



## Physiological and Biochemical Mechanisms of Salinity Tolerance in Tall Fescue: A Comparative Analysis of Cultivars Differing in Salinity Tolerance

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### ABSTRACT

Salinity stress causes an increasingly pervasive threat to agricultural productivity, including turf and forage grasses. This study aimed to reveal how salinity stress affects the physiological and biochemical levels of *Festuca arundinacea* (tall fescue) cultivars differing in salinity tolerance. The salinity stress treatments were assessed through the evaluation of growth measures, biochemical profiles, and the activity of antioxidative enzymes in four tall fescue cultivars having different salinity stress tolerance (tolerant, moderate-high, moderate-low, and sensitive). The results revealed that the tolerant cultivar named 'Titan RX' (tolerant cv.) showed increased root growth and maintained physiological status under salinity stress conditions. This was attributed to the activation of the biosynthesis for the free proline and the antioxidative status (total phenolic and flavonoid contents, DPPH activity, enzymes of CAT, and GR). The other cultivars exhibited lower values in these parameters compared to the tolerant cv., indicating their lesser capacity for salinity tolerance. The results indicated that the cv. 'Titan RX' could potentially be utilized to generate tall fescue crops that can cope with salinity stress conditions.

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## Kamışsı Yumak Bitkisinde Tuzluluk Toleransının Fizyolojik ve Biyokimyasal Mekanizması: Tuz Stresine Farklı Hassasiyet Gösteren Çeşitlerin Karşılaştırmalı Analizi

### ÖZET

Bu çalışma, farklı tuzluluk toleransına sahip *Festuca arundinacea* (kamışsı yumak) çeşitlerinde tuzluluk stresinin fizyolojik ve biyokimyasal düzeydeki etkilerini ortaya koymayı amaçlamıştır. Tuzluluk stresi uygulamaları, farklı tuzluluk stres toleransına (toleranslı, orta-yüksek, orta-düşük ve hassas) sahip dört kamışsı yumak çeşidinde büyüme parametreleri, biyokimyasal profiller ve antioksidan enzimlerin aktivitesinin değerlendirilmesi yoluyla değerlendirilmiştir. Sonuçlar, 'Titan RX' adlı tolerant çeşidin tuzluluk stresi koşullarında kök büyümesini artırdığını ve fizyolojik durumunu koruduğunu ortaya koymuştur. Bu durum, serbest prolin biyosentezinin ve antioksidan mekanizmaların (toplam fenolik ve flavonoid içerikleri, DPPH aktivitesi, CAT ve GR enzimleri) aktivasyonu ile ilişkilendirilmiştir. Diğer çeşitler bu parametrelerde tolerant çeşide kıyasla daha düşük değerlere sahip olmuş ve sonuçlar bu çeşitlerde tuzluluk toleransı kapasitelerinin daha sınırlı olduğunu göstermiştir. Elde edilen bulgular, 'Titan RX' çeşidinin, tuzluluk stresine dayanıklı kamışsı yumak bitkileri geliştirmek için potansiyel bir kaynak olarak kullanılabileceğini göstermektedir.

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## INTRODUCTION

Salinity stress has become more serious year by year, resulting in major yield and quality losses in agricultural production (El Sabagh et al., 2020). Salinity stress has been linked to physiological changes in plants, including turf and forage grasses, which may cause adverse effects, including mortality (Demirkol and Yılmaz, 2023). Therefore, an increasing challenge has been observed in recent studies for turfgrass or forage crop breeders to focus on developing salinity-tolerant cultivars (Oral et al., 2019; Bushman et al., 2020; Liu et al., 2023).

Plants have adapted and changed their metabolic processes to deal with salinity stress (Demirkol, 2020). Stimulating the antioxidant defense mechanism is a crucial strategy for minimizing the damaging effects of salinity stress (Begum et al., 2022). Studies have indicated that the expression levels of functional genes such as WRKY, bZIP, MYB, and DREB were up-regulated in salinity-tolerant plants under stress conditions (Price et al., 2022). These molecular regulatory mechanisms promote the activity of antioxidant enzymes (Kaya et al., 2020). Earlier studies have reported that plants with enhanced antioxidant defense systems are more resistant to salinity stress (Demirkol, 2021). Enhanced antioxidant activity is associated with a variety of physiological strategies, including the biosynthesis of endogenous hormones, closure of stomata, scavenging of reactive oxygen species (ROS), accumulation of protective osmolytes, cell membrane stability, and protein structure maintenance (Hussain et al., 2021; Yılmaz, 2019).

*Festuca arundinacea* Schreb. (tall fescue) It is a main cool-season perennial forage, turfgrass, and cover crop (Sato, 2022). The strong adaptability of tall fescue increases its usage worldwide. However, regional salinization restricts the growth of tall fescue, affecting turf persistence and forage yield (Chen et al., 2022; Kaplan et al., 2017).

The objective of this study was to assess the impact of salinity-induced stress conditions on growth parameters and the physio-biochemical status of tall fescue cultivars characterized by varying degrees of salinity tolerance.

