using the method of Bates et al. (19). Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content in 1 g of fresh leaf weight according to the methods of Lutts et al. (20). The concentration of MDA was calculated from the absorbance at 532 nm (correction was done by subtracting the absorbance at 600 nm for unspecific turbidity) by using the extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$. For enzyme assays, leaf samples were frozen in liquid nitrogen and extracted with ice-cold 50 mM phosphate buffer including 0.1 mM of Na-EDTA. All enzyme assays were based on the method of Çakmak and Marshner (21) and Çakmak (22).

All analyses were done according to a completely randomized design. Each experiment was carried out with 3 replications. Data presented are mean \pm standard errors (SE). The results were subjected to analysis of variance (ANOVA). Data analyses were carried out using SPSS. The results were compared with least significant differences (LSD) at the 5% level.

Results

NaCl caused a reduction in the fresh and dry leaf weight of the tested cultivars (Figure 1). The reduction occurred due to the duration of salt treatments and cultivars. When compared to the controls, except for Çengelköy, the fresh and dry leaf weights decreased significantly ($P < 0.05$) in other cultivars on day 7 of salt treatment. However, at day 14 of treatment, salinity caused a significant ($P < 0.05$) decrease in the fresh and dry weight of the 3 tested cultivars. Less important changes were recorded in Çengelköy. The decrease in the rate of fresh weight compared to the control for Çengelköy was 58.1%, which was the lowest, and 69.7% for Beith Alpha, which was the highest loss rate. Dry weight loss with rate of 30.6% for Çengelköy, 58.2% for Anadolu F1, and 50.3% for Beith Alpha was observed when compared to the control.

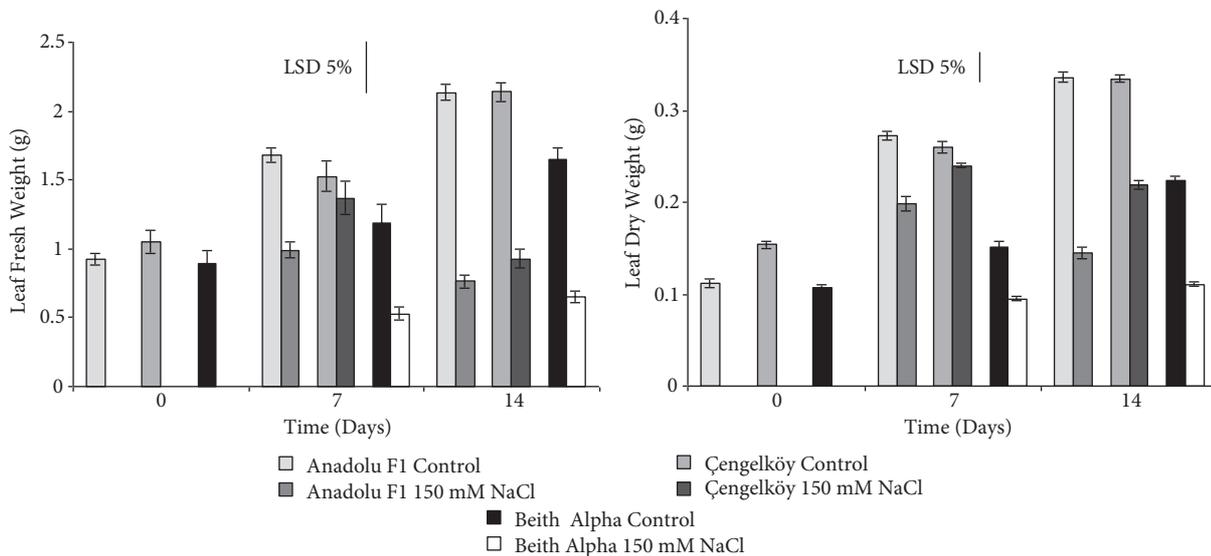


Figure 1. FW and DW of the leaves of 3 cucumber cultivars treated with 150 mM NaCl on days 7 and 14. The values are the means (\pm SE) of 3 replicates.

