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Original article

Determination of Salt Tolerance of Some Barley Varieties Based on Physiological and Biochemical Properties

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Abstract

Salt stress is one of the important environmental factors limiting the growth and productivity of barley (*Hordeum vulgare* L.) worldwide. Increasing salt stress negatively affects plant growth and development, posing a threat to global food security. In this study, some physiological and biochemical effects of salt stress at different concentrations (0, 50, 100, 200 mM NaCl) on 8 barley varieties (*Kıral-97, Harman, Yaprak, Yaba, Larende, Cumhuriyet-50, Kalaycı-97, Çıldır-02*) grown in Turkey were determined. For this purpose, the effects of salt stress on root-shoot length, biomass, pigment content, specific leaf area (SLA), relative water content (RWC), lipid peroxidation content (TBARS), hydrogen peroxide (H_2O_2) (spectrophotometric and histochemical staining) content were determined in 35-day-old seedlings. Our results showed that increased salt stress decreased the root-shoot lengths, biomass, SLA, and pigment contents in *Cumhuriyet-50* and *Çıldır-02* varieties, while increasing the amount of TBARS and H_2O_2. It was determined that *Yaprak* and *Yaba* varieties were less affected by salt stress and were more resistant to salinity compared to other varieties.

Keywords: *Hordeum vulgare* L., Salt stress, Growth, NaCl, Hydrogen peroxide.

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INTRODUCTION

Barley is one of the most important field crops grown in the world and ranks fourth after wheat, corn, and rice in terms of production amount (Payandeh et al., 2021). Barley, which is widely used in the animal feed, food, and malt industry in the world and in Turkey, has spread over wide geography due to its resistance to adverse climatic conditions (Hierro et al., 2021). While it produces 51.6 million hectares, 157 million tons of production, and 3043 kg/ha yield in the world, 3.09 million hectares of cultivation, 8.3 million tons of production, and 2684 kg/ha yield are obtained in Turkey (Anonymous, 2020).

Plants show their healthy growth and development stages in physiologically appropriate growth conditions. However, when suitable growing conditions are disrupted, plants perceive this situation negatively and become stressed. These stresses are abiotic (drought, salinity, temperatures, etc.) and biotic (plants, animals, microorganisms, etc.) factors (Lichtenthaler, 1996).

Salinity is the second most important abiotic factor affecting agricultural productivity in the world. Salinity occurs in arid and semi-arid areas in two ways. The first of these occurs naturally in the soil or the salty groundwater close to the surface, while the second occur due to unconscious irrigation in agricultural areas (Anonymous, 2005). It is well known that salinity is an important problem that reduces productivity in agricultural lands (Munns & Tester, 2008). The effects of salt stress occur in two different types osmotic and ion stress (Munns et al., 1995). Salt accumulation in the soil, the plant restricts the uptake of water from the roots and resulting in osmotic stress. Osmotic stress restricts nutrient intake and causes deterioration of nutrient balance and membrane properties. It reduces photosynthetic activity and stomatal opening. As a result of decreased photosynthetic activity, reactive oxygen species (ROS) formation increases and oxidative damage occurs (Munns & Tester, 2008; Rahnama et al., 2010). As a result of salt presence in the soil, ion stress caused by the accumulation of Na^+ and Cl^- ions in plant tissues. It prevents the uptake of K^+ and Ca^{2+} ions, which are important for Na^+ cells, and the regulation of stomatal conductance. Cl^- causes degradation in photosynthetic activity by causing chlorophyll destruction (Tavakkoli et al., 2011). As a result of excessive ion uptake in roots and leaves, the ion balance is disturbed, and physiological disorders occur. As a result of salt stress, the amounts of ROS such as singlet oxygen ($^1O_2$) superoxide radical ($O_2^{\cdot}$), hydroxyl radical ($OH^{\cdot}$), and hydrogen peroxide (H_2O_2) increase (Abdelgawad et al., 2016).

The antioxidant defence system plays an important role in preventing the excessive increase of ROS in the cell content and the damage caused by this increase. It consists of enzymatic (superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione peroxidase (GPX), guaiacol peroxidase (GOPX), glutathione transferase (GST)) and non-enzymatic

(ascorbic acid (ASH), glutathione (GSH), phenolic compounds, alkaloids, non-protein amino acids, α-tocopherols, etc.) antioxidants (Gill & Tuteja, 2010).

To maintain agricultural productivity in areas where salinity stress exists, it is very important to determine the tolerance of varieties to salinity. In this study, physiological and biochemical effects of salt stress at different concentrations (0, 50, 100, 200 mM NaCl) on 8 barley varieties (*Kıral-97, Harman, Yaprak, Yaba, Larende, Cumhuriyet-50, Kalaycı-97, Çıldır-02*) grown in Turkey were determined. For this purpose, the effects of salt stress on root shoot length, biomass, photosynthetic pigment content, specific leaf area (SLA), relative water content (RWC), lipid peroxidation content (TBARS), hydrogen peroxide content (H_2O_2) (spectrophotometric and histochemical staining) in 35-day-old seedlings were investigated.