## MATERIALS and METHODS

### Plant materials and preliminary study

The seeds of four tall fescue cultivars ('Grande', 'Starlet', 'Titan RX', 'Rendition') were used for the experiment, with the anticipation of observing variation in their resistance to salinity. The seeds were sterilized in a 70% (v/v) ethanol solution and shaken for 1 minute. Afterward, the seeds were washed three times with sterile distilled water.

The salinity stress tolerances of the cultivars were assessed in petri dishes in a preliminary study to confirm their different salinity tolerances. The findings classified 'Titan RX' as tolerant, 'Starlet' as moderate-high, 'Rendition' as moderate-low, and 'Grande' as sensitive to salinity stress (data not shown).

### Growth and stress conditions

The experiment was performed in the greenhouse of the Agricultural Research Center in Tokat Gaziosmanpasa University. The seeds were planted in pots filled with field soil and were subjected to irrigation using Hoagland's nutrient solution at 80% of field capacity in two days. Afterward, three-week-old seedlings with two leaves were separated into five groups, each irrigated with varied salinity levels (0, 50, 100, 150, 200 mM). The samples were harvested at three weeks after the application of salinity stress treatments. During the research period, greenhouse conditions were maintained within the ecological requirements of the tall fescue cultivars to prevent the occurrence of any secondary stress.

### Growth parameters and relative water content

The harvested plants' roots and shoots were measured to determine their lengths (cm) and dried (60°C for 72 h) weight (g/plant) values. The relative water content of the plants was assessed using the formula of Farrant (2000).

### Free proline content

The protocol developed by Bates et al. (1973) was modified to determine the free proline content of the plants. Initially, a 0.3 g sample of fresh leaves was homogenized in 10 milliliters of 3% sulphosalicylic acid, and then it was centrifuged at  $10,000 \times g$  for 10 minutes. After that, 0.1 ml of the solution was taken. The sample was then vortexed with 0.2 ml of ninhydrin reagent (1.25 g ninhydrin, 30 ml glacial acetic acid, and 20 ml 6M phosphoric acid), 0.2 ml of glacial acetic acid, and 0.1 ml of 3% sulphosalicylic acid. After 1 h of incubation at 95°C, the prepared sample was placed in an ice bath for 5 min to stop the reaction. Then, 1 ml toluene was added to the cooled sample, and centrifuged ( $5\,000 \times g$ , 30 sec). After centrifugation, the upper phase was measured at 520 nm in a spectrophotometer.

### Hydrogen peroxide

The hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content was detected using the procedure outlined by Velikova et al. (2000). 5 ml of a 0.1% (w/v) trichloroacetic acid solution was used to homogenize leaf samples weighing 0.5 g in an ice bath. Following this, the homogenate was centrifuged for 15 min at 12 000 × g. After centrifugation, 0.5 ml of the supernatant was added to 1 ml of a 1 M potassium iodide solution and 0.5 ml of a 10 mM potassium phosphate buffer (pH 7.0). The resulting mixture's absorbance was then measured at 390 nanometers with a spectrophotometer.

### Malondialdehyde contents

For the purpose of evaluating membrane damage, the malondialdehyde (MDA) content was detected using the protocol explained by Ohkawa et al. (1979). 0.5 g of fresh leaf sample and 2 ml of 5% trichloroacetic acid were mixed together and homogenized. Centrifugation was performed on the resultant extract for 10 min at 15 000 × g. Subsequently, 0.5 ml was extracted from the supernatant of the centrifuged sample and mixed with 0.5% thiobarbituric acid (TBA) dissolved in 20% TCA. The resulting mixture was then heated to 95°C for 30 minutes. After the incubation period, the mixture was cooled in an ice bath for 5 min, and then centrifugation at 10 000 × g was performed for 15 min to terminate the reaction. Finally, the absorbance of the supernatant that was obtained after centrifugation was measured at 532 and 600 nm.

### Total chlorophyll content and electrolyte leakage

The acetonic extract was filtered and then centrifuged at 10 000 × g for 15 min. Afterward, supernatant was used to measure chlorophyll a and b contents using a spectrophotometer at 645 and 663 nm (Arnon 1949). Electrolyte leakage in the plants was found using a conductivity meter by the formula of Rai et al. (2013).

### Total phenolic content and total flavonoid content

Based on the procedure developed by Slinkard and Singleton (1977), methanolic extracts were diluted and added to 0.1 mL of Folin-Ciocalteu reagent for a 5-min reaction period. Afterward, 0.3 mL of a saturated Na<sub>2</sub>CO<sub>3</sub> solution was added, and the mixture was incubated for 2 h. Absorbance was measured at 760 nm after incubation. Gallic acid was used as the reference standard, and all tests were performed in triplicate. TPC values were presented as mg of gallic acid equivalents per g of dry weight (mg GAE/g DW).

To determine total flavonoid concentrations in the samples, 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 M potassium acetate, and 4.3 mL of 80% ethanol were mixed with 0.5 mL of plant extract (dilution 1:5 in methanol). The mixture was incubated at room temperature for 40 minutes before being detected for absorbance at 415 nm with a UV-Vis spectrophotometer. Calibration was performed using quercetin solutions. All the measurements were performed in triplicate. The total flavonoid concentration was calculated as mg of quercetin equivalents per gram of dry weight (mg QE/g DW), Marcucci et al. (1998).

