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SALINITY-INDUCED TOTAL ANTIOXIDANT, PHENOLIC AND FLAVONOID CONTENTS IN RICE VARIETIES

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Abstract

Salinity has detrimental effects on rice's morphological, physiological, and biochemical traits, which significantly impedes development and yield. This study examined differences in total antioxidant capacity (DPPH), total phenolic (TPC), and flavonoid (TFC) content in four different *Oryza sativa* L. types (Karacadağ, Gala, Tunca, and Aromatik-1) that were exposed to different salt concentrations (0, 100, 200, and 300 mM NaCl). The highest increase in both TPC and TFC was observed in 300 mM NaCl application of Tunca (91 and 83%, respectively). Out of all the applications, the highest decrease in TPC and TFC was at 100 mM NaCl of Aromatik-1 (68 and 60%, respectively) as compared to the control. In terms of the total antioxidant capacity, NaCl applications of Gala, Tunca and Aromatik-1 at 400-1000 µg/ml concentrations were higher than BHT used as positive control. Within the rice varieties, the concentrations of Tunca at 100, 200, and 300 mM NaCl demonstrated high rates of free radical scavenging activity (80.08, 80.18, and 79.67, respectively).

Introduction

The effect of salinity on plant growth is closely related to plant stages of development. The salinity stress impacts negatively to plant growth parameters such as seed growth, flowering, fruit formation resulting decrease in productivity and quality (Jampeetong and Brix 2009, Gorai *et al.* 2010). The salts in the environment impress to water-absorbing ability (osmotic stress) or transpiration flow (ionic stress) of plant. These two factors are accepted as primary effects generated by salinity stress on plants (Yang and Guo 2018).

The negative effect of high salt concentration on plants is due to the osmotic retention of water and the damage of ions on protoplasm. The water potential decreases as linear depend on the increasing salt concentration in the solution and as a result of this, the plant getting less water as the salinity concentration increasing. The increase in the Na^+ cation and the associated Cl^- and SO_4^{2-} anions in the environment leads to the deterioration of the ion balance $K^+/Ca^{2+}/Na^+$) in the protoplasm. In parallel with, the enzyme activity, protein syntheses and membrane permeability are decreased. So, chloroplast and the other structures significantly get harm (Taban and Katkat 2000, Kacar *et al.* 2002).

Expulsion or selective deposition of salt ions, control of ion uptake via roots, some changes in the photosynthetic signal pathway, changes in membrane structure and stimulation of phytohormones take part durability strategies that developed by plants (Parida and Das 2005). In addition of these, plants activate some non-enzymatic systems such as tocopherols, flavonoids, anthocyanins, phenols, carotenoids, gultathione, ascorbate and enzymatic system including Reactive Oxygen Species (ROS) scavenger enzymes towards to kind of stresses. Moreover, it is important to produce phenolics at the early maturation stage for plant defense against abiotic stresses. Phenolics and anthocyanins at whole rice grain relates to decrease of developing in some chronic disease such as obesity, cancer, diabetes risk (Okater and Liu 2010).

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Considering the salinity causing significant damage to plant development, it is considered that the application of rice farming in these areas will be effective and economical in order to reintroduce the salty lands that affect large areas in Turkey (Demiral and Türkan 2005). In addition, it has been stated in many international studies that the salinity tolerance of rice varieties during germination and seedling period can give a complete idea about the salinity tolerance of these genotypes, as in many plants (Lutts *et al.* 1999, Babu *et al.* 2005). Thus, in the present study, changes in chemical compositions including total phenols, flavonoids and total antioxidant in response to salinity stress in four rice varieties were investigated.

Materials and Methods

In the research, a total of 4 different rice seeds, Aromatik-1, Gala, Tunca and local Karacadağ cultivars, were used as plant material. Seeds of rice varieties were obtained from Trakya Agricultural Research Institute. Seeds grouped separately for each variety were left to imbibition after surface sterilization and were planted in pots containing soil:peat:perlite (3:3:1) and allowed to grow in the plant growth room where controlled conditions were provided. Seeds were irrigated with ¼ Hoagland nutrient solution based on field water capacity (65%) for 4 weeks (28 days). After a 4-week growing period, plants were exposed to salt stress factor with prepared NaCl concentrations. For this, it was irrigated with ¼ Hoagland nutrient solution prepared with 0, 100, 200 and 300 mM NaCl for 10 days. Plants in the control groups were watered with ¼ Hoagland nutrient solution without NaCl at the same time and extent. After 10th day, the plants were harvested.

