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Some Physiological and Biochemical Effects of *Bacillus thuringiensis* LU3 Biopriming in Common Wheat (*Triticum aestivum* L.) under Salt Stress

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ABSTRACT
Salt stress is one of the main abiotic stresses limiting sustainable crop production in the world. Biopriming is the technique involving the use of beneficial and environmentally friendly biological agents to improve the physiological functioning of seeds. Plant growth-promoting rhizobacteria (PGPR) are found in the rhizosphere of plants and have the potential to cope with salinity stress. In this study, the effects of *Bacillus thuringiensis* LU3 (Bt LU3) biopriming application on two common wheat (*Triticum aestivum* L.) varieties (Sultan-95 and Tosunbey) under salt stress (0, 100 and 200 mM NaCl) on physiological (root and shoot length, biomass, dry weight, specific leaf area (SLA)), and biochemical parameters (pigment content, total protein content, hydrogen peroxide content (H_2O_2), lipid peroxidation content (TBARS) and antioxidant enzyme activities (peroxidase activity (POX), glutathione reductase activity (GR))) were investigated. As a result, it was determined that salt-sensitive Sultan-95 had better growth with Bt LU3 biopriming compared to salt-tolerant Tosunbey.

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Tuz Stresi Altındaki Ekmeklik Buğdayda *Bacillus thuringiensis* LU3 ile Biyopriming'in Bazı Fizyolojik ve Biyokimyasal Etkileri

ÖZET
Tuz stresi, dünyada sürdürülebilir tarımsal üretimi sınırlayan başlıca abiyotik streslerden biridir. Biyopriming, tohumların fizyolojik işleyişini iyileştirmek için faydalı ve çevre dostu biyolojik ajanların kullanımını içeren bir tekniktir. Bitki büyümesini teşvik eden rizobakteriler (PGPR), bitkilerin rizosferinde bulunur ve tuzluluk stresi ile başa çıkma potansiyeline sahiptir. Bu çalışmada, tuz stresi altında (0, 100 ve 200 mM NaCl) iki ekmeklik buğday (*Triticum aestivum* L.) çeşidine (Sultan-95 ve Tosunbey) *Bacillus thuringiensis* LU3 (Bt LU3) biyopriming uygulamasının fizyolojik (kök ve gövde uzunluğu, biyokütle, kuru ağırlık, spesifik yaprak alanı (SLA)) ve biyokimyasal parametreler (pigment içeriği, toplam protein içeriği, hidrojen peroksit içeriği (H_2O_2), lipid peroksidasyon içeriği (TBARS) ve antioksidan enzim aktiviteleri (peroksidaz aktivitesi (POX), glutatyon redüktaz aktivitesi (GR))) araştırılarak tuza duyarlı Sultan-95'in Bt LU3 biyopriming ile tuza dayanıklı Tosunbey'e göre daha iyi büyüme ve performans gösterdiği belirlenmiştir.

Bitki Fizyolojisi

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INTRODUCTION
Common wheat (*Triticum aestivum* L.) is a strategic food source, with an estimated area of more than 200 million hectares in 2020. The area of cultivation of wheat is expected to remain fairly constant in 2030 (Erenstein et al., 2021). Each year, approximately 200

million tons of wheat are produced (FAO, 2021). Abiotic and biotic stresses are the major factors limiting crop production in the changing global climate (Islam et al., 2016; Hossain et al., 2021). Salt stress is one of the most prominent abiotic stress factors limiting plant yield, it constitutes a threat to global food security by negatively affecting plant growth and development. Nevertheless, osmotic stress occurs when salt accumulated in the soil and the plant restricts the water uptake from the roots, restricting the nutrient uptake, and causing the nutrient balance and membrane properties to be damaged (Munns & Tester, 2008; Rahnama et al., 2010).

Reactive oxygen species (ROS) are produced naturally in plant cells such as photosynthesis and respiration. When the balance between the increase in the content of ROS in cells and detoxification is disturbed, oxidative stress occurs, and the antioxidant defense system plays an important role in the prevention of oxidative damage. Antioxidant defense systems constitute of enzymatic antioxidants (such as Superoxide Dismutase (SOD); Catalase (CAT), Ascorbate Peroxidase (APX), Glutathione Reductase (GR), etc.) or non-enzymatic antioxidants (Ascorbic Acid (ASH), Glutathione (GSH), etc.) (Gill & Tuteja, 2010).

Because of the complexity of basic salt tolerance mechanisms have limited success in the responses of plants to salinity during their growth stages of them (Taie et al., 2013). On the other hand, the seeds are exposed to pre-treatment with different agents via priming (Ali et al., 2017; Subramanyam et al., 2019), thereby improving the metabolic activity, germination, and seedling formation of the seed under adverse conditions. Biopriming is used as an alternative new seed priming method by combining physiological (seed hydration) and biological (seed inoculation with beneficial organisms) mechanisms (Migahid et al., 2019 Rhaman et al., 2020).

It has been reported that plant growth-promoting rhizobacteria (PGPR) and biopriming applications provide an advantage in reducing the negative effects of chemical fertilizers and pesticides on the environment based on sustainable agriculture (Kloepper & Schroth, 1978). In salt conditions, PGPR has been reported to contribute to plant growth in sorghum, chickpeas, maize, wheat, and rice (Sarig et al., 1992; Jacoud et al., 1998; Alam et al., 2001; Hamaoui et al., 2001; Saubidet et al., 2002). Paul & Nair (2008) reported that PGPR bacteria *Pseudomonas flourescens* isolated from coastal agricultural soils, produces proteins that alleviate the effect of salt stress and may be a suitable inoculant for plant growth in saline soils. Similarly, it was determined that wheat seedlings inoculated with indole 3-acetic acid (IAA) producing *Pseudomonas* isolates increased root and shoot growth by 40% and 52%, respectively, under salt stress (100 mmol L^{-1} NaCI) (Egamberdieva, 2009).

Although nickel (Ni) is a heavy metal, it is an essential microelement for plants (Ain et al., 2016). It has been determined that 2% of 35 heterotrophic bacterial isolates obtained from the root rhizosphere of the nickelophilic *Alyssum pinifolium* in Ezine (Çanakkale-Türkiye), which is distributed (Esen, 2016) in soils containing very high Ni (1702 mg g^{-1}) but not saline (0.33 dS m^{-1}), have high salt tolerance (Öztürk & Hacıoğlu Doğru, 2020). It is well known that the content of Na^+ increased with salt stress negatively affects the membrane integrity and the water potential in the root zone. Nevertheless, positive results are obtained with seed pre-treatment in reducing the negative effects of salt stress, which reduces plant growth and yield, or in improving the damage.

