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EVALUATION OF CHANGE IN GENE EXPRESSION AND ANTIOXIDANT ENZYMES ACTIVITY IN SOME EGYPTIAN WHEAT CULTIVARS UNDER SALINITY STRESS

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Abstract

Six Egyptian wheat cultivars were evaluated for their salt tolerance. The results revealed that different salt treatments had significant effects on all growth parameters (Shoots length, Roots length, Fresh weight and Dry weight). The cultivars Gemmiza-9, Giza-11 and Misr-1 revealed high tolerance against salinity stress for all parameters, as compared to the Giza-171, Sids-12 and Giza-168 cultivars. Furthermore, the Gemmiza-9 cultivar revealed the highest significantly activity of SOD and peroxidase where its SOD activity was 0.225 u/g which represent 1.97 multiplier for control (197%) and 0.321 u/g which represent 2.82 multiplier for control (281.58%), respectively and its peroxidase activity was 0.383 µmol mg^{-1} min^{-1} represent 1.42 multiplier for control (142%) and 0.43 µmol mg^{-1} min^{-1} represent 1.6 multiplier for control (159.85%), respectively. Followed by Giza-11 and Misr-1 showed relatively high SOD and peroxidase activity. While, Giza-171, Sids-12 and Giza-168 showed significant decrease in SOD and peroxidase activity with raising salinization level. Moreover, the gene expression of Gemmiza-9 (high salt-tolerant cultivar) showed significantly higher for Δ1-pyrrolin-5-carboxylate synthetase (*P5CS*), sodium hydrogen antiporter (*TNHX1*) and Salt Overly Sensitive (*TaSOS1*) with raising salinization level compared to Giza-171 (high salt-sensitive cultivar) which showed significant decreased expressions of three genes with raising salinization level. These results confirmed that up-regulation of genes for proline accumulation and Na^+ exclusion are related to salinity tolerance in wheat. This information will be beneficial for improvement of wheat cultivars for salinity tolerance.

Key words: Wheat, Salt stress, Antioxidant enzyme, *P5CS*, *TNHX1* and *TaSOS1* Gene expression.

Introduction

Triticum aestivum L. (bread wheat) is fundamental grain crop, which is staple food for many people in the worldwide including Egypt and known as cereals king (Amirbakhtiar *et al.,* 2019; Yassin *et al.,* 2019a). It is the main source of plant carbohydrates and proteins that contain 13% protein as a high protein content compared to other cereal crops. Wheat cereals are the main source of dietary fibers, micronutrients, vitamins, minerals and fats (Giraldo *et al.,* 2019). Therefore, wheat is a strategic crop and plays a major role in the world's food, nutrition, economy and production, moreover it is a major species widely cultivated throughout the world and feeds over a third of the world's population (Barutcular *et al.,* 2017; Yildirim *et al.,* 2018).

Wheat is considered moderately salt tolerant (Saddiq *et al.,* 2019). However, the production of wheat is affected by numerous abiotic and biotic stresses, which lead to decrease in growth and yield of plants. The senior of these stresses is salinity that negatively impacts the quality and production of wheat, which perhaps due to ionic toxicity and osmotic stress (Abdelaal *et al.,* 2018; Out *et al.,* 2018; Jahan *et al.,* 2019; Yassin *et al.,* 2019a; b).

Abiotic stress leads to yield loss, as saline stress is a very harmful stress that reduces crop growth and production up to 60% worldwide (Xie *et al.,* 2016; Barnawal *et al.,* 2017), whereas, the area of salt-affected land overrides 800 million ha of agricultural land that representing 6% of the total land area of the world (Saddiq *et al.,* 2019). Salinity will lead to loss of 50% of cultivated lands in the middle of the twenty-first century (Amirbakhtiar *et al.,* 2019). The proportion of cultivated Egyptian lands is 33%, of which 3% is influenced by salinity (Yassin *et al.,* 2019b).

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There are balance between antioxidant systems and the reactive oxygen species (ROS) production such as the hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), singlet oxygen ($^1O_2$) and superoxide (O_2^-) in plant, but the different types of stresses such as salinity can disable the antioxidant processes and significantly increases oxidative stress (Dudziak *et al.,* 2019a; Wada *et al.,* 2019). Also, it reduces the plant's ability to absorb water and causes an ionic imbalance that leads to increased accumulation of Cl^- and Na^+ ions that reduce the absorption of nutrients such as K^+, Mn, Mg^{2+} and Ca^{2+} in sensitive plants and hence both Cl^- and Na^+ negative impact on plant growth by destroying metabolic systems and reducing photosynthesis efficiency (Amir Bakhtiar *et al.,* 2019; Yassin *et al.,* 2019b). The over-expression of ROS lead to increase in destruction of DNA, lipid peroxidation (LPO) and oxidation of protein, which can lead to apoptosis due to destruction of membrane and inactivated enzymes (Wada *et al.,* 2019).

