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The Role of Tubulin and Thaumatin Genes and Osmotic Factors in Salinity Tolerance of Tomato Plants

Mahmoud S. Abdelmoneim

Egypt-Japan University of Science and Technology (E-JUST)
Assiut University

Elsayed E. Hafez

City of Scientific Research and Technological Applications (SRTA-City)

Mona F. A. Dawood

Assiut University

Sherif F. Hammad

Egypt-Japan University of Science and Technology (E-JUST)
Helwan University

Mohamed A. Ghazy

Egypt-Japan University of Science and Technology (E-JUST)
Ain Shams University

Abstract: Soil salinity is a drastic abiotic factor that affects many physiological processes and whole plants' activities, as well as up-and down-regulating gene expression. Studying the effect of salinity on tubulin and thaumatin relative gene expression as DNA markers for salinity stress in tomato plants is a scarcely studied topic. Thus, the present investigation was conducted to study the impact of different salinity levels (0, 75, 100, and 120 mM NaCl) on the tomato plants' growth, osmotic adjustment, and relative gene expression of both antioxidant and salt tolerance genes. As the salt concentration intensified, the fresh and dry weight of the shoots and the roots reduced significantly, accompanied by a reduction in chlorophyll a and carotenoids. On the other hand, salinity stress significantly decreased the level of osmotica (e.g., soluble sugars and soluble proteins) in tomato tissues compared to non-saline-grown plants, while a significant accumulation of free amino acids was recorded. At the molecular level, it was observed that the relative expression of the polyphenol oxidase, peroxidase, and thaumatin genes was high at the level of 100 mM NaCl, but it was suppressed at 120 mM NaCl. In contrast, salinity down-regulated tubulin gene expression in stressed tomato plants relative to controls, revealing various mechanisms that instigated salinity tolerance, which is concentration-dependent. The study recommended the importance of amino acids as osmotica as well as the relative expressions of PPO, peroxidase, and thaumatin genes in conferring salt tolerance at low to moderate salt levels.

Keywords: Salinity, Growth, Primary metabolites, Gene expression, Tomato

Introduction

Tomato (*Lycopersicon esculentum* Mill.) plants are one of the most frequently grown and consumed crops in the world. Egypt stands out for its tomato output, both for home use and export. It occupies the first rank among vegetables as a processed commodity in Egypt and many other countries (El-Khalifa et al., 2022). Salinity, or salt stress, which interferes with many phonological, physiological, biochemical, and molecular processes, is the second major abiotic factor lowering agricultural productivity worldwide. The tomato is considered to be a

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sensitive, tolerant, or moderately tolerant crop to salinity stress. In this regard, it has been reported that salinity stress lowered the germination percentage in the medium, fresh, and dry weight of the shoot, root length, chlorophyll a, b, and carotenoids contents, and K and Ca levels due to an increment in the tissue content of Na in tomato plants (Aazami, Rasouli, & Ebrahimpzadeh, 2021). Many physiological attributes have been impacted by the damaging effects of salinity in tomato plants, including the increase of reactive oxygen species, membrane degradation, imbalance in ionic status, failure of the antioxidant system, deregulation of secondary metabolites, and hormonal imbalance (Dawood et al., 2022). However, the responses of antioxidant molecules or enzymes vary according to salinity level, exposure period, and plant growth stage (Parvin et al., 2019). The relationship between antioxidant activity and salt tolerance development may be associated with some alterations in their gene expression. As a defense mechanism against the harmful effects of salt, plants accumulate metabolic products, such as sugars, proteins, and proline as osmotica, to up-regulate the osmotic status of tissues (Sheteiwy et al., 2022). Furthermore, at the molecular level, plants tolerate salinity stress by activating stress-related genes (Raza et al., 2022). At present, many functional genes for protecting cells have been characterized, including heat shock protein (HSP), late embryogenesis-abundant (LEA) protein, antioxidant enzymes, and membrane transporters (Liu et al., 2023). Genetic engineering of novel stress-tolerance-related genes from plants in special habitats can provide candidate genes for cultivating crop varieties that can tolerate abiotic stresses. The overexpression of specific stress response genes in plants capable of surviving in each stress environment is evoked as a common adaptive mechanism. To conserve plant cells from oxidative damage induced by abiotic stresses, such as salt stress, the organization of several genes encoding antioxidant enzymes could take into account their payoff in the salt stress response in plants, such as plant peroxidases and polyphenol oxidases (Azzam et al., 2021). The molecular mechanisms of changing cell wall dynamics are highly affected by salt stress. The tubulin proteins are the molecular building blocks of microtubules, which have a remarkably maintained cell shape via conserving the structure of the cell wall (Janke & Magiera, 2020). However, the mechanisms of changing cell wall dynamics through salt stress and cell wall integrity pathways remain unclear (Chun et al., 2021). Thaumatin-like proteins (TLPs) are the products of a huge, very complicated gene family that participates in host defense and a variety of developmental processes in fungi, plants, and animals. The structural diversity of TLPs in plants is connected to a wide range of traits. The majority of their anticipated activities are in relation to biotic challenges, whereas others are in response to abiotic conditions, including drought and osmotic stress. Yet, the roles of certain TLPs superfamily members have not been fully established (de Jesús-Pires et al., 2020). The quality and quantity of crops are influenced by both biotic and abiotic factors; as a result, this research aims to: i) investigate the influence of salinity as a common problem in the Egyptian soil on the osmoprotectants ii) detect antioxidant-related defense gene expression, and iii) study the behavior of tubulin and thaumatin gene expression in tomatoes under salinity stress.

