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CHANGES IN CAT2, NHX-1 GENE EXPRESSION IN TOMATO UNDER SALT STRESS CONDITION

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Abstract

In order to expand tomato cultivation into newly reclaimed land, selection for salt tolerant genotypes of tomato are the main target of many breeding program. Four tomato genotypes was subjected to graded levels of NaCl for two weeks, data on some physiological and molecular parameters were recorded. Salt stress reduced both of shoot and root lengths, fresh and dry weights compared with the control. Remarkable reduction in osmotic potential (Ψs) and an increase in osmotic adjustment among tomato genotypes under salt stress were evident. The increase in proline contents in Edkawi, GS 12 and Alisa was coincided with salt concentration, while, it was decreased in Sama genotype under similar condition. Quantitative (real-time) PCR technique was applied to measure the change in expression pattern of catalase (cat2) and NHX1genes in response to salinity. The expression manners of the both genes under different salt stress was significantly increased (up-regulation) with increasing salt concentration. Edkawi show the highest level of cat2 and NHX1gene expression under salinization compared to the other genotypes used. Based on the present data, Edkawi proved to be the most salt tolerant genotypes and can be used in future breeding programs to improve salt tolerance in tomatoes.

Keywords **:** Salt stress; physiological parameter; free proline content; gene expression; catalase; NHX1gene

Introduction

Tomato (*Solanum lycopersicum* L.) are one of the most significant agricultural vegetable crops in worldwide, that considered as a protective rich food since it contains large amount of vitamins A, B and C, minerals, beta-carotene, lycopene, flavonoids and antioxidants which can inhibit the development of various types of cancer, such as breast, prostate and colon cancers, in addition, tomato adds flavor to the foods and it has also rich in medicinal value , so tomato has achieved high popularity (Abdelgawad *et al.*, 2019; Nizam *et al*., 2019 and Sharma *et al*., 2019). Generality, different tomato cultivars are sensitive to moderate levels of salinity up to 2.5 dSm^{-1}.

Plant is face to a multitude of abiotic stresses, such as high salt stress that cause significant decreased in growth and yield of most agricultural products, as a consequence of ionic strength and osmotic stresses. (Alshareef *et al*., 2019). According to the FAO report in 2015, nearly 40% of agricultural land is affected by salinization worldwide. The effect of soil salinity problem expected to be increased in the future due to a reduction in the quality and quantity of irrigation water and universal climate change (Abdelgawad *et al*., 2019).

In Egypt, the salinization of soils and land waters are increased in recent years, which result from the Nile's weak demineralization of the soil and irrigation during the year without a corresponding drainage system, in addition, about five thousand millions m3 of salt drainage wastewater are yearly used for irrigating about 405.000 ha of land leading to the absorption and accumulation of salts in quantities toxic for plants (El Gamal, 2007 and Allam *et al*., 2018). Annually increasing of soil salinity is leads to by 2050, more than 50% of the land available for agriculture will be gone because of salinization (Hasanuzzaman *et al*., 2014 and Gharsallah *et al*., 2016).

Under salinity stress condition, plants accumulate compatible solutes such as sugars, glycine betaine, proline and several unusual amino acids as an adaptive mechanism to tolerate salinity stress, these components help the plants to regulate their osmotic pressures and to maintain its cellular homeostasis (Yang *et al.,* 2019). Proline plays a crucial role in plant response to salinity, such as, membrane stabilization, osmotic adjustment and detoxification of injurious ions in crops. Moreover, proline is a serious amino acid in determining membrane and protein structures and wash out reactive oxygen species under salinity conditions. Therefore, it not only acts as an osmo-tolerant, but also acts as a nutritional source (Chun *et al*., 2018).

The production of osmoprotectants like glycine-betaine (GB), proline and antioxidant enzymes (superoxide dismutase and catalase) has been shown a protecting role to keep away cells from immediate cellular damage (Fu *et al*., 2018). Transcript abundance of sodium/hydrogen exchanger 1 (NHX1) gene in response to salt stress can excite the activities of tonoplast Na^+/H^+ antiporter (Blumwald *et al*., 2000 and Shi *et al*., 2002). Moreover, increasing the expression of different forms of NHX gene under salinity treatment including At-NHX1, 2 and 5 in Arabidopsis (Yokoi *et al*., 2002). Catalase (CAT) is one of the most important antioxidant enzymes; its main function is changing H_2O_2 to water and oxygen in peroxysome. The expression of antioxidant genes resulted in improved salt tolerance of agricultural crops (Esfandiari *et al*., 2007; Zhou *et al*., 2007; Aflaki *et al*., 2012 and Hadwan, 2018).