Leaf samples of rice varieties left to dry during harvest were pulverized and macerated with methanol for 24 hrs. The solvent part of the extracts filtered through Whatman No:1 filter papers was removed by evaporator. Stock solutions were prepared from the solid extract at a concentration of 1000 µg/ml for total phenolic, total flavonoid and antioxidant activity determinations.

The concentration of total phenolics of methanol extracts were determined by using Folin-Ciocalteu reagent (Slinkard and Singleton 1977) and external calibration with gallic acid. About 0.1 ml of extract solution, 4.5 ml of distilled water and 0.1 ml of Folin-Ciocalteu reagent were added and the contents mixed vigorously. After shaking 3 min, 2% Na_2CO_3 was added, and finally the mixture was allowed to stand for 2 hours at room temperature. The absorbance was measured at 760 nm using UV-VIS spectrophotometer. The concentration of the total phenolics was estimated as µg of gallic acid equivalent by using an equation obtained from gallic acid calibration curve.

Total flavonoid contents of rice were estimated by using the aluminium nitrate colorimetric method as described by Moreno *et al.* (2000). The 100 µl of the stock solutions of the extracts were taken and the volume was made up to 4.8 ml with 80% methanol. Then 100 µl of 1 M potassium acetate and 100 µl of 10% aluminum nitrate added to the mixture. The same procedure was followed for quercetin solution prepared at different concentrations and spectrophotometric measurements were taken at 415 nm wavelength after 40 min incubation time.

The free radical scavenging effects of the methanol extracts were estimated according to the method of Blois (1958). The 1 ml of each sample, prepared at various concentrations (10, 25, 50, 100, 250 and 500 µg/ml) were added to 100 µM DPPH radical solution. The mixture was shaken and allowed to stand for 30 min at room temperature in the dark, and then the absorbance was measured at 517 nm with a spectrophotometer. Ascorbic acid, BHT and BHA were used as positive control. The percentage inhibition activity was calculated by the following equation: Scavenging effect (%) = $[(A_{control} - A_{sample}/A_{control})] \times 100$.

$A_{control}$ and A_{sample} are the absorbance values of the control sample (containing all reagents except the test compound) and the test sample, at particular times, respectively.

All the experiments were done in triplicate. Data were reported as mean ± standard deviation (SD). Statistical analysis were performed using SPSS 20.0 for Windows (SPSS Inc., Chicago, USA). Significance of differences was tested using one-way analysis of variances (ANOVA) and Duncan tests at a 0.05 level of significance.

Results and Discussion

Figure 1 illustrates the variations in the total phenolic content (TPC) of various rice cultivars in response to varying concentrations of NaCl stress, expressed as an equivalent to gallic acid. Because rice cultivars respond differently to varying degrees of salinity stress, there are notable variances at the TPC. Karacadağ variety comparing to the control group, the application of 100mM NaCl salinity stress raised TPC ratio by 56%. Applying 200 mM NaCl significantly raised the TPC ratio in Gala by 64 %. It was observed an increasing at TPC parallel with increase of salty concentration when compared with the control group in Tunca cultivar that applied salinity stress. The 300 mM NaCl stress at 91% ratio showed the largest increase among the applied concentrations. Control, 200 and 300 mM NaCl applications had high values for total phenolic content in Aromatik-1 rice. All salt treatments were observed to lower the phenolic content when compared to the control group, with the largest decline occurring 68% and 100 mM NaCl concentration.