Material and Methods

Experimental Design

A pot experiment was undertaken in the greenhouse of the City of Scientific Research and Technological Application (SRTA-City), Borg El-Arab, Alexandria, Egypt, under natural conditions of humidity, temperature, and light during the year 2022. Seedlings of the tomato hybrid T-186 (45 days old) were transplanted into the experimental pots. Each pot was lined with a plastic sac and filled with one kilogram of soil (1 clay: 2 sandy). Four different concentrations of salinity (sodium chloride) were prepared at four levels: 0, 75, 100, and 120 mM. Five pots per treatment were applied. The plants were irrigated with tap water on a field capacity (40%) basis. Tomatoes were kept under these conditions until harvested after 30 days for phenological, physiological, and gene expression studies.

Plant Analysis

Growth Parameters

Three replicated plants per treatment were sampled for growth measurements. At harvest, roots and shoots were immediately separated, washed with distilled water to remove any additional salt surface contamination, and dried on absorbent paper. Fresh weights were directly recorded, and samples for dry weight determination were taken.

Determination of Photosynthetic Pigments

The fractions of pigments (chlorophyll a, chlorophyll b, and carotenoids) were estimated using the spectrophotometric method recommended by Lichtenthaler (Lichtenthaler, 1987).

Determination of Osmolytes Content

Soluble carbohydrates were measured based on the anthrone sulfuric acid method. (Fales, 1951; Schlegel, 1956). According to the method of Lowery et al., soluble proteins were determined (Lowry, Rosebrough, Farr, & Randall, 1951). Ninhydrin assays were utilized to examine free amino acids (Lee & Takahashi, 1966). Soluble carbohydrates, soluble proteins, and free amino acids content were estimated by the preparation of calibration curves using pure glucose, bovine serum albumin, and glycine, respectively.

Fourier Transmission Infrared Spectroscopy (FTIR) of Tomatoes' Leaf Contents

The Fourier transform infrared (FTIR) spectra for dry-grinded tomato leaves were recorded using a Nicolet iS10 (Thermo Scientific, USA) with 1 cm^{-1} resolution and a range of $500\text{--}4000\text{ cm}^{-1}$. Furthermore, the KBr-Wafer method was used (Hsu & Lo, 1999).

Relative Gene Expression of POD, PPO, Thumatin, and Tubulin Using Quantitative RT-PCR (qRT-PCR)

Extraction of RNA and Synthesis of cDNA from Tomato Plants

One hundred milligrams of tomato leaves were used for total RNA extraction by the TRIzol LS Reagent Kit (Chomczynski & Sacchi, 2006). The purity and concentration of extracted RNA were measured by Nanodrop Thermo Scientific, model 2000c, USA, at A260/A280. In order to synthesize cDNA, $1\text{ }\mu\text{g}$ of DNase I-treated RNA was utilized from each sample as a template in a reverse transcription process. The reaction procedure was carried out with oligo (dT) and random hexamer primers, and the reaction conditions and components were applied in accordance with previously established protocols (Abdelkhalek et al., 2019). The final cDNA product was kept at $-20\text{ }^{\circ}\text{C}$ until utilized as a template for qRT-PCR.