MATERIALS and METHODS

Plant Materials, Seed Sterilization, Salt Stress Treatment, and Growth Condition

Barley (*Hordeum vulgare* L.) from the family *Poaceae* and a member of the grass family, is a major cereal grain grown in temperate climates globally. In this study, barley seeds were used, and the seeds were obtained from Konya Bahri Dagdas International Agricultural Research Institute (*Kıral-97*, *Larende*), Transitional Zone Agricultural Research Institute (*Cumhuriyet-50*, *Kalaycı-97*, *Çıldır-02*), Trakya Agricultural Research Institute (*Harman*, *Yaprak*, *Yaba*). Surface sterilization of seeds was carried out with a 5% sodium hypochlorite solution. At the end of the treatment, the seeds were washed with distilled water and their surfaces were dried at room temperature. After the seeds were germinated in the petri dish for 3 days, they were transferred to pots containing perlite. Barley seedlings were grown with Hoagland nutrient solution (Hoagland & Arnon, 1950) for 21 days in a growth chamber (24±2°C temperature, 16/8 h day/night photoperiod) under controlled conditions. Salt stress (Control (0 mM), 50 mM, 100 mM, 200 mM NaCl) was started on the 21st day following seed sowing and on the 14 days of stress, root-shoot length, biomass, specific leaf area (SLA), relative water content (RWC), photosynthetic pigment content, lipid peroxidation (TBARS) content and hydrogen peroxide (H_2O_2) content (spectrophotometric and histochemical staining), detection of was determined (Figure 1).

Figure 1. Salt stress (Control (0 mM), 50 mM, 100 mM, 200 mM NaCl) was initiated on *Hordeum vulgare* L. seedlings on the 21st days and salt stress was applied for 14 days.

Physiological and Biochemical Analysis

Root and Shoot Length

The green part up to the root was determined as the shoot length (cm) and the root part as the root length (cm) of the wheat seedlings in all groups with the help of a ruler.

Biomass

Biomass the weight of three seedlings from each group was determined by weighing on the precision scale (g plant^{-1}).

Relative Water Content (RWC)

To determine relative water content (RWC), 9 leaves from each group were weighed immediately (FW) after harvesting the plant. Leaves were then placed in distilled water for 4 h and then turgid weight (TW) was measured. Then the leaves were dried in oven at 70°C for 24 h to obtain their dry weight (DW). RWC was calculated by the following formula (Formula 1.) (Smart & Bingham, 1974).

$$RWC\% = (FW - DW) / (TW - DW) \times 100 \quad (1)$$

Specific Leaf Area (SLA)

Specific leaf area (SLA) was calculated using the leaf photos of wheat seedlings in the Image J program. Then the samples are dried in an oven at 70°C for 24 h and weighed on a precision scale. SLA is calculated by the formula (Formula 2.) (Wilson et al., 1999).

$$SLA = Area\ (cm^2) / Dry\ weight\ (mg^{-1}) \quad (2)$$

Photosynthetic Pigment Content

Determination of photosynthetic pigment contents, 0.1 g of tissues taken from the leaves of the plants were homogenized in 80% acetone. The absorbance values determined spectrophotometrically from the homogenate at 663, 645 and 480 nm were calculated according to Arnon (1949) using the following formula 3:

Chlorophyll a (Chla) = (A663 x 12.70) – (A645 x 2.69) x 10/mg

Chlorophyll b (Chlb) = (A645 x 22.90) – (A663 x 4.68) x 10/mg

Total chlorophyll (Chlt) = (20.2 x A645) + (8.02 x A663) x 10/mg

Carotenoid (Car) = ((A480 + (A663 x 0.114) – (A645 x 0.638))/112.5 x 10/mg (3)

Histochemical Staining Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) was detected in leaves by solutions staining immersed with 1 mg mL^{-1} 3′,3′-diaminobenzidine (DAB) in a solution containing at 25°C for 12 h. The incubated leaves were decolorized by immersion in boiling ethanol (90%) for 15 min to visualize the reddish-brown spots of H_2O_2. Then stained leaves were photographed against a contrasting background for proper visual (Kumar et al., 2014).

Hydrogen Peroxide Content (H_2O_2)

Hydrogen peroxide content, a mixture of plant tissue (0.1 g), 3 mL of H_2SO_4 and cold acetone was homogenized with homogenization buffer and centrifuged. Supernatants were determined at 550-800 nm (μg mL^{-1}) spectrophotometric with a reading buffer containing H_2SO_4, purified water, ferrous ammonium sulfate, xylenol orange, sorbitol, and ethanol (e-FOX) (Cheeseman, 2006).

Lipid Peroxidation Content (TBARS)

The thiobarbituric acid reactive substance (TBARS) levels in leaf tissues were measured and analyzed according to the method of Madhava Rao & Sresty (2000) (nmol g fresh weight^{-1}, €=155 mM^{-1} cm^{-1}). Leaf samples (0.1 g) were homogenized in 2.5 mL trichloroacetic acid (TCA 0.1%). The supernatant was mixed with 4 mL trichloroacetic acid (20% TCA) containing thiobarbituric acid (0.5% TBA). The mixture was then exposed to 95°C temperature for 30 min. Following cooling, the absorbance values were recorded at 532 nm and 600 nm.

