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Differential expression of salt tolerance related genes in tomato in response to a low dose of γ irradiation

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Abstract

Using a low dose of gamma rays (30 Gy), the response of twelve salt tolerance-related genes (*SlTAS14*, *SlNCED1*, *SlDREB2*, *SlAREB*, *SlGR*, *SlAPX1*, *SlDELLA*, *SlJAZ1*, *SlCU/ZnSOD* (*SlCSD2*), *SlFSD*, *SlTIR1* and *SlNHX1*) was examined at two concentrations of salt stress (50 and 200 mMNaCl). Real-time reverse transcription-PCR analyses of the examined genes showed different expression profiles in shoot and root tissues. In the case of irrigation by 50 mM NaCl, seven genes (*SlAPX1*, *SlGR*, *SlTAS14*, *SlNCED1*, *SlDELLA*, *SlJAZ1*, *and SlCSD2*) showed a significant increase in their expression in shoot tissues of the irradiated plants. On the other hand, two genes (*SlNCED1* and *SlDREB2*) showed a significant increase in the root tissues at the same concentration. The potential effect of a low dose of gamma rays on enhancing the salinity response of tomato plants can be observed at 200 mM NaCl, where all genes showed a significant increase in shoot tissues of irradiated plants. Interestingly, nine genes (*SlNCED1*, *SlDREB2*, *SlAREB*, *SlAPX1*, *SlDELLA*, *SlJAZ1*, *SlCSD2*, *SlFSD*, and *SlTIR1*) showed a significant increase in the roots of the irradiated plants compared to non-irradiated plants.

Keywords: *Solanum lycopersicum*, salt stress, γ irradiation, qPCR, gene expression.

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Introduction

Salinity affects crop productivity and quality in arid and semi-arid regions (Gharsallah *et al*., 2016). Combining the fact of the increasing world population with the reduction of the available area for cultivation, it is mandatory to improve plants that can withstand salinity, drought, and poor drainage (Taiz, 2013; FAOSTAT, 2017). Moreover, land salinity is annually increasing at a rate of 10% (Flowers, 2004; Foolad, 2007). Also, 20% of the irrigated land is affected by salinity where crop yields were distinctly limited (Qadir *et al*., 2014; Akladious and Mohamed,

2018). It is evaluated that by the year 2050, the increased salinization will lead to devastating global effects as well as to the loss of up to 30% of the cultivated lands (Rengasamy, 2006; FAO, 2011; Aragüés *et al*., 2015; Lal, 2015). Nevertheless, salinity also affects the nutrients uptake of plants (Sofy *et al*. 2020).

Salt stress often leads to the accumulation of ion toxicity due to increased concentrations of Na+ and Cl- ions, such unfavorable conditions, afterward, induce reactive oxygen species (ROS) which cause oxidative stress (Kim *et al*., 2014). ROS are highly harmful to plants due to their ability to oxidize proteins, pigments, lipids, and nucleic acids leading to the alteration of cell structure and causing mutations (Mateos-Naranjo *et al*., 2013). The defense against abiotic stress is thought to be caused by the direct or indirect scavenging of ROS (Vickers *et al*., 2009).

Through salinity stress, plant adaptation to the stress causes adjustments in ion uptake, extrusion as well as the synthesis of compatible solutes to maintain cellular homeostasis (Chaves *et al*., 2009). Furthermore, plants have emerged a complex survival response that involves the harmonious action of many physiological and genetic processes, including ion sequestration, metabolic adjustment, osmotic adjustment, control of water loss through stomata, and antioxidative defense (Orsini *et al*., 2011; Abo-Ogiala *et al*., 2014; Adem *et al*., 2014; Geilfus *et al*., 2015).

Plant responses to environmental stresses are very complex and involve changes at the cellular, physiological, and transcriptome levels to avoid damage and to ensure survival under different stress conditions (Atkinson and Urwin, 2012). Upon stress discernment, transcription factors (TFs) bind to their target genes to regulate their expression and to manage physiological and biochemical modifications critical for stress tolerance and the adaptation of plant growth under different stresses (Hichri *et al*., 2014).

Tomato (*Solanum lycopersicum* L.), family Solanaceae, is considered one of the most economically important horticultural crops worldwide (FAOSTAT, 2016). It is also considered as the model for studying plant development and abiotic stress tolerance (Zouine *et al*., 2017). Fruits of tomato are a rich source of natural pigments such as lycopene, ß-carotene, dietary fiber, potassium, and A, C, and E vitamins (Kashyap *et al*., 2020).