Plants have several protective non-enzymatic and enzymatic mechanisms to tolerate stress, maintain normal metabolism and cell components (Khalil *et al.,* 2016; Kolupaev *et al.,* 2016) like osmotic adjustment, exclusion of sodium ion (Na^+), excess accumulation of some compatible solutes that act as an osmoprotectant like proline and glycine betaine, which reduces osmotic capacity of cytoplasm, accelerates absorption of water and escapes ROS (Abd Elgawad *et al.,* 2016; Gharsallah *et al.,* 2016). There are strong correlation between tolerance to stresses especially salinity and drought and over-accumulation of proline and over expression of P5CS. Proline is principally synthesized under salinity stress by P5CS (Δ1-pyrroline-5-carboxylate synthetase) and P5CR (P5C reductase) from glutamate or ornithine. Induction of the P5CS gene precedes the accumulation of proline, suggesting that the P5CS gene has a main role in the proline biosynthesis under osmotic pressure (Zheng *et al.,* 2014; Khalil *et al.,* 2016; Fu *et al.,* 2018).

Oxidative stress defense system occurs by antioxidant enzymes mechanism which ROS detoxification such as superoxide dismutase (SOD), catalase (CAT), peroxidases (POX), ascorbate peroxydase (APX), mono-dehydroascorbate dehydrogenase (MDHAR) and dehydroascorbate reductase (DHAR) (Al Kharusi et al. 2019). The SOD, POX and CAT are the principal antioxidant enzymes in the living organism. Moreover SOD is the leading scavenger of O_2^- and is the first line of defense mechanism against ROS, where SOD turn O_2^- into H_2O_2 and O_2, subsequently POX or CAT change H_2O_2 into O_2 and H_2O (Geng *et al.,* 2019). The large excess in the activity of SOD, POX and CAT are an indicator of the capacity of plants to resist oxidative stress due to high increase of salt stress (Sarker and Oba 2018; Al Kharusi *et al.,* 2019).

Other mechanisms depends on the ability to efflux excess sodium from the cells which a vital for salinity tolerance. Vacuolar sodium hydrogen antiporter (NHX1) plays a significant role in salinity tolerance via reduction of injurious effects of sodium increase in the cytosol and preserving the balance of osmosis in vacuoles utilizing sodium as an ionic osmolyte (Kumar *et al.,* 2017). Salt Overly Sensitive (*SOS1*) plays a crucial role in the response to oxidative stress, it is a Na^+/H^+ antiporter and participated in the homeostasis of sodium in plants by sodium efflux of the cytoplasm combined with the H^+ influx, therefore, it is considered the only protein for outflow of sodium in the plant's plasma membranes (Feki *et al.,* 2017; Nemat Alla *et al.,* 2018; Fan *et al.,* 2019).

The main goal of this study was to evaluate the response of some Egyptian wheat cultivars to salinity tolerance by measured the expression level of *P5CS*, *TNHX1* and *TaSOS1* genes, determine the SOD and peroxidase activities as antioxidant enzymes and to analyze the parameters related to growth among six wheat cultivars.

Materials and Methods

Germplasm collection

The Seeds of six wheat cultivars (Giza-168, Giza-171, Sids-12, Misr-1, Giza-11 and Gemmiza-9) were obtained from field crop institute, Agricultural Research Center, Ministry of Agriculture, Egypt.

Germination of seeds and salt stress treatments

Seeds were germinated in the green house of the genetic engineering research center (GERC), faculty of agriculture – Cairo University, Egypt. The six Egyptian wheat cultivars seeds were planted in plastic pots (20 cm diameter) containing a mixture of sand and soil (1:1/ w:w). The plantlets were irrigated daily with 450 ml of one tenth of the Murashige and Skoog basal medium solution (Murashige and Skoog 1962), at 25 days after planting, the plants were subjected to salt stress by the addition different concentrations of 0, 75, 125 and 175 mM NaCl to the irrigation solution for 25 days. The temperature was 25°C and normal light intensity with 70 % relative humidity under a 16 hours light/8 hours dark photoperiod. The treatment was planned by five replicates per NaCl treatment per cultivar.

Growth Measurements

At the end of the treatment, Fresh weight (FW), dry weight (DW), shoots and roots length was measured.

The plants were taken from the pots carefully washed using tap water to reduce root loss and remove any soil on plant surfaces then dried by paper towels. Consequently fresh weight (FW) was weighted and shoot and root length were measured. For dry weight (DW) was weighted after the same plants of fresh weight were dried in air-forced draught oven at 80°C (Heraeus-0871, USA) for three days.

Antioxidant enzyme assays

The crude extract for SOD and peroxidase measurements was isolated according to Sarker and Oba (2018). Wheat leaves (0.25 g) were homogenized with 3ml of 100 mM potassium phosphate buffer (pH 7.0) containing 1mM EDTA and 1% (w/v) polyvinyl pyrrolidone (PVP), at 4°C. The homogenate was filtered through four layers of cheesecloth and centrifuged at 15000 x g for 20min.

GPOX (EC 1.11.1.7) Guaiacol peroxidase activity was estimated spectrophotometrically using the guaiacol oxidation as substrate according to Sarker and Oba (2018) by measuring the increase in absorption at 470 nm due to guaiacol oxidation to the formation of tetra-guaiacol. The reaction mixture (3 ml final volume) composed of 100 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 10 mM H_2O_2 and 50µl of crude extract. The enzyme activity was expressed as µmol tetra-guaiacol formed per min per mg protein.