### DPPH assay

To analyze the radical scavenging activity of the samples, DPPH was measured using the method presented in the study of Bektaş et al. (2018). A 50 µl aliquot of methanolic extract solution was mixed with 5 mL of 0.004% (w/v) DPPH radical methanol solution. The reaction mixture was incubated in the dark at 25°C for 30 minutes before measuring absorbance at 517 nm. The inhibition percentage and extract concentration were plotted to determine the concentration required to inhibit DPPH by 50% (IC<sub>50</sub>). The DPPH levels were measured in triplicate and expressed as µg/ml.

### Antioxidative enzymes

In the study, the levels of Ascorbate Peroxidase (APX), Catalase (CAT), Glutathione Reductase (GR), and Superoxide Dismutase (SOD), key antioxidative enzymes, were analyzed to assess their roles in stress response according to the following method. The enzymatic activity of APX, CAT, SOD, and GR was detected using procedures developed by Wang et al. (1991), Chance and Maehly (1955), Beauchamp and Fridovich (1971) and Sgherri et al. (1994), respectively. Antioxidant enzyme activities were normalized to total protein concentration (per mg protein).

### Statistical analyses

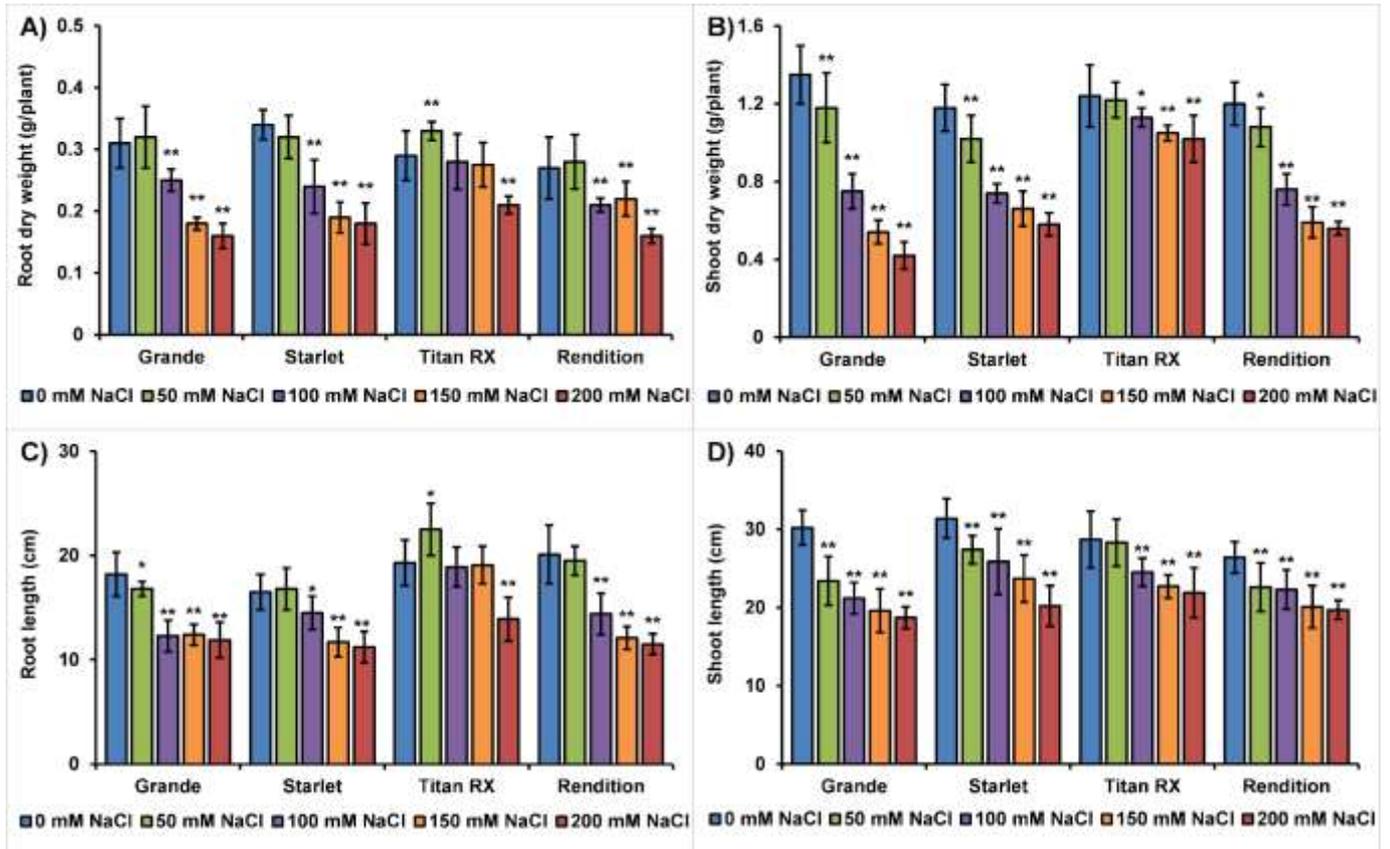
The variables were analyzed statistically using one-way ANOVA with the Tukey test ( $p < 0.05$  and  $p < 0.01$ ), via Minitab software (Minitab Inc., State College, PA).

