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Synthetic Strigolactone Regulates Some Stress Related Genes and Transcription Factors on Tomato (*Lycopersium esculentum* L.)

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Abstract

Türkiye meets about 7% of the world tomato production. The most severe effects of climate change are seen in agriculture. The increase of salinity in agricultural lands reduces the usable area and affects the growth, development and yield of the products grown. The salinity problem in the Iğdır Plain, which has a microclimate feature, causes the region to not be used at full capacity and to obtain sufficient yield from the products grown. The aim of this study is to determine the effects of synthetic strigolactone GR24-rac against salinity at the gene level in H-2274 tomato cultivar grown under saline conditions for the first time. In the experiments carried out for this purpose, the effects GR24-rac applied at 10 nM and 100 nM doses to H-2274 tomatoes grown at 150 mM salt concentration were determined at the gene level. The mRNA levels of the genes encoding the stress enzymes catalase, superoxide dismutase and glutathione reductase and the transcription factors SlWRKY31, ERF84, LeNHX1, HKT1;2 were determined by Real-Time Quantitative Polymerase Chain Reaction. Results showed that GR24-rac application controlled the regulation of genes and transcription factors and helps the plant to cope with high concentration salt. We concluded that the data obtained as a result of the study will open a new avenue for researchers to increase the tolerance to salinity in tomatoes.

Key words: GR24-rac, gene expression, qPCR, H-2247 type tomato.

1. INTRODUCTION

Drought, one of the most obvious consequences of global climate change, has caused a high increase in irrigated agricultural areas. This increase in irrigation needs significantly increases salinity in agricultural areas (Dikilitaş and Karakaş, 2012). In arid and semi-arid agricultural areas, soluble salts accumulate on the soil surface and soil salinity occurs with the increase in water consumption of plants and capillary water movement (Kıran et al., 2014). The osmotic effect of salinity in the soil causes osmotic stress in plants and limits the growth of plants (Okhovatian-Ardakani et al., 2010). Although tomato is a moderately salt tolerant plant, this resistance can vary in different tomato varieties (Kıran et al., 2014). In saline conditions, the germination rate and percentage of tomato seeds decrease (Cuartero and Fernandez-Munoz, 1999), in parallel to vegetative growth and yield decrease (Maas, 1986).

Fourty-six percent of the land on earth is located in climatically arid and semi-arid regions (Karaoğlu and Yalçın, 2018). About half of the irrigated lands are experiencing salinity issue and it is known that the agricultural productivity of approximately 954 million hectares of land worldwide is restricted due to salinity (FAO, 1998).Various researchers have reported that salinity stress on plants negatively affects vegetative growth and yield (Murphy et al., 2003; Mensah et al., 2006; Bressan, 2008).

Strigolactones (SLs) are hormones that has been the subject of rapidly increasing studies in the last decade, has a direct effect on root and stem development architecture in plants, promotes the

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germination of parasitic plant seeds and protects plants against abiotic stress. SLs are a class of terpenoid lactones and are produced in the carotenoid biosynthesis pathway. SLs was first purified from the roots of the parasitic striga plant in 1966 (Cook et al., 1966). Its chemical structure was revealed and defined in 1972 (Cook et al., 1972). With the discovery of SLs, new research opportunities have emerged for researchers on the hormonal regulation of plants and their adaptation to environmental conditions. Besides from its effect on branching architecture, SLs are known to take part in many different biological functions in plants and interact with other hormones to prepare the plant for abiotic conditions such as temperature, coldness, salinity, and light (Saeed et al., 2018). Today, SLs are produced synthetically, giving researchers the opportunity to use this new hormone. GR24 is a synthetically produced SL analogue substance and studies on how this substance affects the plant under abiotic stress conditions in vitro are very popular.

In a study conducted by Karakaş et al., (2013), 50, 100, 150, 200, 250 and 300 mM NaCl were applied to Ayaş, H-2274, Falcon, SC2121 and Rio Grande tomato cultivars under in vitro conditions. According to the results it was found that Rio Grande and H-2274 cultivars were most sensitive to salinity at 150 mM NaCl concentration. It was determined that the vegetative growth of H-2274 cultivar decreased considerably at 150 mM NaCl concentration, and when this dose was exceeded, the plant could not germinate. In the study carried out by Kıran et al., (2014), drought stress was applied to TR-68516, Rio Grande, TR-63233 and H-2274 tomato cultivars. It was reported that TR-63233 and H-2274 varieties were damaged the most by drought stress.

In the last two studies mentioned above, it is seen that the H-2274 tomato variety is sensitive to both salinity and drought stress. Therefore, we aimed to increase salt resistance with GR24 application in H-2274 variety is based on this information in the literature.

There are wide variety of studies showed that abiotic stress factors change the regulation of the genes which are related to stress defence genes (Kurt et al., 2021; Aydin et al., 2021; Kurt et al., 2022). In the study carried out by Gharsallah et al., (2016), 150 mM concentration of NaCl was applied to 20 tomato cultivars grown in Tunisia and the changes in the mRNA levels of 11 transcription factors were examined against time. Moreover, catalase (CAT), ascorbate peroxidase (APX) and glutathione peroxidase (GPOX) antioxidant enzyme activities were also measured by fluorometric methods in the study. Accordingly, it was determined that stress enzymes increased with salinity stress, and transcription factors were expressed differently according to tomato variety. In a study conducted by Wang et al., (2013), it was shown that the *SlBADH* gene, whose activity was increased in salty conditions in a transgenic tomato variety Micro-Tom, confers salt tolerance to the plant. Researchers have shown that the expression of this gene transferred to tomato from the aquatic plant *Suaeda liaotungensis* Kitag. increases with salt, and this increase stimulates the plant to tolerate salt. An important review on the adaptation of SLs to abiotic stress conditions in plants belongs to Mostofa et al., (2018). Using the phrase "the rising path in plant research" for SL and its synthetic analogue, GR24, the researchers argued that the importance of using this substance against abiotic stress in plants, so that an increase in yield is not a dream, especially in agriculture in saline soils.

While there are many studies in the literature on salinity stress in tomatoes, tolerating the salt stress with SL is missing. We think that this deficiency in the literature will be filled with the data obtained from the present study and that new studies on the salinity problem in tomatoes will be pioneered.