Expression of Defense Genes in Treated and Non-Treated Tomato Plants

Quantitative RT-PCR was used to measure the transcript levels of five tomato genes (peroxidase, polyphenol oxidase, thaumatin (TLPs), and tubulin (TUB)) and β -actin for all regimens. Table 1 displays the nucleotide sequences of the primers used. The expression levels of the PPO, POD, TUB, and TLPs tomato genes were adjusted using the β -actin gene as a standard. Each biological treatment's qPCR reactions were conducted in a separate run using a SYBR Green Mix (Thermo Fisher, CA, USA) and a Rotor-Gene 6000 real-time thermocycler (QIAGEN, Germantown, MD, USA). The PCR reaction mixture and the reaction conditions were performed as reported in a previous study (Hafez et al., 2013). Using the $2^{-\Delta\Delta\text{CT}}$ method, relative expression levels for each studied gene were estimated (Livak & Schmittgen, 2001).

Table 1. The nucleotide sequences of the primers used in this study.

Primer and Gene Name	Abbreviation	Direction	Nucleotide Sequence 5'-3'
Peroxidase	POD	Forward	GCTTTGTCAGGGGTTGTG AT
		Reverse	TGCATCTCTAGCAACCAA CG
Polyphenol oxidase	PPO	Forward	CATGCTCTTGATGAGGC GTA
		Reverse	CCATCTATGGAACGGGAAGA
Thaumatococcus	TLPs	Forward	CATGTCTCCACAGAGTAC
		Reverse	ATATAATCCCATTTTCGTGCTTATG
Tubulin	TUB	Forward	AGGATGCTACAGCCGATGAG
		Reverse	GCCGAAGAAGTACGAGAATC
β -actin	β -actin	Forward	TGGCATACAAAGACAGGACAGCCT
		Reverse	ACTCAATCCCAAGGCCAACAGAGA

Statistical Analyses

Using the SPSS 21 software, all data were statistically analyzed using a one-way ANOVA. Tukey's honest significant differences (H.S.D.) at a probability value ($p \leq 0.05$) were applied to the obtained data, where three replicates were applied per treatment.

Experimental Results

The different concentrations of NaCl salinity significantly impacted the fresh and dry weights of shoots and roots. As represented in Table 2, salinity stress caused a reduction in shoot fresh weight (SFW) by 10%, 15%, and 17%, as well as 31%, 55%, and 53% for root fresh weight (RFW) at the levels 75, 100, and 120 mM, respectively, compared to control. Also, the dry weight of shoots and roots was significantly reduced by salinity stress, where the percent reduction of shoots (SDW) was 7%, 30%, as well as 50% and 48% for root dry weight (RDW) at the levels of 100 and 120 mM, respectively.

Table 2. The fresh and dry weight of shoots and roots of tomato plants grown under different levels of NaCl salinity stress (0, 75, 100, and 120 mM); SFW = shoot fresh weight; RFW = root fresh weight; SDW = shoot dry weight; RDW = root dry weight. The significant difference is ** at $p \leq 0.01$ and * at $p \leq 0.05$.

NaCl Treatments	Biomass			
	SFW	RFW	SDW	RDW
0 mM	5.93 ^b ± 0.26	4.30 ^b ± 0.52	0.95 ^a ± 0.13	0.4 ^b ± 0.13
75 mM	5.32 ^{ab} ± 0.1	2.95 ^a ± 0.03	1.27 ^{ab} ± 0.19	0.49 ^b ± 0.07
100 mM	5.05 ^a ± 0.09	1.93 ^a ± 0.19	0.88 ^{ab} ± 0.03	0.20 ^a ± 0.02
120 mM	4.93 ^a ± 0.15	2.00 ^a ± 0.17	0.66 ^b ± 0.08	0.21 ^a ± 0.01
F value	7.48*	14.59**	4.07*	12.19**

As was expected, soil salinization reduced the content of chl a significantly, with percent reductions of 20%, 25%, and 45%, respectively, at 75, 100, and 120 mM compared to control (Figure 1). On the other hand, chlorophyll b (Chl b) content was not affected by the studied concentration of salinity in comparison with chlorophyll a (Chla) and total carotenoids (TC). In this regard, the degradation of carotenoids was maximized by increasing the level of salt, with percent reductions of 21%, 26%, and 44%, respectively, at levels of 75, 100, and 120 mM, compared to control.

Under salinity stress, the primary metabolites of tomatoes are harmfully affected. Statistical analysis revealed that both soluble carbohydrates (SC) and soluble proteins (SP) were significantly decreased at 100 mM and 120 mM levels. The present reduction was 26% and 44% for sugars as well as 37% and 46% for proteins at 100 and 120 mM, respectively, compared to the control. while a reversible situation was estimated for free amino acid (FAA), where highly significant accumulation was denoted at the levels of 74, 100, and 120 mM, recording an increasing percent of 61%, 66%, and 74% (Figure 2).