Statistical Analysis

The results were given as means ± standard error of five replicates. The compiled data were subject to an ANOVA (ONE WAY) and the differences between the means were compared by the Tukey test to assess the effect of physiological and biochemical properties in barley (*Hordeum vulgare* L.) during salt stresses. Those comparisons with $P \leq 0.05$ were taken as significantly different. The data were analyzed by using SPSS 22.0 software (Demirbaş & Acar, 2017).

RESULTS and DISCUSSION

Generally, shoot length declined as NaCl concentration increased. Compared to the control with the treatment of 50 mM NaCl concentrations, shoot length of *Kıral-97*, *Harman* and *Cumhuriyet-50* decreased by 10.65%, 11.87% and 4.28%, respectively. Especially, 200 mM NaCl decreased the shoot length of *Kıral-97* and *Cumhuriyet-50* varieties by 15.79% and 12.45%, respectively, compared to the control. These results indicated that *Kıral-97* and *Cumhuriyet-50* varieties were more susceptible than the other varieties at all NaCl levels based on the shoot growth. However, 200 mM NaCl concentrations shoot length of *Yaprak* and *Yaba* increased 5.12%, 2.15% respectively. Besides, 200 mM NaCl provided more effective protection in *Yaprak* and *Yaba* by reducing the inhibition of root and shoot length caused by salt stress (Figure 2), but the lowest value was determined as 14.59% and 20.29% in *Kalaycı-97* and *Harman* at 200 mM NaCl compared to the control, respectively. On the other hand, the highest values were determined by %14.58 and 41.37% in *Larende* and *Kalaycı-97* with 200 mM NaCl stress compared to control, respectively.

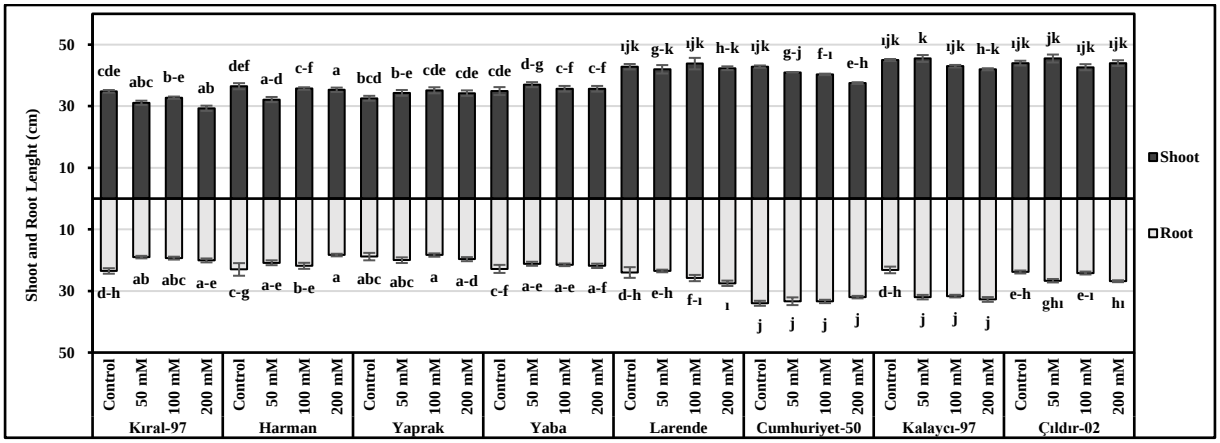


Figure 2. The effects of on root and shoot of 14-day some barley (*Hordeum vulgare* L.) varieties under salt stress (Control (0 mM), 50 mM, 100 mM, 200 mM NaCI). (Means values followed by different letters are significantly different at P <0.05).

Biomass decreased especially in *Kıral-97, Larende, Cumhuriyet-50* and *Çıldır-02* varieties with increasing salt stress compared to control. In addition, compared to the control with the treatment of 200 mM NaCl concentrations, the biomass of *Kıral-97, Larende* and *Cumhuriyet-50* decreased 17.52%, 15.57% and 38.09% respectively. On the other hand, in the application of 200 mM NaCl, it increased by 31.51%, and 27.02% in *Yaprak* and *Yaba*, compared to the control, respectively (Figure 3).