In this study, we focused on effects of *Bacillus thuringiensis* LU3 biopriming applications on physiological (root and shoot length, biomass, dry weight, specific leaf area (SLA)) and biochemical parameters (pigment content, total protein content, hydrogen peroxide content (H_2O_2), lipid peroxidation content (TBARS) and antioxidant enzyme activities (peroxidase activity (POX), glutathione reductase activity (GR))) in two wheat varieties (Sultan-95 and Tosunbey) under salt stress (0, 100 and 200 mM NaCI).

MATERIALS and METHODS

Bacterial Strain

The *Bacillus thuringiensis* LU3 (Bt LU3) strain was used in this study (Öztürk & Hacıoğlu Doğru, 2020). It was previously isolated from *A. pinifolium* rhizospheric soil in the Ezine region of Çanakkale (Türkiye) and showed its ability to synthesize IAA and inorganic phosphate (Karakaş et al., 2022).

Experimental Material and Seed Biopriming

In his study, two common cultivars (*T. aestivum* L.) were used. Salt-tolerant Tosunbey variety (Önay, 2019) was obtained from Ankara Field Crops Central Research Institute (Türkiye) and the susceptible salt-sensitive Sultan-95 variety (Önay, 2019) was obtained from Transitional Zone Agricultural Research Institute (Eskisehir-Türkiye). Surface sterilization of seeds was carried out with a 5% sodium hypochlorite solution (Abdul-Baki et al., 1979). Bt LU3 strain was cultured in nutrient broth at 28°C for 4 days. The bacterial cells were harvested by centrifugation for 4 min at 2000g. The pellets were re-suspended in sterile distilled water (dH_2O). The bacterial culture was adjusted to 10^8 colony-forming units (CFU) mL^{-1}. This bacterial suspension of Bt LU3 was used to biopriming wheat seed for 24 h. Then the seeds were washed and dried with blotting paper. The study was carried out according to the completely randomized block design with three replicates. Plants were grown in triplicate

and each group contained at least 3 petri dishes (Figure 1). Plants were grown in an *in vitro* plant growth chamber at 24±2°C in a photoperiod (light/dark cycle of 16/8 h). Additionally, the seedlings were irrigated with Hoagland nutrient solution (100%) (Hoagland & Arnon, 1950). Following the growth, the tissues were stored in a deep freeze (-20°C) by sampling the leaf tissues of 7d-old seedlings.

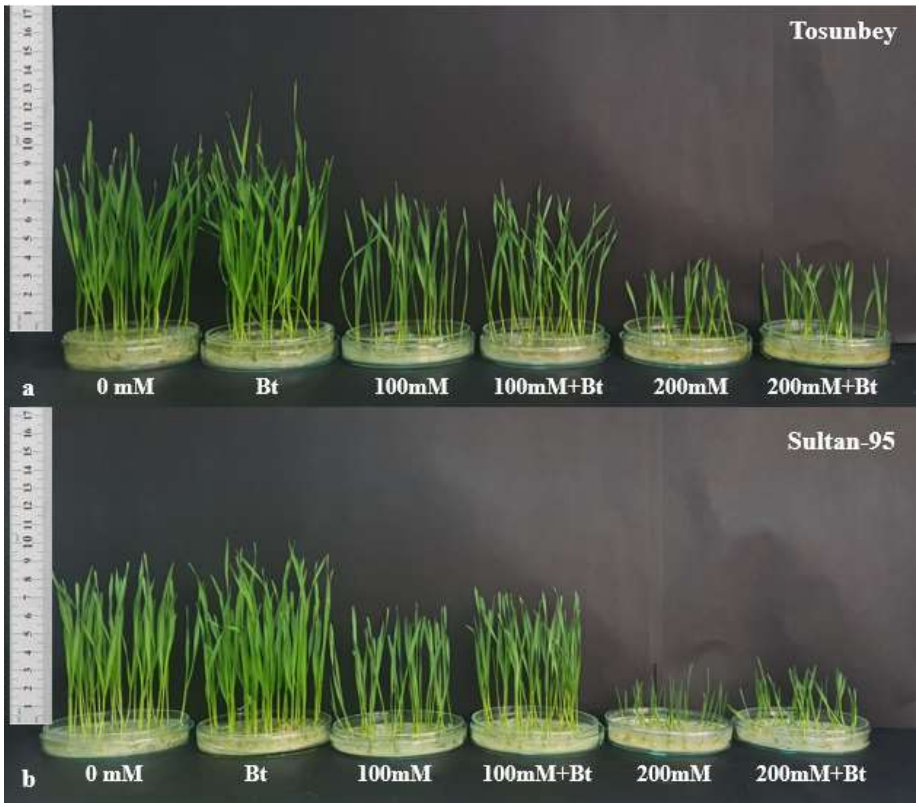

Figure 1. Wheat seedlings inoculated and uninoculated with Bt LU3 at different salt concentrations (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3 +salt stress; 100 mM+Bt, 200 mM+Bt) (a: Salt-tolerant variety; Tosunbey, b: Salt-sensitive variety; Sultan-95).

Şekil 1. Farklı tuz konsantrasyonlarında Bt LU3 aşılı ve aşısız buğday fideleri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (a: Tuz stresine dayanıklı çeşit; Tosunbey, b: Tuz stresine duyarlı çeşit; Sultan-95).

Root and Shoot Length (cm)

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 and Dry Weight (g plant^{-1})

Biomass the weight of three seedlings from each group was determined by weighing on the precision scale (g plant^{-1}). For dry weight, three seedlings from each group were dried in an oven at 80°C for 24 h (g plant^{-1}).

Specific Leaf Area (SLA) (cm^2 mg^{-1})

After the wheat seedlings were visualized in the Image J program, they were dried in an oven at 70°C for 24 h and weighed on a precision balance. It was calculated according to Wilson (1999) using the following formula 1:

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

Pigment Content (mg g^{-1})

Determination of pigment content, was made on 0.1 g 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 2:

Chlorophyll a (Chla) = (A663 x 12.70) – (A645 x 2.69) x 10/mg (2)

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

Total Protein Content (mg mL^{-1})

Leaf tissue samples were homogenized with 50 mM NaP buffer (pH 7.8, 1 mM EDTA) and then centrifuged for protein analysis. 0.1 g of Coomassie Brilliant Blue G 250 was mixed in a tube with a protein reagent containing ethanol (50 mL) and ortho-phosphoric acid (100 mL). The absorbance values determined at 595 nm in the spectrophotometer were used to calculate the total protein content (mg g^{-1}) on the standard graph (Bradford, 1976).