The present study aims to determine the effects of synthetic SL GR24-rac against salt stress on H-2274 tomato type by comparing gene expression levels of the salt stress related genes catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), and abiotic stress related transcription factors SlWRKY31, ERF84, NHX1, and HKT1;2.

2. MATERIALS and METHODS

2.1. Germinating the Tomato Seeds

The seeds of H-2274 tomato type were obtained from commercial seed supplier Arzuman Tohumculuk (Türkiye). The seeds were surface sterilised with 10% (v/v) sodium hypochlorite for 10 minutes and rinsed three times with sterile deionised water. The seeds were placed between sterile filter paper inside petri dishes and incubated at 27°C for two days in dark conditions. When radicle emerged, the plantlets transferred to ½ Hoagland solution (Hi-Media TS1094, India) and the growth conditions were adjusted to 25°C/18°C (16 hours day/8 hours night photoperiod), 40-50% humidity until four-leaved stage. When the plants at four-leaved stage, the plants were transferred to pots including 1:3 (w/w) peat:perlite and watered with ½ Hoagland solution including 50 mM, 100 mM and 150 mM NaCl every other day, respectively. The control group was watered only with ½ Hoagland solution. When the watering reached to 150 mM concentration (3rd day after transferring to pots), two doses of (10 nM and 100 nM) GR24-rac (Strigolab, Italy) were applied as solution.

Fresh leaf tissues were collected for quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) at the 3rd, 6th, 9th, 12th, 24th hours and 7th day after the GR24-rac was applied to the plants. The sampled leaves were frozen immediately using liquid nitrogen and stored at –86°C until RNA extraction.

2.2. Total RNA Extraction and Complementary DNA (cDNA) Synthesis

We used TRIZol® (Invitrogen, USA) following the protocol provided by the manufacturer using ~100 mg of starting material. The RNA pellets were diluted in 0.1 mM EDTA. The concentration of the extracted RNAs was measured by Nanodrop (MaestroNano, Maestrogen, Taiwan), and the integration was checked by 1.5% agarose gel electrophoresis. Prior to cDNA synthesis DNase I enzyme (Invitrogen Cat No: 18047019) applied to 1 µg of RNA to eliminate DNA contamination RNA extracts using the provided protocol. cDNA synthesis from 1 µg RNA was performed using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific Cat No: K1621, USA) following the manufacturers protocol for each RNA extract. The success of cDNA synthesis was validated with end point PCR of *Actin* gene (Gharsallah et al., 2016).

2.3. Designing the Primers, and qRT-PCR Analysis

In the present study we evaluated the effects of GR24-rac on the comparative expression levels of three stress related genes *catalase* (*CAT*), *superoxide dismutase* (*SOD*), *glutathione reductase* (*GR*), and four abiotic stress related transcription factors SlWRKY31, ERF84, NHX1 and HKT1;2 under salinity stress. We used *beta-Actin* gene as reference gene for normalisation of the qRT-PCR data. To perform qRT-PCR, we designed the necessary primer pairs as three sets from the related reference transcripts using National Center for Biotechnology Information (NCBI) Primer BLAST tool (Table 1). The primer efficiency and the correlation coefficient (R^2) were calculated for each primer by the slope of the regression line in the standard curve. We selected the primers with efficiency values between 90–110% and the R^2 value was greater than 0.98 according to the literature (Radonic et al., 2004 and Hürkan et al., 2018).

qRT-PCR was performed on Rotor-Gene-Q 5 Plex HRM instrument (Qiagen, USA) using 72-well carousel. Each qRT-PCR tube (Qiagen Cat. No. 981103) included 5 µL RealQ Plus 2x Master Mix Green (Ampliqon Cat. No. A323402), 0.25 µL of 10 picomoles of each primer, 20 ng cDNA and nuclease free water to 10 µL. Reaction conditions were following 15 minutes at 95°C initial denaturation, 40 cycles of 15 seconds at 95°C denaturation, 30 seconds at 60°C annealing and 30 seconds at 72°C elongation. We added melting curve analysis to validate specifity of the amplicons from the

temperature 55–90°C. qRT-PCR data was analysed with Rotor-Gene-Q 2.3.5 software using relative quantification $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Table 1. *Designed and selected primers for Quantitative Real-Time Polymerase Chain Reaction*

Primer Name	Reference Transcript	Sequence (5´→3´)	T_m (˚C)	Expected Amplicon Size (bp)
Sl_CAT_1_F	NM_001247898.1	CCATCACCATGGATCCCTCTA	58.38	86
Sl_CAT_1_R		GTACACAGGACCACCAGCAT	59.68	
Sl_SOD_1_F	NM_001311084.1	TCTCACTGGTCCACAGTCCA	60.11	108
Sl_SOD_1_R		CCAGCATTTCCGGTGCTTTT	59.68	
Sl_GR_1_F	NM_001247314.2	AATTTTGGGGCTTCGGTTGC	59.97	102
Sl_GR_1_R		TACACATCCCCGAAGCACAC	60.04	
Sl_WRKY31_1_F	NM_001319981.1	TTCGCCTTCCTCTTACTTTGCT	59.96	103
Sl_WRKY31_1_R		CGTCGGAGATGGAAGAAGGTTTG	61.47	
Sl_ERF84_1_F	XM_004237769.4	GCTCAAAGGGGTTATGGAGGT	59.71	84
Sl_ERF84_1_R		CAGGCTTGTATCTTCTTCGAGC	59.14	
LeNHX1_1_F	NM_001246987.1	TCAGCAAATTAAAAGAGACCGAG	57.14	90
LeNHX1_1_R		TGGTTCCAAGTTCAAAACCCATAC	59.66	
HKT1;2_1_F	NM_001302904.1	TGTGTTGTGGAGAAAGACAAAATGA	59.58	96
HKT1;2_1_R		TCCAACTGTCCCATATGCACTTA	59.48	
Sl_ACT_1_F	NM_001330119.1	TGCATTCCCTGACTGTTTGC	59.04	110
Sl_ACT_1_R		TCCTCTCCGTCTGCCATCTT	60.32	

T_m: Melting temperature.

2.4. Statistical Analysis

The experiments were performed as biological (pooled prior to cDNA synthesise) and technical triplicates and each replicate included five plants. The descriptive statistics of the triplicate data were calculated as mean ± standard deviation, and one-way ANOVA with post-hoc analyses (LSD) were used for mean comparison of the gene expression values using RStudio 2022.07.1 with the packages psych (2.2.9) (Rewelle, 2022) and agricolae (1.4) (Felipe de Mendiburu and Yaseen, 2020).