## RESULTS

### The impact of salinity stress treatments on growth parameters

No statistically significant reduction was observed in root development in 'Titan RX' at NaCl stress doses below 200 mM NaCl, while significant decreases were observed in other cultivars (Figure 1A, 1C). In addition, 'Titan RX' showed enhanced root development under 50 mM salt stress conditions (Figure 1A, 1C).

Although significant reductions in shoot weight and length were observed in the 'Titan RX' under salinity stress, these decreases were more pronounced in the other cultivars, especially in 'Grande,' where reductions reached up to 70% under salinity stress (Figure 1B, 1D).



**Figure 1.** Growth parameters: A. Root dry weight (g/plant), B. Shoot dry weight (g/plant), C. Root length (cm), D. Shoot length (cm) of the tall fescue cultivars in different sets. The columns represent means, and the bars represent standard errors of the four replicates. Asterisks indicate a significant difference between control (0 mM) and NaCl-stressed plants in a cultivar \*p < 0.05 or \*\*p < 0.01.

**Şekil 1.** Farklı gruplardaki kamışsı yumak çeşitleri için büyüme özellikleri; A. Kök kuru ağırlığı (g/bitki), B. Sürgün kuru ağırlığı (g/bitki), C. Kök uzunluğu (cm), D. Sürgün uzunluğu (cm). Sütunla gösterilen değerler ortalama değerleri, barlar ise dört tekrarin standart hatalarını göstermektedir. Yıldız işaretleri, bir çeşitte kontrol (0 mM) ile NaCl stresi uygulanan bitkiler arasındaki anlamlı farkı göstermektedir (\*p < 0,05 veya \*\*p < 0,01).

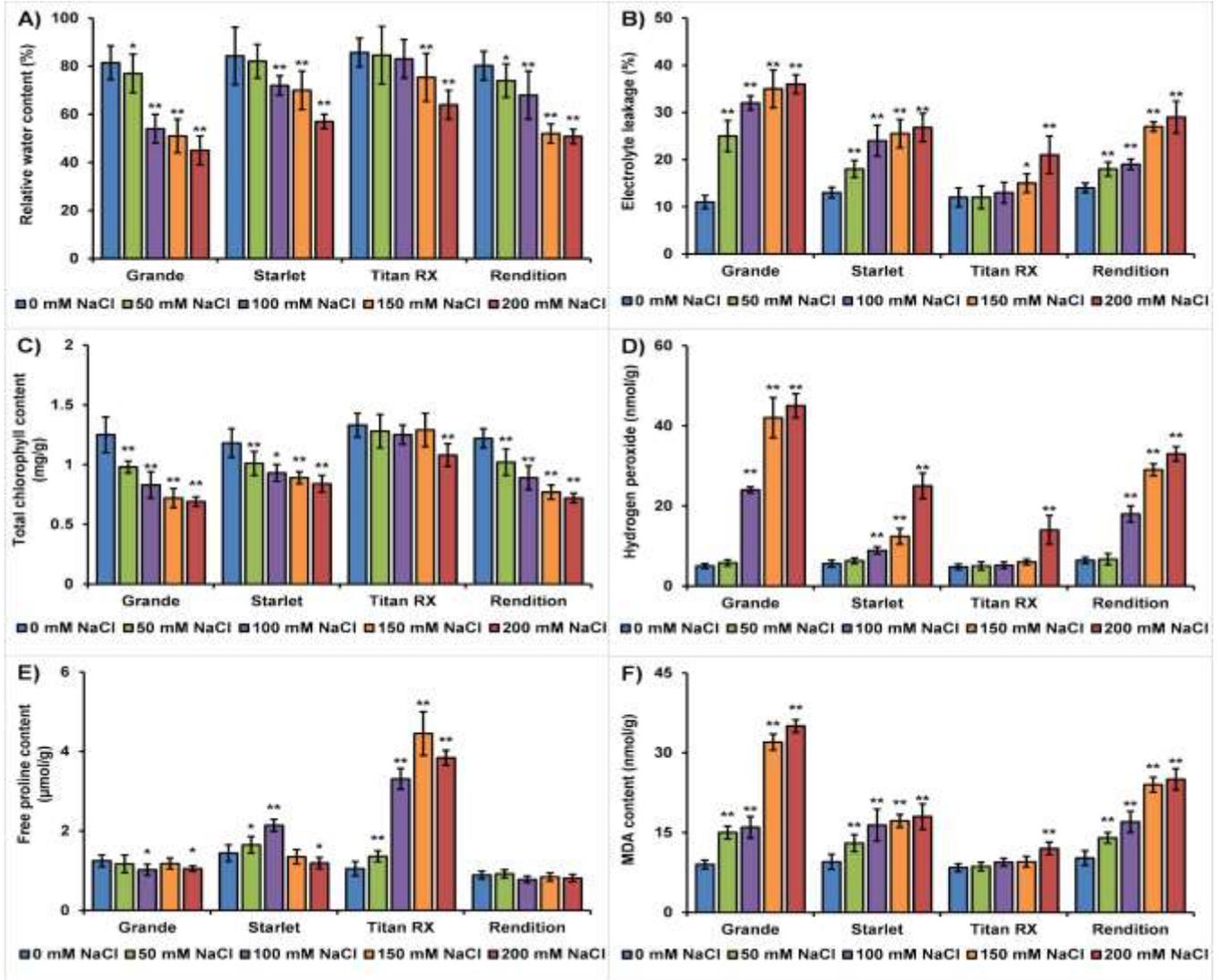
### The impact of salinity stress on biochemical profiles