Hydrogen Peroxide Content (H_2O_2) (µg mL^{-1})

The H_2O_2 content, a mixture of plant tissue (0.1 g), 3 mL of H_2SO_4, and cold acetone were homogenized with homogenization buffer and centrifuged. Supernatants were determined at 550-800 nm (µg mL^{-1}) spectrophotometric with reading buffer containing H_2SO_4, purified water, ferrous ammonium sulfate, xylenol orange, sorbitol, and ethanol (e-FOX) (Cheeseman, 2006).

Lipid Peroxidation Content (TBARS) (nmol g^{-1})

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.

Peroxidase Activity (POX; EC 1.11.1.7)

The POX activity was quantified by homogenizing tissue samples with 2 mL of 0.05 M (pH 6.5) sodium acetate buffer and using 0.05 M (pH 6.5) sodium acetate buffer, 0.1M pyrogallol, and 0.09 M H_2O_2 solutions. It was determined by a spectrophotometer at 300 nm (Kanner & Kinsella, 1983).

Glutathione Reductase Activity (GR; EC 1.6.4.2)

The GR activity, as the decrease in the content of oxidized glutathione in the presence of NADPH, was calculated by the absorbance value by spectrophotometer at 340 nm (€=6.2 mM^{-1} cm^{-1}). The reaction mixture contained 0.025 mM sodium phosphate buffer (pH 7.8), 0.5 mM GSSG, and 0.12 mM $NADPH.Na_4$ and 0.1 mL. 1 enzyme unit was determined as the content of oxidized glutathione (µmol mL^{-1}) per minute (Foyer & Halliwell, 1976).

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 biopriming with Bt LU3 on physiological parameters and biochemical analysis in *T. aestivum* during salt stress. Those comparisons with $P \leq 0.05$ were taken as significantly different. The data were analyzed by using Statistical Package for the Social Sciences (SPSS 27.0) statistical software.

RESULTS

Root and Shoot Length

Bt LU3 application increased the root length by 5% in Sultan-95 compared to the control and no changed it in the Tosunbey variety. While 100 mM NaCl decreased root length by 26-28% in both varieties compared to the control, Bt LU3 application improved this reduction by 11% in Sultan-95 and 3% in Tosunbey. Similarly, 200 mM NaCl reduced root length by 58-63% in both varieties, while Bt LU3 application improved this reduction by 7% in Sultan-95 and 5% in Tosunbey (Figure 2). 100 mM NaCl reduced the shoot length of Sultan-95 by 28%, while Bt LU3 application improved it by 11%. However, this improvement was limited in 200 mM NaCl application. In Tosunbey, both salt concentrations reduced the shoot length by 25-57%. Bt applications did not cause a statistically significant change in these reductions. Our results showed that Bt LU3 biopriming was more effective in Sultan-95 compared to Tosunbey in reducing root and shoot length inhibition caused by salt stress (Figure 2).

Biomass and Dry Weight (g $plant^{-1}$)

Bt LU3 biopriming increased biomass by 9% in Sultan-95 and decreased by 14% in Tosunbey compared to control. Compared to the control with 100 mM NaCl, the biomass decreased by 43% in Sultan-95 and by 55% in Tosunbey, while it increased by 3% in both varieties with Bt LU3 biopriming. Biomass, which decreased 65% in Sultan-95 and 61% in Tosunbey with 200 mM NaCl, increased by 6% in Sultan-95 and 3% in Tosunbey with Bt LU3 biopriming (Figure 3). However, with Bt LU3 biopriming, only 33% of dry weight was recovered in Sultan-95 under 200 mM NaCl stress (Figure 4). Our results indicate that Bt LU3 biopriming has a better effect on the reduction in biomass and dry weight in Sultan-95 under high salt stress (Figure 3, Figure 4).

Specific Leaf Area (SLA) (cm^2 g^{-1})

Biopriming of Bt LU3 in both varieties increased SLA by 3% compared to the control. While 100 mM NaCl decreased SLA by 22% in both varieties, Bt LU3 application improved it by 11% only in Sultan-95.

While 200 mM NaCl stress reduced SLA by 61% in Sultan-95 and 50% in Tosunbey, Bt LU3 biopriming improved this reduction by 29% in Sultan-95 and 18% in Tosunbey (Figure 5). The results show that the reduction in SLA with severe salt stress is significantly improved in both varieties with Bt LU3 (Figure 5).

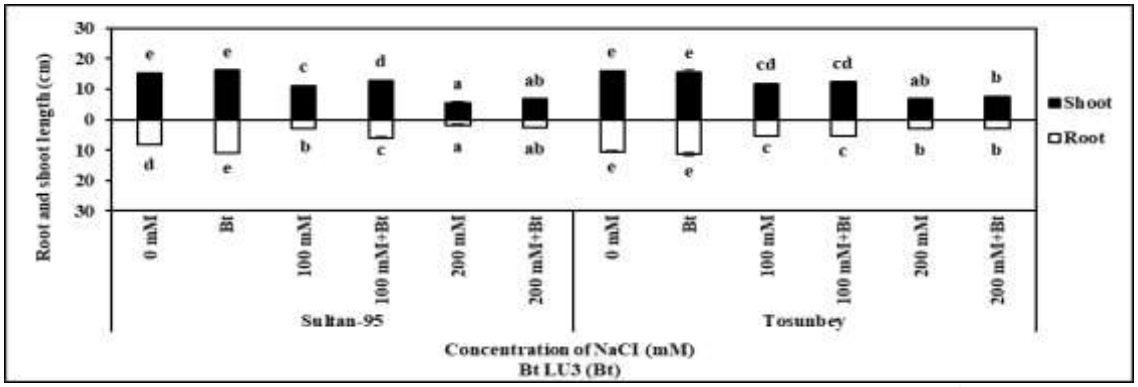

Figure 2. The effects of Bt LU3 biopriming on root and shoot length of two *T. aestivum* L. (Sultan-95 and Tosunbey) varieties under salt stress (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt). (P <0.05).