3. RESULTS and DISCUSSION

3.1. RNA Extraction, cDNA Synthesis and qRT-PCR Analysis

RNA extraction from each leaf tissue were successfully performed to synthesise cDNA (Table 2). The concentration was ranged between 167.19 to 1715.27 ng μL^{-1}. The success of cDNA synthesise was validated with the specific amplicon of Actin reference gene by end-point PCR.

According to the qRT-PCR results, the cycle thresholds were ranged between 16.10–25.79 for CAT, 16.62–22.20 for SOD, 20.67–26.95 for GR, 19.26–28.24 for SlWRKY31, 25.34–30.1 for SLERF84, 26.36–32.40 for LeNHX1 and 21.33–24.98 for HKT1;2, which are in ideal range for qRT-PCR analysis. The melt curve analysis showed only one peak for each studied gene which validated the specific amplification.

Table 2. *Information about the extracted RNAs*

Sample ID*	Concentration (ng µL^{-1})	A260/A230	A260/A280
K0	684.12	1.367	2.007
T3	360.30	0.783	1.874
T6	1715.27	1.483	1.912
T9	697.63	1.685	2.042
T12	705.63	1.606	2.02
T24	1209.70	1.407	2.086
T7d	545.29	1.088	1.683
TS10_3	663.22	1.067	1.861
TS10_6	1322.94	1.271	2.005
TS10_9	360.06	1.546	1.931
TS10_12	1641.59	1.693	2.022
TS10_24	312.31	1.082	1.908
TS10_7d	962.04	1.18	1.749
TS100_3	690.58	1.197	1.97
TS100_6	986.41	0.953	2.063
TS100_9	701.26	1.081	1.603
TS100_12	950.79	1.617	1.97
TS100_24	167.19	0.881	2.004
TS100_7d	1234.49	1.176	1.964

*Abbreviations: K: Control, T: Salt, S: GR24-rac, TS: Salt+GR24-rac, numbers before underscore: GR24-rac doses 10 nM and 100 nM, number after underscore: Sampling times (hour), 7d: Seventh day of sampling.

qRT-PCR results showed that expression of CAT relative to control (K-0) started to increase after ninth hour (10-fold) of salt application and turned to “normal” (similar to control) state on the 24th hour. The expression reached to peak on the seventh day (40-fold) due to salt accumulation (Fig. 1). These results showed that plant tolerated the salinity on the 24th hour. Application of 10 nM GR24-rac increased the expression of CAT on all the samples except 6th hour. The expression of CAT reached to peak on the 7th day (64-fold) due to salt and GR24-rac accumulation on the growth substrate. On the dose of 100 nM GR24-rac the expression of CAT increased until 12th hour and decreased on 24th hour and 7th day.

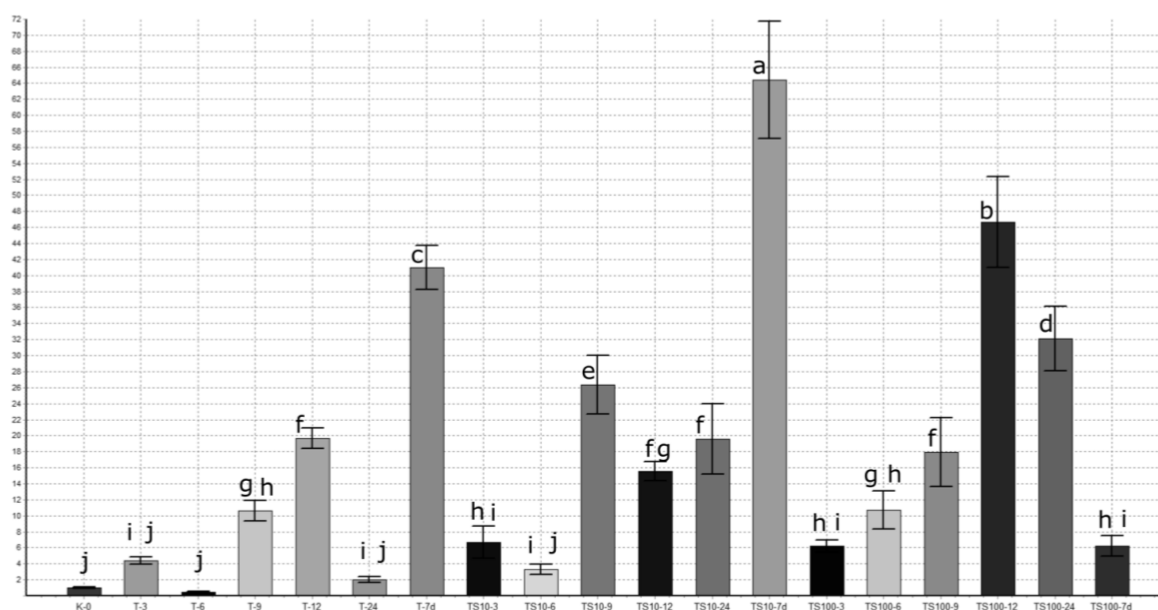

Figure 1. *Relative gene expression changes with standard deviation of catalase encoding gene. Y-axis represents fold changes. Letters above bars represents ANOVA post-hoc (LSD) groupings according to the significance level p ≤ 0.01. Abbreviations: K: Control, T: Salt, S: GR24-rac, TS: Salt+GR24-rac, numbers before underscore: GR24-rac doses 10 nM and 100 nM, number after underscore: Sampling times (hour), 7d: Seventh day of sampling.*

The expression of SOD increased significantly on the 9th hour and 7th day relative to K-0 with salt application (Fig. 2). GR24-rac on 10 nM concentration increased the relative expression 9th hour significantly (9.2-fold) and turn to stabile state on the 12th, 24th hours and on the 7th day. 100 nM GR24-rac increased the expression of SOD on the 9th, 12th, 24th hours and on the 7th day, as well. On the 7th day the expression significantly decreased for both 10 nM and 100 nM GR24-rac concentrations relative to T-7d sample.

