Plant Archives Volume 20 No. 2, 2020 pp. 9339-9347 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

CHANGES IN SOME MORPHOLOGICAL, PHYSIOLOGICAL, BIOCHEMICAL PARAMETERS AND GENE EXPRESSION IN SORGHUM UNDER SALT STRESS CONDITIONS

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Abstract

Knowledge of salt tolerant cultivars and understanding defense mechanism of this plant is more essential to solve the problem. Therefore, six sorghum cultivars were subjected to different concentration of NaCl for two weeks and changes in fresh and dry weight, shoot height and root length, Relative water content, Osmotic potential and osmotic adjustment and free proline contents was determined. The results showed that reduction in morphological and physiological parameters with the increasing of saline conditions. In contrast, osmotic adjustment and proline content increased in the salt-tolerant cultivars (Maka-244 and Horas). Verification of morphological and physiological parameters results was done by quantitative real-time PCR (QRT-PCR). *Betaine aldehyde dehydrogenase* L. and *Glutathione S-transferase* are an important target genes in understanding the regulation of sorghum responses to salt stress. The expression pattern revealed up-regulation of both genes in Maka-244 and Horas cultivars particularly at 150 and 300 mM NaCl. While, PM cultivar showed down-regulation at the same concentration for both genes. The all data supported that Maka-244 and Horas are salt-tolerant cultivars and Giza-420 is moderate salt-tolerant cultivar, while, PM, Special-85 and Special-90 are salt-sensitive cultivars. These findings could be a good indicator of the salt tolerance mechanism present in the studied sorghum cultivars.

Key words: Salt stress; physiological parameter; free proline content; gene expression; Betaine aldehyde dehydrogenase1; Glutathione S-transferase genes.

Introduction

Sorghum bicolor L. is one of the most important crop worldwide and multi-purpose crop which has received great attention for its high productivity, photosynthetic efficiency and increase its sugar content in saline areas, drought, high temperatures, soil toxicity and other stress conditions (Guo *et al.,* 2018, Calone *et al.,* 2020 and Fitrahtunnisa *et al.,* 2020).

Salinity stress is a serious factor limiting the growth and production of plants because it produces toxic salt ions that directly destroy the plant cells and prevents the efficient use of water in the plant which results in negatively affecting crucial biological processes, like metabolism of energy, synthesis of proteins, photosynthesis and metabolism of lipids (Sui *et al.,* 2018 and Yang *et al.,* 2020) and various physiological processes like germination of seeds, growth of leaf cells, growth and vitality of seedling, production of plant biomass, growth of vegetative, flowering and production of fruits (Fahmideh and Fooladvand, 2018).

Under salt stress condition plants develop several tolerance mechanisms among them, the enzymatic scavenging mechanisms which includes a number of enzymes such as peroxidase (POD), catalase (CAT) and glutathione S transferase (GST) enzymes. The nonenzymatic system is accumulation of osmolytes, compatible solutes or osmoprotectants such as amino acids such as glycine betaine (GB) and proline (Pro) and polyols such as glycerol, mannitol and sorbitol (Liu *et al.,* 2019 and Yilmaz *et al.,* 2020). These osmoprotectants are nutrients and metabolites accumulate within the cell with high concentrations without generating metabolic damage and act as signal molecules that activate specific or hormonal transduction pathways and change gene expression and protein patterns (Ahanger *et al.,* 2019;

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Magaña *et al.,* 2019 and Yilmaz *et al.,* 2020).

Proline accumulation in higher plants is a metabolic response and physiological reaction to salinity and other environmental stresses. Proline plays many protective functions such as maintaining biological membranes, carbon and nitrogen storage, osmoprotectant, stabilizing cellular structure, scavenging reactive oxygen species (ROS), keeps up redox balance in stressed plants. In addition, it regulates osmotic adjustment and acts as a protective agent for enzymes (Dien *et al.,* 2019, Ghaffari *et al.,* 2019 and Meena *et al.,* 2019).

The osmolytes Glycine-betaine is one of the most significant compatible solutes that accumulate in the cytosol. It has an essential role in the plant when exposed to varied environmental stresses as salinity because its accumulation helps in water preservation and protecting proteins and vital membranes by efficiently stabilizing the structure and function of proteins and reducing the T_m of DNA that leads to provide protection against several stresses (Mitsuya *et al.,* 2015 and Magaña *et al.,* 2019).