Şekil 2. Tuz stresi altında iki T. aestivum L. (Sultan-95 ve Tosunbey) çeşidinin kök ve gövde uzunluğu üzerine Bt LU3 biyopriming'in etkileri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

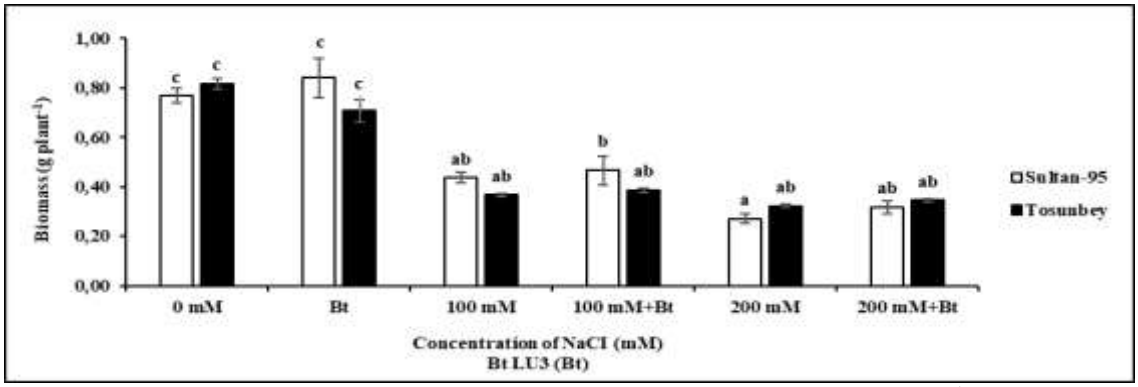

Figure 3. The effects of Bt LU3 biopriming on the biomass of two *T. aestivum* L. varieties (Sultan-95 and Tosunbey) under salt (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt) (P <0.05).

Şekil 3. Tuz stresi altında iki T. aestivum L. çeşidinin (Sultan-95 ve Tosunbey) biyokütlesi üzerine Bt LU3 biyopriming'in etkileri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

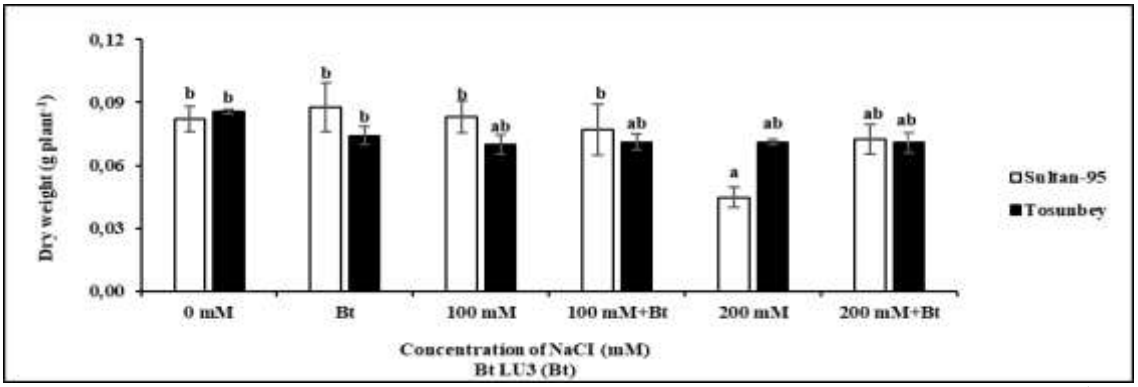

Figure 4. The effects of Bt LU3 biopriming on the dry weight of two *T. aestivum* L. varieties (Sultan-95 and Tosunbey) under salt stress (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt) (P <0.05).

Şekil 4. Tuz stresi altında iki T. aestivum L. çeşidinin (Sultan-95 ve Tosunbey) kuru ağırlığı üzerine Bt LU3 biyopriming'in etkileri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

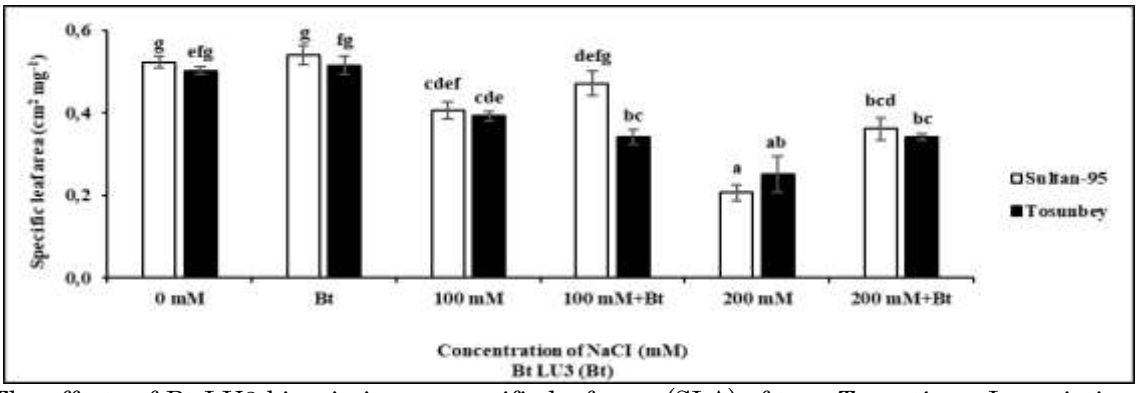

Figure 5. The effects of Bt LU3 biopriming on specific leaf area (SLA) of two *T. aestivum* L. varieties (Sultan-95 and Tosunbey) under salt stress (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt) (P <0.05).

Şekil 5. Tuz stresi altında iki T. aestivum L. çeşidinin (Sultan-95 ve Tosunbey) spesifik yaprak alanı (SLA) üzerine Bt LU3 biyopriming'in etkileri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

Pigment content (mg g^{-1})

Firstly, the content of Chla in Sultan-95 decreased by 28% with 100 mM NaCl and by 36% with 200 mM NaCl compared to the control. It was determined that Bt LU3 biopriming increased the decreased Chla content by 28% and 59%, respectively, with 100 and 200 mM NaCl (Figure 6 a). Secondly, Chlb contents increased by 21% and 22%, respectively, with Bt LU3 application in Sultan-95 under 100 and 200 mM NaCl stress. These increases were determined as 55% and 41% for the Tosunbey variety, respectively (Figure 6 b). Thirdly, it was determined that the number of Car increased by 12% with Bt LU3 biopriming with only 200 mM NaCl application in the Sultan-95 variety, and 24% and 33%, respectively, compared to control with 100 and 200 mM NaCl in Tosunbey variety (Figure 6 c). As a result, the Chlt increased by 22% and 17%, respectively, in the Sultan-95 variety with 100 mM+Bt and 200 mM+Bt applications, while it increased by 44% and 46%, respectively, in the Tosunbey variety (Figure 6 d).

Our pigment results indicate that the content of Chla, Chlb, Car, and Chlt levels increased by 20%, 66%, 45%, and 80%, respectively, in the Tosunbey compared to mild salt stress treatment with Bt LU3 biopriming. However, with Bt LU3 biopriming, Chla, Chlb, and Chlt content increased by 28%, 90%, and 37%, respectively, in the Sultan-95 compared to mild salt stress application. Chla, Car and Chlt contents increased by 28%, 17%, and 90%, respectively, with Bt LU3 biopriming compared to 200 mM NaCl application in the Tosunbey variety. In Sultan-95, these increases were determined as 59%, 24% and 45%, respectively (Figure 6). Accordingly, Bt LU3 biopriming reduced salt stress-induced chlorosis in both varieties compared to control and even increased it dramatically in the Tosunbey.