SOD, Superoxide dismutase (EC 1.15.1.1) activity was assayed spectrophotometrically by estimating its ability to reduce NBT (nitro-blue tetrazolium) according to Sarker and Oba (2018). One unit of SOD activity was assayed as the amount of SOD enzyme which leads to 50% inhibition of nitro-blue tetrazolium assayed at 560 nm in existence of light and riboflavin. The final reaction mixture 3 mL comprised of 50 mM phosphate buffer (pH 7.8), 75 µM nitro-blue tetrazolium, 13 mM methionine, 0.1 mM EDTA, 2 mM riboflavin and 50 µL enzymes extract. The reaction was placed under light for 15 min.

RNA Isolation and quantitative Real Time PCR (qRT-PCR) analysis

Total RNA was extracted from 100 mg of frozen normal and stressed leaf tissue of two plants of Gemmiza-9 (high salt-tolerant cultivar) and Giza-171 (high salt-sensitive cultivar) utilizing the RNeasy Plant Mini Kit (Qiagen, Germany, CAT NOs. 74903 and 74904) according to the manufacturer's instructions. Then, the cDNAs were synthesized utilizing Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, USA, Cat NO. K1621).

The qPCR was utilized to determine gene expression of Δ1-pyrrolin-5-carboxylate synthetase (*P5CS*), sodium hydrogen antiporter (*TNHX1*) and Salt Overly Sensitive (*TaSOS1*) in the shoot tissue using a Maxima SYBR Green/ROX qPCR Master Mix kit (Thermo Scientific, USA, Cat NO. K0221). Primers pairs were designed for three genes using primer 3 software table 1 and the *Actin* gene as internal control gene for data normalization. Genes amplifcation via RT-qPCR (the MX3000PqPCR Machine from Stratagene) were performed in biological triplicates for all genes with the following program conditions: pre-denaturation at 95°C for 10 min followed by 40 cycles of 15 s denaturation at 95°C, 60 s annealing at 60°C, 60 s extension at 72°C and a final 4 min extension at 72°C

Data analysis

The qRT-PCR data was calculated based on the threshold cycle (C_T) method. Consequently, the fold expression changes of target mRNAs over the reference values were determined using the $2^{-\Delta\Delta Ct}$ method according to Livak and Schmittgen (2001), where $\Delta\Delta C_T$ was calculated by subtracting ΔC_T value of the corresponding internal control from ΔC_T of specific targets. 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

Estimation of the salinity effect on some growth characteristics

In the present study, an experiment has been conducted to evaluate the potentiality of six wheat cultivars (Giza-168, Giza-171, Sids-12, Misr-1, Giza-11 and Gemmiza-9) to withstand salt stress. Plants germinated under normal conditions and at 25 days after planting, they were subjected to salt stress of 0, 75, 125 and 175 mM NaCl, to test their ability to tolerate different levels of salt stress and determine the most sensitive and tolerant cultivars. Plant fresh weight, dry weight, shoot and root length were measured Figs. 1, 2, 3 and 4 and calculated the ratio of the effect of salinity stress on these parameters in table 2. Wheat cultivars differed in their

Table 1: Gene-specific primers used in qPCR.

Reverse primer (5' to 3')	Forward primer (5' to 3')	Gene
AGACCTTCAACACCCACAG	ACAGAGATAAAGTAGCAGAGAC	*P5CS*
CACCGAAAGAATCCCAAGAG	GCCTGGTTCACCCATAGAGA	*TNHX1*
TTTCCTCGAGCAACCCAGTC	ATTCCCTCAGGTGCTTCGTG	*TaSOS1*
GGGCGGAAAGAATTAGAAGC	TGGCATCTCTCAGCACATTC	*Actin*

response to different levels of salt stress and the effects were easily observed on plant leaves.

At salt concentration of 125 mM NaCl and 175 mM NaCl, the growth of Gemmiza-9 revealed the highest significantly of all growth parameter compared to the other cultivars, where 85.92% F.W., 85.00% D.W., 91.42 % shoot length and 91.79 % root length at 125 mM NaCl as compared to their control but, at 175 mM NaCl the F. W. was 80.79%, D. W. was 82.50, shoot length was 85.82 % and root length was 82.53 % as compared to their control, followed by Giza-11 then Misr-1. While the Giza-171, Sids-12 and Giza-168 showed significantly decrease in all growth parameter. At 175 mM NaCl concentration the Giza-171 revealed the highest significantly decrease in all growth parameter, where the F. W. was 31.80%,

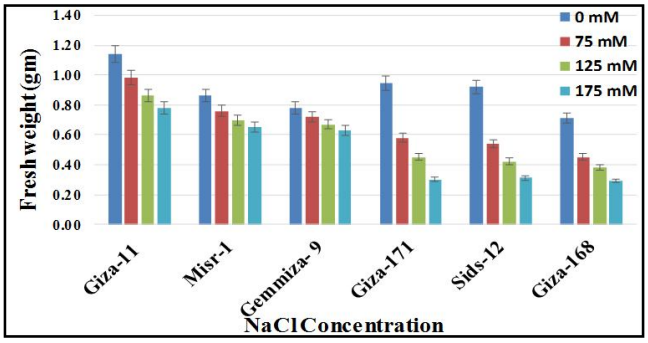

Fig. 1: Histogram illustrating means of fresh weight for the six wheat cultivars under salt stress and control. Bars represent mean values ± standard error. P<0.05 is reflect significant.