Tomato production has been limited due to the high level of salinity of the irrigation water or the soil (Zhai *et al*. 2015). Moreover, the quality and yield of tomatoes are greatly affected by other biotic and abiotic stresses such as pathogen infection, low temperature, and drought (Zhao *et al*., 2020).

Cultivated tomato is, in general, considered as moderately tolerant (~70 mM NaCl) to salinity stress (Pérez-Alfocea *et al*., 1996). However, soil salinization strictly affects tomato fruit yield and quality by decreasing photosynthetic efficiency and affecting some physiological metabolism due to osmotic stress, ion toxicity, and nutrient deficiency (Pineda *et al*., 2012; Wu *et al*., 2020). Identifying key salt-responsive genes that induce salt tolerance and facilitate plant survival during high salinity would facilitate developing tolerant plants (Flowers and Colmer, 2015; Larrieu and Vernoux, 2016).

Gamma radiation is one of the most studied ionizing radiation types (Lind *et al*., 2019) due to its high penetration power that helps in their wider application for the improvement of various plant species compared to other ionizing radiations (Moussa, 2011). Gamma rays compose of high-energy photons, are high-frequency electromagnetic

radiation, and can pass through most types of materials (Richardson, 2004). Artificial sources of radiation are man-made gamma rays emitters such as Cobalt-60 (^{60}Co) and cesium-137 (^{137}Cs) cells (Charles, 2001). Irradiation with low doses is known to have stimulatory effects on plant growth, a concept referred to as hormesis (Calabrese, 2002). The possibility of using gamma rays to improve the growth and productivity of plants was investigated by Akshatha *et al*., (2013) and Jaipo *et al*., (2019). In addition, gamma rays would urge plants to resist changes in environmental conditions through the induction of genomic, biochemical, physiological, and morphogenetic changes in plant cells and tissues (Geras'kin, 2016).

The exposure of a biological system specifically seeds to ionizing radiation can cause different changes in living cells. Given the high energy of gamma and its greater penetrability into exposed cells and tissues, the DNA of the subject material may undergo severe alterations (Zaka *et al*., 2004; Majeed *et al*., 2017). Scientists interpreted the mechanism of gamma radiation effect on living materials through two theories; the direct effect caused in direct physical strikes of gamma irradiation on the genetic material in the cell and the indirect effect by the reaction with a water molecule, which is omnipresent in organisms causing in an ionized water molecule (H_2O^+) and the radicals H and OH due to the primary reactions of excitation and ionization followed by production the reactive O_2 species such as hydrogen peroxides, hydroxyl ions and other active atomic oxygen which can further interact with DNA and other cellular components and biomolecules resulting in the ionization, functional changes of proteins and enzymes and overall metabolic activities (Ward, 1995; Esnault *et al*., 2010; Majeed *et al*., 2017).

Altering plants' DNA after espousing seeds to radiation can be a potential source of variation in descendants. Mutant offspring resulted from irradiated seeds can exhibit either abnormality in germination, morphology, and growth or these traits may be positively influenced which generally depend on repairing the DNA and rearrangement of genes, in addition to the changes in hormonal balance, metabolic pathways, and enzymatic alterations (Melki and Marouani, 2010).

We studied the possibility of a low dose of gamma irradiation to enhance the salinity response of tomato at the seedling stage, by investigating the differentiation level of gene expression for twelve salinity-related genes in both shoot and root tissues in the presence of two salt concentrations (50 and 200 mM NaCl).

Materials and Methods

Plant materials

Seeds of tomato plants (*Solanum lycopersicum* L. cv. Castle Rock) were divided into two groups. The first one was used as the control and the second was exposed to a low dose of gamma rays (30 Gy). Gamma irradiation was carried out at the National Center for Radiation Research and Technology, Cairo, Egypt. Irradiation was achieved by a ^{60}Co gamma unit (Indian gamma cell) which delivers 2.25 kGy per hour.