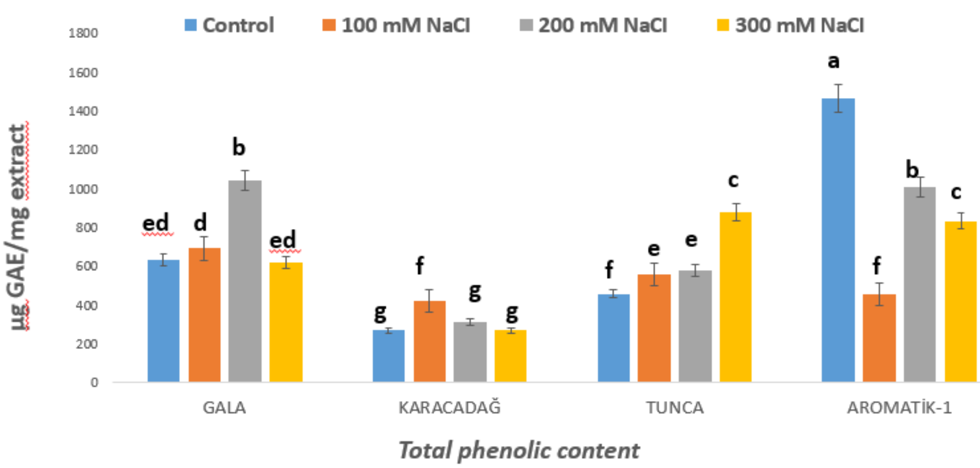

Fig. 1. Effect of salinity on total phenolic contents in rice varieties. Vertical bars indicate ± SD. Letters (a-h) indicate significant differences $p \leq 0.05$ compared with control group.

Figure 2 displays the total flavonoid content (TFC) of the methanol extract from the rice cultivars Karacadağ, Gala, Tunca, and Aromatik-1 at varying salinity concentrations, which is comparable to quercetin. When compared to the control group, TFC was seen to be declining at 300 mM concentration and rising at 100 and 200 mM salinity concentration in Karacadağ. At Gala, it was discovered that the salt concentrations raise the TFC in comparison to the control. The increase was 64% at 200 mM solution of NaCl and roughly 20% at 100 mM concentration. It was shown that raising the TFC in Tunca rice correlated with an increase in salt content. The largest increase, of 83%, happened at a concentration of 300 mM NaCl. All of the salt amounts that were administered to the Aromatik-1 variety reduced the TFC in comparison to the control group. The highest decreasing occured at 100mM NaCl concentration of Aromatik-1 with 60% among the all varieties and salinity application.

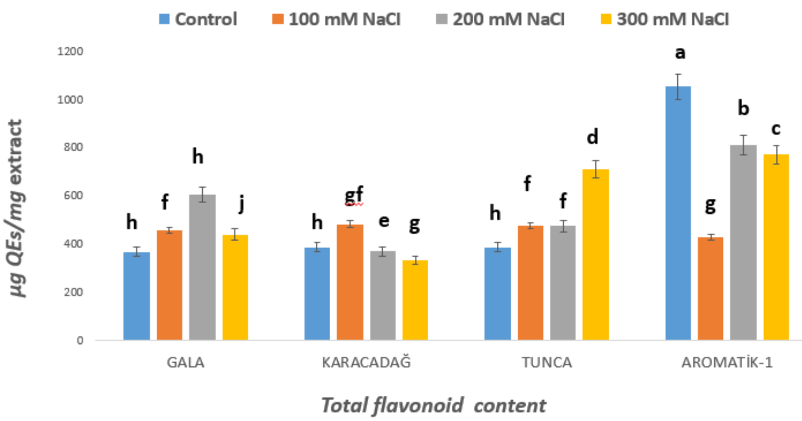

Fig. 2. Effect of salinity on total flavonoid contents in rice varieties. Vertical bars indicate ± SD. Letters (a-h) indicate significant differences $p \leq 0.05$ compared with control group.

BHT, BHA, and ascorbic acid were utilized as positive control for DPPH free radical scavenging activity (Fig. 3). All applications of the Aromatik-1 rice variety, as well as at 200 µg/ml concentration 200 mM NaCl of Gala, 100 and 300 mM NaCl of Tunca were found to have greater radical scavenging activities than BHT, which was utilized as a positive control. Furthermore, it was discovered that all NaCl concentrations used in the rice types Gala, Tunca, and Aromatik-1 at 400–1000 µg/mL concentrations were greater than BHT. On the other hand, free radical scavenging activities of all cultivars that NaCl applied was found lower than other positive controls (BHA and ascorbic acid). When the free radical scavenging activity in rice cultivars was evaluated, the highest rates were found in 200 mM, 100 mM and 300 mM NaCl concentrations of Tunca (80.184%; 80.082; 79.671, respectively).