While no significant changes were observed in the relative water content of 'Titan RX' up to 150 mM NaCl, significant decreases were detected in other cultivars under salinity stress, most notably in 'Grande' (Figure 2A). Similar results were observed in the electrolyte leakage ratio in 'Titan RX', while extreme increases were detected in other cultivars under salinity stress (Figure 2B).

While the 'Titan RX' maintained its total chlorophyll content at NaCl stress doses below 200 mM NaCl, significant decreases were observed in other cultivars under all NaCl stress levels compared to their control (Figure 2C). Similar results were observed in H<sub>2</sub>O<sub>2</sub> and MDA content in 'Titan RX', while extreme increases were detected in other cultivars under salinity stress (Figure 2D, 2F).

Although some increases were observed in free proline content in 'Starlet' under salinity stress, extremely higher

increases were found in 'Titan RX' (4.2 and 3.7-fold, compared to the control under 150- and 200-mM salinity, respectively) (Figure 2E). In contrast, the other cultivars either showed a decrease or no notable change in proline content compared to their control. (Figure 2E).

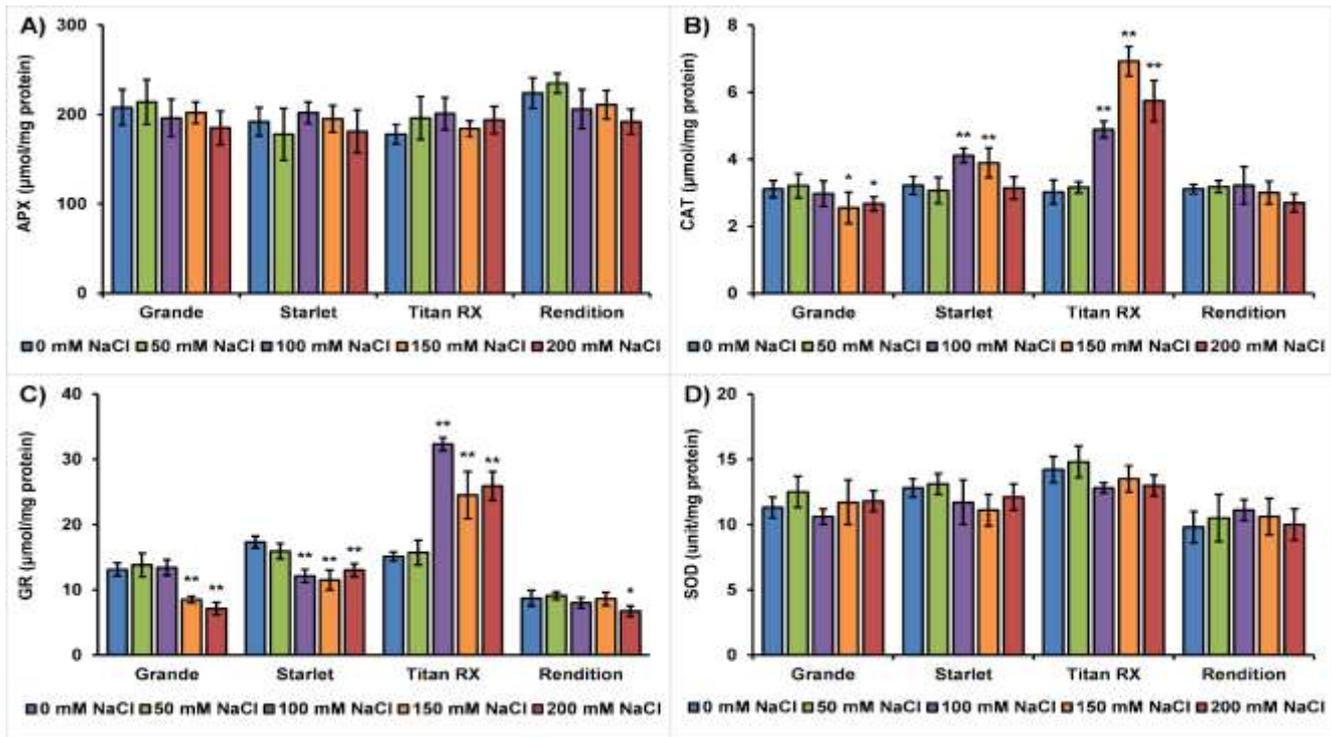


**Figure 2.** Biochemical status: A. Relative water content (%), B. Electrolyte leakage (%), C. Total chlorophyll content (mg/g), D. Hydrogen peroxide (nmol/g), E. Free proline content ( $\mu\text{mol/g}$ ), F. MDA content (nmol/g) of the tall fescue cultivars in different sets. The columns represent means, and the bars represent standard errors of the four replicates. Asterisks indicate a significant difference between control (0 mM) and NaCl-stressed plants in a cultivar \*p < 0.05 or \*\*p < 0.01.

