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Biochemical and Molecular Evaluation of Some Tomato Hybrids in Salt Stress At The Growth Stage

Abstract

Growing stages of plants are the most sensitive phase to abiotic stress which leads to decrease in yield. The object of this study was to evaluate the biochemical and molecular properties of seven tomato cultivars and to observe their responses to salinity during growth stages. Greenhouse experiment was performed to study differences in genotypes response to salinity with four doses (50 mM, 100 mM, 150 mM and 200 mM). Differences in growth parameters, proline accumulation, lipid peroxidation and hydrogen peroxide were tested in order to put forward the relative tolerance or sensitivity of tomato cultivars. These results were also supported by expression using semi quantitative RT-PCR of two stress related genes, *SIVOZI*, and *SIWRKY8*. Overall results demonstrated that, all evaluated parameters increased with 200 mM NaCl treatment for all cultivars. Although the effects of salinity stress differentiated in different genotypes, our results provided some evidence that Seyran, Ciko and Indigo Rose Black tomato cultivars had higher salt tolerance potential than rest of the cultivars. This is the first report of evaluation of Turkish tomato hybrids based on biochemical and molecular data and results could be useful for determination of future breeding strategies for salt tolerance in tomato.

INTRODUCTION

Tomato is the second most fundamental vegetable crop after potato, with global production exceeding 180 million tons from 5.03 million hectares. (FAO, 2019). Identification of novel tomato varieties which accumulate higher levels of antioxidants and secondary metabolites is a priority objective that increases tomato consumption (Hou et al., 2020). In Turkey, tomato is very important for the diet and Turkey is the third in the world (about 13 million tons) for tomato production (for fresh and processing goals) following China, and India (FAO, 2019). Multiple environmental factors influence plant growth and development, which are broadly divided into biotic and abiotic components. Abiotic stressors in plants are caused by salinity, high temperatures, cold, or drought, resulting in enormous agricultural losses around the world (Parihar et al., 2015). Among those factors, salinity is one of the most important constraints in environmental and agricultural crop production (Gharsallah et al., 2015). It is currently estimated that 1125 million hectares of land and 32 million ha of agricultural area are salt-affected, and the world's irrigated land is decreasing by 1-2% each year (Hossain, 2019). To address these limitations, it is critical to improve crop plant salt tolerance by better understanding the biochemical, physiological, and molecular mechanisms that plants have adapted to overcome salinity stress. During cultivation, tomatoes are susceptible to some abiotic stresses which ultimately affects the quality and output of fruits (Chakma et al., 2021). Tomato is a fruit moderately resistant to saline environments with a saturated paste EC threshold of 1.3~6 dS m⁻¹ (Maggio et al., 2004). Higher salinity, on the other hand, has an impact on fruit output, germination, and plant vigor (Yang et al. 2019; Abdelgawad et al., 2019). Furthermore, salinity affects the plant's physiology and nutritional health, which is linked to lower yields, such as smaller or fewer fruits (Negrao et al., 2017). Salt

resistance can be strongly influenced by a variety of factors, including developmental stage, plant genotype, and crop tolerance to salt stresses in the soil (Amjad et al., 2019; Zhang et al., 2017). The salinity tolerance of plants should be increased to a certain level in order to make semi/arid regions agriculturally productive (Karan et al., 2012). Evaluation of the current tomato cultivars used in tomato production is important to determine breeding strategies for salt tolerance in tomato. The present study aimed to evaluate salt tolerance potential of some Turkish hybrids of tomato cultivars based on biochemical and molecular parameters. This study evaluates available resources in terms of salt resistant trait for the development of new varieties that are more tolerant to salinity stress. To achieve this aim, proline content, lipid peroxidation and H₂O₂ content which have been used as biochemical markers (Kongngern et al., 2012), and the expression levels of *SIVOZI* and *SIWRKY8* genes used as molecular markers (Bai et al., 2018) were determined to evaluate salt tolerance level of the tomato hybrid cultivars.