The present investigation has been carried out to address the response of tomato cultivars to increasing salt stress during the early seedling stages, where tolerance to salinity at the seedling stage is positively correlated with tolerance to salinization in adult plants. Also, to investigate the expression pattern of catalase (cat2) and NHX1genes under different salt stress concentrations as component involved in plant tolerance mechanism against salinity.

Materials and Methods

Four Egyptian tomato genotypes were used in this investigation, namely Edkawi, Sama, GS 12 and Alisa genotypes obtained from the Agricultural Research Center (ARC).

Salt stress treatments

Green house experiment was conducted to determine the effects of different salinity concentration (0 mM as control, 100, 150, 200 mM NaCl) on some of the physiological parameter and expression of salt stress responsive genes (cat2 and NHX1) in tomato genotypes. Ten seeds were germinated in plastic (28 cm diameter) each containing a mixture of sand, soil and peatmoss (1:1:1/ w:w).

Seedlings were irrigated daily with 500 ml of one tenth of Murashige and Skoog basal medium (Murashige and Skoog 1962) to the bottom of the outer pot. One-month-old plants were subjected to salt stress by the addition of different concentration from NaCl to the daily supply of nutrients for 15 days. The temperature was kept at 25°C and relative humidity at 70 % with normal light intensity under a 16/8 hours light/dark photoperiod. There were five replications per each NaCl treatment and the control.

Plants were harvested fifteen days after initiation of the salt treatment. Fresh weight (FW), shoot and root length was measured. For dry weight (DW) the same plants of fresh weight were dried at 80°C in an air-forced draught oven (Heraeus-0871, USA) for three days, and then weighted.

Determination of osmotic potential and osmotic adjustment

Osmotic potential (ψs) and osmotic adjustment (OA) were determined as described by Jones and Turner (1978). Leaf tissues were collected and stored in liquid nitrogen at -20°C. Samples were thawed then, centrifuged at 1,500 ×g for 20 minutes at 4°C to extract the cell sap. Osmotic potential of the cell sap was determined using a vapor pressure osmometer (model 5,500, Wescor, Logan, UT, USA). To measure the (OA) the differences in (ψs) between salt treated and control plants were calculated.

Free proline Assay

Proline content was measured according to the protocol which described by Bates *et al.* (1973). Frozen leaves was grind in 3% of sulfosalicylic acid. Then, the mixture was centrifuged at 6000 g for 20 min. The supernatant was transferred in new test tube and then 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent were added. Then, the solution was boiled at 99°C for 1hr. The reaction mixture was cooled, then four ml of toluene was added and vortex. And the absorbance was measured at 520 nm in spectrometer compared to reference blank of pure toluene. Proline conc. was estimated from a calibration curve.

RNA extraction and quantitative PCR analysis

Total ribonucleic acid (RNA) was isolated from 100 mg of frozen leaf tissue using the RNeasy Plant Mini Kit (Qiagen, Germany, CAT NOs.74903 and 74904) according to the guidelines instructions. Then, the total RNA was reversed to cDNA using Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, USA, Cat NO. K1621).The quantitative PCR was used to estimate messenger RNA expression levels of both genes (cat2 and NHX1) in the shoot tissue using a Maxima SYBR Green/ROX qPCR Master Mix kit (Thermo Scientific, USA, Cat NO. K0221). Primer nucleotides were designed using primer 3 software (Table 1). The β- tubulin gene was used as a normalize gene. The program profile of PCR was started with initial denaturation at 95°C for 10 min., then 40 cycles of denaturation at 95°C for 15 s., followed by annealing at 60°C for 60 s and followed by an extension at 72°C for 60 s., and a final extension at 72°C for 4 min. The reaction was carried out with three replicates for all genes. The analyses were performed using the MX3000PqPCR Machine from Stratagene.

Table 1 : Gene-specific primers used in qPCR.

Gene	Forward primer (5` to 3`)	Reverse primer (5` to 3`)	Product size (bp)
Cat2	GCACAGGGATGAGGAGATCG	TCTGTCGGGTGTGAATGAGC	175
NHX1	GGCTAGTTGCAATCATGGGG	AAGAGCGGTGATGGAATCGT	179
β-tubulin	GGATCTGGCATGGGAACACT	TCATCGGCATTCTCCACCAA	164

Data analysis

The comparative Ct (ΔΔCt) was measured by subtracting ΔCt of calibrator from ΔCt of treated samples. Relative expression fold changes were also calculated by using the formula 2-ΔΔCT which was proposed by Livak and Schmittgen (2001).To determine the significance (P-value was <0.05) between the mean differences of the genotypes, the independent unpaired student's t-test was used.