Salt treatment caused a significant decrease in RWC in all cultivars (Figure 2). Differences between duration of salt treatments and between responses in cultivars were significant ($P < 0.05$). When compared to the controls, the relative decrease of RWC in Çengelköy was lower than in the other cultivars. The decrease was about 3% and 9% of control at days 7 and 14 of salt treatment in Çengelköy, respectively. On the other hand, in Beith Alpha, the reduction in RWC reached about 27% and 36% of the control on days 7 and 14 of salt treatment, respectively.

Salt treatment caused a significant ($P < 0.05$) decrease in total chlorophyll contents, depending on the duration of salt treatment and cultivars (Figure 3). When compared to the controls, the decrease was lower in Çengelköy (24%) than in Anadolu F1 (67%) or Beith Alpha (90%) on day 7 of salt treatment. At the end of 14 days, Beith Alpha showed a lower total chlorophyll content (88%) than Anadolu F1 (79%) or Çengelköy (74%).

The presence of NaCl in the rooting medium induced significant changes in the Na^+ , K^+ , Ca^{2+} , and Cl^- concentrations in the leaves (Table). Salinity caused an increase in the Na^+ and Cl^- contents of all the cultivars. When compared to the control, Beith Alpha had the highest Cl^- content and Anadolu F1

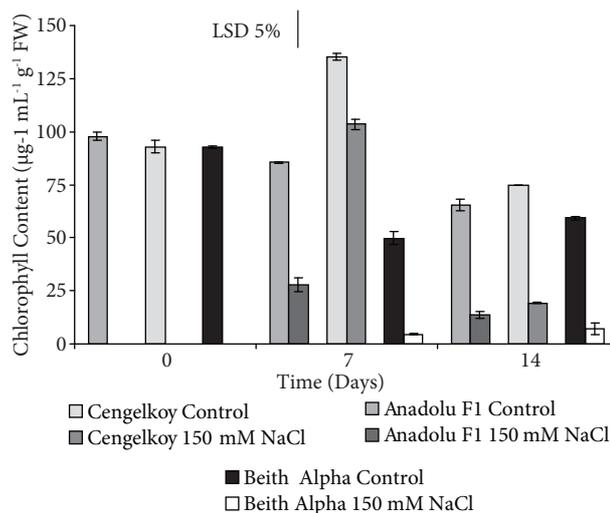


Figure 3. Total chlorophyll content of the leaves of 3 cucumber cultivars treated with 150 mM NaCl on days 7 and 14. The values are the means (\pm SE) of 3 replicates.

had the highest Na^+ contents, but the relative increase was the lowest in Çengelköy on day 14 of salt treatment. K^+ concentration gradually decreased in response to salinity in Beith Alpha and Anadolu F1, and increased in Çengelköy, although the increase in Çengelköy was not significant. After 14 days of salt treatment, the K/Na ratio of Beith Alpha and Anadolu F1 was lower than that of Çengelköy. The Ca^{2+} concentration of all the cultivars significantly decreased with NaCl treatments compared to their controls. The decrease in Ca^{2+} concentration was lower in Çengelköy on day 14 of salt treatment than in the others.

Proline contents were similar in the control groups of all cultivars, but salt treatment caused a significant ($P < 0.05$) increase in proline content depending on the cultivars and treatment periods (Figure 4). When compared to the controls, Beith Alpha had the highest proline content and Çengelköy had the lowest on days 7 and 14 of salt treatment.

The MDA (malondialdehyde, a product of lipid peroxidation) contents in the leaves of the 3 cucumber cultivars after 7 and 14 days of salt treatment are shown in Figure 5. Salinity caused an increase in the MDA content on days 7 and 14 of salt treatment in all cultivars compared to the controls; the increase in MDA was significant on day 14. The increase in MDA