MATERIALS and METHODS

Plant materials and salt stress application

Six tomato hybrids Alkis F1, Cigdem F1, Admin F1, Ciko F1, Seyit F1, Seyran F1 and one Indigo Rose Black genotype were used as plant materials. Tomato hybrids obtained from different origins were given in Table 1. The seedlings were grown under glasshouse conditions in pots at 28 °C/20°C day/night temperatures at Manavgat Vocational School of Higher Education, Akdeniz University, Antalya, Turkey. Experiments were designed in randomized blocks design with three replicates (five plants in each replication). Plants were grown two months with Hoagland solution and salinity stress has been constantly increased (50 mM, 100 mM, 150 mM and at final 200 mM NaCl) to avoid osmotic shock every other day intervals (Akbudak and Filiz 2019). Control

plants were grown non-salinize conditions at the same time in the experiment. After 24h of the final treatment of 200 mM NaCl, plant leaves were picked for RNA isolation.

200 mM salt stress for tomato plants at seedling stage showed great differences in a short time (Dasgan et al., 2002), therefore we have used this concentration.

Table 1. Plant material and origin of the tomato genotypes used in the experiment

Genotype	Species	Origin	Specific fruit weight
Alkis F1	<i>S. lycopersicum</i>	Multi Seed	35-50 g
Cigdem F1	<i>S. lycopersicum</i>	White Seed	170-200 g
Admin F1	<i>S. lycopersicum</i>	Sera Seed	220-240 g
Ciko F1	<i>S. lycopersicum</i>	Yuksel Seed	150 g
Seyit F1	<i>S. lycopersicum</i>	Yuksel Seed	50 g
Seyran F1	<i>S. lycopersicum</i>	Nunhems Seed	200-220 g
Indigo Rose Black	<i>S. lycopersicum</i>	Zengarden	70-90 g

Biochemical characterization

The amount of proline in the sample was determined using the method previously reported (Bates et al., 1973). The samples were measured at 520 nm (Epoch Microplate Spectrophotometer, Biotek). The thiobarbituric acid (TBA) method to determine the malondialdehyde (MDA) level was used to test lipid peroxidation, as described by Ali et al. (2005). The absorbance was measured at 450, 532, and 600 nm (Epoch Microplate Spectrophotometer, Biotek). MDA levels of the samples were calculated using the equation specified in (Uluisik and Oney-Birol, 2021). Hydrogen peroxide (H₂O₂) level was determined as reported by Junglee et al. (2014) with minor alterations. 100 mg of leaf samples (control and 200 mM salt treatment) were mixed with 1 ml of solution containing 0.25 ml trichloroacetic acid (TCA) (0.1% (w:v), 0.5 ml 1 M KI and 0.25

ml potassium phosphate buffer (10 mM, pH 7.4) at 4°C for 10 mins. The absorbance of the samples was taken at 390 nm.

Molecular characterization

RNA extraction and semi q-RT-PCR

Total RNA was extracted from fine powdered of control and 200 mM NaCl treated tomato leaves using CTAB method described by Meisel et al. (2005). Total RNA was reverse transcribed into cDNA and the related gene primers were used from the work carried out (Bai et al., 2018). The *ELONGATION FACTOR 1- α* gene, (*LeEF-1*, GenBank accession X14449) (Pokalsky et al., 1989) was used as an internal expressed gene. PCR Master Mix (2X) (Thermo Fisher Scientific) was used to perform the semi-q-RT-PCR reaction in a total volume of 20 μ L and was conducted in a Thermal Cycler (Applied Biosystems Veriti Dx 96 Well). Primers used for the analysis were listed in Table 2.

Table 2. Primers used for RT-PCR validation

Gene	Forward Sequence	Reverse Sequence
SIVOZ1	CACGGCAAAGTCTCCTTGGA	GTGCCAACCCCGTCCATTAT
SIWRKY8	TAATTCTGCCGAAAGCCTC	ATGCTTATTGCCGTACTCGA
LeEF-1	ACCTTTGCTGAATACCCTCCATTG	CACAGTTCACTTCCCCTTCTTCG

Based on the expression of *LeEF-1* gene, equal density of their respective PCR products in the control and salt treated

samples were testified to induce *SIVOZ1* (Ganie et al., 2020) and *SIWRKY8*

(Bai et al., 2018) if the respective PCR products showed different intensities.