Results

Effect of salt stress on some physiological parameter

In this study, various levels of NaCl concentrations (0, 100, 150 and 200 mM NaCl) were used to assess the effect of salt stress on growth and development parameters of tomato genotypes. The plant fresh weight (FW), dry weight (DW), shoot and root length were measured (Table 2). Exposing tomato genotypes to100 mM NaCl reduced the plant fresh and dry weight compared to the control. In general, the results indicated no significant differences in FW and DW among the different genotypes under normal growth conditions. While, there were significant differences between tomato genotypes with increasing salt concentration to 200 mM. Moreover, at 200 mM NaCl, Edkawi showed the highest plant dry weight compared to the other genotypes. Whereas, the dry weight of the Sama was inhibited by increasing NaCl concentration. Although, the bad effects of salt on roots, the length of roots in tomato appears to be less affected (Table 2). Visual symptoms of salt injury after 15 days appeared on tomato plants except for Edkawi. As shown in Figure (1), the symptoms included: reduction in plant size than normal, the plant stopped growing, became completely wilted at concentration 200 mM NaCl.

Table 2 : The effect of salinity stress on the physiological parameters of tomato genotypes exposed to different NaCl concentration for 15 day.

		Parameter			
Genotypes	**NaCl treatment (mM)**	**Shoot length (cm)**	**Root length (cm)**	**F.W (g)**	**D.W (g)**
Edkawi	0	35.33 ± 1.04	14.33±0.76	6.84±0.83	0.47±0.13
	100	29.33± 1.15	13.50±0.76	5.59±0.62	0.43±0.15
	150	25.17± 0.58	10.33± 0.58	4.06± 0.72	0.42±0.08
	200	23.33± 0.76	9.33±0.29	3.94± 0.06	0.35± 0.01
Sama	0	25.17±0.76	10.33±0.58	11.90±0.73	0.80± 0.06
	100	19.00± 1.0	8.00± 0.50	6.53± 0.91	0.58±0.08
	150	14.83±1.04	6.17±0.29	4.19±0.79	0.37± 0.06
	200	11.00± 0.50	4.16± 0.29	2.56±0.29	0.31± 0.02
GS 12	0	27.67±0.29	7.00±0.58	5.19±0.59	0.34±0.04
	100	20.33± 1.04	5.83±0.29	3.15± 0.19	0.25±0.04
	150	18.00± 1.00	5.50±0.50	2.71± 0.36	0.24±0.04
	200	15.50±0.50	3.67±0.76	2.49± 0.12	0.19± 0.01
Alisa	0	30.67±0.58	10.00±0.58	12.68±0.79	1.04±0.30
	100	22.67±0.58	7.67± 0.58	6.76± 0.70	0.72±0.09
	150	17.83±0.58	6.67±0.29	5.64±0.13	0.62±0.03
	200	15.33± 0.58	4.50±0.50	5.39± 0.80	0.56± 0.02