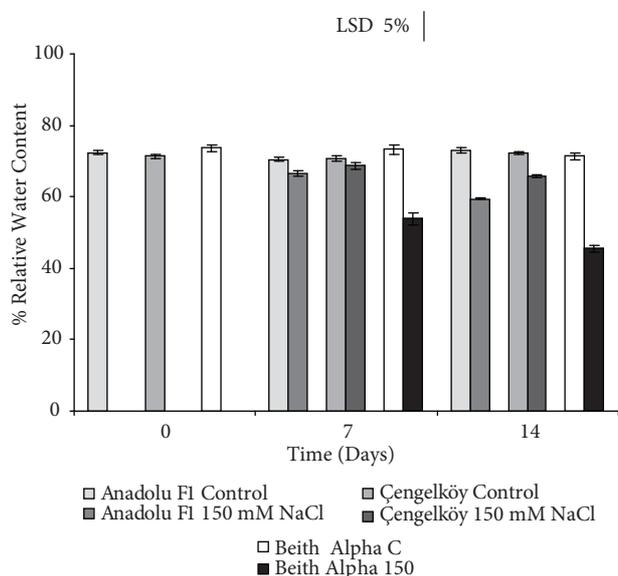


Figure 2. RWC of the leaves of 3 cucumber cultivars treated with 150 mM NaCl on days 7 and 14. The values are the means (\pm SE) of 3 replicates.

Table. Changes in Na⁺, K⁺, Cl⁻, Ca²⁺, and K/Na levels (mg g⁻¹ DW) of the leaves of 3 cucumber cultivars treated with 150 mM NaCl concentration on days 7 and 14. The values are the means (\pm SE) of 3 replicates.

Cultivar		Na ⁺	K ⁺	Cl ⁻	Ca ²⁺	K/Na
Anadolu F1	Day 0	0.441 \pm 0.036	1.889 \pm 0.153	0.005 \pm 0.002	2.093 \pm 0.136	4.295 \pm 0.241
	Day 7 Control	0.920 \pm 0.005	31.670 \pm 1.820	0.009 \pm 0.000	26.540 \pm 0.146	34.430 \pm 2.110
	Day 7 150 mM NaCl	20.400 \pm 0.510	26.670 \pm 3.340	0.087 \pm 0.006	26.070 \pm 0.937	1.317 \pm 0.198
	Day 14 Control	1.331 \pm 0.068	34.360 \pm 2.642	0.005 \pm 0.002	22.570 \pm 1.477	25.750 \pm 0.686
	Day 14 150 mM NaCl	28.510 \pm 1.035	22.180 \pm 0.406	0.174 \pm 0.005	18.545 \pm 0.536	0.780 \pm 0.031
Çengelköy	Day 0	0.425 \pm 0.039	1.615 \pm 0.172	0.006 \pm 0.003	2.273 \pm 0.062	3.880 \pm 0.561
	Day 7 Control	1.174 \pm 0.092	21.620 \pm 1.540	0.006 \pm 0.001	50.400 \pm 3.508	18.510 \pm 1.184
	Day 7 150 mM NaCl	12.600 \pm 1.630	25.530 \pm 3.950	0.079 \pm 0.010	36.100 \pm 1.450	2.060 \pm 0.312
	Day 14 Control	1.480 \pm 0.068	34.815 \pm 1.576	0.005 \pm 0.003	37.760 \pm 2.867	33.660 \pm 3.624
	Day 14 150 mM NaCl	24.040 \pm 1.461	37.450 \pm 2.664	0.147 \pm 0.009	30.000 \pm 1.644	1.580 \pm 0.197
Beith Alpha	Day 0	0.422 \pm 0.024	2.048 \pm 0.093	0.005 \pm 0.000	2.161 \pm 0.103	4.897 \pm 0.445
	Day 7 Control	1.251 \pm 0.138	29.900 \pm 2.560	0.006 \pm 0.001	36.180 \pm 1.370	25.070 \pm 5.340
	Day 7 150 mM NaCl	17.060 \pm 0.800	29.490 \pm 1.120	0.117 \pm 0.009	37.320 \pm 1.114	1.730 \pm 0.035
	Day 14 Control	1.272 \pm 0.086	33.675 \pm 3.090	0.005 \pm 0.002	32.670 \pm 1.341	28.770 \pm 0.706
	Day 14 150 mM NaCl	26.890 \pm 0.493	20.250 \pm 2.233	0.191 \pm 0.006	20.085 \pm 0.186	0.755 \pm 0.093