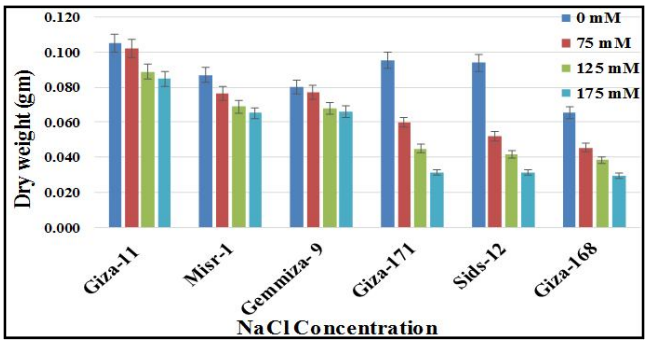

Fig. 2: Histogram illustrating means of dry weight for the six wheat cultivars under salt stress and control. Bars represent mean values ± standard error. P<0.05 is reflect significant.

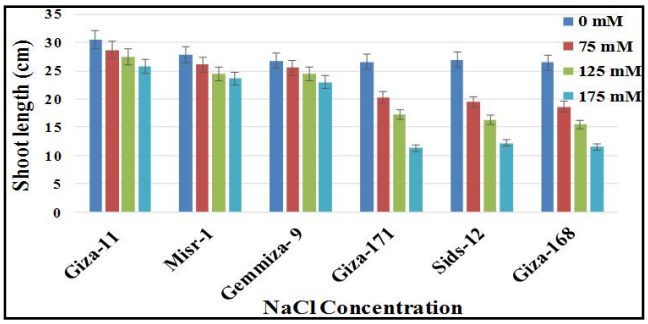

Fig. 3: Histogram illustrating means of shoot length for the six wheat cultivars under salt stress and control. Bars represent mean values ± standard error. P<0.05 is reflect significant.

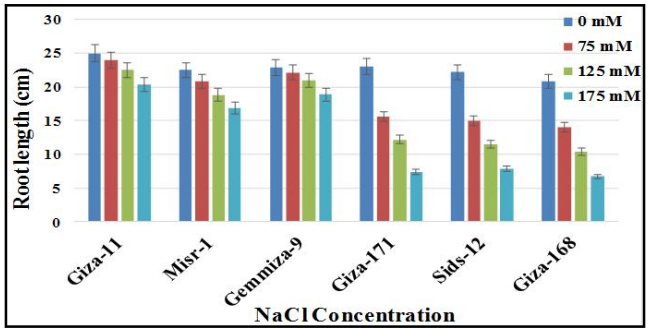

Fig. 4: Histogram illustrating means of root length for the six wheat cultivars under salt stress and control. Bars represent mean values ± standard error. P<0.05 is reflect significant.

Table 2: The percentage difference of the effect of salinity stress on the growth parameters and antioxidant enzyme activity of six wheat cultivars expressed by the stressed plants as compared to their control wheat cultivars.

Cultivars	Growth Parameter				Antioxidant enzyme		
	NaCl treatment (mM)	Shoot length %	Root length %	F.W. %	D.W. %	Peroxidase %	SOD %
Giza-11	0	100.00	100.00	100.00	100.00	100.00	100.00
	75	94.10	96.00	86.00	97.21	124.52	154.37
	125	90.16	90.00	75.47	84.54	140.23	194.68
	175	84.59	81.20	68.45	80.74	152.87	278.73
Misr-1	0	100.00	100.00	100.00	100.00	100.00	100.00
	75	93.55	92.44	88.26	87.73	118.29	138.73
	125	87.81	83.56	81.18	79.10	133.07	184.62
	175	84.59	75.11	75.60	74.86	149.81	258.82
Gemmiza-9	0	100.00	100.00	100.00	100.00	100.00	100.00
	75	95.15	96.51	92.38	96.25	126.21	155.26
	125	91.42	91.79	85.92	85.00	142.38	197.37
	175	85.82	82.53	80.79	82.50	159.85	281.58
Giza-171	0	100.00	100.00	100.00	100.00	100.00	100.00
	75	76.32	67.83	61.24	62.89	111.71	114.74
	125	64.66	53.04	47.70	47.17	87.84	82.47
	175	42.48	32.17	31.80	32.49	64.86	28.97
Sids-12	0	100.00	100.00	100.00	100.00	100.00	100.00
	75	72.22	67.57	58.82	55.32	100.81	98.65
	125	60.37	51.80	45.65	44.15	77.24	84.62
	175	45.19	35.59	33.70	33.44	65.45	30.58
Giza-168	0	100.00	100.00	100.00	100.00	100.00	100.00
	75	70.19	67.31	63.20	69.47	105.56	115.68
	125	58.49	50.00	53.37	58.63	84.72	80.25
	175	43.40	32.21	40.73	44.89	65.28	30.00