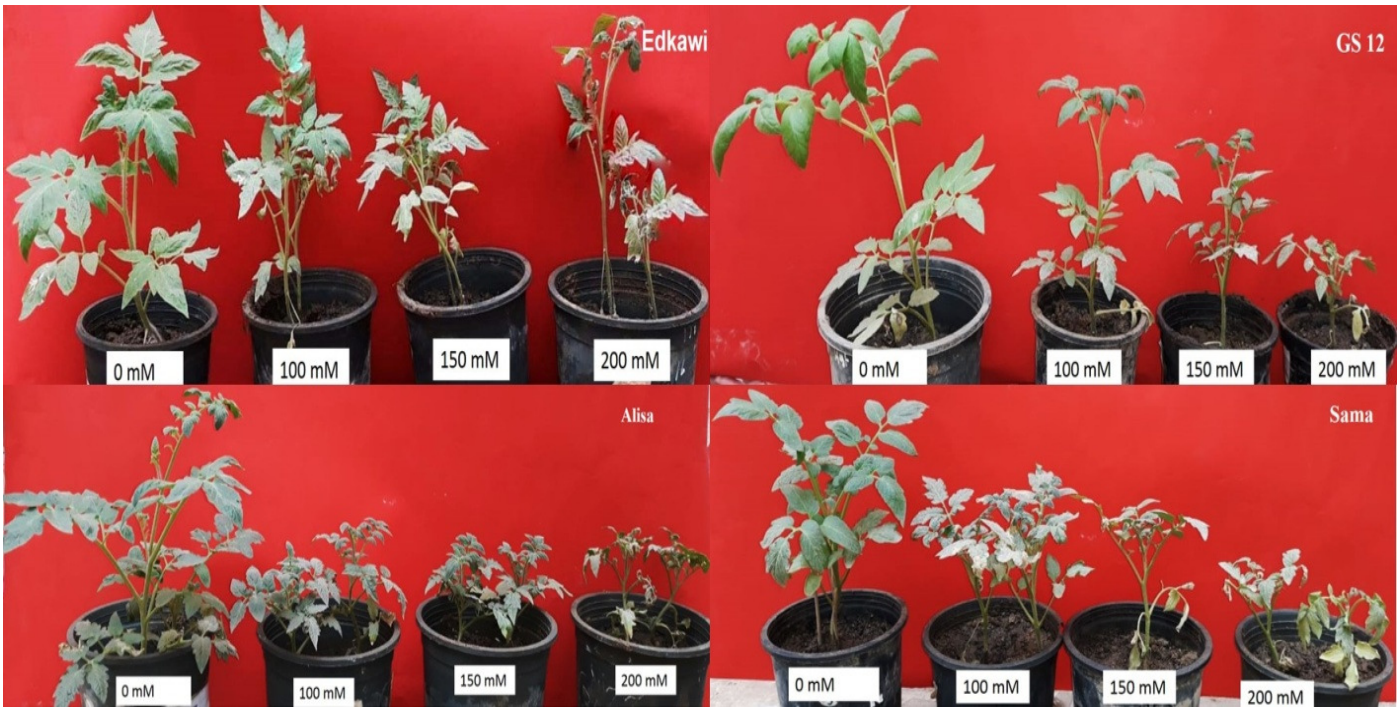

Fig. 1 : Effects of salt stress on the growth of tomato plants.

Osmotic potential and osmotic adjustment

Osmotic potential and osmotic adjustment were estimated from tomato plants grown under different salt treatments (Table 3). The results of osmotic potential (Ψs) showed that the values were decreased with increasing saline stress conc. in the irrigation water, the decrease was more pronounced in Sama with respect to control plants. Plants that treated with 200 mM NaCl concentration suffered a greater salt stress than those which treated with 100 and 150 mM NaCl. For osmotic adjustment, total OA increased with increasing severity of salt stress. Edkawi exhibited a higher OA value (3.60) at 200 mM NaCl concentration compared to control followed by GS 12 (2.57) and Alisa (2.08).Whereas, Sama showed lowest OA value (1.63).The results of osmotic adjustment indicated a higher capacity of the Edkawi and GS 12 for osmotic adjustment as a potential trait for improved salinity tolerance.

Table 3 : The effect of salinity on water potential (Ψs) and osmotic adjustment (OA) of leaves from tomato genotypes.

	NaCl Treatment (mM)	Parameter	
Genotypes		**Ψs (MPa)**	**O A**
Edkawi	0	-3.99	
	100	-5.90	1.91
	150	-5.92	1.93
	200	-7.58	3.60
Sama	0	-3.52	
	100	-4.83	1.31
	150	-5.23	1.71
	200	-5.15	1.63
GS 12	0	-3.67	
	100	-4.73	1.07
	150	-5.5	1.83
	200	-6.24	2.57
Alisa	0	-3.57	
	100	-5.24	1.67
	150	-5.48	1.91
	200	-5.65	2.08

Free Proline Content

The content of proline was assayed in four tomato genotypes by reading the absorption of chromophore at 520 nm using spectrophotometer (Fig. 2). Results from these assays revealed different levels of proline content among different genotypes. The concentration of proline was increased with increase in NaCl concentration in Edkawi, GS 12, Alisa genotypes and the opposite results were found in Sama .

The highest content of proline was 190 µmol gm^{-1} which has been recorded in Edkawi following by175 µmol gm^{-1} in GS 12 and 157 µmol gm^{-1} in Alisa while the lowest values was recorded in Sama (50 µmol gm^{-1}) at the 200 mM NaCl.