Plant growth and treatment

Solanum lycopersicum cv. Castle Rock seeds were grown in the greenhouse under controlled conditions of light (500 µmol m^{-2} s^{-1}), photoperiod (16/8 h day/night), humidity (60-65%), and temperature (25˚C) after being exposed to γ irradiation. One month later, plants were designated for salt treatment. Seedlings were randomly divided into two groups and were subjected to different salinity stress of 50 or 200 mM, each treatment had three biological replicates. The first group was divided into three subgroups; one was kept without exposure to irradiation (control), the

second was treated with 50 mM NaCl (S 50) and the third was treated with 200 mM NaCl (S 200). The second group was exposed to γ irradiation before being treated with 50 mM NaCl (iRS50) and 200 mM NaCl (iRS 200). Samples were collected after 24 hours from salinity treatment and seedlings from both groups were frozen in liquid nitrogen and stored at -80˚C until further used.

RNA isolation and cDNA synthesis

Total RNA was isolated from frozen tissues (shoots and roots) using TRIzol reagent according to the manufacturer's protocol (Invitrogen, USA). RNA concentration and purity were evaluated using NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA). First-strand cDNA was synthesized using 1.2 µg of total RNA using M-MLV reverse transcriptase (Promega, USA) according to the manufacturer's instructions. Then, the reaction was diluted 10 times for further use as a qPCR template.

Expression analysis of target genes by quantitative PCR

The expression levels of the salt-responsive selected genes (*SlAPX1, SlFe-SOD, SlDREB2, SlTAS14, SlAREB1, SlTIR1, SlSOD2, SlNCED1, SlDELLA, SlJAZ1, SlNHX1* and *SlGR*) were analyzed by quantitative real-time PCR (qRT-PCR). Primer sequences are presented in Table (1). The *SlActin* gene was used as a housekeeping gene and the actin gene was used as a reference gene for mRNA normalization and analysis. Q-PCR reactions were carried out using a Stratagene MX3005P Real-Time System (Agilent, United States) with HERA SYBR® Green qPCR (willow fort, UK) as manufacturer's instructions. Three independent biological replicates were measured in the qPCR analysis. Relative gene expression levels for mRNA were calculated using the $2^{-\Delta\Delta CT}$ method (Schmittgen and Livak, 2008). Heatmap of the gene expression levels was performed using the ClustVis web tool (Metsalu and Vilo, 2015).

Quantitative real-time PCR analysis

The statistical analysis was calculated using SPSS (20.0, IBM) and significant differences were analyzed by one-way analysis of variance (ANOVA) followed by the Duncan post hoc test. RNA quantification data are presented as mean and standard error (SE) of means (n = 3).

Table (1): Primer names and sequence for the selected twelve salinity tolerance-related genes used in the q-PCR analysis.

Primer name	Forward	Reverse
SlAPX1	5'-CCATTTGGAACAATCAGGCACCCG-3'	5'-CGGGGCCTCCCGTAACTTCA-3'
SlFSD	5'-TAAATAGAGACTTTGGTTCC-3'	5'-TATATTTGCCTCTTAACCCT-3'
SlDREB2	5'-TGCATTCTGCTGCCTACGATGC-3'	5'-TCGGCATTGTCCAACTGGTATGC-3'
SlTAS14	5'-AGATGGCACAATACGGCAAT-3'	5'-ACCAGTACCCATGCCTTGAG-3'
SlAREB1	5'-TAATTTGCCACTGAATGTGAATGGG-3'	5'-CCCAACTGACCACTATTTGGGATA-3'
SlTIR1	5'-AGGGGTCCTCCAGATACAAG -3'	5'-CGCTAATACCTGCCCATCTTT-3'
SlCSD2	5'-AATCTCCGGGAACGATAGTG-3'	5'-AAGGCATGGATATGGAAAGC-3'
SlNCED1	5'-AGGCAACAGTGAAACTTCCATCAAG-3'	5'-TCCATTAAAGAGGATATTACCGGGGAC-3'
SlDELLA	5'-TGATGCGACTATACTTGATATAAG-3'	5'-GGGTTAATCTGTTTAATAGAGTTC-3'
SlJAZ1	5'-TTCCCTCAAGGTGGAATGAAGGCT-3'	5'-TCCGAAACTCGGAACCACCAAATC-3'
SlNHX1	5'-GACAGTCCTGGAAAATCT-3'	5'-GGTTATCAGCCCAAACACC-3'
SlGR	5'-TTGGTGGAACGTGTGTTCTT-3'	5'-TCTCATTCACTTCCCATCCA-3'
SlActin	5'-TGTCCCTATTTACGAGGGTTATGC-3'	5'-CAGTTAAATCACGACCAGCAAGAT-3'