There are two main effects of soil's high salinity on the plants: the first is the osmotic effect, which stops water from entering the soil solution, and the second is the harmful ion effect, which alters some physiological states (James *et al.* 1982). The stress came from salinity may cause oxidative stress by triggering the formation of active oxygen species. Accumulation of phenols in plants have important function that cope with oxidative stress came from salinity (Hichem *et al.* 2009). For this purpose, changing of contents in total phenolics, flavonoid and antioxidant (DPPH) of 4 different rice cultivars were examined that exposed salinity stress in present study.

By controlling ion and water activity, salt-tolerant plant species offer a stronger antioxidant defense system against reactive oxygen species (Hussain *et al.* 2012). Minh *et al.* (2016) determined the relationship between salinity stress and total phenolic and flavonoid on growth in rice. Total phenolics, flavonoids, vanillin and protocatechuic acid contents were strongly increased in tolerant cultivars, whereas they were significantly decreased in sensitive cultivars. Similar findings were made by Danai-Tambhale *et al.* (2011), who found that under salinity stress, the tolerant rice variety accumulated more total polyphenols and other secondary metabolites than the sensitive. Researchers have reported that the adaptation mechanism of rice under salt stress may be responsible for the rise in phenolic and flavonoid synthesis in tolerant types. In the present study, higher increases in total phenol and flavonoid contents were detected in Tunca rice variety compared to other varieties.

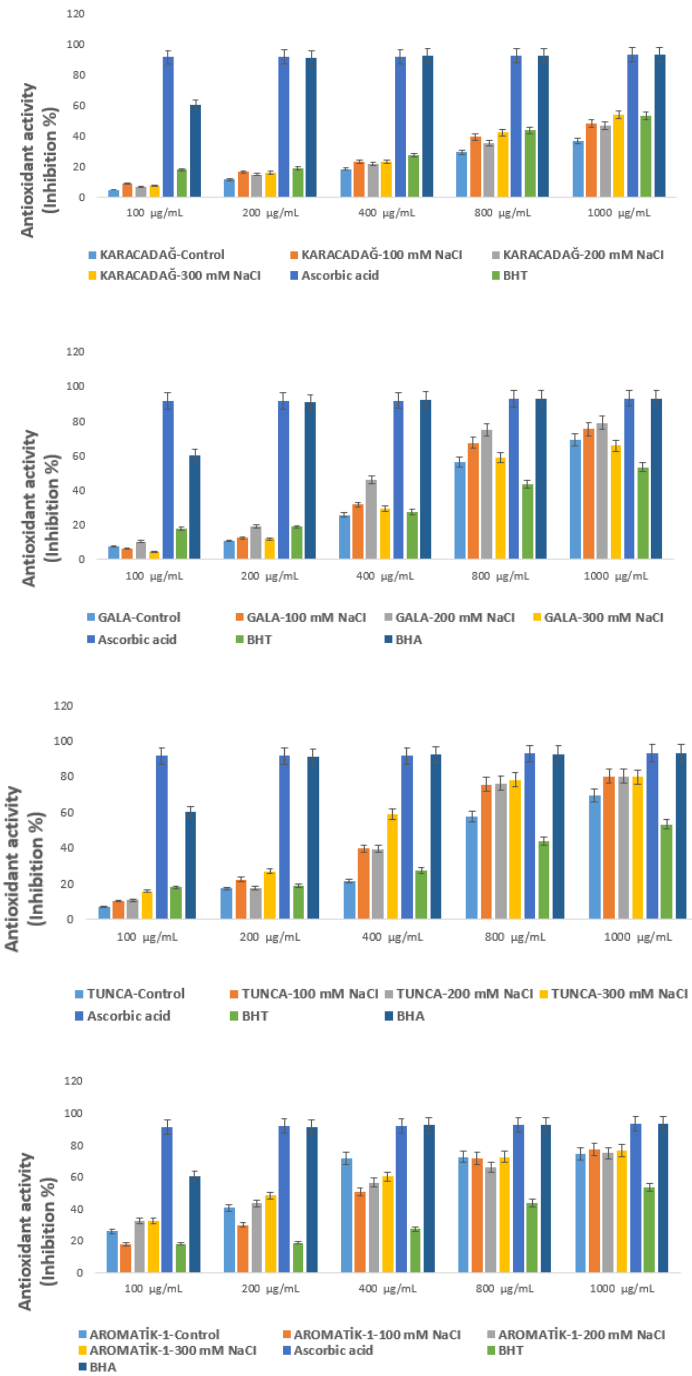

Fig. 3. Effect of salinity on DPPH radical scavenging activities in *Karacadağ, Gala, Tunca* and *Aromatik-1* rice varieties. Vertical bars indicate ± SD.