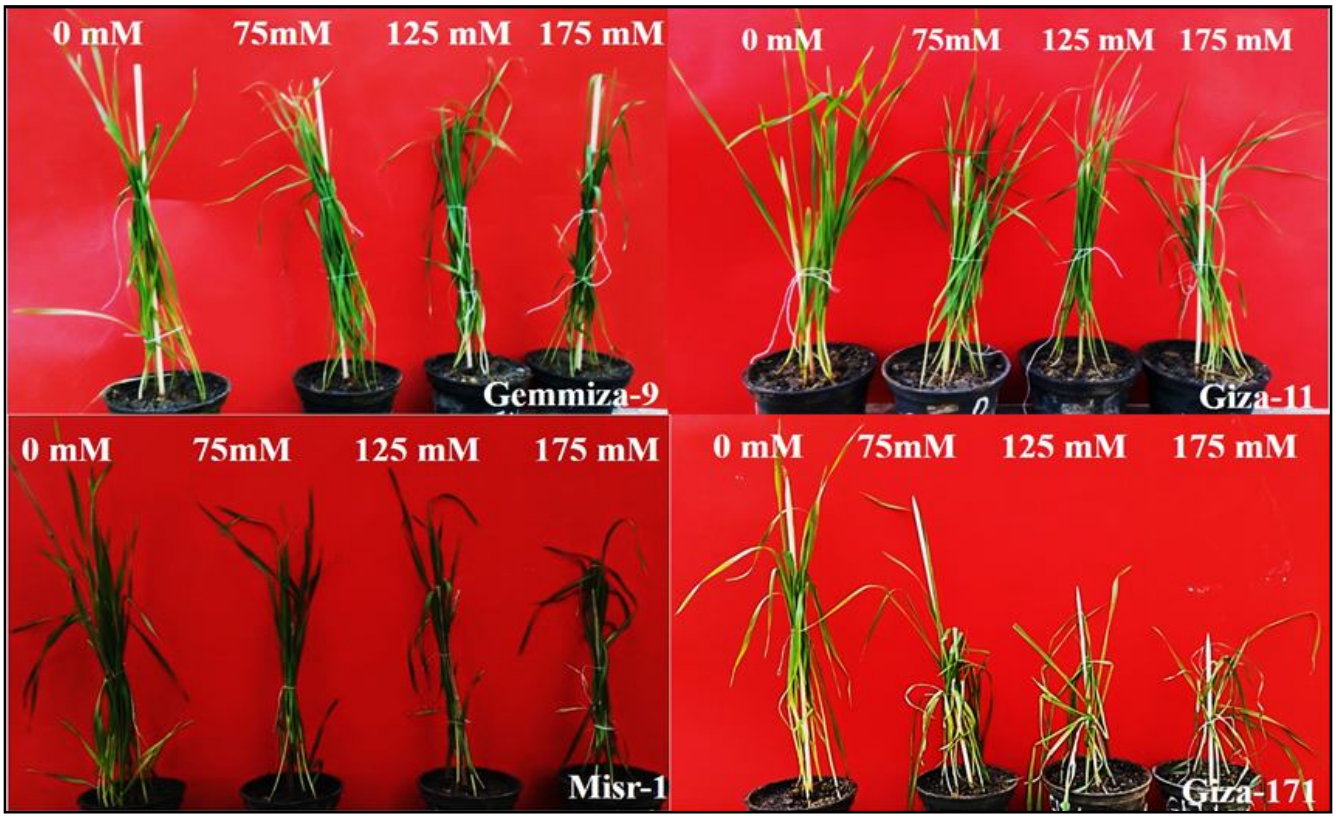

Fig. 5: Effects of salinity on the growth of wheat plants.

D.W. was 32.49, shoot length was 42.48 % and root length was 32.17 % as compared to their control.

Optical symptoms of salinity injury after 25 days showed on sensitive cultivars only, these symptoms were shown in Fig. 5 such as decrease in plant size than normal, the plant atrophy turn into completely wilted and plant died at the high concentration.

Antioxidant enzyme assays

In the present investigation, levels of total SOD and peroxidase activities were assayed in six wheat cultivars using NBT and guaiacol as a substrate, respectively. Results from these assays revealed different levels of catalytic activities among six wheat cultivars. The histograms in Figs. 6 and 7 represent the means of the SOD and the peroxidase enzymes activity and the table 2 represent the percentage of the effect of salinity stress on the SOD and the peroxidase enzymes activity of six wheat cultivars expressed by the stressed plants as compared to their control wheat cultivars.

At 125 mM and 175 mM NaCl concentration, the Gemmiza-9 cultivar revealed the highest activity of SOD and peroxidase where its SOD activity was 0.225 u/g which represent 1.97 multiplier for control (197%) and 0.321 u/g which represent 2.82 multiplier for control

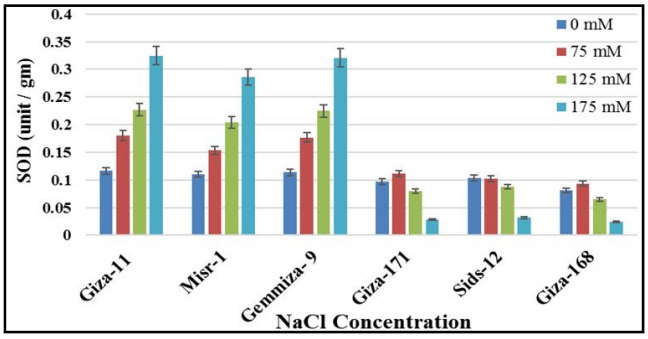

Fig. 6: Histogram representing the means of SOD enzyme activity for the six wheat cultivars under salt stress and control. Bars represent mean values ± standard error. P<0.05 is reflect significant.