Glutathione S transferase enzymes (GSTs) are a large group of multi-functional protective enzymes that play an important role in the defense against oxidative damage and peroxidative products of DNA or lipids generated by environmental stresses (Liu *et al.,* 2017 and Abdul *et al.,* 2018). GSTs affect on plant growth and development by involvement in plant primary and secondary metabolism, cell signal transduction and stress tolerance by scavenging ROS (Kumar and Trivedi, 2018; Horváth *et al.,* 2019 and Yang *et al.,* 2019). Also GSTs act as ligands or binding proteins and can function as nonenzyme carriers in intracellular transport (Du *et al.,* 2019).

At the molecular level, various genes related to saline stress responses are expressed differently after plants sense external signals of saline stress to enhance the tolerance of the plant to salinity. The differential expressions of these genes then push the saline stress responses at the physiological and biochemical levels (Yuan *et al.,* 2016; Kong *et al.,* 2016; Cui *et al.,* 2018; Leng *et al.,* 2018 and Zhang *et al.,* 2019).

Estimation of gene expression became highly vital in understanding molecular mechanism and getting insights about gene function. Quantitative Real-Time PCR (qRT-PCR) is the gold standard for quantifying gene expression because of its sensitivity, accuracy, specificity, reproducibility and widespread use in investigating targeted genes (Hossain *et al.,* 2019).

In this study salinity tolerance among six sorghum cultivars was investigated. The relative salinity tolerance at the seedling stage was determined based on morphological, physiological and biochemical parameters. The expression level of *Betaine aldehyde dehydrogenase 1* and *Glutathione S-transferase genes* under salinity stress condition was measured.

Materials and Methods

Seeds collection

Six sorghum cultivars used in this study, namely Horas, Maka-244, Giza -420, PM, Special-85 and Special-90 were obtained from the Sorghum Department, Field Crops Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

Salt treatments

Seeds was germinated in plastic pots that putted in outer trays each containing a mixture of sand, soil and peat moss (1:1:1/ w: w). Seedlings was irrigated with one tenth of Murashige and Skoog basal medium (Murashige and Skoog, 1962) to the bottom of the outer pot. One-month-old plantlets was subjected to graded levels of NaCl as follows 0 mM as control, 75 mM, 150 mM and 300 mM, under a 16/8 hours light/dark photoperiod at 25°C. Five replications will be performed per each NaCl treatment in addition to the control.

Samples were collected fifteen days past the salt treatments for the following measurements: plant fresh and dry weight, shoot height and root length, Relative water content, Osmotic potential and osmotic adjustment, free proline contents and gene expression analysis.

Morphological parameters measurement

The plantlets were collected after the 15 days of the salt treatments to measure several morphological parameters including fresh and dry weight of roots and shoots. For dry weights, the plantlets were dried in air-forced draught oven at 80°C (Heraeus-0871, USA) for three days.

Physiological parameters measurement

Osmotic potential and osmotic adjustment

Osmotic potential was measured on leaf tissues using a vapor pressure osmometer (model 5500, Vapro, Wescor, Inc. USA). Leaf was collected in a 1.5 mL eppendorf and stored at -20°C. Leaf tissue was thawed and centrifuged at 5000 rpm for 15 minutes at 4°C to extract the cell sap. Osmotic adjustment was calculated by the differences in osmotic potential between salt treated and control plants according to Jones and Turner (1978) protocol.

Determination of Relative water content (RWC)

The water content (%) of the shoots was calculated using the following formula: RWC = ((fresh weight - dry

weight)/fresh weight)×100 (Lindenberg *et al.,* 2015, Tian *et al.,* 2015 and Alharby *et al.,* 2019).

Biochemical parameters measurement

Free Proline content assay

Proline accumulation was assayed according to Bates *et al.,* (1973). Leaf samples of 0.2g from each treatment were homogenized in 10 mL of 3% sulfosalicylic acid followed by centrifugation at 12000 rpm for 20 min. The 2 mL of supernatant was transferred in a new tube and then added 2 mL of the acid ninhydrin reagent and 2 ml of glacial acetic acid. The mixture was reacted for 60 min. at 100°C to develop the colors then cooled in ice. Four mL of toluene was added and vortexed to separate chromophores. The changes in color were measured by a spectrophotometer for absorbance at 520 nm compared to toluene as blank. The calibration curve was developed based on the proline standard for assessing proline concentrations in plant leaves.