Total Protein Content

The protein content of the Sultan-95 variety increased with all treatments compared to the control. The protein content increased by 2% compared to the control with only 200 mM NaCl+Bt application in the Tosunbey variety (Figure 7).

Hydrogen Peroxide Content (H_2O_2) (µg mL^{-1})

The H_2O_2 content decreased by 21-27% with Bt LU3 biopriming compared to control in both varieties. In contrast, it increased 31-33% with 100 mM NaCl and 51-58% with 200 mM NaCl. Bt LU3 application decreased H_2O_2 production by 26% in Sultan-95, 8% in Tosunbey under 100 mM NaCl stress, 63% in Sultan-95 under 200 mM NaCl stress, and 39% in Tosunbey (Figure 8).

Our results show that the content of H_2O_2, which increased with salt stress, decreased in both varieties with Bt LU3 biopriming, and remained below the control level in 200 mM+Bt application, especially in Sultan-95 (Figure 8).

Lipid Peroxidation Content (TBARS) (nmol g^{-1})

Lipid peroxidation levels increased 1.3 times and 2.1 times in Sultan-95 with 100 mM and 200 mM NaCl, respectively. These increases were determined as 49% and 87% in the Tosunbey, respectively. Bt LU3 application alone caused a non-significant increase in both varieties. However, TBARS levels in Sultan-95 plants under 100 mM and 200 mM NaCl stress were decreased by Bt LU3 by 59% and 106%, respectively. Similarly, Bt LU3 application in the Tosunbey variety decreased TBARS levels by 11% and 40%, respectively (Figure 9). Our results indicate that lipid peroxidation developed by salt stress decreased in both varieties with Bt LU3 application, and the most effective reduction occurred in the salt-sensitive Sultan-95 (Figure 9).

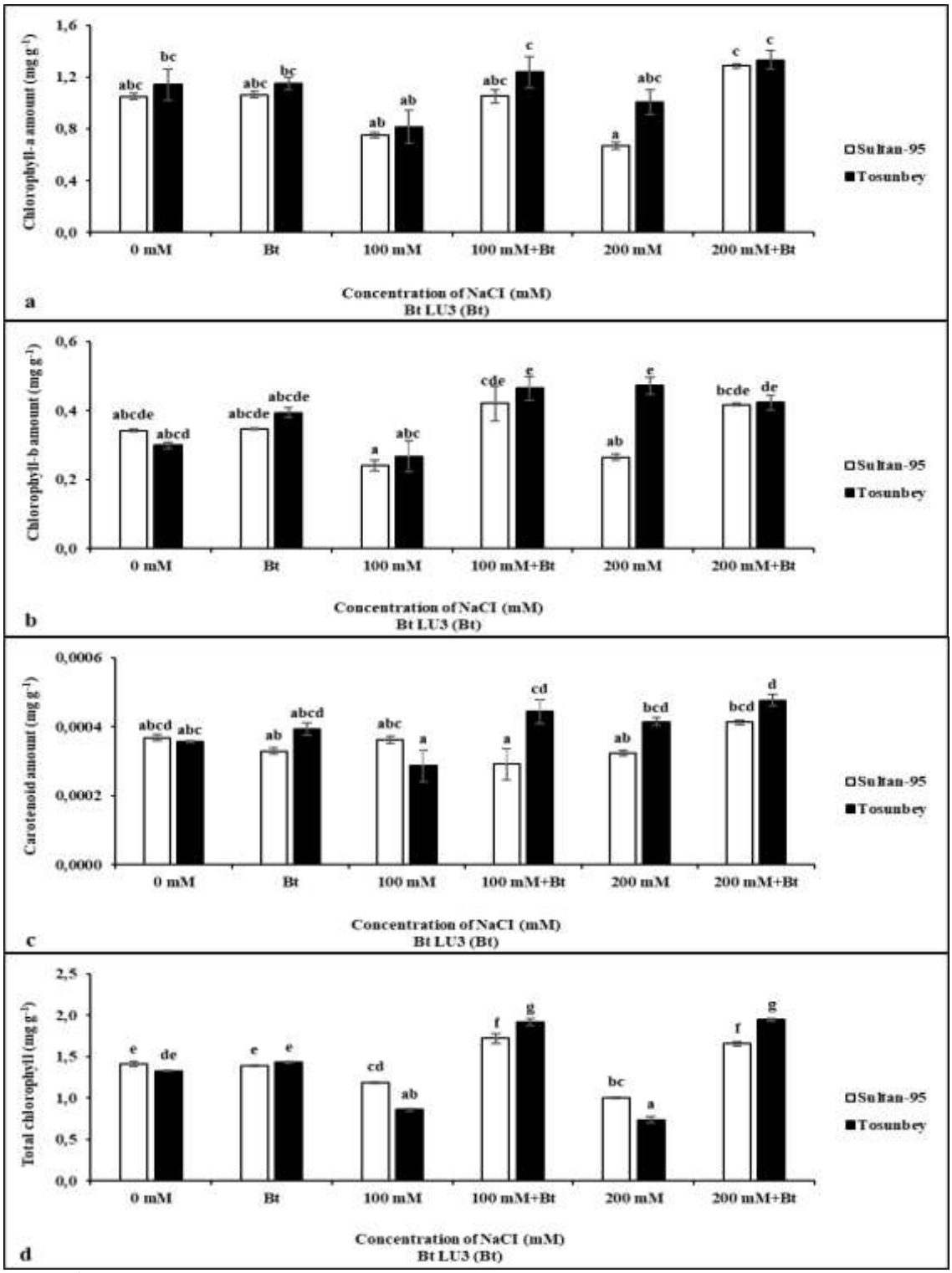

Figure 6 (a, b, c, d). The effects of Bt LU3 biopriming on pigment content of two *T. aestivum* L. varieties (Sultan-95, Tosunbey) under salt stress (Control; 0 mM, Bt LU3; Bt, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt). (a; Chlorophyll a content (Chla), b; Chlorophyll b content (Chlb), c; Carotenoid (Car), d; Total chlorophyll content (Chlt)) ($P < 0.05$).

Şekil 6 (a, b, c, d). Tuz stresi altında Bt LU3 biyopriming'in iki T. aestivum L. çeşidinin (Sultan-95, Tosunbey) pigment içeriği üzerine etkileri (Kontrol; 0 mM, Bt LU3 grubu; Bt, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt). (a; Klorofil a içeriği (Chla), b; Klorofil b içeriği (Chlb), c; Karotenoid (Car), d; Toplam klorofil içeriği (Chlt)) (P <0.05).