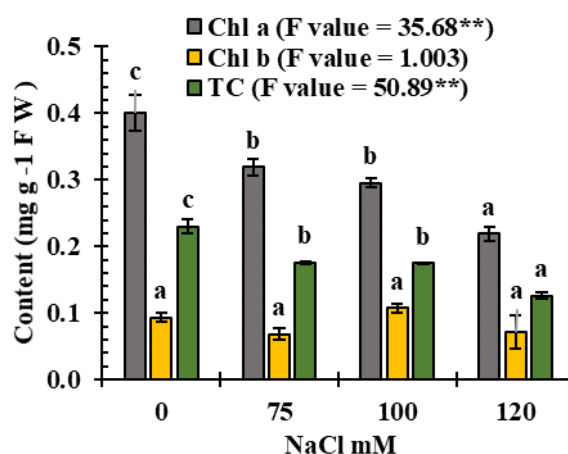


Figure 1. Content of chlorophyll a (Chl a), chlorophyll b (Chl b), total carotenoids (TC) (mg g⁻¹ FW). Values are means ± SE, n= 3. Mean values with different letters are significantly different at $P \leq 0.05$ according to Tukey's test. * and ** = Significant difference at $P \leq 0.05$ and $P \leq 0.01$ confidence level, respectively.

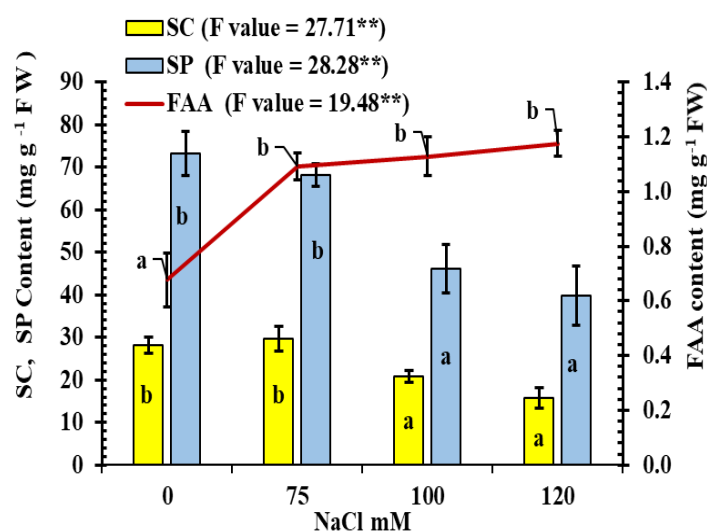


Figure 2. Content of soluble carbohydrates (SC), soluble proteins (SP), free amino acids (FAA) (mg g⁻¹ FW). Mean values with different letters are significantly different at $P \leq 0.05$ according to Tukey's test. * and ** = Significant difference at $P \leq 0.05$ and $P \leq 0.01$ confidence level, respectively.

The impacts of four different concentrations of salinity (0, 75, 100, and 120 mM) on the characteristics of tomatoes' leaves and their functional groups were investigated using FTIR analysis (Fig. 3) (Table 2). The FTIR spectra showed that no new peaks appeared at 75 and 100 mM compared to the control. While at 120 mM, peaks at 1320 cm⁻¹, and 1380 cm⁻¹ were recorded for O–H bending, referring to phenols. The strong peak at 3400 cm⁻¹ belongs to O–H and N–H stretching vibrations, referring to alcohol and aliphatic primary amines. The peaks at 2920 cm⁻¹ are recorded for C–H stretching, indicating alkanes. Also, the peaks of the C=C functional group were recorded at 1630 cm⁻¹ referring to alkene compounds. The peaks at 1430 cm⁻¹ have been identified as O–H bending, which pertains to the carboxylic group. The tertiary alcohols and amines, as C–O and C–N functional groups, respectively, were assigned at 1150 cm⁻¹. At 1080 cm⁻¹, the primary alcohol was recorded as the C–O stretching functional group. In addition, the S=O stretching functional group as sulfoxide was noticed at 1030 cm⁻¹.