Results and Discussion

The effect of salinity on gene expression in non-irradiated plants

Differential gene transcription was measured in the shoot and root tissues of non-irradiated plants as a response to the two used salt concentrations. When plants were treated with 50 mM NaCl, eight of the studied genes (*SlAPX1*, *SlAREB*, *SlGR*, *SlTSA14*, *SlDELLA*, *SlJAZ1*, *SlFSD*, and *SlTIR1* genes) showed an increasing transcription pattern in shoot tissues compared to the control. This increase was significant for *SlAREB*, *SlTSA14*, and *SlDELLA* genes but non-significant for *SlDREB2*, *SlAPX1*, *SlGR*, *SlJAZ1*, *SlFSD*, and *SlTIR1* genes. However, a non-significant decrease was recorded for *SlNCED1*, *SlCSD2*, and *SlNHX1* genes. The highest gene transcription value was recorded for the *SlDELLA* gene (2.63-folds), while the lowest one was that for the *SlNCED1* gene (0.855-folds) as shown in Figure (1f).

On the other hand, under the same salt concentration (50 mM NaCl), the expression of the selected genes in root tissues showed an increasing trend for all the studied genes except for the *SlDREB2* gene which showed a non-significant decrease in the transcription value compared to the control. This increase was significant for *SlGR*, *SlCSD2*, *SlFSD* and *SlNHX1* genes and non-significant for *SlAPX1*, *SlAREB*, *SlTAS14*, *SlNCED1*, *SlDELLA*, *SlJAZ1* and *SlTIR1* genes. The highest value of gene transcription was recorded for the *SlGR* gene (5.12-folds) (Figure 2d), while the lowest number was observed for the *SlDREB2* gene (0.799-folds) as shown in Figure (2c).

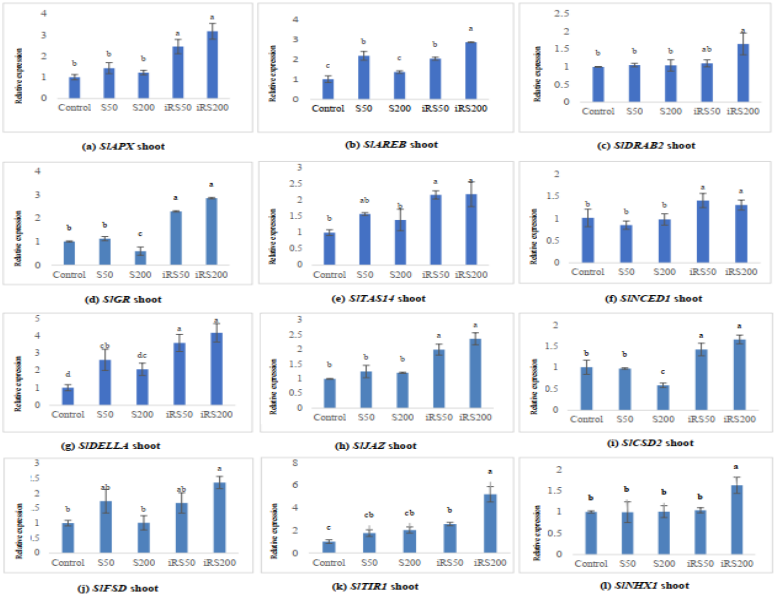

Figure1: qRT-PCR analysis of *SlAPX1*, *SlAREB*, *SlDREB*, *SlGR*, *SlTAS14*, *SlNCED1*, *SlDELLA*, *SlJAZ*, *SlCu/ZnCSD2*, *SlFSD*, *SlTIR1* and *SlNHX1* expression pattern in response to salinity stress (50mM and 200mM NaCl) in shoot tissues of irradiated and non-irradiated tomato seedling.
Different letters indicate statistical differences at $p<0.05$ determined by Duncan's multiple range tests.