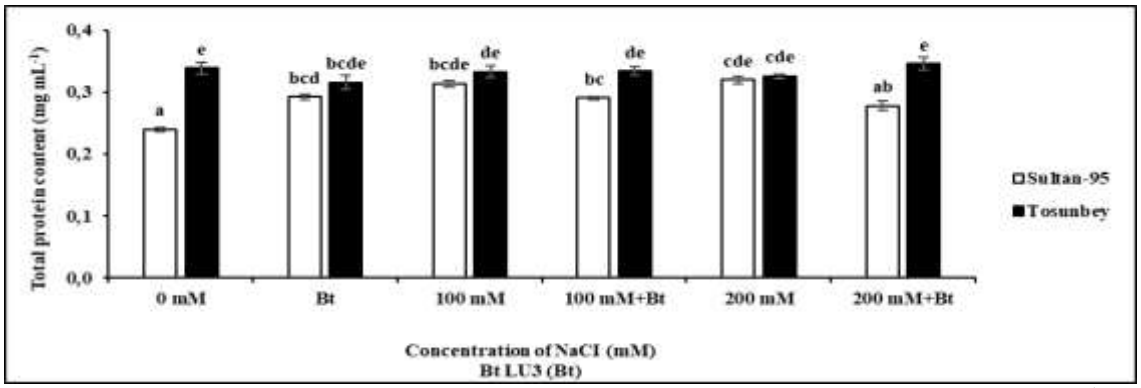

Figure 7. The effects of Bt LU3 biopriming on total protein content of two *T. aestivum* L. varieties (Sultan-95 and Tosunbey) under salt stress (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt) (P <0.05).

Şekil 7. Tuz stresi altında iki T. aestivum L. çeşidinin (Sultan-95 ve Tosunbey) toplam protein içeriği üzerine Bt LU3 biyopriming'in etkileri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

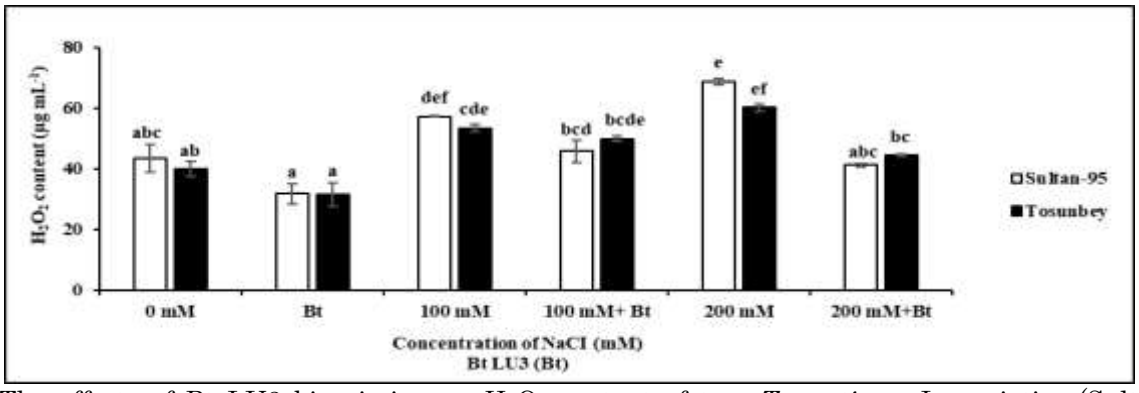

Figure 8. The effects of Bt LU3 biopriming on H_2O_2 content of two *T. aestivum* L. varieties (Sultan-95 and Tosunbey) under salt stress (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt) (P <0.05).

Şekil 8. Tuz stresi altında Bt LU3 biyopriming'in iki T. aestivum L. çeşidinin (Sultan-95 ve Tosunbey) H_2O_2 içeriği üzerindeki etkileri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

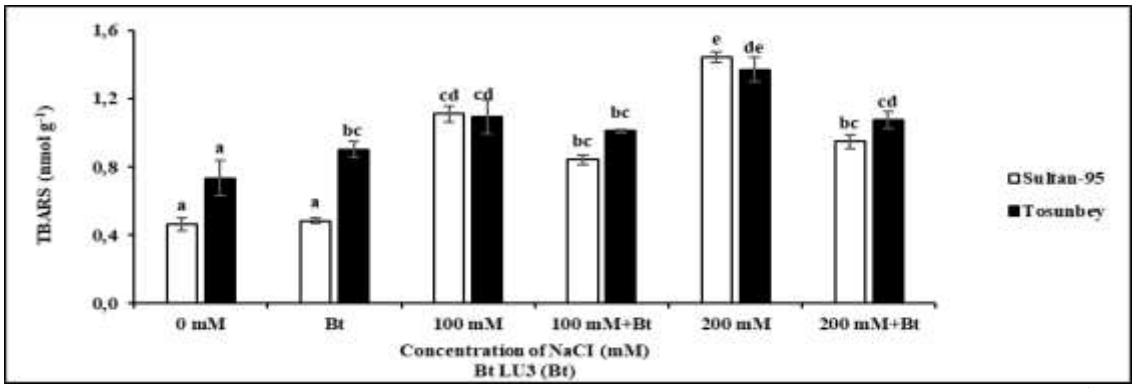

Figure 9. The effects of Bt LU3 biopriming on TBARS content of two *T. aestivum* L. varieties (Sultan-95 and Tosunbey) under salt stress (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt) (P <0.05).

Şekil 9. Tuz stresi altında iki T. aestivum L. çeşidinin (Sultan-95 ve Tosunbey) TBARS içeriği üzerine Bt LU3 biyopriming'in etkileri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

Peroxidase Activity

POX activity increased by 43% and 65% in Sultan-95 with 100 mM and 200 mM NaCl, respectively. These increases were determined as 9% and 19% in Tosunbey, respectively. In both varieties, the Bt LU3 application alone increased POX activities by 56-59%. However, the application of 100 mM and 200 mM NaCl+Bt increased the POX activities in Sultan-95 by 32% and 94%, respectively. Similarly, Bt LU3 application in the Tosunbey variety increased the POX activities by 11% and 24%, respectively (Figure 10). Our results showed that Bt LU3 application increased salt stress-induced POX increases in both varieties and the most effective increase occurred in the salt-sensitive Sultan-95 (Figure 10).