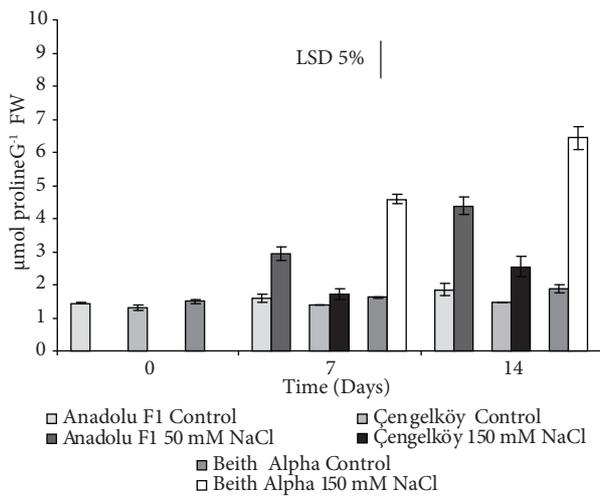


Figure 4. Proline content of the leaves of 3 cucumber cultivars treated with 150 mM NaCl on days 7 and 14. The values are the means (\pm SE) of 3 replicates.

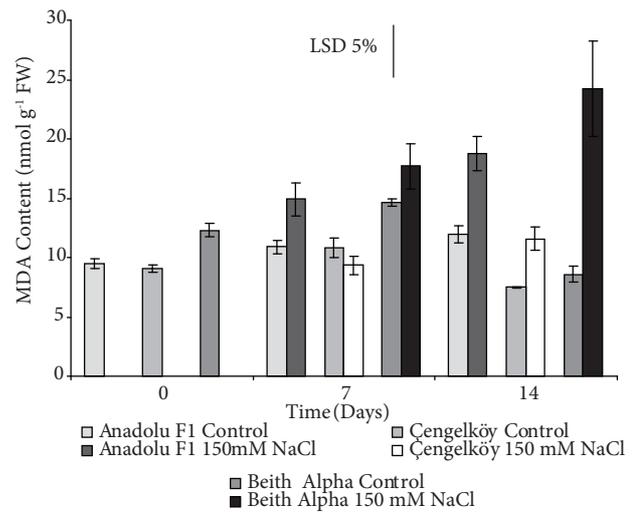


Figure 5. MDA contents of the leaves of 3 cucumber cultivars treated with 150 mM NaCl on days 7 and 14. The values are the means (\pm SE) of 3 replicates.

contents were 182% in Beith Alpha, 57% in Anadolu F1, and 53% in Çengelköy, as compared to the controls.

SOD, CAT, and APOX activities in all cucumber cultivars increased with salinity in respect to the

cultivars and duration of salt treatment. When compared to the controls, 7 days of NaCl treatment caused a significant increase in SOD activity in Anadolu F1 and Çengelköy, but no significant change occurred in Beith Alpha. After 14 days of salt

treatment, SOD activity significantly increased in all tested cultivars. The highest rate of increase in SOD activity was found in Çengelköy on days 7 and 14 of salt treatment (Figure 6).

On day 7 of salt treatments, CAT activity showed a significant increase only in the leaves of Anadolu F1 and Çengelköy. However, at day 14, CAT activity significantly increased in all cultivars (Figure 7). CAT activity was the highest in Çengelköy with an increase of 123% and 134% compared to the control plants at days 7 and 14 of salt treatments, respectively.

After day 7 of salt treatment, APOX activity significantly increased only in Çengelköy as compared to the controls (Figure 8). However, 14 days of salt treatment caused a significant increase in APOX activity in both Anadolu F1 and Çengelköy, with an increase of 60% and 312% compared with the control plants, respectively.

Discussion

Increasing salinity is accompanied by significant reductions in the fresh and dry weights of leaves, stems, and roots; plant height; and the number of leaves per plant (23). Decline in vegetative growth in cucumbers with increasing salinity stress was

reported by Chartzoulakis (12). Abd-Alla et al. (14) reported observed reductions in plant height, leaf area, leaf numbers, and fresh and dry weight of cucumber plants in response to salinity. The results of this study demonstrated the significant effect of salinity on cucumber cultivars. The fresh and dry weight of leaves was reduced depending on the duration of salt treatments. The reduction of the fresh and dry weights due to increased salinity may be a result of a combination of osmotic and specific ion effects of Cl and Na, as reported by Al-Rwahy (24). Similarly, Greenway and Munns (7) reported that the reduction in growth could be a combined effect of osmotic stress, which is more harmful to plants during the succulent seedling stage, and the higher ion uptake.