Research on how stress signals affect plants' secondary metabolites is becoming more and more prevalent. Compounds like phenolics, flavonoids, and phenolic acids have been found to be the most prevalent important groups of plant secondary metabolites since the middle of the 20th century (Bourgaud *et al*. 2001). These molecules have been identified as markers of biotic and abiotic stress tolerance in plants (Balasundram *et al.* 2006, Lattanzio *et al.* 2006). Plants exposured to particular stress conditions produce more of these compounds than that not under stress (Selmar 2008).Du *et al.* (2021) examined the total phenol, flavonoid, and antioxidant capabilities of two rice types (Qiutianxiaoting and 93-11) after gradually exposing them to cold stress (4 °C) for 0,12,18,36, and 48 hrs.The findings demonstrated that 93-11 had more antioxidant activity than Qiutianxiaoting following the chilling treatment. According to Zhang *et al.* (2016), when rice seedlings were exposed to low temperatures, their phenol and flavonoid content increased. In three distinct rice varieties, Rayee *et al.* (2018) investigated the effects of water deprivation stress applied after anthesis. In their investigation, two Japonica cultivars, K1 and K3, as well as an Indica subtype, K4 , were subjected to water deficiencies at intervals of two and three days following till harvest. The researchers reported that in DPPH radical scavenging assays, rice grain's antioxidant capacity increased to 59.1%. In another related study, the protective mechanisms of antioxidants against drought stress in three different rice types (PB, IR-29, and Pokkali) were investigated. When varieties under drought condition were compared, increasing of content of phenolics and flavonoid were determined related with drought in especially PB and IR-29 varieties. It has been reported by researchers that the Pokkali rice variety has a higher potential than other types to transition from drought-related oxidative stress (Basu *et al.* 2010). Drought-tolerant cultivars of rice (Guo *et al.* 2006), caper (Ozkur *et al.* 2009), coffee (Lima *et al.* 2002), and wheat (Lascano *et al.* 2001) have also reported of exhibiting higher antioxidant activity than sensitive ones.

Also high antioxidant capacity under salt, temperature, drought etc. stress can prevent cell damage and correlate with the plant's tolerance to salt stress (Shon *et al.* 2004, El-Beltagi *et al.* 2008, Salama *et al.* 2009). It was reported by many studies that the ability to tolerate drought, salinity, etc. stress of phenolic, flavonoid and antioxidant capacities are affected by varies greatly between plant genotypes. The impact of several solvents on the efficient recovery and antioxidant activity of phenolics in 10 different types of rice was studied by Zubair *et al.* (2012). Their research on ferrous ion chelating activity, reducing power, and antioxidant activity showed that the rice cultivars Basmati Pak, Basmati 2000, and Basmati 515 have high antioxidant potential. Using six different solvent, the DPPH radical scavenging activity of extracts from rices ranged from 6.26 mg/ml (Basmati 515) to 2.22 mg/ml (Basmati 2000).

The enzymes peroxidase and catalase are responsible for removing extremely poisonous H_2O_2. Catalase catalyzes the transformation of H_2O_2 into molecular oxygen and water in peroxisomes. By oxidizing substrates like phenolic compounds or antioxidants, the peroxidase enzyme carries out the same task (Pan *et al.* 2006). The Tunca and Aromatik-1 rice varieties' higher total phenolic, flavonoid, and antioxidant capabilities could be because they were better at scavenging the reagent H_2O_2 in the present study.

The development of salt tolerance in plants is mediated by a variety of physiological and biochemical processes. Because of this, it is quite challenging to comprehend why plants tolerate salt. Furthermore, these systems by themselves are insufficient to give plants salt tolerance. It is necessary to reveal to disclose how different processes interact and what role each plays in the development of salt tolerance. Acquiring this data will also be helpful for breeding research and the creation of genotypes that are resistant to salt. The present study's data can provide useful evidence for developing protect rice production against biotic stresses. Additionally, it might aid in the creation of certain flavonoids, phenolic acids, and bioactive reagents to the generation of rice that can withstand salinity.

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