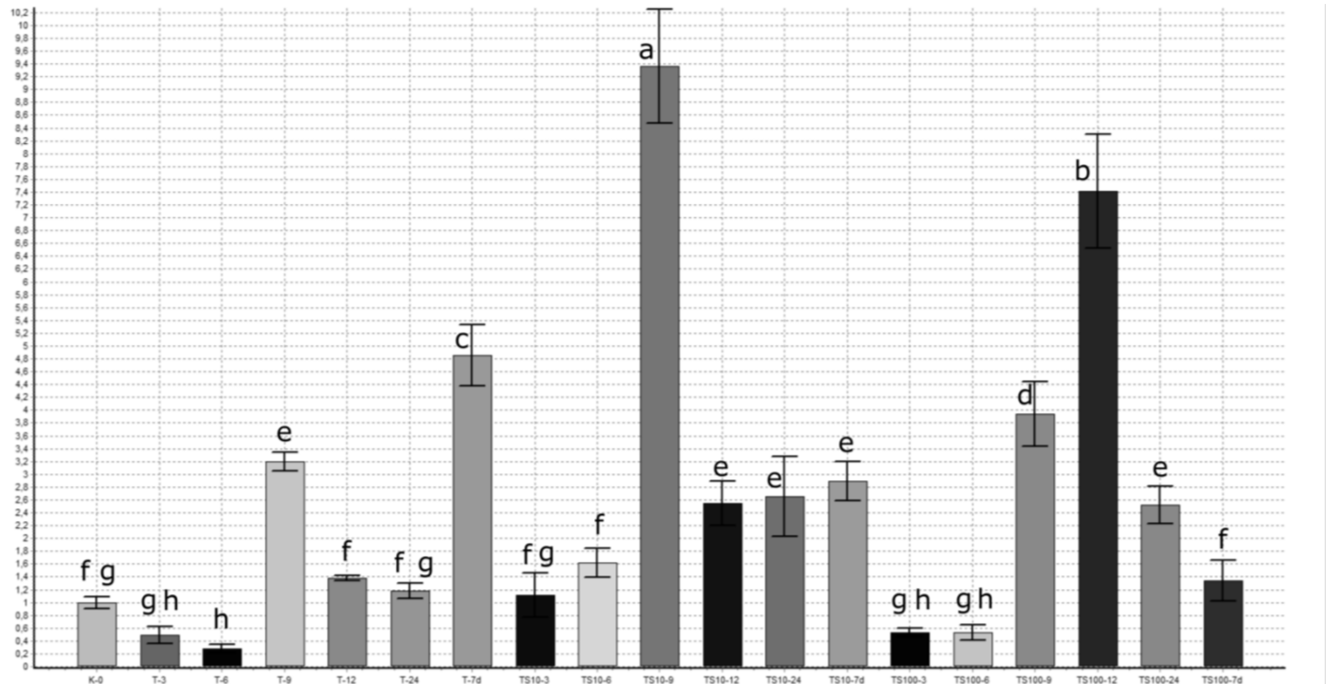

Figure 2. *Relative gene expression changes with standard deviation of superoxide dismutase encoding gene. Y-axis represents fold changes. Letters above bars represents ANOVA post-hoc (LSD) groupings according to the significance level $p \leq 0.01$. Abbreviations: K: Control, T: Salt, S: GR24-rac, TS: Salt+GR24-rac, numbers before underscore: GR24-rac doses 10 nM and 100 nM, number after underscore: Sampling times (hour), 7d: Seventh day of sampling.*

High level of salinity causes the formation and accumulation of reactive oxygen species (ROS) in cells. Oxidative stress results activation of enzyme-based defence systems such as catalase, superoxide dismutase, ascorbate peroxidase and peroxidase (Chawla et al., 2013). In the present study, significant increases on the expression of *CAT* and *SOD* observed meaning that the 100 mM salt application caused stress on the plant. GR24-rac application of 100 nM concentration was increased the expression more than 10 nM concentration. Therefore, we can say that 100 nM GR24-rac application was the optimum dose for GR24-rac to prepare the plant for saline conditions.

Comparative GR expression analysis showed that there was limited statistically significant change among K-0, salt (T), salt+GR24-rac 10 nM (TS-10), and salt+GR24-rac 100 nM (TS100) groups (Fig. 3). GR expression reached to peak on the 7th day with salt accumulation. GR24-rac application did not change the expression level of GR significantly. In the plant antioxidant defense pathway, dehydroascorbate is converted to dehydroascorbate and oxidized glutathione via glutathione (GSSG). GSSG is reduced by glutathione reductase (GR) enzyme using NADPH (Bray et al., 2000). The duty of GR is to scavenge the H_2O_2 free radical in plant cells. The increase in the GR enzyme in the cells means that the plant tolerates oxidative stress. In the present study, it was determined that the expression of the gene encoding the GR enzyme did not show a significant change by salt and GR24-rac applications. We think that the most important reason for this situation is that the tomato cultivar H-2274 was not stressed enough to increase the GR activity at the 150 mM salt dose we applied.