When plants were treated with higher salt concentration (200 mM NaCl), the transcription patterns of nine genes in the shoot tissues showed a significant decrease comparing with the transcription patters of the shoot tissues that were treated with 50 mM NaCl. This downregulation was non-significant in six genes (*SlAPX1*, *SlDREB2*, *SlTAS14*, *SlDELLA*, *SlJAZ1*, and *SlFSD*) but it was significant for *SlGR*, *SlAREB*, and *SlCSD2* genes. However, *SlNHX1*, *NCED1*, and *SlTIR1* were upregulated comparing to their transcription values when treated with the lower salt concentration. It is worth mentioning that, *SlAPX1*, *SlAREB*, *SlDREB2*, *SlTAS14*, *SlDELLA*, *SlJAZ1*, *SlFSD*, *SlTIR1*, and *SlNHX1* genes showed no significant increase in the transcription level in shoot tissues at 200 mM NaCl compared to the control. While *SlGR* and *SlCSD2* showed a significant decrease. Moreover, the transcription value of the *SlNCED* gene showed a non-significant decrease compared to the control.

On the other hand, the transcription patterns of eight genes in the root tissues significantly increased upon treatment with 200 mM NaCl compared to their transcription patterns when treated with 50 mM NaCl. This increase was significant for *SlTAS14* and *NHX1* genes and non-significant for *SlAPX*, *SlAREB*, *SlDREB2*, *SlDELLA and SlJAZ1*, and *SlTIR1*. Moreover, the transcription patterns of *SlGR*, *SlNCED1*, *SlCSD2*, and *SlFSD* genes showed a decreasing trend at 200 mM NaCl compared with their transcription patterns when treated with 50 mM NaCl. The studied genes were upregulated except the *SlCSD2* gene that was down-regulated compared with the control. The increase in the transcription level was significant for *SlGR*, *SlTAS14*, *SlFSD*, *SlTIR1*, and *SlNHX1*. On the contrary, the increase in the transcription value was non-significant for *SlAPX*, *SlAREB, SlDREB2, SlNCED1*, *SlDELLA*, and *SlJAZ1* genes compared to the control.

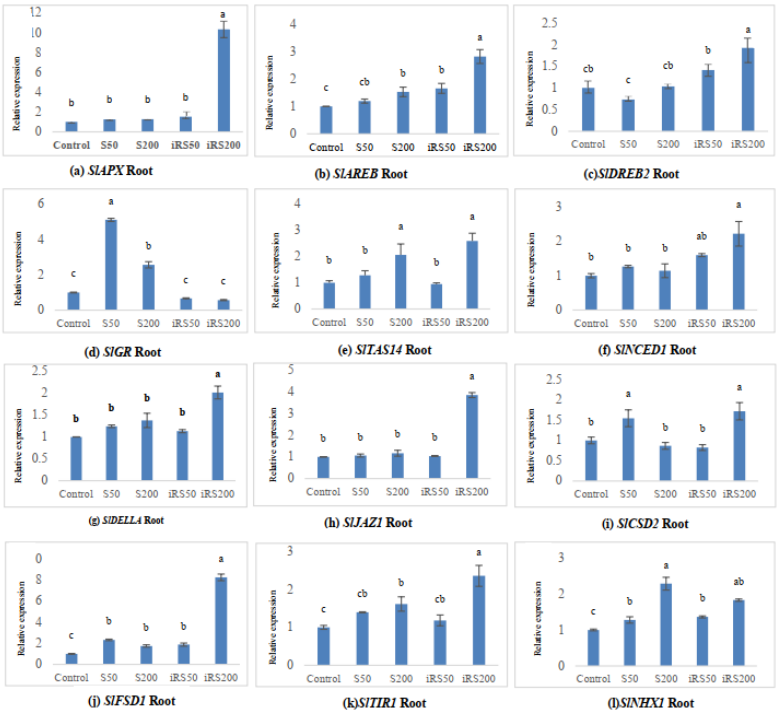

Figure2: qRT-PCR analysis of *SlAPX1*, *SlAREB*, *SlDREB*, *SlGR*, *SlTAS14*, *SlNCED1*, *SlDELLA*, *SlJAZ*, *SlCu/ZnCSD2*, *SlFSD*, *SlTIR1* and *SlNHX1* expression pattern in response to salinity stress (50 mM and 200 mM NaCl) in root tissues of irradiated and non-irradiated tomato seedling.
Different letters indicate statistical differences at $p<0.05$ determined by Duncan's multiple range tests.

The highest increase in the transcription patterns was that recorded at 200 mM NaCl by the *SlDELLA* gene (2.08-folds) in shoots and with the *SlGR* gene (2.57-folds) in roots, while the lowest number was observed with the *SlCSD2* gene in both shoots and roots (0.589 and 0.869, respectively) compared with the level of expression of the other genes in root tissues.