**Şekil 2.** Farklı gruplardaki kamışsı yumak çeşitleri için büyüme özellikleri; A. Bağlı su içeriği (%), B. Elektrolit sızıntısı (%), C. Toplam klorofil içeriği (mg/g), D. Hidrojen peroksit (nmol/g). Sütunla gösterilen değerler ortalama değerleri, barlar ise dört tekrarin standart hatalarını göstermektedir. Yıldız işaretleri, bir çeşitte kontrol (0 mM) ile NaCl stresi uygulanan bitkiler arasındaki anlamlı farkı göstermektedir (\*p < 0,05 veya \*\*p < 0,01).

### The impact of salinity stress on total phenolics, DPPH activity, and total flavonoids

The increases were observed in total phenolics and total flavonoid contents under salinity stress, primarily in 'Titan RX', followed by 'Starlet', compared to their controls, in contrast to the others, except for 'Rendition' under 50 mM NaCl (Figure 3A, 3B). The contrary results were obtained in DPPH activity, where declines were observed under salinity stress primarily in 'Titan RX', followed by 'Starlet', compared to their controls, while no change was observed in the others (Figure 3C). Notably, a 70% decline was detected in 'Titan RX' under a 200 mM NaCl stress level (Figure 3C).



**Figure 3.** Biochemical status: A. Relative water content (%), B. Electrolyte leakage (%), C. Total chlorophyll content (mg/g), D. Hydrogen peroxide (nmol/g), E. Free proline content ( $\mu\text{mol}/\text{g}$ ), F. MDA content (nmol/g) of the tall fescue cultivars in different sets. The columns represent means, and the bars represent standard errors of the four replicates. Asterisks indicate a significant difference between control (0 mM) and NaCl-stressed plants in a cultivar \* $p < 0.05$  or \*\* $p < 0.01$ .

**Şekil 3.** Farklı gruplardaki kamışı yumak çeşitleri için büyüme özellikleri: A. Toplam fenolik miktarı (mg GAE/g), B. Toplam flavonoid miktarı (mg QE/g), C. DPPH aktivitesi ( $\mu\text{g}/\text{ml}$ ). Sütunla gösterilen değerler ortalama değerleri, barlar ise ise dört tekrürün standart hatalarını göstermektedir. Yıldız işaretleri, bir çeşitte kontrol (0 mM) ile NaCl stresi uygulanan bitkiler arasındaki anlamlı farkı göstermektedir (\* $p < 0,05$  veya \*\* $p < 0,01$ ).

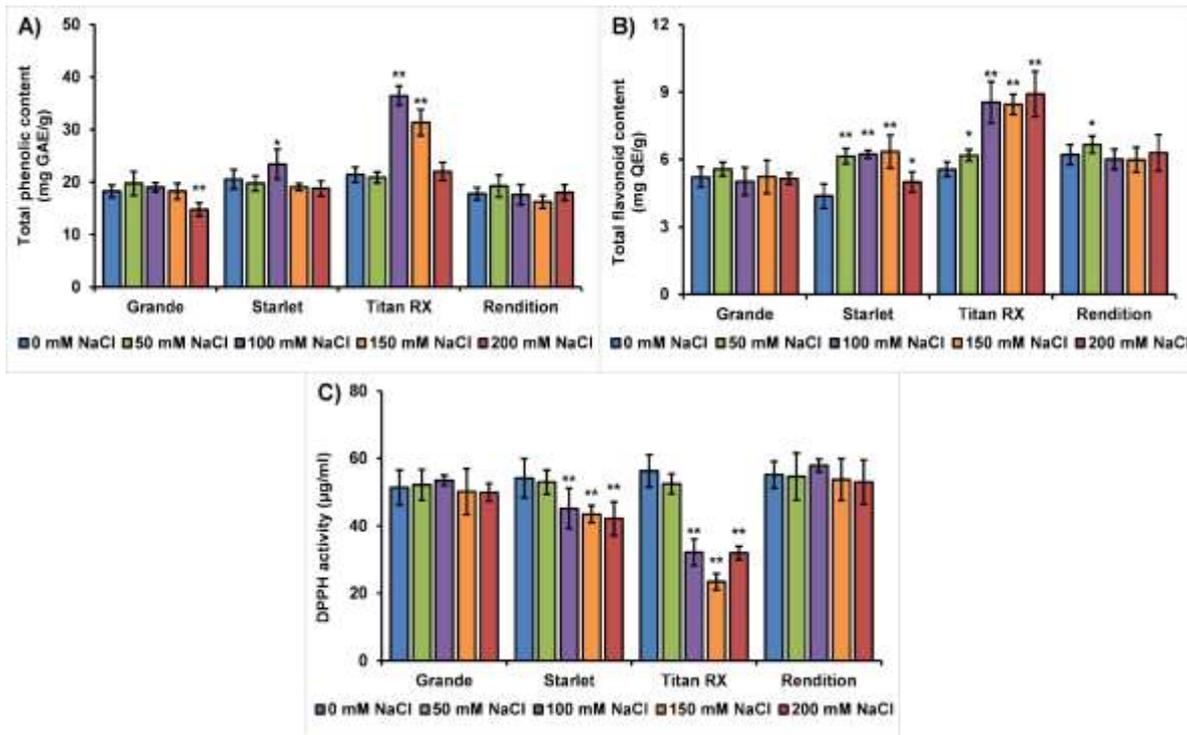
### The impact of salinity stress on antioxidative enzymes