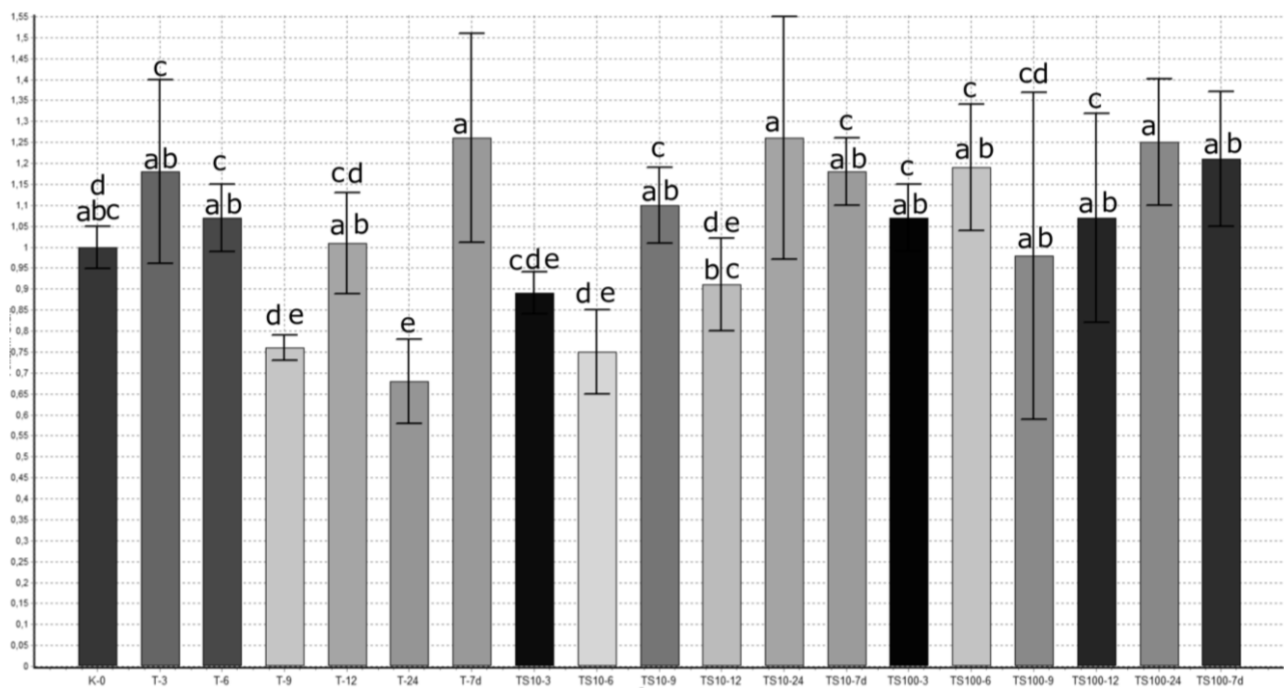

Figure 3. *Relative gene expression changes with standard deviation of glutathione reductase encoding gene. Y-axis represents fold changes. Letters above bars represents ANOVA post-hoc (LSD) groupings according to the significance level p ≤ 0.01. Abbreviations: K: Control, T: Salt, S: GR24-rac, TS: Salt+GR24-rac, numbers before underscore: GR24-rac doses 10 nM and 100 nM, number after underscore: Sampling times (hour), 7d: Seventh day of sampling.*

WRKY is a group of transcription factors that regulates tolerance to biotic and abiotic stress, senescence, seed dormancy, seed germination and some developmental processes in algae and plants and is also involved in secondary metabolism Schluttenhofer and Yuan, (2014). qRT-PCR results showed that the gene expression of SlWRKY31 transcription factor increased by salinity stress (Fig. 4). Expression level reached its maximum level at the 3rd hour after salt application, decreased at the 6th, 9th and 12th hours, and increased again in response to salt accumulation caused by repeated salt applications on the 7th day. As a dose of 10 nM GR24-rac, it was determined that SLWRKY31 expression decreased only at the 3rd hour compared to the salt group, and there was no significant change at other sampling times. The use of 100 nM GR24-rac as a dose showed parallel results with the use of a 10 nM dose. In previous studies, it was determined that the WRKY transcription factor increased in many plants, including tomato plants, in drought and salt applications (Bakshi and Oelmululler, 2014; Huang et al., 2012; Jiang and Deyholos, 2009; Liu et al., 2011; Peng et al., 2012; Sun et al., 2014). The data we obtained in the present study and the literature data show similar results in terms of the response of SlWRKY31 transcription factor in tomato plant exposed to salinity stress. It has been determined that GR24-rac application protects the plant by decreasing SlWRKY31 gene expression in the first stage of salinity at the doses tried in the study, and it is not effective in the progressive process and increased salt accumulation.

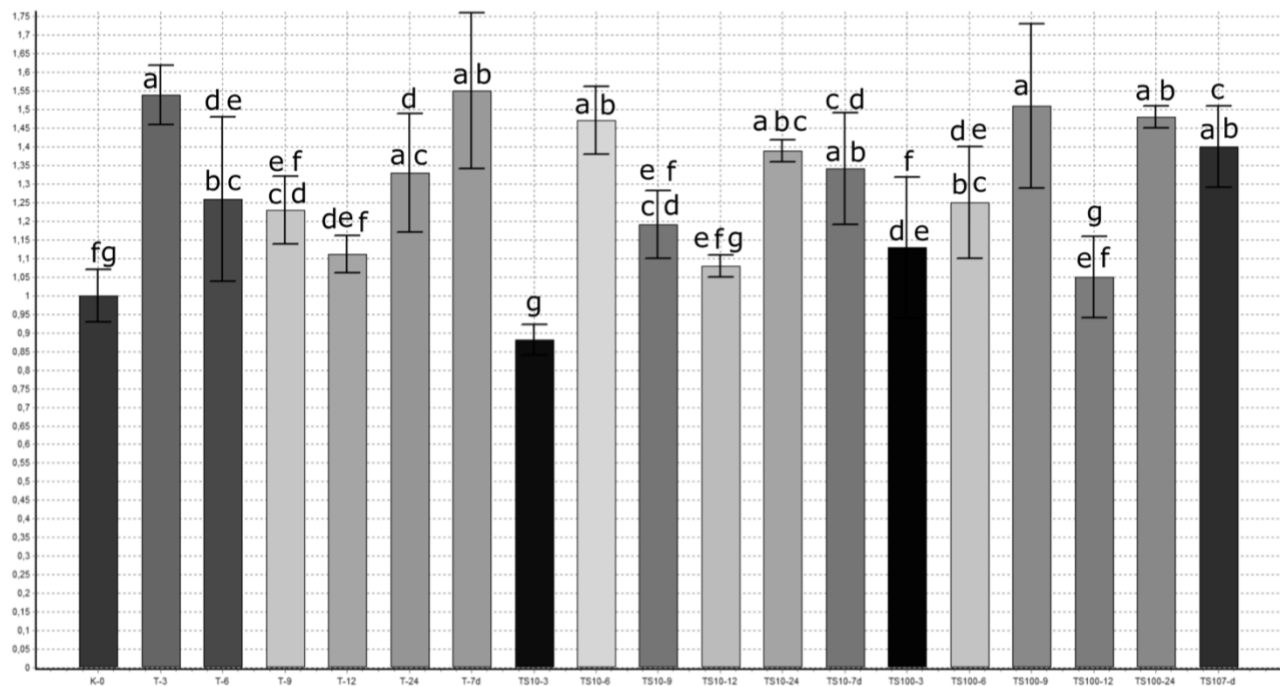

Figure 4. *Relative gene expression changes with standard deviation of SlWRKY31 encoding gene. Y-axis represents fold changes. Letters above bars represents ANOVA post-hoc (LSD) groupings according to the significance level $p \leq 0.01$. Abbreviations: K: Control, T: Salt, S: GR24-rac, TS: Salt+GR24-rac, numbers before underscore: GR24-rac doses 10 nM and 100 nM, number after underscore: Sampling times (hour), 7d: Seventh day of sampling.*