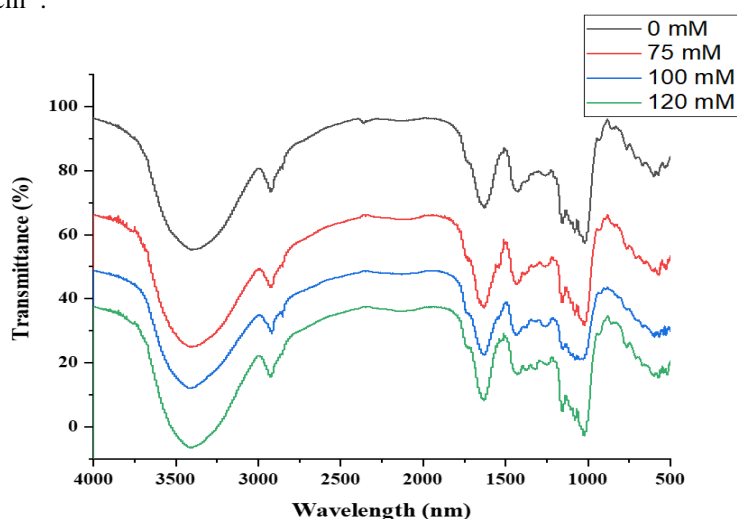


Figure 3. FTIR spectrum of tomatoes' leaves under four levels of salinity (NaCl): 0, 75, 100, and 120 mM.

The data represented in Figure 4 revealed that there is a significant difference in the relative gene expression of polyphenol oxidase (PPO), peroxidase (POD), and thaumatin-like proteins (TLps) in tomatoes under three different levels of salinity. The relative gene expression of PPO was significantly increased (3.61-fold) at the level of 100 mM compared to the control, without significant effect at the levels of 75 or 120 mM. It was denoted as a reduction in relative gene expression of POD at levels of 75 or 120 mM and an increase of 0.71-fold at 100 mM relative to control. Regarding the relative gene expression of TUB, a non-significant reduction was observed under salinity stress, especially at the lowest saline level applied. However, the relative TLps gene expression was highly significant at levels 75 and 100 mM, about 50.34-fold relative to 120 mM and the control.

Table 3. The functional groups of tomato leaves under four levels of salinity (NaCl 0, 75, 100, and 120 mM) were investigated using FTIR analysis.

Functional groups	Class	Salinity levels mM			
		0	75	100	120
O–H stretching, N–H stretching	Alcohol, Aliphatic primary amines	3400	3420	3420	3430
C–H stretching	Alkanes	2940	2930	2930	2940
C=C stretching	Alkenes	1640	1650	1630	1630
O–H bending	Carboxylic group	1420	1440	1430	1440
O–H bending	Phenol	-	-	-	1380
O–H bending	Phenol	-	-	-	1320
C–O stretching, C–N stretching	Tertiary alcohol, Amines	1150	1160	1160	1160
C–O stretching	Primary alcohol	1070	1080	1080	1070
S=O stretching	Sulfoxide	1030	1030	1020	1030