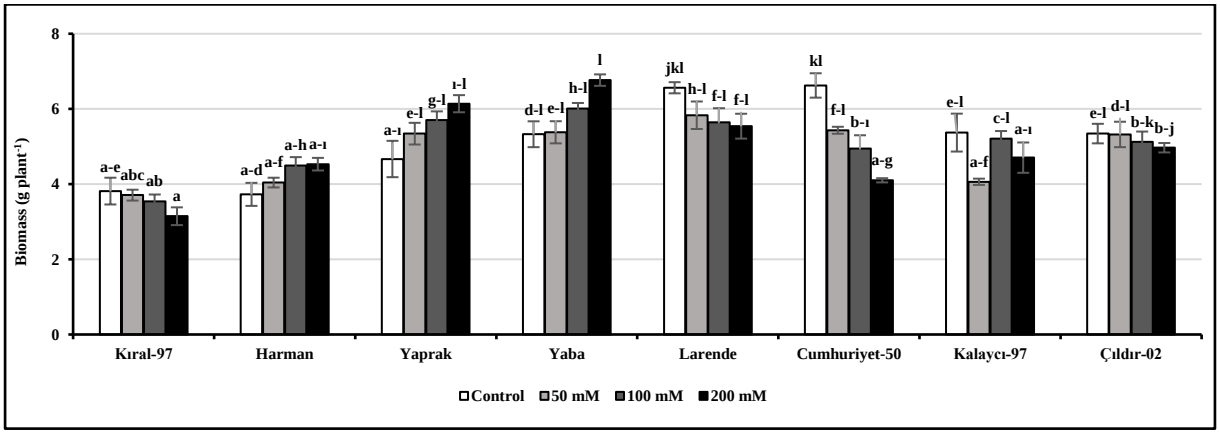


Figure 3. The effects of on biomass of 14-day some barley (*Hordeum vulgare* L.) varieties under salt stress (Control (0 mM), 50 mM, 100 mM, 200 mM NaCI). (Means values followed by different letters are significantly different at P <0.05).

Relative water content decreased in all barley varieties with increased salt stress application compared to control. In addition, in the application of 200 mM NaCl, it decreased by 8.39% and 15.55% in *Harman* and *Kalaycı-97* compared to the control, respectively (Figure 4).

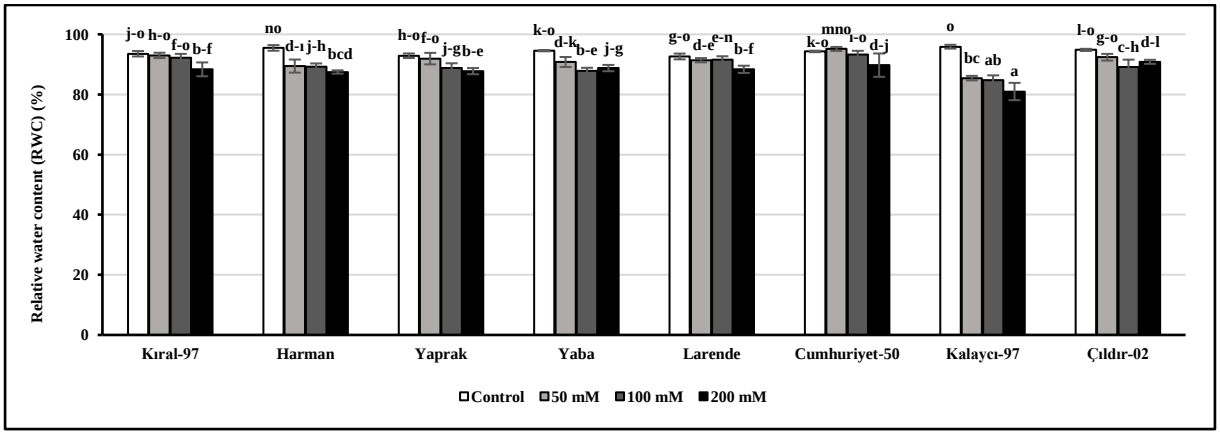


Figure 4. The effects of on relative water content (RWC) of 14-day some barley (*Hordeum vulgare* L.) varieties under salt stress (Control (0 mM), 50 mM, 100 mM, 200 mM NaCI*).* (Means values followed by different letters are significantly different at P <0.05).

Specific leaf area decreased in *Kıral-97*, *Larende*, *Cumhuriyet-50, Kalaycı-97* and *Çıldır-02* varieties with increased salt stress application compared to control. Especially, in the application of 200 mM NaCl, it decreased by 25.34% and 21.20% in *Cumhuriyet-50* and *Çıldır-02* varieties compared to the control, respectively. However, in the application of 200 mM NaCl, it increased by 20.83% and 7.28% in *Yaba* and *Yaprak* compared to the control, respectively (Figure 5).