Statistical analysis

All of the studies were carried out in triplicate ($n=3$), and the findings are given as mean and standard error (SE). The experimental data was analysed using Microsoft Excel 2016 (Microsoft Corp., Redmond, Washington, USA). Data for p -values were analysed by Student's t test at a significance level of 0.05.

RESULTS

Biochemical characterization

Proline, which helps plants withstand stress conditions, is admitted to be composed in higher amounts under stress conditions (Siddique et al., 2018). A clear relationship has been found between 200 mM NaCl treatment and an increase in the amount of proline (Figure 1) ($P<0.05$).

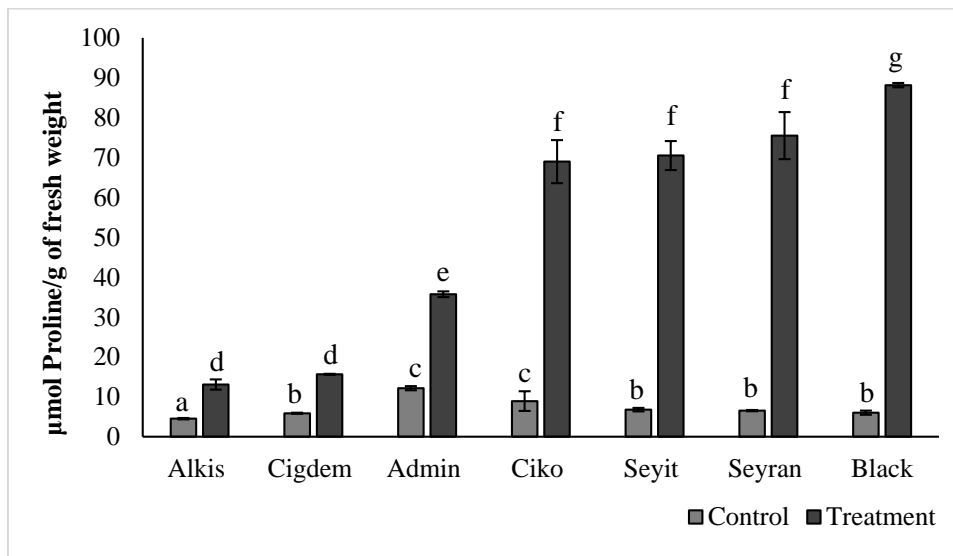


Figure 1. Effect of salt stress on leaf proline content of ($\mu\text{mol Proline/g}$ of FW) seven tomato cultivars subjected to control and 200 mM NaCl concentration. Values represent means \pm SE ($n=3$). Different letters indicate significant differences (Student's t -test; $P<0.05$)

Proline accumulation increased significantly 24 h after the 200 mM NaCl treatment, and there was much dramatic increase in Ciko, Seyit, Seyran and Indigo Rose Black tomato genotypes. Although there was a significant increase ($P<0.05$) in proline content of Alkis and Cigdem under salt stress, this up-regulation was only one-fifth of those which were the first four genotypes. For example, while control plants of Alkis were 4.5 $\mu\text{mol Proline/g}$ of FW, it increased to 13 $\mu\text{mol Proline/g}$ of FW under 200 mM salt stress. However, when we looked at the tomato Indigo Rose Black variety at same time, the amount of

increased from 6 $\mu\text{mol Proline/g}$ of FW in control to 88.1 $\mu\text{mol Proline/g}$ of FW in 200 mM salt stressed plants. The highest proline content was produced by Indigo Rose Black tomato variety among all genotypes at 200 mM NaCl concentration and indicating that this variety has a better capacity to protect macromolecules such as proteins and cell membranes. The concentration of MDA evaluated by TBA reaction. In response to salt stress, all genotypes studied showed a steady increase in MDA content in their leaves when compared to their respective controls (Figure 2).