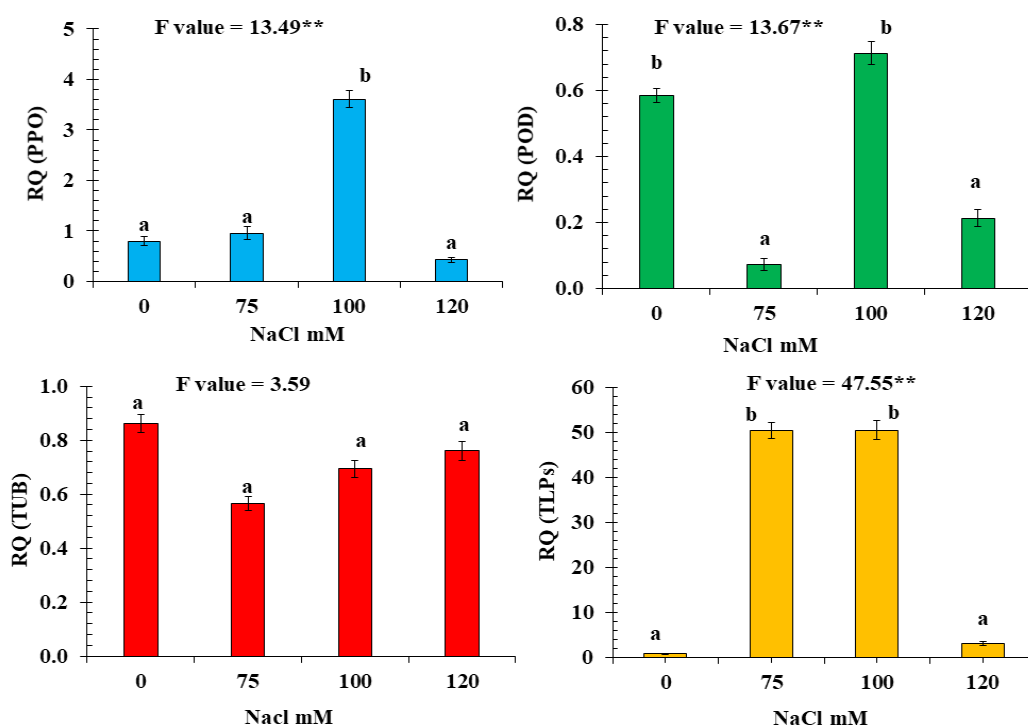


Figure 4. Relative quantitative expression analysis (RQ) of four genes: **A**: polyphenol oxidase (PPO), **B**: peroxidase (POD), **C**: tubulin (TUB), and **D**: thaumatin (TLPs) in tomato plants exposed to salinity at four levels: 0 mM, 75 mM, 100 mM, and 120 mM. Mean values with different letters are significantly different at $P \leq 0.05$, according to Tukey's test. * and ** = significant differences at $P \leq 0.05$ and $P \leq 0.01$ confidence levels, respectively.

Discussion

The saline environment is a persistent stress in arid and semi-arid areas such as Egypt, which is extremely stressful to all life features of sensitive crops such as tomatoes. In the present investigation, salt stress reduced the performance of the shoots and roots of tomato plants, and the reduction rate was intensified by increasing salinity from 100 to 120 mM, while little effect was recorded at 75 mM. In addition, the roots were highly affected by salt stress compared to shoots where the roots were in direct contact with a salt medium. This result is corroborated by observations made during an investigation into the effect of salt stress on avocado root growth, which indicated that root growth in avocados may be more hampered by salinity than shoot growth (Bernstein *et al.*, 2004). Therefore, stress-induced inhibition of root growth and root proliferation could significantly reduce the surface area of juvenile roots and, consequently, the root system's absorption capacity (Wolstenholme & Whiley, 1999). Similar intensification of the damaging impact of salinity on plant growth was reported by Yurtseven *et al.*, who demonstrated that the biomass yield was already diminished at a salinity level of 2.5 dS m^{-1} and that the reduction increased as the salinity rose from 2.5 to 10.0 dS m^{-1} . The average decrease

in biomass yield induced by a salinity increase from 2.5 to 5.0 dS m⁻¹ was approximately 37%; as the salinity increased to 10.0 dS m⁻¹, the yield decreased by approximately 60% (Yurtseven et al., 2005). This reduction in tomato biomass could be related to the stress that salt causes on the metabolic activities of cells, which diminishes water potential due to ionic and osmotic stress (Sofy et al., 2022). As most crop plants are sensitive to salinity, which affects plant growth, development, and productivity, improving salt tolerance is crucial for sustaining global agricultural productivity (Raza et al., 2022). Biomass was determined for the reason that this parameter and yield are crucial variables utilized for informed decision-making and production management in agriculture. In addition, biomass measurements offer information on a plant's ability to absorb sunlight, water, and minerals and convert them into plant material, as well as aid in the determination of crop fertilizer and irrigation needs (Johansen et al., 2019). Salt stress usually decreases photosynthesis, but these effects vary with dose and species (Zhang et al., 2018). Salinity stress differentially affected the pigment composition of tomato plants, where Chl b showed high resistance to the applied salt stress, recording no reduction in its content, while Chl a degraded highly significantly even at the lower level of salinity. It was shown that salinity can inhibit chlorophyll production (Khan, 2003), trigger chlorophyllase (Sultana et al., 1999), and cause protein-pigment combinations to be unstable (Jaleel et al., 2008). Also, osmotic stress induced by salinity reduced the water potential and decreased stomatal conductivity, which may limit the flow of CO₂ to the leaves, affecting the CO₂ : O₂ ratio in the chloroplasts and hence lowering photosynthesis (Sarker & Oba, 2019). Furthermore, carotenoids were also drastically affected by salt stress, but the damaging impacts showed a moderate response compared to that of Chl a. During salt stress, β-carotene degrades and zeaxanthin synthesis is diminished, resulting in decreased carotenoid levels (Sultana et al., 1999). Thus, tomato plants showed sensitivity to salinity stress, especially at the levels of 100 mM and 120 mM.