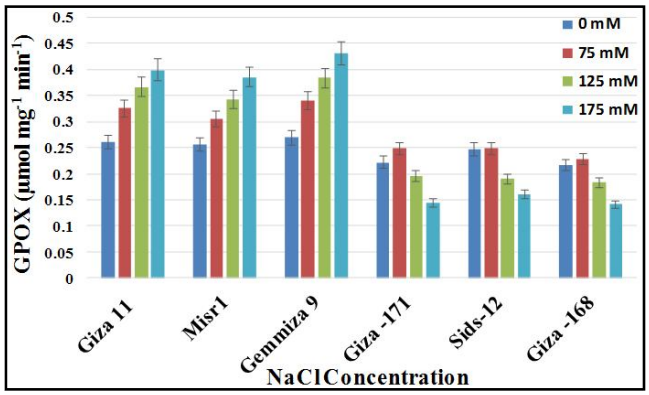

Fig. 7: Histogram representing the means of Peroxidase enzyme activity for the six wheat cultivars under salt stress and control. Bars represent mean values ± standard error. P<0.05 is reflect significant.

(281.58%), respectively and its peroxidase activity was 0.383 µmol mg^{-1} min^{-1} represent 1.42 multiplier for control (142%) and 0.43 µmol mg^{-1} min^{-1} represent 1.6 multiplier for control (159.85%), respectively. Followed by Giza-11 then Misr-1 showed relatively high SOD and peroxidase activity.

At 125 mM NaCl concentration, the SOD activity of Giza-11 and Misr-1 was 0.227 u/g represent 1.95 multiplier for control (195%) and 0.204 u/g represent 1.85 multiplier for control (185%), respectively and the peroxidase activity of them was 0.366 µmol mg^{-1} min^{-1} represent 1.4 multiplier for control (140%) and 0.342 µmol mg^{-1} min^{-1} represent 1.33 multiplier for control (133%), respectively.

Whereas, at 175 mM NaCl concentration, the SOD activity of Giza-11 and Misr-1 was 0.325 u/g represent 2.78 multiplier for control (278.73%) and 0.286 u/g represent 2.58 multiplier for control (258.8%), respectively and the peroxidase activity of them was 0.399 µmol mg^{-1} min^{-1} represent 1.53 multiplier for control (152.87%) and 0.385 µmol mg^{-1} min^{-1} represent 1.5 multiplier for control (149.8%), respectively.

While, Giza-171, Sids 12 and Giza -168 showed significantly decreased in SOD and peroxidase activity with raising salinization level compared to the control. Whereas, at 175 mM NaCl concentration the Giza-171 revealed the highest significant decrease in SOD and peroxidase activity, where the SOD activity decreased to 0.0281u/g represent 28.97% and the peroxidase activity decreased to 0.144 µmol mg^{-1} min^{-1} represent 64.86 % as compared to their control.

Change in gene expression under salt stress

The level of gene expression was measured by the quantitative Real Time PCR (qRT-PCR) for three salt responsive genes related to proline accumulation such as Ä[1]-pyrrolin-5-carboxylate synthetase (*P5CS*) and related to Na^+ exclusion such as sodium hydrogen antiporter (*TNHX1*) and Salt Overly Sensitive (*TaSOS1*) in the shoot tissue for two Egyptian wheat cultivars Gemmiza-9 (high salt-tolerant cultivar) and Giza-171 (high salt-sensitive cultivar) to verify the effect of saline stress. The results of quantitative PCR for the *P5CS*, *TNHX1* and *TaSOS1* expression patterns genes under difierent salinity stress levels for two wheat cultivars showed in Figs. 8, 9 and 10 respectively. The results showed significant variances in each gene expression between wheat cultivars at different salinity stress levels. Moreover, Gemmiza-9 (salt tolerant cultivar) showed significantly higher ($P<0.05$) expressions of three genes with raising salinization level compared to Giza-171 (salt stress sensitive cultivar) which showed significantly decrease ($P>0.05$) expressions of three genes with raising salinization level.