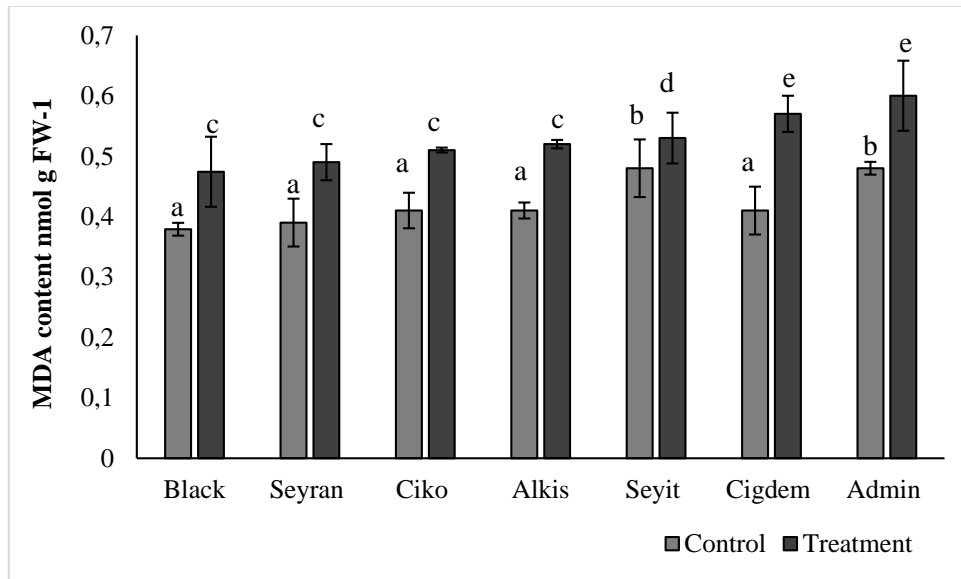


Figure 2. Effect of salt stress on leaf MDA content of (MDA content nmol g FW⁻¹) seven tomato cultivars subjected to control and 200 mM NaCl concentration. Values represent means \pm SE (n=3). Different letters indicate significant differences (*Student's t-test*; $P < 0.05$)

Two-hundred mM salinity stress treatment showed statistically significant increase ($P < 0.05$) in lipid peroxidation in all varieties, although the effects were greater in Cigdem and Admin. Generally, exposure of plants to 200 mM NaCl caused more than a %25 increase in MDA content over the control plants. However, Seyit (%10 increase, the smallest) and Cigdem (%40 increase, the biggest) differentiated compared to other genotypes. Although the MDA values of Indigo Rose Black, Seyran,

Ciko and Alkis are very close to each other, Indigo Rose Black variety have the least MDA value by a small difference. These results indicated that the genotypes Indigo Rose Black and Seyran with the lowest MDA levels have higher antioxidant levels and are more resistant to salt stress. The activity of H₂O₂ elevated significantly in all genotypes ($P < 0.05$), except Ciko, with the treatment of 200 mM NaCl similar to proline and MDA contents (Figure 3).