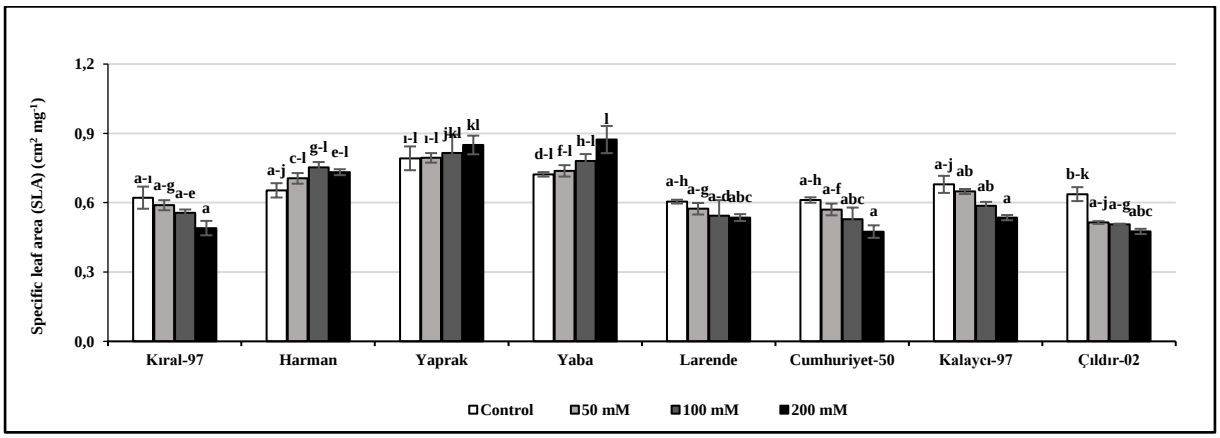
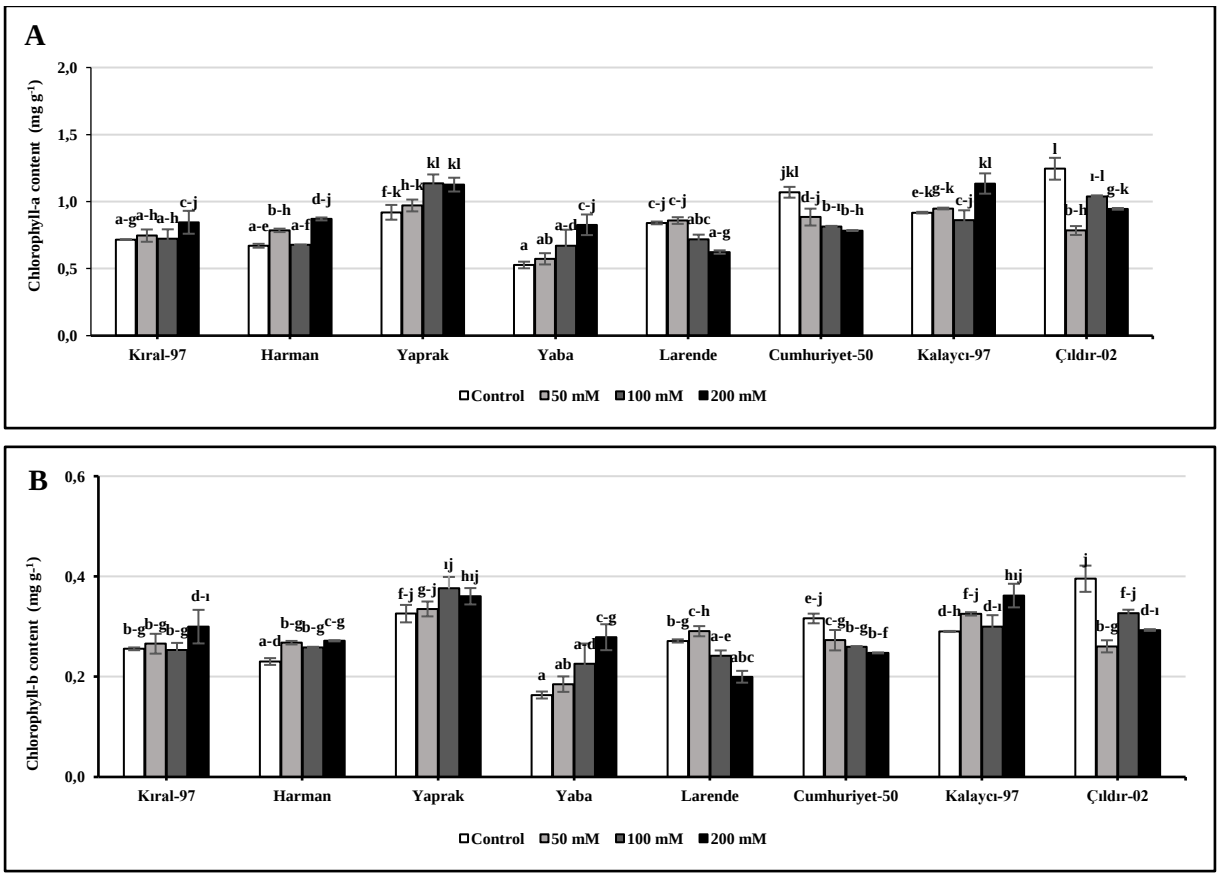


Figure 5. The effects of on specific leaf area (SLA) of 14-day some barley (*Hordeum vulgare* L.) varieties under salt stress (Control (0 mM), 50 mM, 100 mM, 200 mM NaCI). (Means values followed by different letters are significantly different at P <0.05).