Gene expression analysis (Quantitative PCR analysis)

RNA isolation and c-DNA preparation

The fresh leaf tissue (100 mg) was stored in liquid nitrogen (-80^{0}C), then the total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, Germany, CAT NOs. 74903 and 74904) according to the guidelines instructions. Samples of RNA were quantified using the Nano Drop ND-1000 spectrophotometer (NadroDrop Technologies, Wilmington, DE, USA).

One microliter from total RNA was reversed to cDNA using Revert Aid First Strand cDNA Synthesis Kit for RT-PCR and oligo dT primers (Thermo Scientific, USA, Cat NO. K1621) according to manufacturer's instructions. After that, the product of this step was diluted 1:12 with nuclease-free water and used for real-time PCR analysis. Two genes were selected to investigate the effect of salt stress on expression level of mRNA in shoot tissue besides *Actin gene* as a reference gene. Primers were designed using the primer 3 software and described in table 1.

PCR condition

The reactions were performed in a total volume of 20 µl containing 2 µl diluted cDNA template, 10 µl Maxima SYBR Green/ROX qPCR Master Mix kit (Thermo Scientific, USA, Cat NO. K0221), 0.25 µl each forward and reverse primers and 7.5 µl nuclease-free water. Amplification conditions of qpcr were programmed with 95°C for 10 min followed by 40 cycles of 95°C for 15 s., then annealing at 60°C for 60 s and an extension step at 72°C for 60 s. and a final step at 72°C for 4 min. The all reactions were carried out with triplicates for all genes. The reaction was done using the Stratagene MX3000P Multiplex Quantitave PCR System (California, USA).

Data analysis

The comparative Ct (ΔΔCt) was measured by subtracting ΔCt of calibrator from ÄCt of treated samples. Relative expression fold changes were also calculated by using formula 2-ΔΔCT which was proposed by Livak and Schmittgen (2001) and calculated as: $\Delta\Delta Ct = \Delta Ct_{target} - \Delta Ct_{reference}$. To determine the significance ($P \leq 0.05$) between the mean differences of the groups, the independent unpaired student's t-test was used.

Results

Effect of salinity on morphological and Physiological parameter

A gradual increase of NaCl concentrations (0, 75, 150 and 300 mM NaCl) were used to investigate the effect of salt stress on morphological and physiological parameters of sorghum cultivars. The changes in FW, DW, RWC, shoot and root length were measured table 2. The results revealed that, salt stress was significantly affects on plant growth and the effect was increased with increasing NaCl stress concentration. There were variation in shoot length and root length among the studied cultivars. The highest shoot length was recorded in Maka-244 (53.50 cm) and the shortest shoot length observed in Horas (36.83 cm), in control plants. After application of salinity, there were significant differences between sorghum cultivars with increasing NaCl concentration.

The FW, DW, shoot and root length of salt treated plants decreased with increasing NaCl concentration to 300 mM. Maka-244 and Horas cultivars showed the highest morphological parameters (0.98g and 0.64g for FW, 0.13g and 0.09g for D.W., 40 cm and 29.5 cm for Sh. L. and 12.33 cm for and 12 cm for R.L., respectively) followed by Giza-420 (0.18g for FW, 0.05g for D.W., 23 cm for Sh.L. and 7.5 cm for R.L., respectively) compared to the other cultivars at 300 mM NaCl. Whereas, the

Table 1: Gene-specific primers used in quantitative PCR.