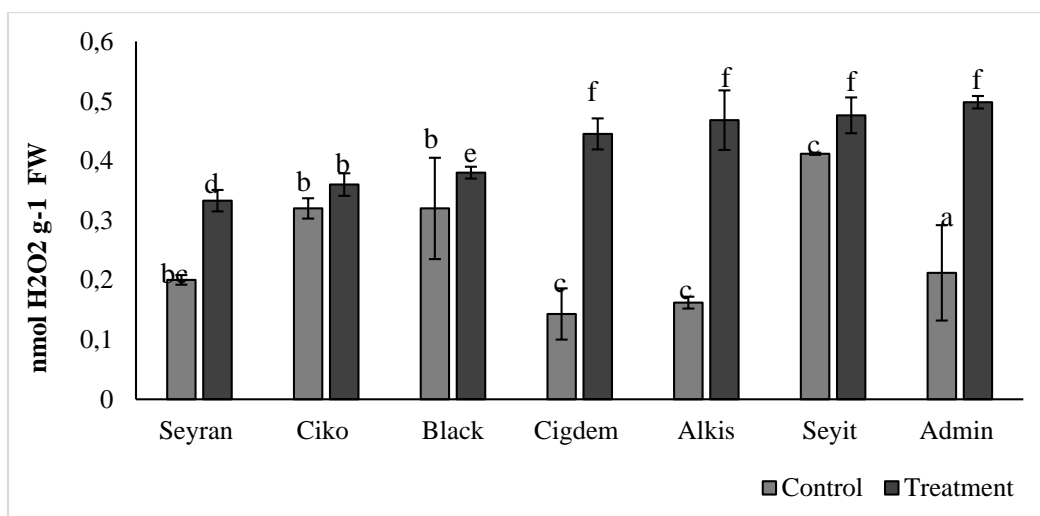


Figure 3. Effect of salt stress on leaf H₂O₂ content of (nmol H₂O₂ g⁻¹ FW) seven tomato cultivars subjected to control and 200 mM NaCl concentration. Values represent means \pm SE (n=3). Different letters indicate significant differences (*Student's t-test*; $P < 0.05$).

There is a statistical difference ($P < 0,05$) between control and 200 mM salt treatment for all genotypes. As a result, it's possible that salt stress can easily cause H_2O_2 levels to rise to various amounts. For example, the H_2O_2 level increased by 12% in Ciko variety compared to its control, while this level increased 188% in Alkis variety. Increased lipid peroxidation accompanied the increase in H_2O_2 activity in salinity-treated plants, as demonstrated by the shift in MDA levels (Figure 2). Interestingly, Alkis variety showed the highest increase in MDA values after Cigdem compared to control. Based on these results it can be said that there is a positive correlation between H_2O_2 and lipid peroxidation. These results somehow confirm the high proline and low MDA contents in resistant cultivars, and vice versa. Therefore, it can be assured that genotypes Seyran and Ciko might have a better antioxidative defence system to scavenge H_2O_2 against salinity stress.

Molecular characterization

The expression *SIVOZI* gene in all tomato cultivars change when the 200 mM was applied. However, this increment was clearer in Ciko and Indigo Rose Black cultivars with sharper bands than other treated plants. There was no dramatic up-regulation of *SIVOZI* in the cultivars Alkis and Cigdem cultivars against salt stress (Figure 4). In response to salt stress, *SIWRKY8* gene was showed elevated expression and displayed distinct patterns in different cultivars (Figure 4). Similar to the *SIVOZI*, the expression intensity was significantly sharper in Ciko and Indigo Rose Black cultivars compared to their controls and rest of the treated plants. In overall, it can be said that the expression of salt stress related genes, *SIVOZI* and *SIWRKY8* showed much more up-regulation in Ciko and Indigo Rose Black tomato cultivars in 200 mM NaCl treatment compared to other cultivars.

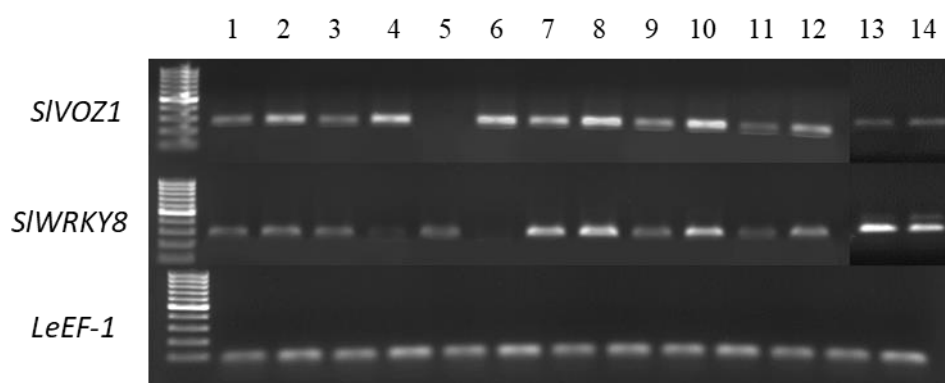


Figure 4. Gene expression of *SIVOZI* and *SIWRKY8* in response to 200 mM salt stress in leaves of seven different tomato cultivars. Lane 1-2 (Admin C-Admin T), 3-4 (Seyit C-Seyit T), 5-6 (Seyran C- Seyran T), 7-8 (Ciko C-Ciko T), 9-10 (Indigo Rose Black T- Indigo Rose Black C), 11-12 (Alkis C-Alkis T), 13-14 (Cigdem C-Cigdem T). C: Control, T: 200 mM NaCl Treatment. (Note: Lane 13-14 for Cigdem, since there was not enough room in the gel, the PCR product was loaded on a different gel and the image was taken from there)