The AP2/ERF superfamily is the largest group of plant-specific transcription factors, and the AP2/ERF domain is 60-70 amino acids in size. This superfamily is divided into three groups as AP2, RAV and ERF. Ethylene responsive factor (ERF), which we analysed in the study, is especially specialised for the stress response in plants (Yamada et al., 2020). In the study, it was determined that the expression of the SlERF84 transcription factor increased at the 3rd hour with salt application, and decreased at the 6th, 9th, and 12th hours and returned to the same level as the control group at the 24th hour (Fig. 5). SlERF84 was found to rise again with salt accumulation in the 7th day sampling. It was observed that the expression of the transcription factor decreased at the 3rd hour and increased again at the 6th hour with the application of 10 nM GR24-rac. It was determined that there was an increasing trend in the following sampling times. In the application of 100 nM GR24-rac, it was determined that the expression reached the highest level at the 3rd and 24th hours. It has been observed that a significant change in the expression of the SlERF84 transcription factor can be achieved with GR24-rac application and the tolerance of the plant against salinity stress can be increased by increasing the expression of this transcription factor with 100 nM GR24-rac.

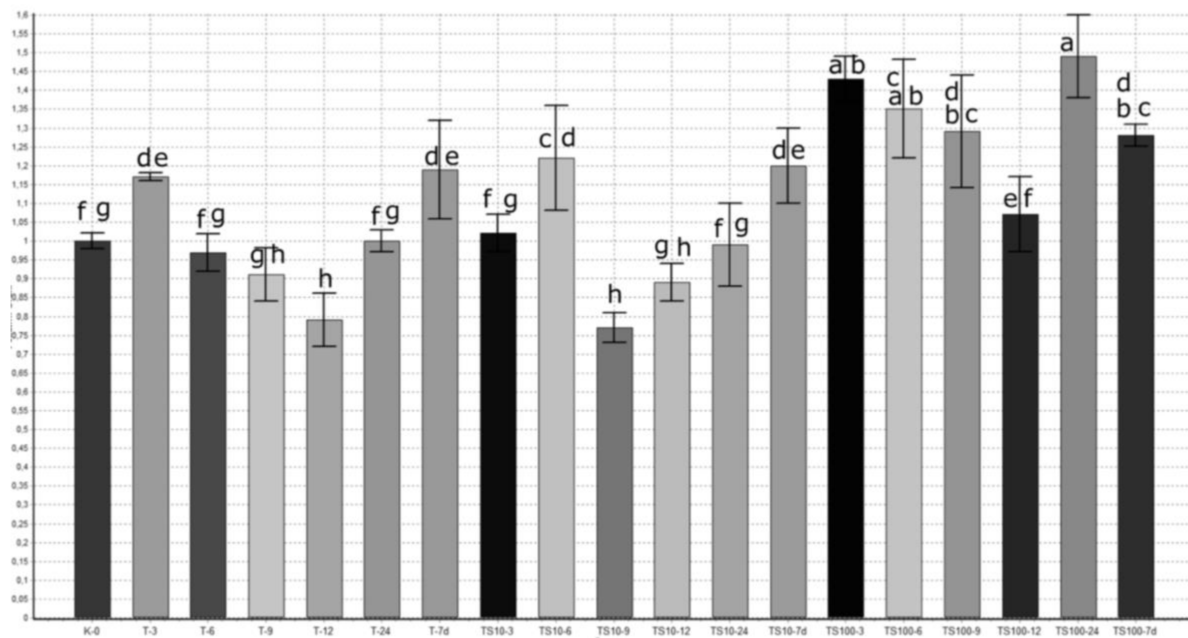

Figure 5. *Relative gene expression changes with standard deviation of SlERF84 encoding gene. Y-axis represents fold changes. Letters above bars represents ANOVA post-hoc (LSD) groupings according to the significance level p ≤ 0.01. Abbreviations: K: Control, T: Salt, S: GR24-rac, TS: Salt+GR24-rac, numbers before underscore: GR24-rac doses 10 nM and 100 nM, number after underscore: Sampling times (hour), 7d: Seventh day of sampling.*

Ion channels in plant cells are very important in adapting to and resisting salinity stress. Cation transporters such as HKT and LeNHX increase salt tolerance by regulating the Na^{+} ion balance in the cell (Gharsallah et al., 2016). LeNHX1 and LeNHX2 are transporters on the tonoplast that are actively involved in K^{+} ion uptake, turgor regulation and stomatal functions (Barragan et al., 2012). qRT-PCR results showed that LeNHX1 expression increased at the 3rd and 6th hours in salinity condition, but this increase was not statistically significant (Fig. 6). It was observed that there was no significant expression difference between the samples after the application of 10 nM GR24-rac. In the application of 100 nM GR24-rac, a significant increase was observed only on the 7th day compared to the control group. According to these results, it was understood that the plant did not reached to the turgor state in the salt dose applied in the study.