The gene expression levels of Gemmiza-9 were significantly increased by 5.3, 17.4 and 35.5 fold higher than control under 75, 125 and 175 mM of NaCl, respectively for *P5CS* gene, 5.9, 15.7 and 32.1 fold higher than control under 75, 125 and 175 mM of NaCl, respectively for *TNHX1* gene and 2.5, 6.5 and 16.5 fold higher than control under 75, 125 and 175 mM of NaCl, respectively for *TaSOS1* gene. While, the expression was

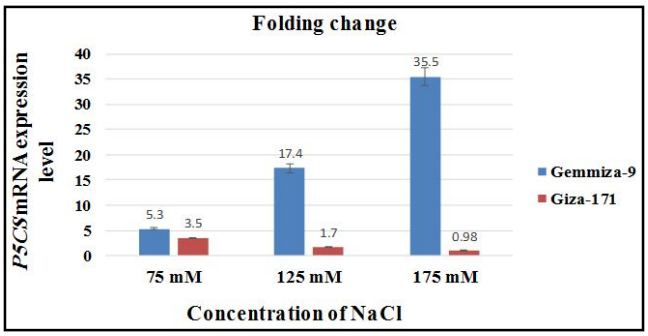

Fig. 8: Histogram represent expression level of *P5CS* gene under salinity. The qRT-PCR analysis results to measure the relative mRNA expression level of *P5CS* gene of the wheat cultivars. Bars represent mean values ± standard error. $P<0.05$ is reflect significant.

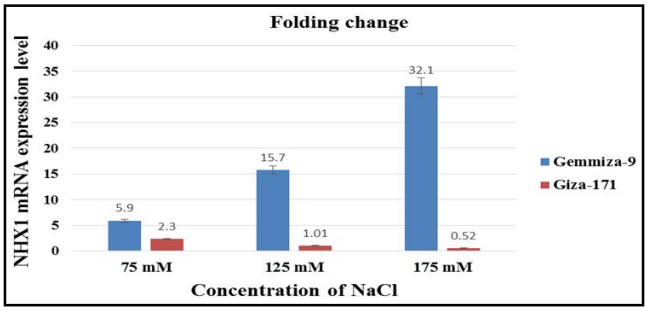

Fig. 9: Histogram represent expression level of *NHX1* gene under salinity. The qRT-PCR analysis results to measure the relative mRNA expression level of NHX1 gene of the wheat cultivars. Bars represent mean values ± standard error. $P<0.05$ is reflect significant.

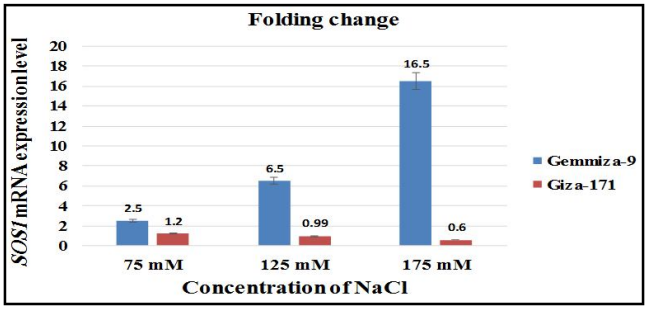

Fig. 10: Histogram represent expression level of *SOS1* gene under salinity. The qRT-PCR analysis results to measure the relative mRNA expression level of *SOS1* gene of the wheat cultivars. Bars represent mean values ± standard error. $P<0.05$ is reflect significant.

significantly decrease for Giza-171 which reaching 3.5, 1.7 and 0.98 folds under 75, 125 and 175 mM of NaCl, respectively for *P5CS* gene, 2.3, 1.01 and 0.52 folds under 75, 125 and 175 mM of NaCl, respectively for *TNHX1* gene and 1.2, 0.99 and 0.59 folds under 75, 125 and 175 mM of NaCl, respectively for *TaSOS1* gene.

Discussion

Salinity stress is a destructive environmental stress factor that affects seeds germination, plant growth and reduction of wheat productivity (Barnawal *et al.,* 2017; Saddiq *et al.,* 2019; Khan *et al.,* 2019). These effects causes hyperosmotic and hypertonic stresses which lead to negative effect on developmental, morphological, biochemical, photosynthetic and physiological processes resulting in metabolic dysfunction and membrane damage which lead to plant death (Oyiga *et al.,* 2019; Zhu *et al.,* 2019).

The main goal of the present study was to estimate the genetic response of six Egyptian wheat cultivars to salinity tolerance. For this purpose, the plants were subjected to different levels of NaCl, then analyzed of the parameters related to growth, assayed the enzymes activity and measured the expression level of some genes.

Estimation of the salinity effect on some growth characteristics

The harmful effects of salinity on the growth traits can be observed as decreased plant growth or plant death, therefore, decreases the productivity (Roy *et al.,* 2014; Negrão *et al.,* 2017). According to several studies growth responses to salinity depend on the plant species or cultivars, levels of salinity and ionic composition of the salts (Yadav *et al.,* 2010; Cokkizgin 2012; Negrão *et al.,* 2017) and can be characterized in two major stages: the first response is the shoot ion-independent and is believed to be related to Na^+ signalling, it occurs in minutes to days (Gilroy *et al.,* 2014; Roy *et al.,* 2014). The second response is the ion-dependent response to salt stress, it occurs within days to weeks, that include the accumulation of ions to the toxic concentration in the shoot especially in old leaves, which causes premature aging of the leaves resulting in plant death and reduced productivity (Negrão *et al.,* 2017).

In this study, the results revealed significant differences among wheat cultivars with raising salinization level compared to the control. The values of whole-plant fresh weight, whole-plant dry weight, shoot length and root length of the Giza-11, Misr-1 and Gemmiza-9 were slightly decreased with raising salinization level compared to the control. While, these values of the Giza-171, Sids-12 and Giza-168 were significantly decreased with raising salinization level compared to the control.