DISCUSSION

Biochemical characterization

To adapt to environmental changes, plants have various physiological biochemical and molecular response mechanisms and defence systems to withstand a variety of stress conditions (Soltabayeva et al., 2021). By imposing toxic effect and water stress,

salinity plays a crucial role in limiting cellular functions, resulting in lower crop quality and yield. In Turkey, it is expected that salinization will cause a land loss of 50% by 2050 (Yazar and Kaya, 2014). On the other hand, according to climate change models and predictions, irrigation demand for agricultural products in Turkey's

Mediterranean Region would grow in the 2070s due to a decrease in precipitation and an increase in temperature (Akça et al., 2020). In this context, understanding their adaptations and effective production in saline land areas requires screening and selection of novel tomato genotypes resistant to salinity stress. In this study, six tomato hybrids and an Indigo Rose Black genotype grown in Turkey were treated with 200 mM NaCl stress. Our findings indicated that different genotypes vary considerably in their response and sensitivity to salinity stress. Plants that are subjected to salt stress accumulate osmoprotective solutes as a ROS scavenger such as proline which can protect cells from disruptive effect of NaCl on cell membrane (Rahneshan et al., 2018). Under salinity treatment, the proline concentration and amplitude of response were shown to differ considerably among the genotypes. Higher proline levels are well established to be the result of a stress reaction, and that the tomato plant uses proline to protect its metabolism from the damage caused by salinity (De la Torre-González et al., 2018). Although it has been reported that salt-tolerant plants have higher proline content than salt-sensitive plants, the specific role of proline in salt tolerance is still not clear (Szabados et al., 2010; Irfan Dar et al., 2016). For example, the proline contents in the salt-sensitive barley lines were remarkably higher than in salt-tolerant lines (Zhu et al., 2020). In a study carried out by 20 different tomato genotypes identified that proline accumulation increases greatly within the tolerant genotype (Gharsallah et al., 2016). Apart from all of these conjectures, based on our findings, Indigo Rose Black and Seyran, are the most two tolerant cultivars to salinity, while Alkis and Cigdem much less tolerant to moderate salt stress. Under salinity stress, lipid peroxidation is a well-known reflection of stress-induced membrane deterioration (Katsuhara et al., 2005). As a result, lipid peroxidation is frequently employed as a biomarker of oxidative damage and has

proven to be a decent indicator of salt tolerance (Furtuna and Tıprıdamaz, 2010). As we expected, salinity affected all cultivars via lipid peroxidation (Figure 2). Indigo Rose Black, Seyran, and Ciko show lower levels of elevated MDA, suggesting that these cultivars may be more resistant to NaCl, whereas the genotypes which have the highest MDA contents, Cigdem and Admin, might have the least effective antioxidative system and salinity tolerance. Low MDA content, like this result, is crucial in terms of salt tolerance, according to various research (Zhu et al., 2020; Ashraf, 2008). Overexpression of *PpSnRK1*, SNF-related Kinase 1 (*SnRK1*), a critical component of the cell signaling network in tomato, improved salt tolerance by increasing proline content and lowering MDA levels as compared to WT under salinity, according to a recent study (Wang et al., 2020). These results of MDA levels clearly support the idea that cultivars Indigo Rose Black, Seyran and Ciko could be more tolerant to salt stress. Antioxidant enzymes including SOD, CAT, POD, and APX may quickly scavenge ROS and protect cells from oxidative stress. Higher enzyme activity and lower MDA levels indicate greater anti-oxidative capabilities, which may imply higher salinity resistance (Pouya, 2015). H₂O₂ is one of the active oxygen species that has been identified as a crucial signaling molecule in the abiotic stress response signaling pathway (Niu and Liao, 2016). In our study, the results indicated that the level of H₂O₂ was higher compared to their controls in all cultivars with 200 mM salt treatment (Figure 3). Although, the cultivar Ciko had higher H₂O₂ activity compared to its control, but it was not significant. The study's findings revealed that different tomato cultivars responded to salinity stress in different ways in terms of H₂O₂ concentration. Based on H₂O₂ levels Seyit, Admin, Alkis and Cigdem were sensitive which were already thought as sensitive tomato cultivars in proline and MDA analysis. In contrast, Seyran, Ciko and Indigo Rose Black