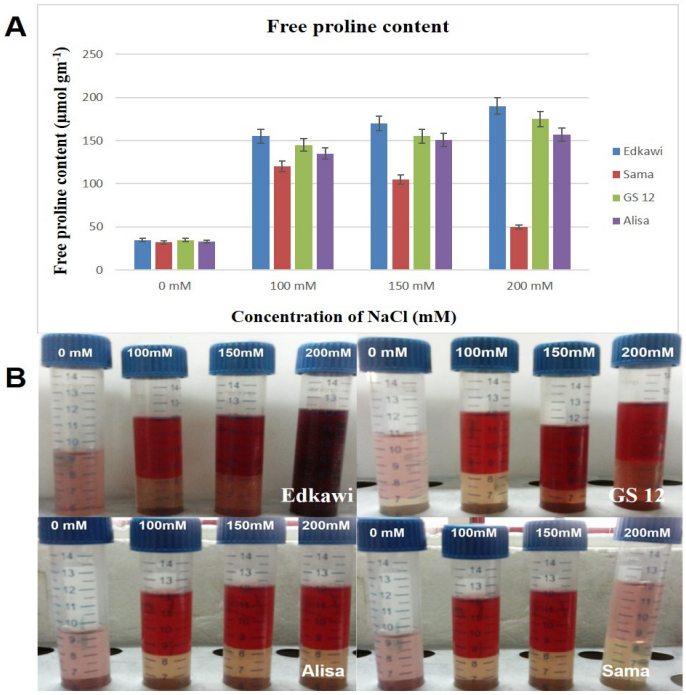

Fig. 2. (A) Histogram representing the means of proline content for four tomato genotypes. **(B)** Effect of different concentrations of NaCl on proline content.

Expression level of NHX1 gene under salinity conditions

To investigate the impact of salinity stress on the expression manner ofNHX1 gene, total ribonucleic acid was isolated under different salt concentrations. The quantitative NHX1 gene expression patterns were estimated by real-time PCR (Fig. 3). The results indicated that significant gain in NHX1 transcript levels in tomatoes with increasing salt concentrations. The NHX1 expression levels of Edkawi was increased by 4.59, 12.62 and 38.91 fold higher than control under 100, 150 and 200 mM of NaCl respectively while, the expression level in GS 12 was 1.73,6.52 and 15.7 fold increase than control plants, respectively. Otherwise, the expression was dramatically lower for Sama (salt sensitive) which reaching 1.07, 3.39 and 7.29 folds under 100, 150 and 200 mM NaCl, respectively. Moreover, comparison of the NHX1 gene expression level indifferent tomato genotypes according to t-test analysis, showed significant differences ($P<0.05$) comparing Edkawi with GS 12and Sama genotypes (150 and 200 mM NaCl). Furthermore, no significant differences ($P>0.05$) in the NHX1 transcription levels in GS 12 and Sama genotypes at 100, 150 and 200 mM NaCl.

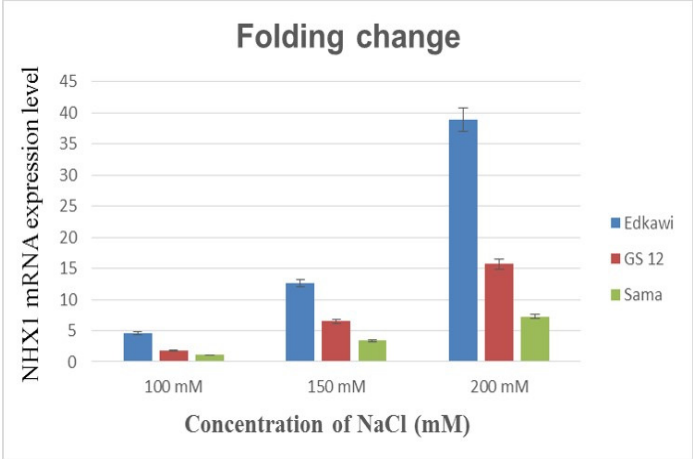

Fig. 3 : The effect of salt stress on the expression level of NHX1gene. The qRT-PCR analysis results to measure the relative mRNA expression level of NHX1 gene of the tomato genotypes. Bars represent mean values ± standard error. $P<0.05$ is reflect significant.

Expression level of catalase gene under salinity stress

The results of quantitative (real-time) PCR of the cat2 expression manner gene in different tomato genotypes which exposed to different treatments of salt showed a significant increased in all genotypes (Fig. 4). The highest level of cat2 gene expression was found Edkawi compared to the other genotypes. The cat2 gene expression in Edkawi increased by 15.27, 47.01 and 71.56-fold higher than control at 100,150 and 200 mMNaCl respectively. Nevertheless, the growing in catalase transcription levels under salt stress were not similar in GS 12 and Sama genotypes. The increasing in expression of this gene of the GS 12 at 150 and 200 mM NaCl were 2.52-and 5.22-fold than control, respectively, while, Sama showed 2.29 and 3.80-fold relative to the control under 150 and 200 mM NaCl. The expression manner of cat2 (catalase) gene at 150 mM salt treatment was not significantly different ($P>.05$) between all genotypes. Both of the genotypes GS 12 and Sama exhibited no significant difference ($P>0.05$) under various salt treatments. While, a significant difference ($P<0.05$) noticed between the Edkawi genotype (salt tolerant) and other genotypes at 200 mM NaCl concentration.