Salinity is important environmental stress that causes damage to the plant, in part, through ionic and osmotic stress (Munns, 2002; Zhu, 2002). Generally, plants differ in their response to salinity; each plant applies a unique defense mechanism that enables it to resist and grow (AbdElgawad *et al*., 2016). This type of tolerance is the result of a number of interdependent series of molecular events comprising of gene activation and/or regulation of a range of salt stress-responsive genes (Passaia *et al*. 2013). In our study, the studied genes showed different response patterns to different salt concentrations in the tissues of shoots and roots of the irradiated and non-irradiated tomato plantlets. In response to salinity, *SlDELLA* and *SlTIR1* genes expression increased in shoot tissues than root tissues of non-irradiated and irradiated plants at both 50 and 200 mM NaCl. On the other hand, *SlNCED1*, *SlFSD*, and *SlNHX1* genes were upregulated due to higher salinity in roots tissues compared to their response in shoot tissues in non-irradiated and irradiated plants at both 50 and 200 mM NaCl. Similar results were reported by Zhang *et al*. (2008) and Moshaei *et al*. (2014) who found that the transcripts of *VHA-c* and *NHX1* genes in the roots of *Aeluropus littoralis* were higher than their expression in shoots tissues at different salt treatments (100, 200 and 400 mM NaCl).

In addition, *SlAPX* and *SlTAS14* showed a higher response to salinity in shoot tissues than roots of both irradiated and non-irradiated plants at 50 mM NaCl and vice versa at 200 mM NaCl. While the *SlGR* gene showed a higher response to salinity in the roots of tomato plants than in shoots of the non-irradiated plants at both 50 and 200 mM NaCl, it showed the opposite trend in tissues of the irradiated plants.

The effect of salinity on gene expression in irradiated plants

The gene expression profile of the studied genes in irradiated tomato plants showed different responses at the same concentrations of salinity compared with non-irradiated plants and the corresponding control in both shoots (Figure 1) and roots (Figure 2).

At 50mM NaCl; irradiated plants were upregulated in shoot tissues compared to the control. This upregulation was significant for ten genes (*SlAPX1*, *SlAREB*, *SlGR*, *SlTAS14*, *SlNCED1*, *SlDELLA*, *SlJAZ1*, *SlCSD2*, *SlTIR1 and SlNHX1)* and non-significant for *SlDREB2* and *SlFSD* genes. The transcriptional level of ten genes (*SlAPX1*, *SlDREB2*, *SlGR*, *SlTAS14*, *SlNCED1*, *SlDELLA*, *SlJAZ1*, *SlCSD2*, *SlTIR1* and *SlNHX1*)increased in the irradiated plants compared to the non-irradiated plants. However, the increase was non-significant for *SlTIR1*gene.

It is worth mentioning that the *SlDELLA* gene showed the highest transcription increase in shoots tissues at 50 mM NaCl in both irradiated and non-irradiated plants (Fig. 1g). However, the level of upregulation was higher in the irradiated plants than in the non-irradiated plants (3.602, 2.626 fold, respectively), while the lowest level of upregulation in the shoot tissues was that of the *SlDREB2* gene (1.10-folds), (Figure 1b).

In roots, nine genes (*SlAPX1*, *SlAREB*, *SlDREB2*, *SlNCED1*, *SlDELLA*, *SlJAZ1*, *SlFSD*, *SlTIR1* and *SlNHX1* were upregulated upon treatment with 50mM NaCl. This increase was significant for *SlAREB*, *SlNCED1*, *SlFSD*, and *SlNHX1* genes and non-significant for *SlAPX1*, *SlDREB2*, *SlDELLA*, *SlJAZ1*, and *SlTIR1*, while *SlGR*, *SlTAS14*, and

SlCSD2 genes showed non-significant downregulation compared with the control. The highest value of upregulation was that of the *SlFSD* gene (1.869 folds) as shown in Figure(2j), while the lowest number was observed for the *SlGR* gene (0.67 folds) compared with the other genes in root tissues (Figure 2d).