The production of suitable osmolytes is one way that plants react to salt stress. Through osmotic adjustment, cells are protected from the oxidative damage induced by reactive oxygen species (ROS) in reaction to high salinity (Abdallah, Abdelgawad, & El-Bassiouny, 2016). The salinity-tolerant ability of plants is controlled by many physiological processes; among them, photoassimilate is inhibited under salt stress, and the degree of reduction in photoassimilate is positively proportional to stress strength (Azzam et al., 2021). This finding is concurrent with the finding of the present study, where soluble sugars have been reduced in response to moderate and high saline treatments. In this respect, researchers found that salt stress reduces photosynthetic pigment and total carbohydrates, which is attributed to the low efficiency of photosystem II (Abdallah et al., 2016). Also, proteins were found to be deregulated by salinity stress at moderate and higher salinities. This reveals the osmotic stress encountered by plants experiencing 100 and 120 mM NaCl salinity. This drift has been reported previously by other researchers; for instance, compared to control seeds at 2, 4, and 6 days, it was found that NaCl-treated seeds had a 16.13%, 26.78%, and 29.49% drop in soluble protein concentration, respectively (L. Chen et al., 2020). In addition, at 12 ds m⁻¹ salt stress, soluble protein concentrations were significantly reduced by 38% due to salinity stress (Khan et al., 2022). In contrast, amino acids have accumulated at salinity levels (75, 100, and 120 mM NaCl salinity). In this regard, the accumulation of amino acids could be at the expense of proteins. As the biosynthesis of proteins switches to the accumulation of free amino acids, the faster rate of protein degradation is accompanied by a high amino acid production affinity. It has been reported that amino acids are involved directly or indirectly in controlling plant responses to environmental signals linked to abiotic salinity in terms of osmotic adjustment or scavenging of reactive species (Ragaey et al., 2022). The total content of free amino acids in plants treated with salinity was shown to increase by a statistically significant amount (Selem, 2019). Thus, the level of 75 mM showed little damage from salinity, with no osmotic stress symptoms paralleling the low reduction of tomato performance under stress. These alterations in the contents of the primary metabolites under salt treatments may be associated with the improvement or retardation of the synthesis, accumulation, or expenditure of these cellular metabolites to face the deteriorations induced by salinity stress.

In this study, FTIR analysis revealed that phenolic compounds were generated in tomatoes' leaves to cope with salt stress at 120 mM in comparison to 0, 75, and 100 mM. According to another study, it was shown that salinity stress had an effect on total phenolic chemicals. It was discovered that as plants were irrigated with salt water, their amount increased (Bistgani et al., 2019). It is hypothesized that normal saline tolerance pathway induction occurs in response to moderate salinity stress by elevating total phenolic compound levels (Salem, Msaada, Dhifi, Limam, & Marzouk, 2014). The total phenolic content in red pepper rose under moderate salinity stress, as reported by Navarro et al. In reality, genetics and the environment interact to determine phenolic levels (Navarro, Flores, Garrido, & Martinez, 2006). For protecting against biotic and abiotic stressors, phenolic chemicals are crucial. These metabolites reduce oxidative stress and remove reactive oxygen species (ROS) from various plant tissues, serving as antioxidants (Selmar & Kleinwächter, 2013).

Salinity stress causes secondary stresses on the plants, where activation of oxidative damage is encountered. To protect plant cells from oxidative damage caused by abiotic stresses, such as salt stress, many genes encoding antioxidant enzymes are regulated or deregulated based on the degree of salt stress. Therefore, analyses of the transcriptional levels of antioxidant defense genes could take into account their payoff in the salt stress response in plants. Plant peroxidases have been used as biochemical markers for various types of biotic and abiotic stresses due to their role in very important physiological processes, such as the control of growth by lignification, the cross-linking of pectins and structural proteins in the cell wall, and the catabolism of auxins. In this regard, the damaging impact of high salinity showed a reduction in the level of the relative gene expression of POD, revealing high oxidative damaging impacts at this level compared to control or the level of 100 mM, which kept a higher level of the relative gene expression of POD compared to control but was not significant. On the other hand, the salinity level at 75 mM showed a reduction of relative gene expression of POD compared to the control, revealing low oxidative damage encountered at this level, and maybe other antioxidant genes were stimulated. Regarding the relative gene expression of PPO, the data of the present work revealed that the level of 100 mM showed tolerance to salinity stress, and a high increase in the relative gene expression of PPO was connected to some improvement in POD gene expression. This reveals that the level of 100 mM NaCl adapted to salt stress via increasing PPO and POD relative gene expressions compared to the level of 120 mM, where the oxidative damage reached a threshold that could not be controlled by the cells. In this regard, Azzam *et al.* reported that changes in peroxidase and polyphenol oxidase have a positive correlation with high levels of abiotic stress tolerance (Azzam *et al.*, 2021). It was demonstrated that the expression of POD and PPO genes increased in faba bean cultivars with increasing NaCl concentrations but decreased at high salt concentrations (El-Flaah *et al.*, 2021).