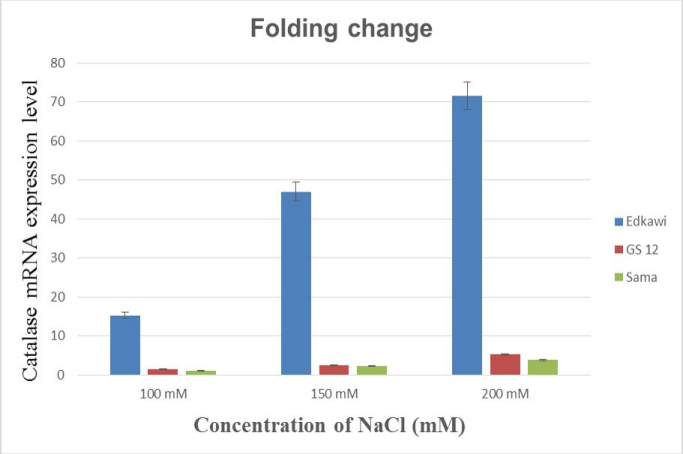

Fig. 4 : The effect of salt stress on the expression level of catalase gene. The qRT-PCR analysis results to measure the relative mRNA expression level of catalase gene of the tomato genotypes. Bars represent mean values ± standard error. P<0.05 is reflecting significant.

Discussion

It was previously reported that salinity is the major abiotic stress factors that cause significant reduction in yield, plant growth and development of most agricultural products, due to ionic and osmotic stresses (Parida and Das, 2005; Moghaieb *et al.,* 2011; Zhang *et al.,* 2016 and Alshareef *et al.,* 2019).

In the present study, one-month-old plants were exposed to salinity by the addition of different concentration from NaCl to the supply of nutrients every day for 15 days. According to the phenotypic and physiological parameter, plants grown under different level of salt stress showed shoot and root growth that was lower than that appeared in the control plants. Edkawi was more tolerant than other genotypes at 200mM NaCl. While, both of GS 12 and Alisa showed a similar response to salt stress. In general increasing NaCl up to 200 mM significantly decrease both of plant FW and DW in all genotypes compared to control. This finding is in harmony with Abdelgawad *et al*. (2019). Nevertheless, salinity resulted in inhibition of osmotic potential (Ψs) and encourages OA because the maintenance of osmotic adjustment resulting in increased compatible solute concentration of cells in with a view to maintain the water potential (Ψw) gradients needed to ensure continued absorption of water during the stage of stress, and permits cell to maintain the turgor, which is necessary for plant growth and various other physiological processes. These results were similar with other researchers (Singh *et al*., 2012; Rivero *et al.,* 2014; Filová and Krivosudská, 2017 and Nahar and Ullah, 2017) in tomato. Moreover, Aranda *et al.* (2001) detected that, salinity inhibited cell expansion in tomato plants, which it was related to a reduced osmotic and water potential and an increase in the turgor potential. Furthermore, the amount of proline was increased with increase in NaCl concentration in Edkawi, GS 12, Alisa. While, Sama showed declined in proline content. Chun *et al*. (2018) described that the alternative response of plant to salinity are fundamentally proline accumulation which controls stress struggle mechanisms due to sustainable production in the field of agricultural production even with high salinity. Where, proline content plays a preponderant role in preserving plants from osmotic stress, because it is an important nitrogen source that is available for plant repair from abiotic stress, restoration of growth and it can act as an osmolyte that reduces the osmotic potential (Ψs) of the cell and the uptake of toxic ions. So, the increased level of proline in plants under salinity condition due to the activation of proline biosynthesis which enhances protein rotation (Zhang *et al.,* 2016).

There are plenty of salt responsive genes play important role in salt tolerance. The expression of these genes was increased with increasing salinity (Horie *et al*., 2004; Avsian-Kretchmer *et al*., 2004 and Zhou *et al*., 2007).

The results indicated that there are significantly up-regulation of the NHX1 and catalase genes at different levels of salt stress. These results agree with (Blumwald *et al*., 2000; Shi and Zhu., 2002 and Fukuda *et al*., 2004) they illustrated that transcript pattern of NHX1 gene in response to salinization can excite the activities of tonoplast Na^+/H^+ antiporter, and increased the expression of different forms of NHX gene under NaCl treatment which including At-NHX1, 2 and 5 in different plants. Furthermore, Ghorbani *et al.* (2018), used RT-PCR to analysis the expression levels of different genes involved in Na^+ compartmentation and ROS detoxification (BFRUCT3, NHX1, OMT and PEAMT genes) in the root of barley. They concluded that salt stress increased the expression of the different genes. This shows long-term response of these genes to salinity stress. Dezfuli *et al*. (2017) examined the role of transcription factors such as bHLH, Zpt2-1 and CBF4 in salt tolerance by investigating their expression patterns under salt stress in the leaf and root tissues of the salt tolerant genotype (Yazdi) and the sensitive genotype (Diablo Verde) of Alfalfa. They results revealed that salt treatments for short time leads to induction of expression patterns of the bHLH, Zpt2-1and CBF4 genes in both genotypes. In the tomato tissue which salt treated, the oxidative stress enzymes (catalase and peroxidase) were induced and influenced by salt treated. Keeping cellular redox homeostasis is essential for normal cellular processes (Zhou *et al*., 2007). They results are in accordance with that observed in this study.