The expression level of 10 genes (*SlAPX1, SlDREB2, SlGR, SlTAS14, SlNCED1, SlDELLA, SlJAZ1, SlCSD2, SlTIR1,* and *SlNHX1* genes) was higher in the shoots of irradiated plants compared to that of the non-irradiated plants. The increase was significant for *SlAPX1, SlGR, SlTAS14, SlNCED1, SlDELLA, SlJAZ1, SlCSD2* and *SlNHX1* genes and non-significant for *SlDREB2* and *SlTIR1* genes. On the other hand, the transcriptional level of five genes (*SlAPX1, SlAREB, SlDREB2, SlNCED1,* and *SlNHX1*) was higher in the roots of irradiated plants compared with the non-irradiated plants. This increase was significant for *SlDREB2* and *SlNCED1* genes.

When plants were irrigated by 200 mM NaCl, the transcription level of the studied genes significantly increased in the shoots of the irradiated plants compared with the control plants. *SlDELLA* and *SlTIR1* genes showed the highest transcription level in the shoots at 200 mM (4.19 and 5.21 folds, respectively) compared to the control. Eleven genes; *SlAPX1, SlAREB, SlDREB2, SlTAS14, SlNCED1, SlDELLA, SlJAZ1, SlCSD2, SlFSD, SlTIR1* and *SlNHX1*; showed significant transcription increase in roots tissue of irradiated plants compared to the control. On the other hand, the *SlGR* gene showed a non-significant downregulation. Moreover, *SlAPX* and *SlFSD* genes showed the highest transcription increase in roots at 200 mM salt concentration (10.78 and 8.23 folds, respectively) compared to the control (1.00-fold).

With a comparison between irradiated and non-irradiated plants at the same level of salinity, we could note that all genes showed a significant increase in their transcription in shoots of irradiated plants compared to non-irradiated plants, while nine genes (*SlAPX1, SlAREB, SlDREB2, SlNCED1, SlDELLA, SlJAZ1, SlCSD2, SlFSD,* and *SlTIR1*) showed an increase in their level of transcription in the roots of irradiated plants compared with that of non-irradiated plants. This increase was significant for *SlDREB2* and *SlNCED1* genes.

From another perspective, the heatmap exhibits a visual appearance for the differential expression of the studied genes in response to salinity stress in irradiated and non-irradiated tomato plants in both shoot (Figure 3a) and root (Figure 3b) tissues comparing with the corresponding control.

The possible role of γ-irradiation in alleviating salinity stress can be expressed through the obvious effect of gamma irradiation on increase the gene expression significantly principally under the high dose of salinity

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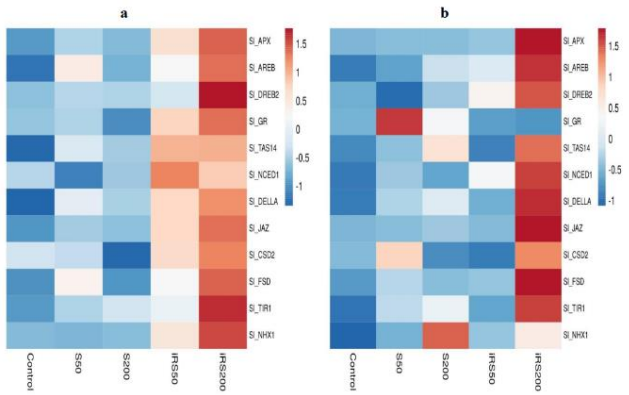

Figure3: Heatmap visualization shows the differential expression of *SlAPX1*, *SlAREB*, *SlDREB*, *SlGR*, *SlTAS14*, *SlNCED1*, *SlDELLA*, *SlJAZ*, *SlCu/ZnCSD2*, *SlFSD*, *SlTIR1* and *SlNHX1*genes in response to salinity stress (50mM and 200mM NaCl) in shoot (a) and root (b) tissues of irradiated and non-irradiated tomato seedling.

At higher salinity concentrations, plants activate the ROS-scavenging machineries to tolerate the oxidative stress caused by ROS accumulation (Zhao *et al*., 2020). ROS-scavenging machineries include an enzymatic agent, such as catalases (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR) (Hanin *et al*., 2016). Cu/Zn-SODs and Fe-SODs are two types of SODs that were recognized in plants (Bowler *et al*., 1994). SODs were known as the first line of enzymatic defense against oxidative damage in plant cells through catalyzing the conversion or dismutation of toxic O_2 radical dot- radicals to H_2O_2 and molecular oxygen (O_2) (Gill *et al*., 2015). Consequently, they are considered essential for stress tolerance.