While Chla content decreased by 17.30% and 37.01% in *Cumhuriyet-50* and *Çıldır-02* compared to the control 50 mM NaCl application respectively, it increased by 6% and 4% in *Yaprak* and *Yaba* varieties compared to the control, respectively. On the other hand, in the application of 200 mM NaCl, it decreased by %26.71 and 24.03% in *Cumhuriyet-50* and *Çıldır-02*, compared to the control, respectively. However, in the application of 200 mM NaCl, it increased by 22.45%, 56.87% and 25.86% in *Yaprak*, *Yaba* and *Larende* compared to the control, respectively (Figure 6A). While Chlb contents increased, in *Kıral-97, Harman*, *Yaprak*, and *Yaba* compared to the control, under 200 mM NaCl stress,

it decreased in *Larende*, *Cumhuriyet-50*, *Kalaycı-97* and *Çıldır-02* varieties compared to the control. However, this increase was mostly 70% in the *Yaba* variety compared to the control (Figure 6B). It was Car contents in the application of 50 mM NaCl, it increased by 12.24% in *Harman*, compared to the control. However, in the application of 50 mM NaCl, it decreased by 14.57% and 31.51% in *Cumhuriyet-50* and *Çıldır-02*, compared to the control, respectively. On the other hand, compared to the control with treatment 200mM NaCl concentrations, Car content of *Yaprak* and *Yaba* increased by 19.97%, and 45.49% respectively (Figure 6C). Chlt decreased by 16.48% and 36.31% in *Cumhuriyet-50* and *Çıldır-02* varieties compared to the control, respectively. However, in the application of 200 mM NaCl, it increased by 60.10% especially in *Yaba*, compared to the control (Figure 6D). Similarly, PSII in the salt tolerant barley variety was shown to be less damaged by salt stress compared to the sensitive one (Akhter et al., 2021). In addition, El Goumi et al. (2014) supports the physiological changes in barley varieties due to increased salt concentration in our study.



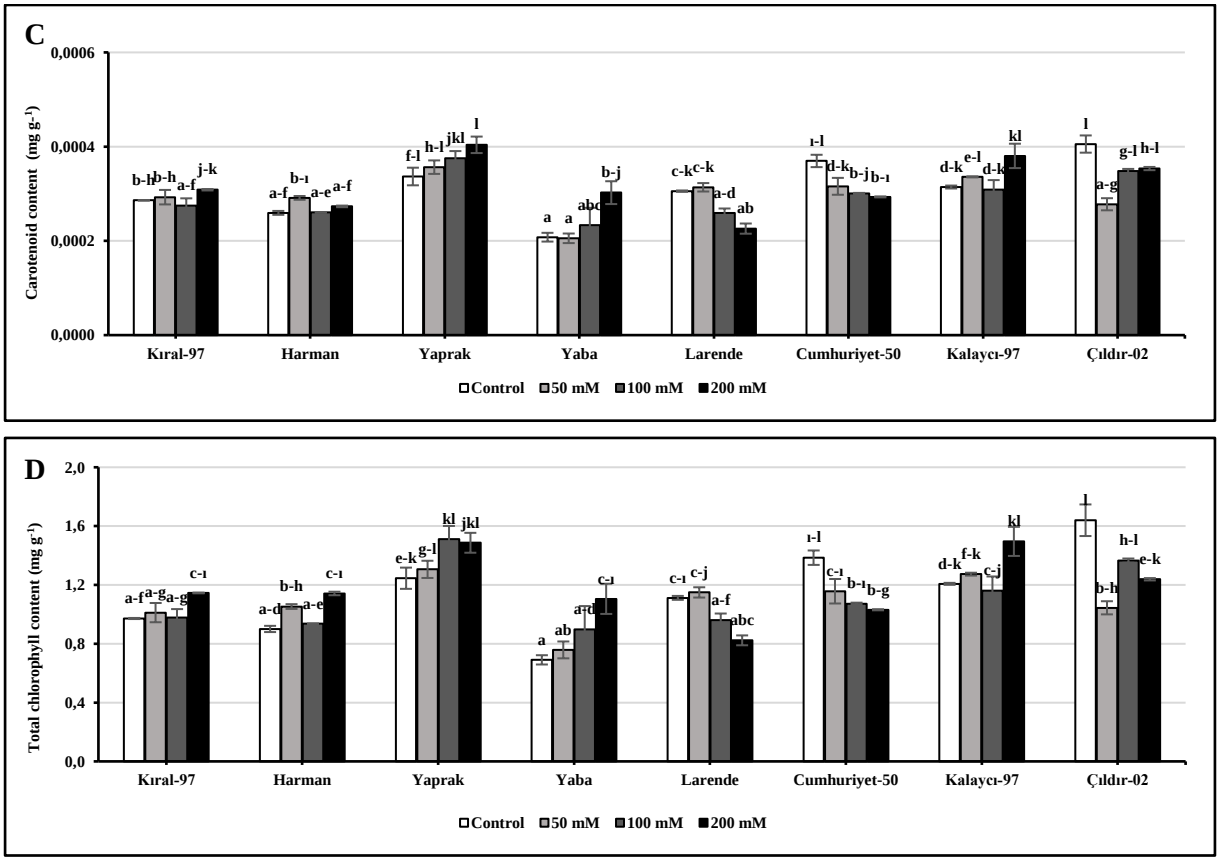


Figure 6. The effects of on photosynthetic pigment contents of 14-day some barley (*Hordeum vulgare* L.) varieties under salt stress (Control (0 mM), 50 mM, 100 mM, 200 mM NaCI). (A; Chl-a content, B; Chl-b content, C; Car, D; Chlt). (Means values followed by different letters are significantly different at P <0.05).