Conclusion

Tomato genotype differed genetically in their response to different levels of salt stress and the effects on growth parameters were easily observed. The plant growth was reduced by salinity in all genotypes in relation to the salt concentration. The stressed plants revealed a gain in the expression patterns of salt stress responsive genes (cat2 and NHX1) compared to control when analyzed by qRT-PCR. The transcript levels of NHX1were stimulated by NaCl treatment. Salt stress responsive genes, especially catalase gene were significantly higher (P<0.05) and showed the quickest response to salt treatments.

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References

Abdelgawad, K.F.; El-Mogy, M.M.; Mohamed, M.A.; Garchery, C. and Stevens, R.G. (2019). Increasing ascorbic acid content and salinity tolerance of cherry

tomato plants by suppressed expression of the ascorbate oxidase gene. Agronomy, 9: 1-14.

Aflaki, F.M.; Sedghi, M. and Pessarakli, M. (2012). Effects of phytohormones on proline content and antioxidant enzymes of various wheat cultivars under salinity stress. J Plant Nutrition. 35: 1098–1111.

Allam, N.G.; Kinany, R.; El-Refai, E. and Ali, W.Y. (2018). Potential Use of Beneficial Salt Tolerant Bacteria for Improving Wheat Productivity Grown in Salinized Soil. J Microbiol Research, 8: 43-53.

Alshareef, N.O.; Wang, J.Y.; Ali, S.; Al-Babili, S.; Tester, M. and Schmöckela, S.M. (2019). Over expression of the NAC transcription factor Jungbrunnen1 (JUB1) increases salinity tolerance in tomato. Plant Physiol and Biochemistry, 140: 113–121.

Aranda, R.R.; Soria, T. and Cuartero, J. (2001). Tomato plant-water uptake and plant-water relationships under saline growth conditions. Plant Science, 160: 265 272.

Avsian-Kretchmer, O.; Gueta-Dahan, Y.; Lev-Yadun, S.; Gollop, R. and Ben-Hayyim, G. (2004). The salt-stress signal transduction pathway that activates the gpx1 promoter is mediated by intracellular H_2O_2, different from the pathway induced by extracellular H_2O_2. Plant Physiol, 135: 1685-1696.

Bates, L.S.; Waldron, R.P. and Tears, J.D. (1973). Rapid determination of free proline for water stress studies. Plant and Soil, 39: 205-207.

Blumwald, E.; Aharon, G.S. and Apse, M.P. (2000). Sodium transport in plant cells. Biochimica et Biophysica Acta. 1465: 140–151.

Chun, S.C.; Paramasivan, M. and Chandrasekaran, M. (2018). Proline accumulation influenced by osmotic stress in arbuscular mycorrhizal symbiotic plants. Frontiers in Microbiol, 9: 1-13.

Dezfuli, A.A.S.; Dehcheshmeh, S.M.; Rafiei, F. and Shiran, B. (2017). Gene expression patterns of some transcription factors (Zpt2-1, CBF4, bHLH) under salt stress in Alfalfa by using qPCR. J Appl Biotechnol Reports, 4: 541-546.

El-Gamal, F. (2007). Use of non-conventional water resources in irrigated agriculture. Options Méditerranéennes, 56: 33-43.

Esfandiari, E.; Shekari, F. and Esfandiari, M. (2007). The effect of salt stress on antioxidant enzymes' activity and lipid peroxidation on the wheat seedling. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 35: 48–56.

Filová, A. and Krivosudská, E. (2017). Significance of the osmotic adjustment of soybean (glycine max l.) selected genotypes during increasing drought conditions. R J AS. 49: 188-195.

Fu, Y.; Ma, H.; Chen, S.; Gu, T. and Gong, J. (2018). Control of proline accumulation under drought via a novel pathway comprising the histone methylase CAU1 and the transcription factor ANAC055. J Exper Botany, 69: 579–588.