NaCl treatment induced a reduction in leaves' RWC. This reduction was more important in the less tolerant cultivar, Beith Alpha, than in the more tolerant one, Çengelköy. According to Katerji et al. (25), the decrease in RWC indicated a loss of turgor that resulted in limited water availability for cell extension processes. Ghoulam et al. (26) reported that salt treatment induced a reduction in leaves' RWC and that the growth inhibition in the less tolerant cultivar could be related to the decrease of RWC provoked by salt treatment for the sugar beet.

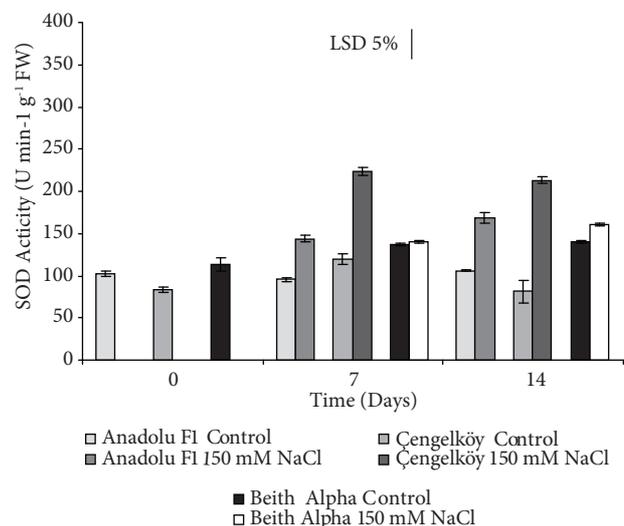


Figure 6. SOD activity of the leaves of 3 cucumber cultivars treated with 150 mM NaCl on days 7 and 14. The values are the means (\pm SE) of 3 replicates.

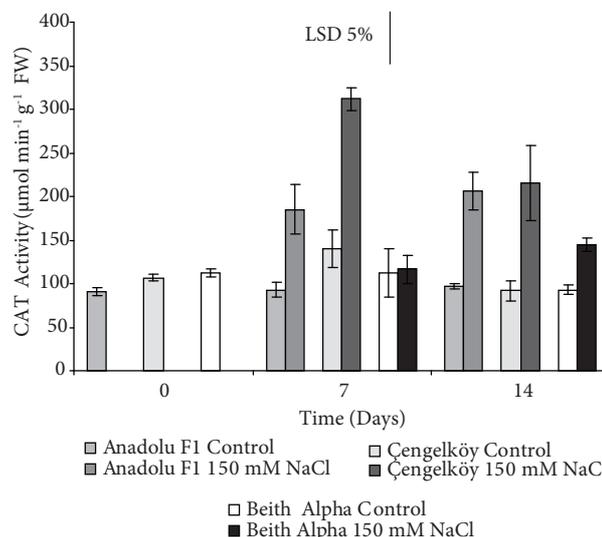


Figure 7. CAT activity of the leaves of 3 cucumber cultivars treated with 150 mM NaCl on days 7 and 14. The values are the means (\pm SE) of 3 replicates.

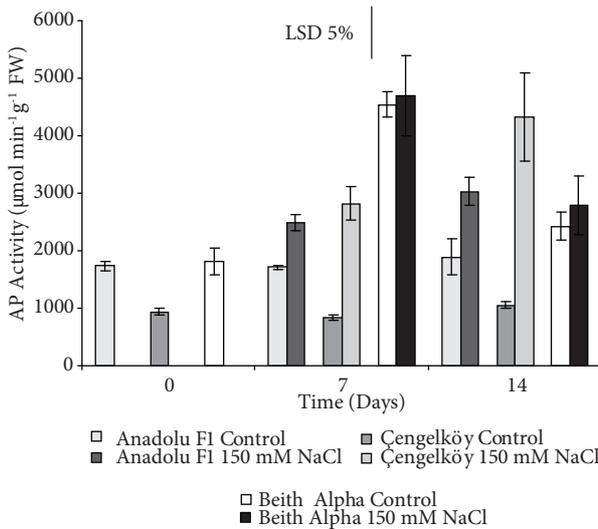


Figure 8. APOX activity of the leaves of 3 cucumber cultivars treated with 150 mM NaCl on days 7 and 14. The values are the means (\pm SE) of 3 replicates.