Gene name	Sequence 5'-3'	Product size (bp)
Betaine aldehyde dehydrogenase 1	F: TTTAGCTGGTGCCGTGATCTR:5'TGGTGACTTGCTTCACGGTC	200bp
Glutathione S-transferase	F: GAGCATCCTCTCCTGCCTAAR: CTGGGCTCATCCTACCCTCA	133bp
β-Actin	F: TGGCATCTCTCAGCACATTCR: GGGCGGAAAGAATTAGAAGC	104

decrease was more pronounced in PM and Special-85 cultivars (0.10g and 0.069g for FW, 0.07g and 0.04g for D.W., 21 cm and 15.33 cm for Sh.L. and 6 cm and 6.67 cm for R.L., respectively). Moreover, the highest RWC was observed in Maka-244 and Horas cultivars (86.46% and 86.56%, respectively) followed by Giza-420 (70.29%), while the highest reduction of 34.68 % for RWC was observed in PM cultivar followed by a reduction of 42.21 % in Special-90 cultivar at 300 mM NaCl table 3.

Nevertheless, the data of osmotic potential (Ψs) and osmotic adjustment (OA) exhibit that table 3, Ψs was declined with increasing in concentration of salt stress. The reduction was more noticeable in Maka-244 with respect to control plants. Plants that treated with 300 mM NaCl suffered a considerable salt stress than those which treated with 75 and 150 mM NaCl. Whereas, osmotic adjustment was increased in all treated plants and was greater in Maka-244 and Horas cultivars and lowest in PM cultivar.

Visible symptoms of salinity injury appeared after 15 days on sensitive sorghum plants only. As appeared in Fig. 1, these symptoms like: reduction in plant size from the normal, the plant stopped growing and at concentration 300 mM NaCl, the plants were completely wilted.

Effect of salinity on accumulation of proline

The proline accumulation in response to salinity in leaves of salt treated plants and control of six sorghum cultivars were determined by reading the absorption of chromophore with spectrophotometer at 520nm. Results presented in Fig. 2 demonstrated that the proline accumulation were almost undetectable in control groups of all cultivars, while revealed that different levels of proline content among various cultivars with raising salinization level. Rising concentrations of NaCl from 75 to 300 mM progressively increased the proline concentration in the leaf tissue of Maka-244 and Horas cultivars by about fivefold (230 µmol gm^{-1} and 235 µmol gm^{-1}, respectively at 300 mM NaCl) compared to the control.

The Giza -420 cultivar, the increase was about twofold in 100 and 150 mM NaCl concentration (150 µmol gm^{-1} and 130 µmol gm^{-1}, respectively), while rising concentration to 300 mM NaCl, decreased the proline concentration in the leaf tissue where almost non-detectable. While, the other cultivars (PM, Special-85 and Special-90 cultivars) revealed that the proline concentration were almost undetectable in all NaCl concentration.

Effect of salinity on gene expression

Quantitative Real-time PCR (qPCR) is a simple, sensitive, efficient, accurate, low-cost and reproducible technique that molecular biologists often use to determine