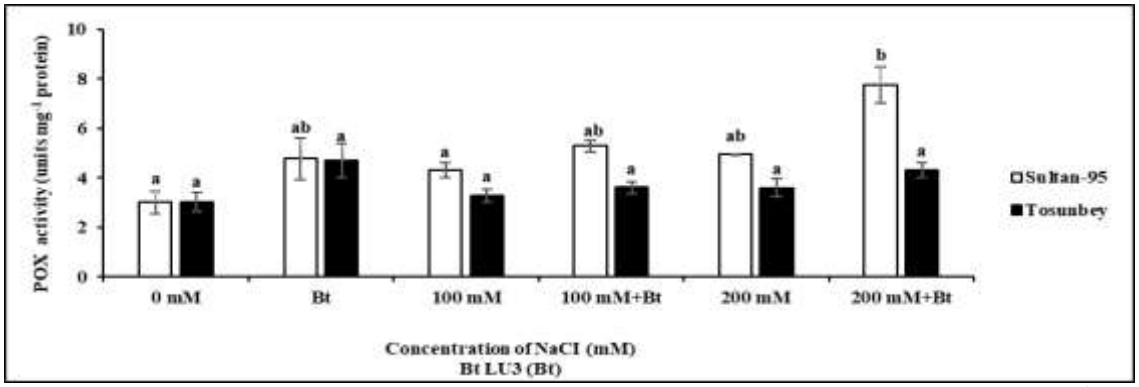

Figure 10. The effects of Bt LU3 biopriming on POX activity of two *T. aestivum* L. varieties (Sultan-95 and Tosunbey) under salt stress (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt) (P <0.05).

Şekil 10. Bt LU3 biyopriming'in iki T. aestivum L. çeşidinin (Sultan-95 ve Tosunbey) tuz stresi altındaki POX aktivitesi üzerindeki etkileri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

Glutathione Reductase Activity

GR activity increased by 23% and 34%, respectively, with 100 mM and 200 mM NaCl applications in Sultan-95. Similarly, these increases were determined as 36% and 61% in the Tosunbey, respectively. The Bt LU3 application in both varieties increased GR activities by 7% in Sultan-95 and by 41% in Tosunbey. However, 100 mM and 200 mM NaCl+Bt application increased GR activities by 49% in Sultan-95. Similarly, the Bt LU3 application in the Tosunbey variety increased GR activities by 26% and 81%, respectively (Figure 11). Our results showed that the Bt LU3 application increased salt stress-induced GR increases in both varieties and the most effective increase occurred in the salt-tolerant Tosunbey (Figure 11).

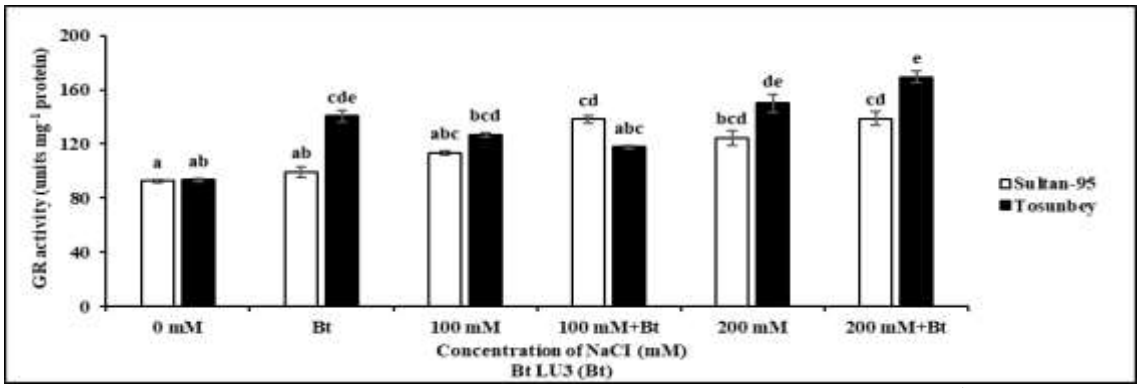

Figure 11. The effects of Bt LU3 biopriming on GR activity of two *T. aestivum* L. varieties (Sultan-95 and Tosunbey) under salt stress (Control; 0 mM, Bt LU3; Bt, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt) (P <0.05).

Şekil 11. Tuz stresi altındaki iki T. aestivum L. çeşidinin (Sultan-95 ve Tosunbey) GR aktivitesi üzerine Bt LU3 biyopriming'in etkileri (Kontrol; 0 mM, Bt LU3 grubu; Bt, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

DISCUSSION and CONCLUSION

PGPR promotes plant growth and is known to increase tolerance to environmental stresses (Stefan et al., 2013; Pandey et al., 2018). Salt stress limits root and shoot length in wheat. In our study, Bt LU3 biopriming increased root and shoot lengths in salt-sensitive Sultan-95 under 100 mM NaCl but not in salt-tolerant Tosunbey varieties under all salt stress. Inoculation with *Bacillus halodenitrificans* in soils under high salt stress has been reported to improve growth parameters in wheat (Ramadoss et al., 2013). On the other hand, it is known that root length increases dramatically with *Bacillus thuringiensis* AZP2 biopriming in wheat under drought stress (Timmusk et al., 2014; Raheem et al., 2018).

Besides, salt stress causes a decrease in the biomass and dry weight of the common wheat (*T. aestivum* L.) plant. Our obtained results showed that decreased biomass (g plant^{-1}) and dry weight (g plant^{-1}) with high salt stress had a better healing effect on salt-sensitive Sultan-95. Similarly, it was reported that the dry weight increased by 40-52% in 120-days wheat seedlings inoculated with *Bacillus* sp. compared to the control (Hajiabadi et al., 2021). In addition, it has been reported that *Bacillus thuringiensis* AZP2 biopriming significantly increased the dry weight (g plant^{-1}) of wheat under drought stress (Timmusk et al., 2014).