In this study, total chlorophyll content decreased with salt treatments in all cultivars. The decrease in chlorophyll content under salinity stress is a commonly reported phenomenon in various studies, because of its adverse effects on membrane stability (27,28). The decrease of chlorophyll in the presence of salt ions has also been described by Loggini et al. (29), Meloni et al. (30), and Parida and Das (23). According to these authors, the reduction in chlorophyll content might be due to the enhancement of chlorophyllase activity at higher salinity levels. Garcia-Sanchez et al. (31) also reported that the observed decrease in chlorophyll content in cucumber plants grown under saline conditions may be attributed to both an increased degradation and inhibited synthesis of chlorophyll pigment. Salt stress induces a reduction in chlorophyll content, affects photosynthetic electron transport, and inhibits PSII activity as a consequence of the accumulation of salts in chloroplasts (32). This reduction in photosynthetic activity has been directly related to the reduction in yield (30). In our study, whereas all cultivars accumulated Na^+ and Cl^- ions in the leaves, Çengelköy, in which the total chlorophyll content was the highest but the MDA content was the lowest, accumulated fewer Na^+ and Cl^- ions than the others.

Salinity exhibits its adverse effects on plants through the decreased water potential of the root medium, through ion toxicity due to excessive Na^+ or Cl^- uptake, and through nutrient ion imbalance by the disturbance of essential intracellular ion concentrations. It also affects plant growth by inducing nutrient (notably K^+ , Ca^{2+} , and Mg^{2+}) deficiency (33). In our study, all cultivars accumulated Na^+ and Cl^- ions in the leaves, but Çengelköy accumulated fewer ions than the others (Table).

Slama et al. (34) reported that salinity only led to a decrease in the K^+ concentration of roots in pepper plants, although under saline conditions the K^+ content in many glycophytes is severely reduced. That may have resulted from the substitution of Na^+ for K^+ , or an increased K^+ efflux out of the roots to the surrounding medium. This suggests that pepper plants are able to maintain high K^+ levels in leaf lamina, which may act as the major monovalent cationic osmoticum in the presence of external salt. The decrease of K^+ recorded in pepper root, which resulted in a low K/Na ratio, may also provide a mechanism by which pepper achieves ionic balance following the uptake of high Na^+ concentrations in its roots. Our results showed that the ability to maintain a relatively high K^+ concentration in leaves may provide the cucumber with a successful mechanism for tolerance to low and moderate salinity levels. Perez-Alfocea et al. (35) reported that, in *L. esculentum*, different degrees of salt tolerance are associated with different responses to salinity. Some cultivars can show an inclusion mechanism, allowing the replacement of K^+ for Na^+ ; however, some others can show an exclusion mechanism with K^+ selectivity.

Osmoregulation can occur in plants by active uptake of inorganic ions (such as Na^+ , K^+ , and Cl^-) or synthesis of organic solutes (such as sugars, organic acids, free amino acids, and proline), depending on the species (5). Salt-induced injuries can occur not only due to osmotic and oxidative effects, but also toxic and nutrient deficiency effects of salinity. Accumulation of Na^+ changes ion balances, such as Na/Ca and K/Na , in plant cells under saline conditions. While the change in the Na/Ca balance results in increased cell permeability, the change in the K/Na balance causes decreased use of metabolic energy (5). In our study, depending on the species, salt

stress resulted in the reduction of K^+ and Ca^{2+} concentrations and the increase of Na^+ concentration, resulting in a K/Na ratio inadequate for plant growth.

The concentration of proline in many salt-tolerant plants has been found to be higher than that of salt-sensitive plants. However, the role of proline in salt tolerance has generally been questioned (36). Lutts et al. (20) did not find a significant role of proline in salt-stressed rice and reported that proline did not take part in osmotic adjustment. In tomatoes, salt-tolerant wild cultivars accumulated less proline in their leaves than the salt-sensitive cultivars (37). Moftah and Michel (38) reported that proline contributed less than 1% to osmotic adjustment in salt-stressed soybeans. Ashraf (39) concluded that contrasting reports on the role of proline in salt tolerance and its use as a selection criterion for salt tolerance have been questioned. In our study, except for Çengelköy, in which the proline content did not change significantly, the NaCl treatment induced a marked increase in proline content in the leaves of all cultivars. This result suggests that proline accumulation may be a response to leaf damage in the tested cucumber cultivars.