cultivars produced significantly less H₂O₂ which might be removed by higher activities of antioxidant enzymes at a specific time or tissue in those lines. In other words, there were generally significant positive associations between cell injury-related features (H₂O₂ and MDA) and antioxidant enzyme activity. (Saed-Moucheshi et al., 2019).

Molecular characterization

In response to salinity stress, a large number of salt-responsive genes/transcription factors (TFs) are either activated or downregulated (Bakshi and Oelmüller, 2014). Vascular plant one zinc-finger (VOZ) TFs provide important roles in the development and stress response of plants (Koguchi et al., 2017). As far as we know, there is not any characterization of the *VOZ* gene in tomato. Therefore, for the first time, the expression of *SIVOZI* (Solyc02g077450) was analysed under salt treatment in tomato. The expression intensity of *SIVOZI* was significantly higher in Ciko and Indigo Rose Black which was a supportive data of biochemical analysis. The involvement of *VOZ* genes to abiotic stress conditions has been studied in *Arabidopsis* (Prasad et al. 2018) and rice (Ganie et al., 2020). In the light of these studies, the results of our study have shown that functional characterization of the *SIVOZI* gene is very important in developing new varieties against various abiotic stress conditions. In plants, WRKY TFs provide a variety of biological functions, but they are most notable for their roles in plant responses to a/biotic stressors. In tomato, WRKY TFs is a very large family with 83 gene members (Bai et al., 2018). Based on previous studies, we thought that it would be worth seeing the expression level of *SIWRKY8* gene in 200 mM NaCl treated tomato cultivars. In the present study, transcription of *SIWRKY8*, was induced with a similar pattern of *SIVOZI*, especially in Ciko and Indigo Rose Black varieties. In contrast, the expression was weak and quite similar in control and treated plants for Admin and Seyit cultivars.

At all periods during the treatment and even in the absence of salt stress, this gene displayed significant transcript increase, especially in the tolerant genotype (Gharsallah et al., 2016). The alleviation of wilting or chlorosis phenotype in plants overexpressing *SIWRKY8* under drought and salt stresses revealed that *SIWRKY8* works as a positive regulator in drought and salt stress responses (Feng Gao et al., 2019). Therefore, we can clearly say that, based on the expression levels of two salt stress related genes, the cultivars Ciko and Indigo Rose Black could be more tolerant against salinity than rest of the cultivars used in this experiment.

CONCLUSION

Due to the drought and low quality of water irrigation, salinity is becoming one of the primary abiotic stresses around the world. A complex system at the plant level is involved in the ability to tolerate abiotic stresses. This is the first study to look at the biochemical and molecular responses to salinity in seven tomato cultivars cultivated in Turkey. Salinity stress was found to be responsible for the generation of oxidative stress and thus associated damage, as evidenced by an increase in MDA content, H₂O₂, and proline build-up in our study. Our preliminary results identified Seyran, Ciko and Indigo Rose Black tomato cultivars as the most tolerant, and Seyit and Alkis as the most salt sensitive cultivar in 200 mM salinity treatment. However, these results should be confirmed for a wider range of salt concentrations as well as over a wider range of environmental conditions. Furthermore, novel candidate genes could be found by transcriptome profiling in future studies to generate novel tomato varieties that are more tolerant to salinity. To enhance selection response under salt stress, novel candidate genes/sequences can be used in breeding programs for mutant screening, changing the expression of the most promising genes, generating transgenic plants, and combining natural alleles with knowledge of phenotypes.

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