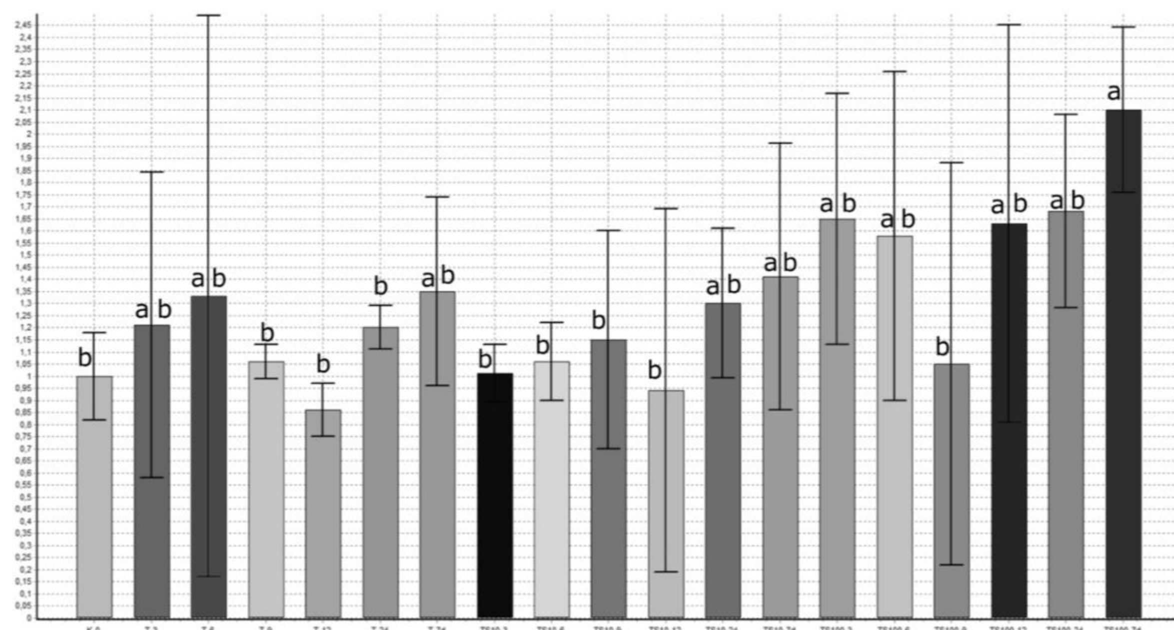

Figure 6. *Relative gene expression changes with standard deviation of LeNHX1 encoding gene. Y-axis represents fold changes. Letters above bars represents ANOVA post-hoc (LSD) groupings according to the significance level p ≤ 0.01. Abbreviations: K: Control, T: Salt, S: GR24-rac, TS: Salt+GR24-rac, numbers before underscore: GR24-rac doses 10 nM and 100 nM, number after underscore: Sampling times (hour), 7d: Seventh day of sampling.*

Histidine Kinase Transporter (HKT1;1 and HKT1;2) is a selective Na^+ transporter in tomato and provides the internal balance of Na^+ and K^+ (Hauser and Horie, 2010). According to the qRT-PCR results HKT1;2 expression increased at the 3rd, 6th and 9th hours after salt application, returned to the normal level at the 12th hour, and increased again at the 24th hour and 7th day (Fig. 7). In the application of 10 nM GR24-rac, it was observed that expression decreased at the 6th hour and was at its lowest level at the 24th hour. It was seen that the lowest expression was observed at the 24th hour in the application of 100 nM GR24-rac. It was determined that the expression differences detected in the HKT1;2 gene compared to the other genes analysed in the study occurred in an extremely narrow range (1.7 fold).

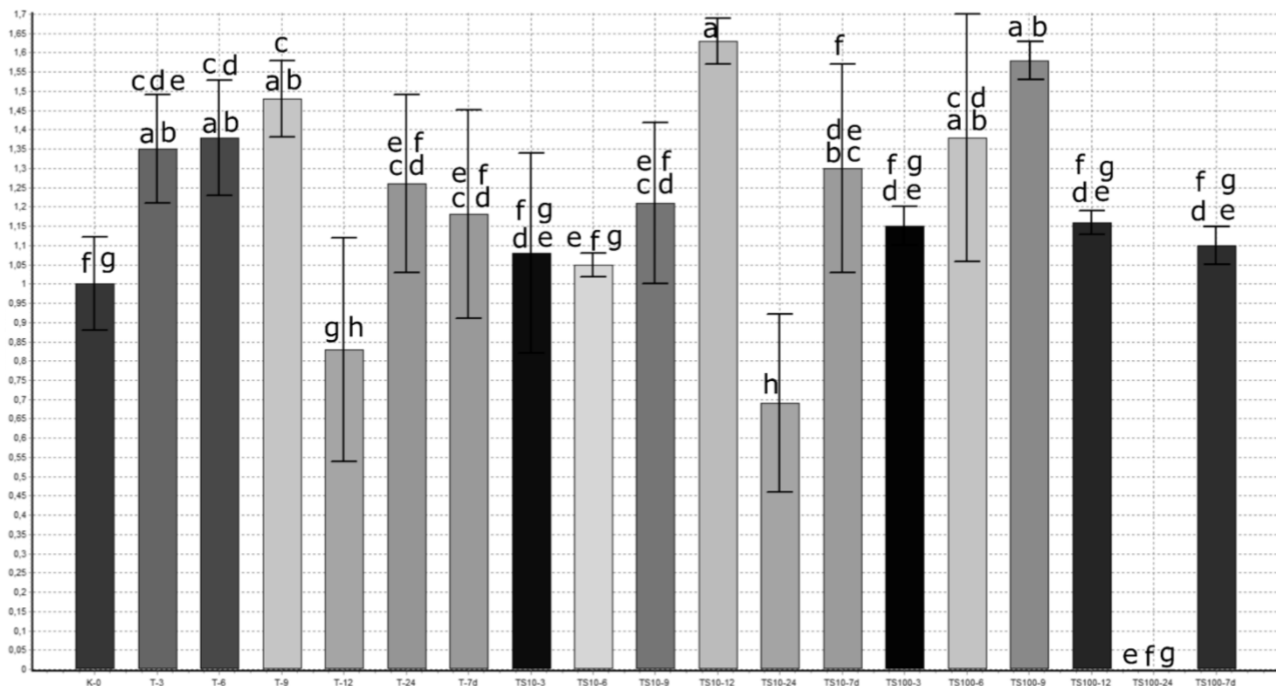

Figure 7. *Relative gene expression changes with standard deviation of HKT1;2 encoding gene. Y-axis represents fold changes. Letters above bars represents ANOVA post-hoc (LSD) groupings according to the significance level p ≤ 0.01. Abbreviations: K: Control, T: Salt, S: GR24-rac, TS: Salt+GR24-rac, numbers before underscore: GR24-rac doses 10 nM and 100 nM, number after underscore: Sampling times (hour), 7d: Seventh day of sampling.*

To obtain a general view of how salinity and GR24-rac applications effected the tomato we generated a heatmap view with the qRT-PCR data (Fig. 8). On the heatmap it is seen that the genes and transcription factors are divided into two main groups according to the changes in expression rates. The first group consists of SlERF84 and LeNHX1, the second group consists of SlWRKY31, HKT1;2, *GR*, *CAT* and *SOD*. We think that the reason for these two main groupings in the heatmap is that the changes in the expressions of the genes in the first group are shallower than the genes in the second group. Considering the experimental groups, it is seen that the heatmap is again divided into two main groups. It is noteworthy that the groups containing salt and 100 nM dose of GR24-rac differed from the other groups, except for exceptional cases.