Under salinity stress conditions, CAT and GR activity levels changed significantly, with both enzymes demonstrating significant increases or declines. In contrast, no significant alterations were observed in the activity of APX and SOD (Figure 4A, 4B, 4C, 4D). Impressively, CAT and GR activities showed extreme increases in 'Titan RX', compared to the control. In contrast, these antioxidative enzymes exhibited a significant decrease in the sensitive one, 'Grande' (Figure 4B, 4C).

This study investigated the morphological, physiological, and biochemical reactions under increasing levels of salinity in four tall fescue cultivars, previously identified in preliminary tests as having varied salt stress sensitivity. The sensitivity levels of the cultivars from the preliminary study were confirmed via the evaluation of growth parameters under salinity stress conditions.

'Titan RX' showed increased or maintained root development at NaCl concentrations below 200 mM, unlike the other cultivars, which showed significant reductions (Figure 1A, 1C), indicating that in 'Titan RX', the root zone remained unaffected by osmotic and ionic stress under saline conditions. Maintaining or enhancing root development under saline conditions is a key adaptation strategy in crops to minimize the detrimental consequences of salt stress (Demirkol et al., 2019; Zou et al., 2022). In a study conducted on *Lolium multiflorum*, it was concluded that low levels of salt stress either enhanced or did not affect root development, which was suggested to be an adaptation to osmotic stress (Simsek Soysal et al., 2021).

'Titan RX' exhibited less reduction in shoot growth under salinity stress compared to other varieties (Figure 1B, 1D). Reduced plant development under salt stress could be caused by insufficient water or the harmful effects of sodium chloride, both of which can threaten physiological reactions. (Dugasa, et al. 2019). The lower reduction in root and shoot development in the 'Titan RX' under salt stress confirmed its higher tolerance to salinity compared to other cultivars.



**Figure 4.** Biochemical status; A. Relative water content (%), B. Electrolyte leakage (%), C. Total chlorophyll content (mg/g), D. Hydrogen peroxide (nmol/g), E. Free proline content ( $\mu\text{mol/g}$ ), F. MDA content (nmol/g) of the tall fescue cultivars in different sets. The columns represent means, and the bars represent standard errors of the four replicates. Asterisks indicate a significant difference between control (0 mM) and NaCl-stressed plants in a cultivar \* $p < 0.05$  or \*\* $p < 0.01$ .

**Şekil 4.** Farklı gruplardaki kamışsı yumak çeşitleri için büyüme özellikleri; A. APX ( $\mu\text{mol/mg protein}$ ), B. CAT ( $\mu\text{mol/mg protein}$ ), C. GR ( $\mu\text{mol/mg protein}$ ), D. SOD (ünite/mg protein). Sütunla gösterilen değerler ortalama değerleri, barlar ise dört tekrarin standart hatalarını göstermektedir. Yıldız işaretleri, bir çeşitte kontrol (0 mM) ile NaCl stresi uygulanan bitkiler arasındaki anlamlı farkı göstermektedir (\* $p < 0,05$  veya \*\* $p < 0,01$ ).

'Titan RX' maintained its relative water and total chlorophyll contents up to 150 and 200 mM NaCl, respectively (Figure 2A, 2C). Other cultivars showed noteworthy declines in relative water and total chlorophyll levels in other cultivars, especially in 'Grande' and 'Rendition' (Figure 2A, 2C). Studies have revealed that reducing water in the plant leaves causes degradation chlorophyll pathway, leading to a decrease in the quantity of chlorophyll (Demirkol and Yılmaz 2023; Pour-Aboughadareh et al., 2017).

The tall fescue cultivars exhibited a higher electrolyte leakage ratio under salinity stress conditions (Figure 2B). Studies showed that salinity stress could modify the biological mechanism of cell membranes and cause increased membrane instability (Demirkol and Yılmaz 2023; Jothimani and Arulbalachandran 2020). In this study, 'Titan RX' maintained its electrolyte leakage ratio up to 150 mM NaCl, indicating that its enhanced salinity tolerance may be associated with the maintenance of cell membrane stability and the ability to rapidly repair damage. A study concluded that under salt stress, salt-tolerant plant species maintained their electrical conductivity more effectively compared to the sensitive group (Hniličková et al., 2019)

The findings showed serious up-regulations in  $\text{H}_2\text{O}_2$  production in the tall fescue cultivars under 100, 150, and 200 mM NaCl stress, except for 'Titan RX' (Figure 2D). This indicates that the level of ROS increased in salinity-sensitive cultivars under saline conditions. Several studies have shown that excessive accumulation of ROS, particularly  $\text{H}_2\text{O}_2$ , which causes toxic substances to cells, reduces plant chlorophyll content and damages biological membranes during salinity stress (Kim et al., 2005; Zeeshan et al., 2020).