Our results indicate that the Bt LU3 biopriming application, which decreased with all salt stress applications, increased significantly in Sultan-95, while it was effective only at 200 mM NaCl in Tosunbey. Similarly, our biomass results are consistent with root and shoot length of Sultan-95 under salt stress results. Salt-tolerant plants have unchanged or increased chlorophyll levels under salt stress, while chlorophyll content is reduced in salt-tolerant plants (Rahneshan et al., 2018). In our study, it was shown that while the pigment contents (Chla, Chlb and Chlt) of both varieties dramatically decreased with all salt stress applications. Despite of salt stresses, Bt LU3 biopriming increases all pigment amounts to control levels. Interestingly, the increases in pigment contents of the salt-sensitive variety with Bt LU3 biopriming increased to the salt-tolerant variety. But Bt LU3 biopriming dramatically increased the pigment contents, especially in the salt-tolerant variety. Inoculation with *Bacillus* sp. has been reported to induce chlorophyll biosynthesis in soybean (*Glycine max*) limiting the reductions in chlorophyll content caused by salt stress and reducing the adverse effect (El-Esawi et al., 2018). On the other hand, Chla and Chlb contents decrease as a result of insufficient absorption of elements such as K^+, Mg^{+2}, Fe and P, increase in Na^+ content, decrease in 5-aminolevulinic acid accumulation or deterioration of some enzymes (such as chlorophyllase, peroxidase) due to phenolic compounds in common wheat under the salt stress (Hajiabadi et al., 2021). The use of Bt LU3 biopriming and PGPR increased the pigment content of both varieties in our study. It has been stated that this may be related to the production of siderophores in plants (Hajiabadi et al., 2021). During the growth of plants, intermediate products of oxygen are constantly produced in the processes of photosynthesis and respiration. Under normal conditions, electron transfer to oxygen and associated intermediate toxic radical molecules (OH, H_2O_2, O_2^{-} and O_2, etc.) are formed (Parida & Das, 2005). As a result of salt stress, the water potential decreases and causes osmotic stress with ion accumulation in plants. Water scarcity causes oxidative stress by increasing the production of ROS such as OH, H_2O_2, O_2^{-} and O_2 (Desingh & Kanagaraj, 2007). Plants form an antioxidant defense system against ROS resulting from salt stress with the activity of certain antioxidant enzymes such as CAT, POX and SOD (Desingh & Kanagaraj, 2007). ROS production in wheat is triggered by unsuitable conditions such as salt stress. Our results showed that POX activities increased in both varieties for ROS detoxification, especially in the Sultan-95 variety. However, GR activity in the ascorbate-glutathione pathway was effectively increased in the Tosunbey variety. Besides, Bt LU3 biopriming significantly increased GR activity in Sultan-95 under moderate salt stress. According to these results, it can be said that the Bt LU3 biopriming application induced an increase in antioxidant activity in the salt-sensitive variety.

On the other hand, our results indicate that salt stress and the increase in antioxidant enzyme activities in both varieties are associated with decreases in the content of TBARS and H_2O_2. All data showed that Sultan-95 had a more effective antioxidant capacity with Bt LU3 biopriming compared to Tosunbey. Our results are consistent with the findings of El-Esawi et al. (2018). PGPR-promoted antioxidant enzyme production is known to reduce excessive ROS production and maintain membrane stability (Afridi et al., 2019). In addition, *in vitro* application of the *B. amyloliquefaciens* SQR9 strain on maize (*Zea mays*) seedlings has been reported to increase POX and GR activities (Wang et al., 2016). Moreover, the increase in antioxidant enzyme activities (APX, SOD, CAT, POX) has been shown to play a role in reducing the harmful effects of high salinity on soybean (*Glycine max*) growth (El-Esawi et al., 2018). On the other hand, it is stated that the activation of antioxidant enzymes increases plant growth and performance through the up-regulation of genes that mediate tolerance under salt stress conditions (El-Esawi et al., 2018).

Table 1. Correlations between physiological and biochemical parameters in wheat seedlings.
Çizelge 1. Buğday fidelerinde fizyolojik ve biyokimyasal parametreler arasındaki korelasyonlar.

Variable	Root	Shoot	Biomass	DW	SLA	Chla	Chlb	Car	Chlt	Protein	H_2O_2	TBARS	POX	GR
Root	1													
Shoot	,887**	1												
Biomass	,819**	,868**	1											
DW	,527**	,417*	,632**	1										
SLA	,809**	,802**	,832**	,635**	1									
Chla	0,065	0,146	0,035	0,225	0,143	1								
Chlb	-0,127	-0,057	-0,229	0,083	-0,062	,583**	1							
Car	0,172	0,081	-0,005	0,128	0,182	,407**	0,112	1						
Chlt	-0,175	-0,111	-0,263	-0,081	-0,302	,677**	,629**	0,055	1					
Protein	-,250*	-0,187	-,364*	-0,155	-,413*	-0,007	0,104	-0,041	0,154	1				
H_2O_2	-,596**	-,735**	-,697**	-,555**	-,761**	-,354*	-0,261	-,432**	0,031	0,202	1			
TBARS	-,639**	-,640**	-,799**	-,557**	-,731**	-0,148	0,049	-,276*	0,094	,356**	,712**	1		
POX	-0,225	-0,15	-0,162	-0,058	0,007	0,052	0,096	0,208	-0,007	-0,159	-0,171	0,137	1	
GR	-,514**	-,428**	-,540**	-,339*	-,380*	0,212	,549**	0,09	,430**	,283*	0,048	,486**	,291*	1

Root: Root length, Shoot: Shoot length, DW: Dry weight, SLA: Specific leaf area, Chla: Chlorophyll a content, Chlb: Chlorophyll b content, Car: Carotenoid, Chlt: Total chlorophyll content, Protein: Total protein content, H_2O_2: Hydrogen peroxide content, TBARS: Lipid peroxidation content, POX: Peroxidase activity, GR: Glutathione reductase activity.
Kök: Kök uzunluğu, Gövde: Gövde uzunluğu, DW: Kuru ağırlık, SLA: Spesifik yaprak alanı, Chla: Klorofil a içeriği, Chlb: Klorofil b içeriği, Car: Karotenoid, Chlt: Toplam klorofil içeriği, Protein: Toplam protein içeriği, H_2O_2 : Hidrojen peroksit içeriği, TBARS: Lipit peroksidasyon içeriği, POX: Peroksidaz aktivitesi, GR: Glutatyon redüktaz aktivitesi
**: Significant correlations P<0.01, and *: P<0.05

Triple interaction (Variety x NaCl x Bt LU3) was statistically significant for all parameters except all pigment results and POX activities (Table 1). This result indicated that there was not any effect of Bt biopriming on pigment contents and POX levels. The correlation between the physiological parameters showed that there was a positive relationship between Root, Shoot, SLA, Biomass and DW. The correlation between also the biochemical parameters showed that there was a positive relationship between GR, TBARS, Protein, H_2O_2. According to these results physiological parameters shown strictly correlation together via Bt biopriming. On the other hand, GR activity more induced than the POX activity against salt stress (Table 1). Our results, a strictly correlation was determined with Bt biopriming application in physiological parameters. Additionally, the positive correlation between salt stress-induced H_2O_2 amount, and GR activity indicates that GR works more effectively than POX in antioxidant defense with Bt biopriming (Table 1).

Consequently, it was determined that the salt-sensitive Sultan-95 cultivar had better growth parameters with Bt LU3 biopriming application compared to the salt-tolerant Tosunbey variety. It can be said that the responses in growth are related to the induction of the antioxidant capacity of the tolerant variety and promoted by the Bt LU3 biopriming application. As a result, more comprehensive studies on biopriming applications of rhizobacteria are required in the context of plant-bacteria-soil relations research for the development of sustainable agricultural practices.

Author Contribution Rates

The authors declare that they contribute equally to the article.

Conflict of interest/Competing interests

The authors declare that there is no conflict of interest.

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