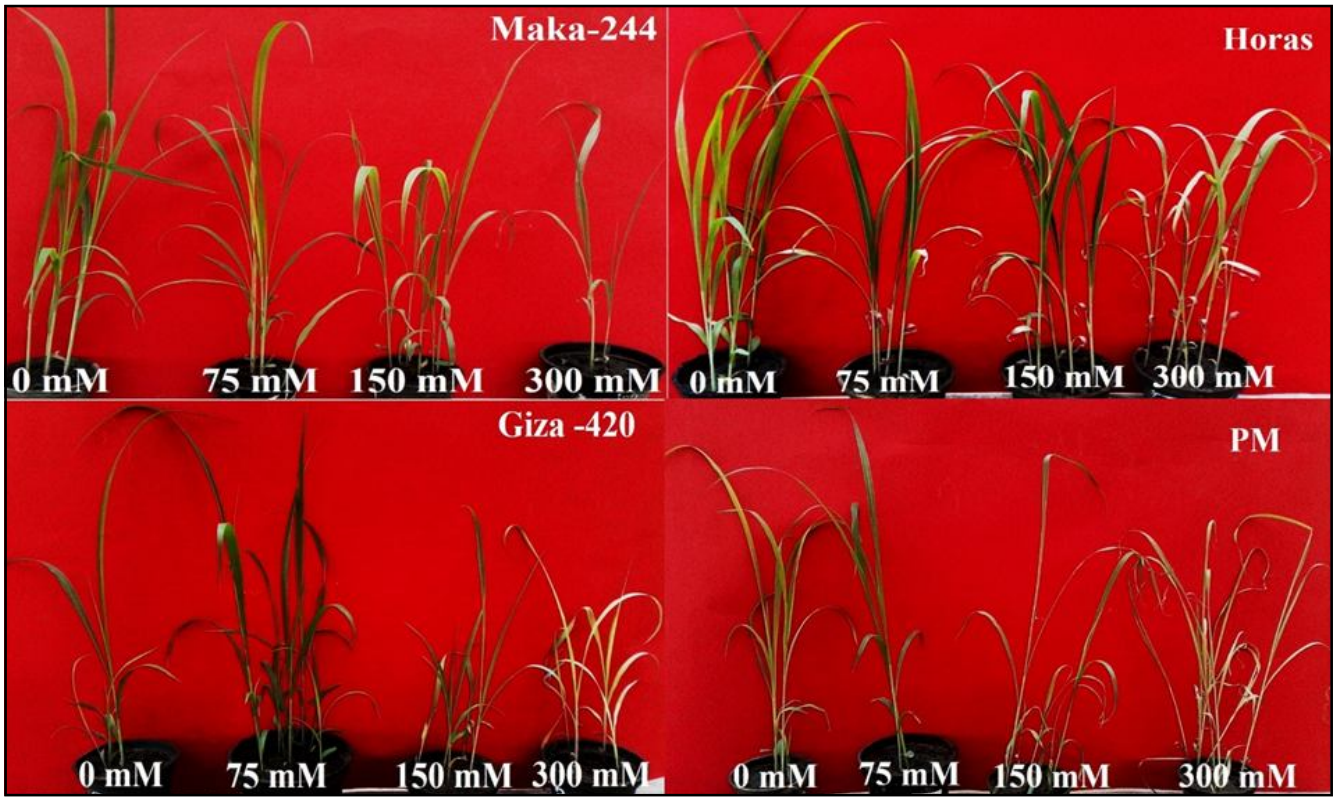

Fig. 1: Effects of salinity on the growth of sorghum plants.

Table 2: The effect of salinity stress on some of the morphological and physiological parameters of sorghum cultivars exposed to different NaCl concentration.

Parameter						
Geno-types	NaCl treat-ment(mM)	Shoot len-gth (cm)	Root len-gth (cm)	F.W (g)	D.W(g) (g)	RW C%
Maka-244	0	53.50±0.67	19.00±0.60	2.28±0.62	0.25±0.08	88.88±0.60
	75	51.33±0.52	15.00±0.04	1.82±0.16	0.23±0.02	87.16±0.57
	150	45.33±0.05	14.00±0.51	1.59±0.26	0.21±0.04	87.09±0.19
	300	40.00±0.50	12.33±0.57	0.98±0.19	0.13±0.04	86.46±0.57
Horas	0	36.83±0.35	23.00±0.60	1.14±0.54	0.12±0.06	89.84±0.28
	75	35.00±0.1	18.00±0.50	0.96±0.41	0.11±0.05	88.5±0.57
	150	34.50±0.52	15.00±0.57	0.86±0.06	0.11±0.01	87.06±0.83
	300	29.50±0.08	12.00±0.57	0.64±0.21	0.09±0.03	86.56±0.60
Giza-420	0	44.33±0.08	18.25±0.22	0.83±0.24	0.11±0.04	86.97±0.37
	75	34.50±0.58	11.00±0.1	0.37±0.21	0.05±0.03	85.82±0.60
	150	33.00±0.21	9.00±0.37	0.30±0.24	0.06±0.03	78.64±0.57
	300	23.00±0.15	7.50±0.52	0.18±0.09	0.05±0.01	70.29±0.69
PM	0	49.00±0.75	22.00±0.55	1.99±0.44	0.22±0.05	88.95±0.32
	75	38.33±0.68	13.67±0.30	0.50±0.02	0.11±0.04	77.2±0.28
	150	29.67±0.16	9.00±0.08	0.21±0.17	0.10±0.05	53.81±0.83
	300	21.00±0.1	6.00±0.5	0.10±0.02	0.07±0.01	34.68±0.69
Special-85	0	40.00±0.75	20.00±0.64	1.26±0.31	0.07±0.03	94.18±0.57
	75	26.33±0.36	10.00±0.04	0.27±0.15	0.07±0.02	73.33±0.37
	150	19.17±0.32	7.83±0.09	0.085±0.07	0.03±0.01	66.59±0.60
	300	15.33±0.57	6.67±0.57	0.069±0.08	0.04±0.01	48.89±0.28
Special-90	0	42.5±0.83	19.00±0.3	1.02±0.30	0.13±0.02	87.63±0.83
	75	27.5±0.69	9.33±0.57	0.26±0.19	0.07±0.04	71.87±0.69
	150	22.00±0.3	9.33±0.57	0.19±0.15	0.06±0.02	68.40±0.37
	300	20.67±0.15	8.17±0.28	0.11±0.00	0.06±0.00	42.21±0.60