Fukuda, A.; Chiba, K.; Maeda, M.; Nakamura, A.; Maeshima, M. and Tanaka, Y. (2004). Effect of salt and osmotic stresses on the expression of genes for the vacuolar H+-pyrophosphatase, H+ ATPase subunit A, and Na+/H+ antiporter from barley. J Exper Botany, 55: 585–594.

Gharsallah, Ch.; Abdelkrim, A.B.; Fakhfakh, H.; Salhi-Hannachi, A. and Gorsane, F. (2016). SSR marker-assisted screening of commercial tomato genotypes under salt stress. Breeding Science, 66: 823–830.

Ghorbani, R.; Mohammadi, S.A.; Toorchi, M. and Ghafarian, S. (2018). Transcriptional changes of BFRUCT3, NHX1, OMT and PEAMT genes in root of barley (*Hordeum vulgare* L.) under salinity stress. Pakistan J Botany, 50: 735-740.

Hadwan, M.H. (2018). Simple spectrophotometric assay for measuring catalase activity in biological tissues. BMC Biochemistry, 19: 1-8.

Hasanuzzaman, M.; Nahar, K.; Alam, M.D.M.; Bhowmik, P.C.; Hossain, M.D.A.; Rahman, M.M.; Prasad, M.N.V.; Ozturk, M. and Fujita, M. (2014). Potential use of halophytes to remediate saline soils. Bio Med Research Inter, 1-12.

Horie, T. and Schroeder, J.I. (2004). Sodium transporters in plants diverse genes and physiological functions. Plant Physiol. 136: 2457–2462.

Jones, M.M. and Turner, N.C. (1978). Osmotic adjustment in leaves of sorghum in response to water deficits. J Plant Physiol., 61: 122-126.

Livak, K.J. and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods, 25: 402-408.

Moghaieb, R.E.A.; Abdelhadi, A.A. and Talaat, N.B. (2011). Molecular markers associated with salt tolerance in Egyptian wheat's. African J Biotechnol, 10: 18092-18103.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiology, 15: 473-497.

Nahar, K. and Ullah, S.M. (2017). Fruit Quality and Osmotic Adjustment of Four Tomato Cultivars under Drought Stress. Asian J Soil Science and Plant Nutrition, 2: 1-8.

Nizam, R.; Hosain, M.D.T.; Hossain, M.D.E.; Islam, M.D.M. and Haque, M.D.A. (2019). Salt stress mitigation by calcium nitrate in tomato plant. Asian J Medical and Biological Research, 5: 87-93.

Parida, A.K. and Das, A.B. (2005). Salt Tolerance and Salinity Effects on Plants: A Review. Ecotoxicology and Environmental Safety, 60: 324-349.

Rivero, R.M.; Mestre, T.C.; Mittler, R.; Rubio, F.; Sanchez, F.G. and Martinez, V. (2014). The combined effect of salinity and heat reveals a specific physiological, biochemical and molecular response in tomato plants. Plant, Cell and Environment, 37: 1059–1073.

Sharma, P.; Thakur, S. and Negi, R. (2019). Recent Advances in Breeding of Tomato- A Review. Inter J Current Microbiol and Appl. Sciences, 8: 1275-1283.

Shi, H. and Zhu, J.K. (2002). Regulation of expression of the vacuolar Na^+/H^+ antiporter gene AtNHX1 by salt stress and ABA. Plant Molecular Biology, 50: 543–550.

Singh, J.; Sastry, E.V.D. and Singh, V. (2012). Effect of salinity on tomato (*Lycopersicon esculentum* Mill.) during seed germination stage. Physiol and Molecular Biology of Plants. 18: 45–50.

Yang, H.; Shukla, M.K.; Mao, X.; Kang, S. and Du, T. (2019). Interactive Regimes of Reduced Irrigation and Salt Stress Depressed Tomato Water Use Efficiency at Leaf and Plant Scales by Affecting Leaf Physiology and Stem Sap Flow. Frontiers in Plant Science.10: 1-17.

Yokoi, S.; Quintero, F.J.; Cubero, B.; Ruiz, M.T.; Bressan, R.A.; Hasegawa, P.M. and Pardo, J.M. (2002). Differential expression and function of *Arabidopsis thaliana* NHX Na^+/H^+ antiporters in the salt stress response. Plant J., 30: 529–539.

Zhang, P.; Senge, M. and Dai, Y. (2016). Effects of salinity stress on growth, yield, fruit quality and water use efficiency of tomato under hydroponics system. Reviews in Agricultural Science, 4: 46- 55.

Zhou, S.; Wei, S.; Boone, B. and Levy, S. (2007). Microarray analysis of genes affected by salt stress in tomato. African J Environmental Science and Technol. 1: 014-026.