Cellular membrane integrity, evaluated by electrolyte leakage and lipid peroxidation and measured as MDA, appeared to be a more reliable index of salt tolerance (20,40). In this study, salinity caused an increase in MDA content on days 7 and 14 of salt treatment in all cultivars when compared to the controls, but the increase in MDA was only significant on day 14. A lesser increase observed in the MDA content of Çengelköy than the other cultivars points to its higher tolerance to oxidative damage caused by salinity.

To scavenge reactive oxygen species (ROS) produced under conditions that favor increased oxidative stresses such as extreme temperatures, water deficits, pollutants, and salinity, plants possess enzymatic and nonenzymatic antioxidant systems. Among the antioxidant enzymes, SOD decomposes $O_2^{\cdot-}$ to H_2O_2 , which is later decomposed by CAT and APOX to H_2O and $O_2^{\cdot-}$ (41). Significant evidence underlines the intimate relationship between enhanced antioxidant enzyme activities and increased resistance to environmental stresses (42), and comparatively higher activity has been reported in tolerant cultivars than in the susceptible ones (43). In

this study, there were also striking differences in antioxidant enzyme activities between the 3 cultivars according to NaCl concentration. Salt treatment caused a significant increase in SOD, but the percentage of increase in activity was higher in Çengelköy than the other cultivars. A significant increase in SOD activity could increase the ability of the leaves to scavenge $O_2^{\cdot-}$ radicals, which could cause membrane damage. Increases in SOD activity and differential varietal salt sensitivity have also been reported in salt-stressed wheat (44) and rice (42,45). A higher constitutive and/or induced activity of SOD was also reported in wild beet and bean species (40,46), confirming a correlation between increased SOD activity and relative tolerance to salt in different genotypes. Zaefyzadeh et al (47) reported that SOD production is one of the stress confrontation system under oxidative stress that is activated in drought and salinity conditions.

APOX, an enzyme that belongs to the Halliwell-Asada pathway, plays a key role in the removal of H_2O_2 from the chloroplasts and cytosol of higher plants (48). In our study, APOX activity significantly increased with increased NaCl concentration in the leaves of all cucumber varieties and, as with SOD, its activity was highest in Çengelköy, reaching 312% of that of the control. Sairam et al. (49) also found that decreased levels of lipid peroxidation were associated with a higher activity of APOX in drought tolerant wheat genotypes.

CAT eliminates H_2O_2 by decomposing it directly to water and oxygen (50). In the present study, NaCl induced an increase in CAT activity in the leaves of the 3 tested cultivars. However, the greater increase in CAT activity in Çengelköy than Anadolu F1 or Beith Alpha may suggest that Çengelköy has a more effective H_2O_2 dismutation capacity outside the chloroplasts under salinity. Confirming our results, Sudhakar (43) and Sairam et al. (44) also reported a higher CAT activity in salt-resistant varieties of mulberry and wheat than in sensitive varieties, respectively. Considering that similar results were also obtained for APOX activities, it may be suggested that CAT and APOX, which are both responsible for detoxification of H_2O_2 , are probably equally important in the detoxification step in Çengelköy. Similar results have been reported by Perez-Lopez et al. (51). They

researched the oxidative stress caused by salinity in 2 barley cultivars, Iranis and Alpha. They concluded that the parallel enhancement of APOX and CAT activity suggests that both routes are important for the elimination of H₂O₂ under salinity, and that Iranis might be the more salt-tolerant cultivar because of its higher constitutive SOD and CAT activities.

Based on the experimental results, we concluded that all of the considered parameters were affected by salinity with a varietal difference. Çengelköy was found to be the cultivar more tolerant to salinity because it had lower Na⁺ and Cl⁻ concentrations and proline content, and higher K⁺ concentrations, RWC, and total chlorophyll content than the other cultivars. Çengelköy's response to salinity could be related to its

osmotic adjustment. Additionally, the higher SOD, CAT, and APOX activities and the lower degree of membrane damage, as indicated by the low MDA content observed in Çengelköy, indicate a relationship between salt tolerance and the antioxidant defense system.

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