Lipid peroxidation contents (TBARS) were increased in *Kıral-97, Larende, Cumhuriyet-50, Kalaycı-97 and Çıldır-02* varieties in 50 mM NaCl application compared to control. Similarly, TBARS content of *Cumhuriyet-50* and *Çıldır-02* varieties increased by 74.59% and 70.38%, respectively, in 200 mM NaCl application compared to the control. In contrast, compared to the control, it decreased by 60% and 36.29%, respectively, in *Yaprak* and *Yaba* varieties with 200 mM NaCl application. (Figure 7).

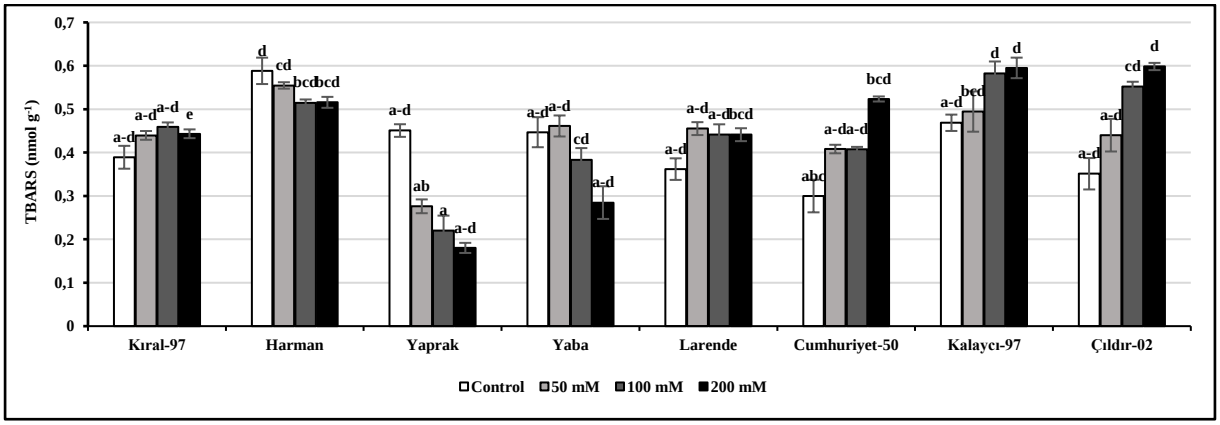


Figure 7. The effects of on TBARS content of 14-day some barley (*Hordeum vulgare* L.) varieties under salt stress (Control (0 mM), 50 mM, 100 mM, 200 mM NaCI). (Means values followed by different letters are significantly different at P <0.05).

Excessive production of ROS is one of the fatal effects of salinity that leads to oxidative stress. Hydrogen peroxide (H_2O_2) content in the tissues was determined by the histochemical staining method used for the direct detection of H_2O_2 in plant leaf tissue (Figure 10 A, B). Accordingly, the H_2O_2 content was determined that the spectrophotometric method increased in *Kıral-97*, *Harman*, *Larende*, *Cumhuriyet-50, and Çıldır-02*, compared to the control, and decreased in *Yaba*, *Yaprak*, and *Kalaycı-97* (Figure 10A). Especially, in the application of 200 mM NaCl, it increased by 59.35% and 65.35% in *Kıral-97* and *Harman* compared to the control, respectively. Barley plants under salt stress exhibit symptoms of oxidative stress, as shown by increased ROS production. Histochemical analysis was, thus, used to investigate the effect of ROS including H_2O_2, using 3,3′-diaminobenzidine (DAB) staining. Under salt stress, the accumulation of brown spots was much lower in the *Yaprak* and *Yaba* than in the other barley varieties. These results suggest that the improved salt stress tolerance of *Yaprak* and *Yaba* plants may be due to reduced ROS production. On the other hand, more brown spots accumulation of other barley varieties that it is indicative of increased ROS production (Figure 10B). Our findings also supported by Seçkin et al. (2010) who reported the salt tolerance mechanism in natural salt-tolerant barley species works with more effective biochemical pathways than the agricultural barley varieties.