Under salinity stress, the cultivars 'Starlet' and 'Titan RX' showed a slight and dramatic increase in free proline content, respectively. However, the other two salinity-sensitive cultivars showed declines or no significant changes when compared to their controls (Figure 2E), suggesting that the increased accumulation of free proline plays a key role in determining salinity stress resistance in tall fescue. According to studies, free proline enables plants to deal with a variety of stressors, including salinity, by maintaining turgor and redox homeostasis (Al Hinai et al., 2022; Rajasheker et al., 2019). Similar results were obtained in a study of salt-tolerant forage pea genotypes

(Demirkol, 2020).

Significant increases in MDA content were observed in the tall fescue cultivars under all NaCl stress treatments compared to their controls, except for 'Titan RX' (Figure 2F). MDA is a well-known biomarker of lipid peroxidation level and oxidative damage in plants under abiotic stress conditions (Demirci-Cekic et al., 2022). The increased MDA under salt stress has been reported in salt-susceptible crops in various studies (Jan et al., 2017; Liu et al., 2022). The detrimental impacts of NaCl stress on lipid peroxidation have been found in several plant species, and MDA has been accepted as a reliable marker of salinity tolerance (Zeeshan et al., 2020). The current data indicate that 'Titan RX' exhibited better protection against oxidative damage under salinity stress than the other cultivars.

Significant increases were detected in total phenolics and total flavonoid contents in 'Titan RX' and 'Starlet' under salinity stress treatments (Figure 3A, 3B), suggesting that these cultivars activated the antioxidative defense system to mitigate oxidative damage caused by salinity stress. In a study, moderate salinity stress was reported to increase total polyphenols in *Thymus vulgaris* (Zrig et al., 2016). These results agree with earlier studies showing that salinity stress stimulates the biosynthesis of phenolics and flavonoids as a part of a protective mechanism in plants (Bistgani et al., 2019). The enhanced biosynthesis of the compounds of total phenolics and flavonoids in 'Titan RX' and 'Starlet', highlighting their potential ability to cope with salinity stress.

Significant decreases were observed in DPPH activity in 'Titan RX' and 'Starlet' under salinity stress treatments (Figure 3C), suggesting a reduction in the radical-scavenging capacity of these cultivars under salinity stress. The previous studies have reported that increased phenolic content has led to a decrease in DPPH (IC50) activity (Demirkol and Tarakci 2018; Kainama et al., 2020). The decreases in 'Titan RX' and 'Starlet' may show the protective roles by increased total phenolics, flavonoids, or antioxidative enzymes (CAT and GR) in these cultivars.

Significantly increased values were observed in CAT in 'Titan RX' and 'Starlet' under salinity stress treatments, compared to their controls (Figure 4B). Plants' antioxidant defense mechanism relies heavily on CAT, which converts H<sub>2</sub>O<sub>2</sub> into water and oxygen (Riseh et al., 2024). This process is crucial in reducing the negative impacts of ROS, which accumulate under a variety of stress conditions, including salinity (Hasanuzzaman et al., 2020). The results suggest that 'Titan RX' and 'Starlet' demonstrated enhanced resistance to salinity stress compared to the other two cultivars, attributed to an upregulation of their CAT activity.

In the study, similar results were observed for GR activity, which paralleled the findings with CAT in 'Titan RX' (Figure 4C).

The recent studies have shown that GR uses NADPH to react with ROS, including hydroxyl and superoxide radicals. This process protects cells against injury caused by oxidation via maintaining redox balance (Mushtaq et al., 2020).

Although significant increases in APX and SOD activities have been identified as essential indicators of salt stress resistance in several plant species (Demirkol, 2021; Hasanuzzaman et al., 2020; Shafi et al., 2015), this impact was not detected in the tall fescue cultivars studied.

## CONCLUSIONS

The results revealed that the salinity-tolerant cultivar 'Titan RX' exhibited enhanced root growth while maintaining shoot biomass, relative water content, H<sub>2</sub> O<sub>2</sub>, MDA, total chlorophyll content, and electrolyte leakage rate under salinity stress. This enhanced tolerance was attributed to the activation of biosynthetic pathways for free proline, total phenolics, total flavonoid contents, and antioxidative enzymes (CAT and GR). Thus, the physiological mechanism underlying salinity stress tolerance in tall fescue has been identified, along with the critical function of antioxidative enzymes in this process. In addition, these findings suggest that 'Titan RX' has the potential to be used under saline conditions.

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## Contribution Rate Statement Summary of Researchers

The contribution of the authors is equal.

## Statement of Conflict of Interest

The authors have declared no conflict of interest.

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