The salt concentration of 125 mM NaCl was critical in determining the more sensitive cultivars. Where the Gemmiza-9, Giza-11 and Misr-1 revealed highly tolerant cultivars, while the Giza-171, Sids-12 and Giza-168 were the sensitive cultivars.

Similarly, Abou El-Maaty *et al.,* (2014) demonstrated that the Gemmiza-9 cultivar was more tolerant to salt stress compared to the other cultivars under high salt concentration. Bhutto *et al.,* (2019) reported that shoot length and root length are one of the major factors affected by salinity because root is in direct contact with the soil and it absorb the water and minerals from the soil and provide them to other parts of the plant, thus it affects the whole-plant fresh weight, whole-plant dry weight. Shoot length, roots length, fresh weight and dry weight measurement are dominant indicators that highly determine the plant's response to salinity. Furthermore, these parameters allow useful selections of cultivars for future breeding programs. Where evaluation of genetic diversification under salt stress conditions is compulsory in defining the tolerance of plants to salt stress.

Antioxidant enzyme assays

Salt stress is the utmost disturbing in plant and an adverse effect on gas exchange lead to low CO_2 absorption for photosynthesis resulting in significant decrease of electron transportation lead to an increase ROS production, which are extremely toxic to DNA, lipid, protein and other cell organelles (Rohman *et al.,* 2019). To reduce oxidative damage by ROS, plants have an antioxidant defense system containing three major enzymatic antioxidant which are SOD (Superoxide dismutase), POD (peroxidase) and CAT (catalase) responsible for scavenging ROS (Wei *et al.,* 2015; Jakovljeviæ *et al.,* 2017; Dudziak *et al.,* 2019b). However, the effectiveness of protective mechanism varies among cultivars (Srinieng *et al.,* 2015; Dudziak *et al.,* 2019b).

Enzyme assays results were in consistent with the all growth parameter. These results confirmed that the Gemmiza-9, Giza-11 and Misr-1 revealed highly tolerance cultivars, while the Giza-171, Sids-12 and Giza-168 were sensitive cultivars.

Similarly, Bajpai and Srivastava (2015) reported that SOD activity increases stimulate the O^-_2 conversion to H_2O_2, which is scavenged by POX and CAT. Moreover, SOD activity increases to a determined level, where POX and CAT may be unable to disassembling all of the H_2O_2 which leads to its accumulation resulting in disrupts the

SOD. Consequently, the antioxidative defense mechanism turn into unbalanced, affecting growth criteria.

Change in gene expression under salt stress

The results revealed that significant variation in *P5CS, TNHX1* and *TaSOS1* genes expression between the two cultivars of wheat with raising salinization level. There is significantly up-regulation of the three genes with raising salinization level in Gemmiza-9 cultivar, but, there is significantly down-regulation of the same genes with raising salinization level in Giza-171 cultivar. These results were in consistent with the FW, DW, shoot length, root length and enzymes assay results. These results confirmed that the Gemmiza-9 revealed more significantly tolerance cultivar, while the Giza-171 were sensitive cultivars. These results confirmed that up-regulation of genes for proline accumulation and Na^+ exclusion are related to salinity tolerance in wheat. This information will be beneficial for improvement of wheat cultivars for salinity tolerance.

The current results are similar to the results of Tavakoli *et al.,* (2016) and Dudziak *et al.,* (2019a), where they demonstrated that the expression levels of *P5CS* and *P5CR* genes responsible for biosynthesis of proline in wheat under different levels of salinity stress (Tavakoli *et al.,* 2016) and drought conditions (Dudziak *et al.,* 2019a). Their results revealed an increase level of *P5CR* and *P5CS* genes expression (up-regulation) in the cultivar of drought tolerant wheat compared to the cultivar of drought sensitive. Similar, Rana *et al.,* (2016) reported that gene expression of *TaNHX1* and *TaSOS1*which responsible for Na^+ exclusion and *P5CR* and *GOGAT* (glutamate synthase) which responsible for proline accumulation in seedlings of wheat cultivars under salinity stress conditions. The results were significantly higher expression of *TaSOS1, TaNHX1, P5CR* and glutamate synthase genes in salt tolerant cultivar as compared to salt sensitive cultivar.

The results of this study confirm that the Wheat cultivars differed genetically for their response to salt stress and the effects were easily observed on plant leaves. The values of growth parameter confirmed that the Gemmiza-9, Giza-11 and Misr-1 revealed high significantly tolerance cultivars, while the Giza-171, Sids-12 and Giza-168 were sensitive cultivars. Moreover, enzymes assay confirmed this results, where, these salt-tolerant cultivars have stimulate the antioxidant enzymes SOD and peroxidase more efficiently than the other salt-sensitive cultivars. Furthermore, the gene expression of the three genes were significantly up-regulation with raising salinization level in Gemmiza-9 cultivar while, Giza-171 cultivar had significantly down-regulation of the same genes with raising salinization level. This information will be beneficial for improvement of wheat cultivars for salinity tolerance.

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