Numerous TLPs have potent antifungal activity and have been engaged in defense mechanisms against a wide range of biotic stressors (de Jesús-Pires *et al.*, 2020). In the present study, we were challenged to study the relative expression of TLPs under salinity stress in tomato plants. Interestingly, the mild and moderate salinity levels folded the relative expression of TLPs compared to the control; however, the expression of the TLP gene was significantly decreased at severe salinity levels compared to 75 and 100 mM salinity levels. This reveals the importance of TLPs in conferring salt tolerance at mild and moderate salinity levels. Some recent studies showed that TLPs were also involved in plant responses to abiotic stress. For example, ectopic expression of a TLP gene in peanuts enhances the tolerance of tobacco seedlings to salt and oxidative stress (Liu *et al.*, 2023). It was demonstrated that turning off TLP6 in rose leaves renders the plants less tolerant to salt stress. Thus, the present studies recommend the importance of TLPs as a salinity tolerance-induced gene at mild and moderate levels (Su *et al.*, 2021).

Osmotic stress is caused by salinity stress, which reduces water availability and hence inhibits plant growth. Salt stress causes significant mechanical stress on plant cells during this phase by raising the pressure threshold for cell walls in growing cells in root and stem meristematic tissues (Neumann, 2011). This mechanical stress modifies cytoskeletal proteins such as tubulin, actin, and kinesins, which assist plants in adjusting to salt stress (Kosová *et al.*, 2013). In the present study, the relative expression of the tubulin gene was included to characterize its role in the salinity tolerance of tomato plants. It is worth mentioning that the tubulin relative expression gene is reduced under soil salinization, especially at a mild level, but such effects are non-significant. It was stated that the importance of tubulin and kinesin in regulating microtubule (MT) organization and ionic homeostasis increases the survival of rice plants under salt stress, thus providing novel genes for salt-insensitive rice breeding in areas with high soil salinity (Chen *et al.*, 2022). The failure of tomato plants to activate the relative expression of the tubulin gene revealed its sensitivity to the applied salinity levels and the damaging impacts of salt on the tomato cells.

Conclusion

To detect the tolerance or sensitivity of tomatoes to salinity, enzyme activity, such as biochemical and gene expression, as molecular markers of peroxidase and polyphenol oxidase, could be used. However, some enzyme activation is responsible for protecting plants against oxidative damage. In addition, the PPO, POD, and TLPs play a vital role in the adaptation of tomatoes against salinity stress at the 100 mM level. In contrast, the expression of the TUB gene in tomatoes does not appear to play a significant role under the studied level of salinity. Such investigations have revealed the important relationship between antioxidant activity and osmolytes, which may be commensurate with its ability to withstand salt stress or because of salt stress, which may cause some change in gene expression.

Recommendations

The research found that severe salinity harmfully affected tomato hybrid T-186. Therefore, it is recommended to transplant tomatoes hybrid T-186 in soils that have salinity not exceeding mild or moderate levels.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgments or Notes

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Authors Information

Mahmoud Abdelmoneim

Biotechnology program, Basic and Applied Science Institute,
Egypt-Japan University of Science and Technology (EJUST),
New Borg-Elarab, Alexandrina, Egypt
Botany and Microbiology Department, Faculty of Science,
Assiut University, Assiut, Egypt
Contact e-mail: Mahmoud.samir@ejust.edu.eg

Elsayed Hafez

Plant Protection and Bimolecular Diagnosis Department,
Arid Lands Cultivation Research Institute, City of Scientific
Research and Technological Applications,
New Borg-Elarab, Alexandrina, Egypt

Mona Dawood

Botany and Microbiology Department,
Faculty of Science,
Assiut University,
Assiut, Egypt

Sherif Hammad

Pharm D program,
Egypt-Japan University of Science and Technology (EJUST),
New Borg-Elarab, Alexandrina, Egypt
Pharmaceutical Chemistry Department, Faculty of
Pharmacy,
Helwan University, Ain Helwan, Cairo, Egypt

Mohamed Ghazy

Biotechnology program, Basic and Applied Science Institute,
Egypt-Japan University of Science and Technology (EJUST),
New Borg-Elarab, Alexandrina, Egypt
Biochemistry Department, Faculty of Science,
Ain Shams University, Cairo, Egypt

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