Badawi *et al*. (2004) found that plants with overexertion of the *APX* gene exhibit enhanced tolerance to salt and drought stresses, which was confirmed later by Asada (2006) who reported that, *APX* is required for the conversion of H_2O_2 to H_2O. Our results indicated a significant upregulation of *APX* gene both in shoots or roots. In addition, the *GR* gene significantly increased in the shoots of irradiated plants under high salinity, a fact that may led to enhancing the plant's tolerance to salinity. Similar results were obtained by Macovei *et al*. (2014) who evaluated the expression and activity of *APX* and *GR* genes in rice seedlings and plantlets grown after γ-irradiated seeds under 100 mM NaCl. They reported higher transcriptional levels and enzymatic activity of *APX* and *GR* genes in the irradiated rice plants.

Abscisic acid (ABA) plays important role in osmotic stress-responsive gene expression mainly through three transcription factors, AREB1, AREB2, and ABF3 (Yoshida *et al*., 2014). The expression of *NCED1* (ABA synthesis) and *TAS14* (ABA response) genes in roots generally correspond to the changes in root ABA levels during salt stress onset. A positive relationship between ABA accumulation and mitigate the inhibitory effect of salinity on photosynthesis, growth, and translocation of assimilates has long been recognized in *Hordeum vulgare* L. (Popova *et al*., 1995) and *Ricinus communis* L. (Jeschke *et al*., 1997).

Li *et al*. (2014) found that JAZ proteins play an important role in the response to biotic stress, development, and fibers initiation in cotton. DELLA proteins interact with JAZ proteins to regulate plant growth and defense (Hou *et al*. 2010). The interaction between JAZ and MYC2 also plays an important role in the JA crosstalk with other phytohormones, such as ABA, salicylic acid, gibberellins, and auxin (Kazan and Manners 2013). At the same time, JAZ

proteins act as repressors of several transcription factors (TFs) (Chini *et al*., 2016).

Jasmonate (JA) and the expression of *JAZs* genes were linked in many plant species. In rice, the physical interaction between JasmonateZim-Domain (JAZ) proteins and class C basic-helix-loop-helix (bHLH) transcription factors, promotes root call elongation under salt stress. Losing the function of this complex results in the upregulation of *JA*-responsive genes and act as the negative regulator of the JA pathway (Toda *et al*., 2013). This may lead us to conclude that, as shown in our results, the significant increase in *JAZ1* transcriptions in both shoots and roots of the irradiated plants may be attributed to the increase of JA ratio as a response to high salinity under the irradiation conditions. This suggestion is consistent with that of Kazan (2015) who reported that the importance of the JAZ protein relies on mediating JA-regulated responses, as well as their transcriptional upregulation in response to JA accumulation. In addition, the results of Chini *et al*. (2017) confirmed that JA regulates the expression of several *JAZ* genes in tomatoes. Moreover, the *JAZ* genes, induced by JA treatment in the roots are also triggered in the leaves except for *JAZ11*, *JAZ1*, and *JAZ3* that are strongly induced by JA treatment.

Conclusion

In the non-irradiated tomato plants; *SlAPX1*, *SlAREB*, *SlTAS14*, *SlDELLA*, *SlJAZ*, *SlFSD*, and *SlTIR1* genes are analogously upregulated in both shoots and roots of tomato plants at the two used salt concentrations. However, other genes are differentially expressed (i.e., *SlGR*) that was upregulated in shoots and roots of tomato plants with 50 mM NaCl while down-regulated in shoots and up-regulated in roots with 200 mM NaCl and (i.e., *SlNCED1*, *SlCSD2*, and *SlNHX1*) were downregulated in shoots and upregulated in roots at 50 mM NaCl while *SlCSD2* was downregulated in both shoots and roots. Moreover, *SlNHX1* gene was upregulated at 200 mM NaCl compared to the control plants.

In the irradiated plants, *SlAPX1*, *SlAREB*, *SlDREB2*, *SlNCED1*, *SlDELLA*, *SlJAZ*, *SlFSD*, *SlTIR1*, and *SlNHX1*genes were upregulated in an analogous in both shoots and roots of the tomato plants at the two salt concentrations. Also, *SlGR*, *SlTAS14*, and *SlCSD2* were differentially upregulated in the shoots and downregulated in the roots of tomato plants at 50 mM NaCl. Interestingly, they were upregulated in both shoots and roots of tomato plants at 200 mM NaCl compared to the control.

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