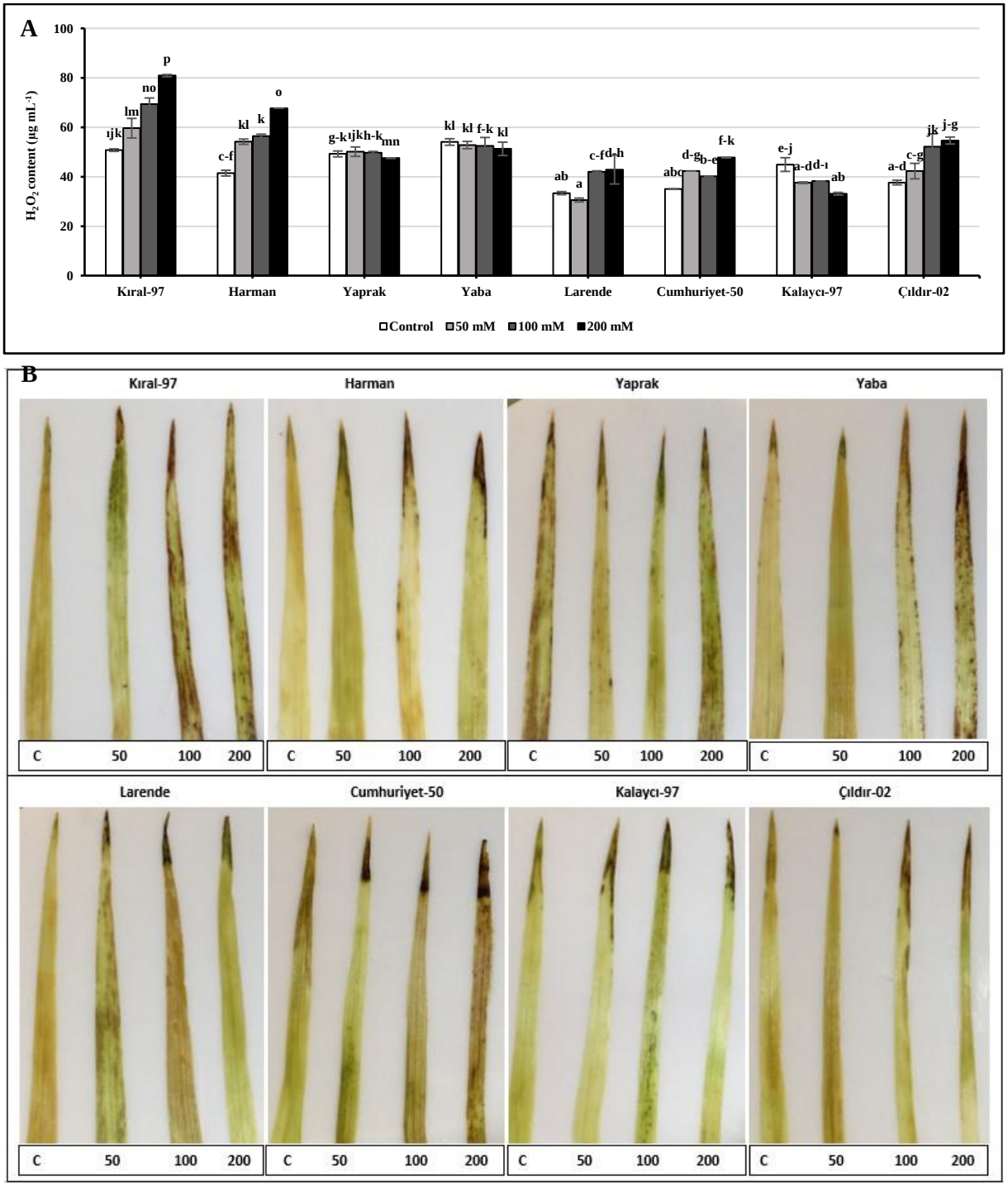


Figure 8. The effects of on hydrogen peroxide content (H_2O_2) (µg/ml) (A) and detection of H_2O_2 by DAB (DAB, 3,3′-diaminobenzidine) staining (B) of 14-day some barley (*Hordeum vulgare* L.) varieties under salt stress ((A); Control (0 mM), 50 mM, 100 mM, 200 mM NaCI); (B); C: Control, 50: 50 mM NaCI, 100: 100 mM NaCI, 200: 200 mM NaCI). (Means values followed by different letters are significantly different at P <0.05).

Conclusion

Salt stress adversely affects plant growth and development through physiological and biochemical processes. This study provides new information about the salt tolerance of some barley cultivars grown

in Turkey based on their physiological and biochemical properties. Biomass, shoot and root lengths reflect plant growth. Our research results showed that the growth of *Kıral-97* and *Cumhuriyet-50* cultivars were inhibited under severe salt stress. On the other hand, it was determined that *Yaprak* and *Yaba* varieties tolerated the highest salt concentration in this study. In addition, the decrease in chlorophyll content of *Larende, Cumhuriyet-50* and *Çıldır-02* cultivars due to salt stress was found to be compatible with the lipid peroxidation and hydrogen peroxide data of these cultivars.

According to our results, *Cumhuriyet-50, Larende* and *Çıldır-02* were most sensitive to salt stress than other varieties. Secondly, *Kıral-97*, *Harman* and *Kalaycı-97* varieties were found moderate tolerant under salinity. Finally, it can be concluded that *Yaprak* and *Yaba* varieties are tolerant based on physiological and biochemical parameters.

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