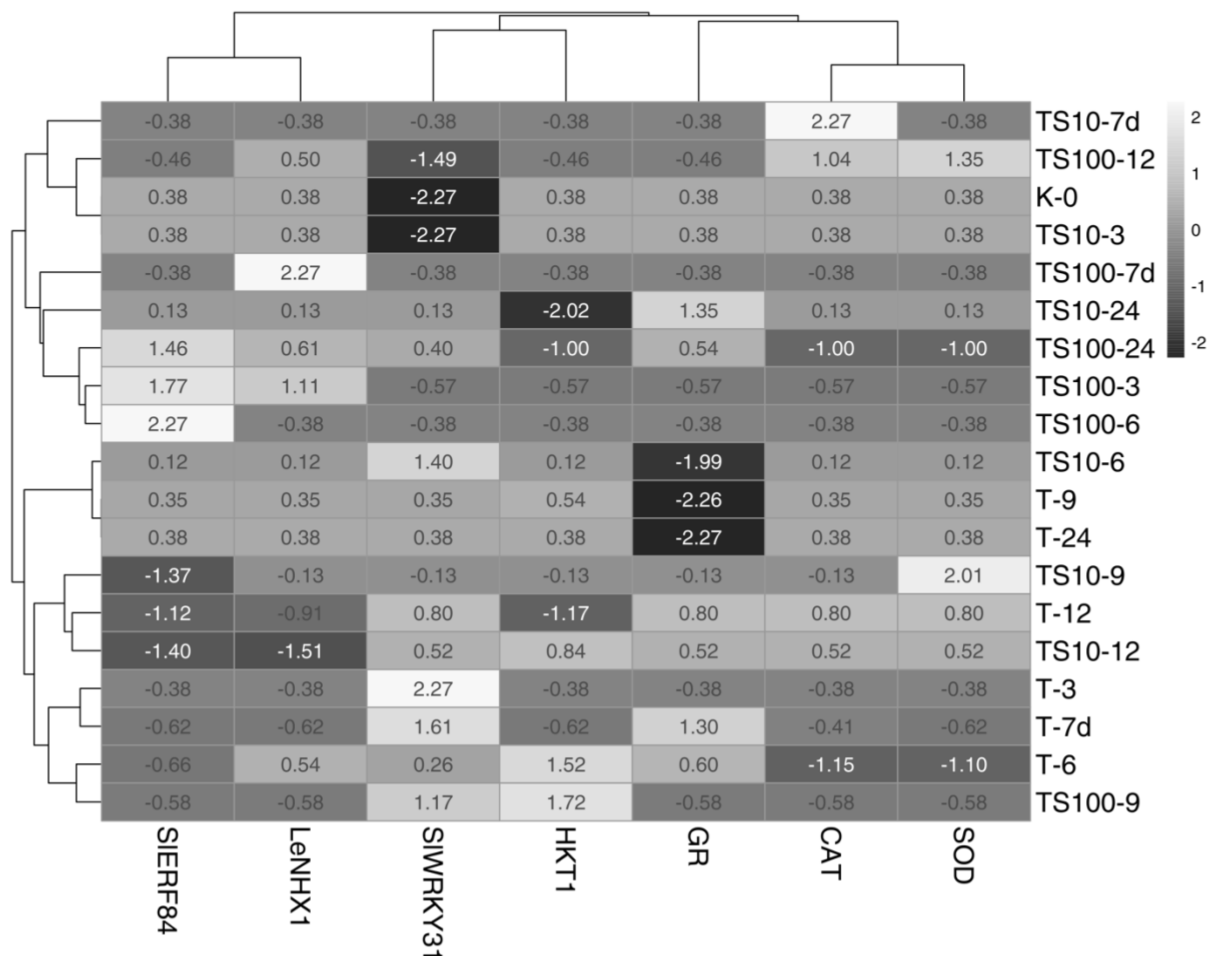

Figure 8. *Heatmap view of the studied genes, and applications. K: Control, T: Salt, S: GR24-rac, TS: Salt+GR24-rac, numbers before underscore: GR24-rac doses 10 nM and 100 nM, number after underscore: Sampling times (hour), 7d: Seventh day of sampling.*

In the present study, we evaluated the effects of synthetic strigolactone GR24-rac to expression levels of the genes encoding abiotic stress-related enzymes CAT, SOD and GR, and transcription factors SlWRKY31, ERF84, LeNHX1, and HKT1;2 under saline conditions in H-2274 commercial tomato cultivar. It was determined that the stress enzymes SOD and CAT genes upregulated with salt application, and this increase was even higher with GR24-rac application. It has been observed that GR24-rac, especially applied at a concentration of 100 nM, activates stress enzymes in the plant and triggers the plant to cope with salinity stress. It is understood that the 150 mM salt application applied in the study did not threaten the life of the plant, since there was no significant change in the GR enzyme. Transcription factors are proteins that bind to gene-coding regions on DNA to regulate the transcription of genes. Among these proteins, SlWRKY31, ERF84, LeNHX1 and HKT1;2, which we examined in the study, are transcription factors and genes that play a key role in adapting and resisting abiotic stress in plants directly. According to the results obtained in the study, it was determined that the expression rates of these transcription factors and genes increased at certain time intervals after salt application and decreased after adapting the plant to stress conditions. It was determined that this increase was higher with the GR24-rac application. In the previous studies, it is reported that SLs positively affected plants under various stress conditions such as heat and cold stresses (Chi, et al., 2021), drought recovery (Visentin, et al., 2020), and salt resistance (Liu et al., 2022). According to the results obtained in this study and literature knowledge, we concluded that GR24-rac application could help to obtain healthy H-2274 tomato seedlings under saline conditions at as low as 100 nM concentration.

4. ACKNOWLEDGEMENTS

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5. AUTHOR CONTRIBUTIONS

The authors have contributed equally to this study.

6. CONFLICT of INTEREST

The authors declare there is no conflict of interest.

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