Data are mean values of 5 replicates ± SE

gene expression (Freitas *et al.,* 2019).

Betaine aldehyde dehydrogenase1 gene expression level under salt stress conditions

The results of qPCR of the *Betaine aldehyde dehydrogenase1* gene expression manner in different sorghum cultivars which exposed to different treatments of salt revealed that increasing in expression level for some cultivars Fig. 3. Maka-244 and Horas cultivars showed similar expression profile 7.467 and 7.773-fold higher than control at 100 mM NaCl, respectively. While, PM showed 2.124-fold relative to the control. On the other hand, the increasing of salt stress concentration lead to significant induction in Betaine aldehyde dehydrogenase 1 transcription levels, at 150 and 300 Mm NaCl. The increasing in expression of this gene of the Maka-244 cultivar at 150 and 300 mM NaCl were 15.085- and 27.990-fold than control, respectively. Besides, Horas showed 21.561 and 29.241-fold higher than control under 150 and 300 mM NaCl. Nevertheless, the expression profile of this gene under salt stress were not similar in PM cultivar at 150 and 300 mM NaCl that revealed down regulation (0.025 and 0.014-fold compared to control, respectively).

Glutathione S-transferase gene expression level under salt stress conditions

The quantitative expression profile for *glutathione S-transferase* (GST) gene under different salt concentrations are shown in Fig. 4. The GST expression levels of Maka-244 cultivar was increased by 4.153, 11.631 and 21.793-fold higher than control under 75, 150 and 300 mM of NaCl respectively. Whereas, the expression level in Horas cultivar was 4.761, 16.583 and 29.592-fold increase than control plants, respectively. Furthermore, the expression was decreased dramatically for PM which reaching 2.130, 0.095 and 0.003-folds under

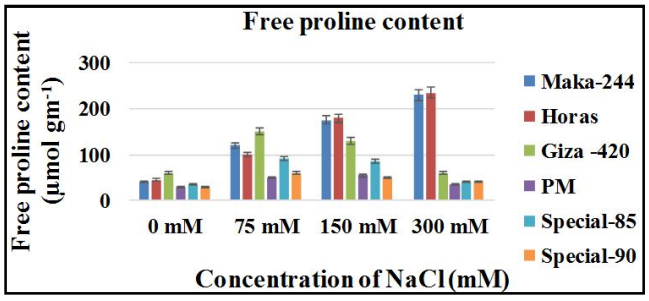

Fig. 2: Histogram representing the means of proline content for six sorghum cultivars and effect of rising Na Cl concentration in the irrigation medium on proline accumulation in leaves. Data are mean values of 5 replicates ±SE.

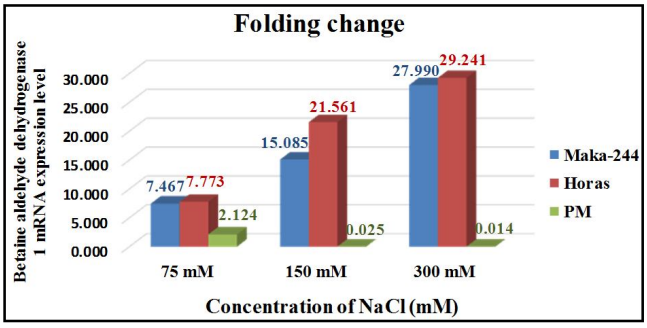

Fig. 3: The effect of the different salt stress concentration (control, 75, 150 and 300 mM NaCl) on the expression level of *Betaine aldehyde dehydrogenase1* gene using qRT-PCR analysis of the sorghum cultivars (Maka-244, Horas and PM).

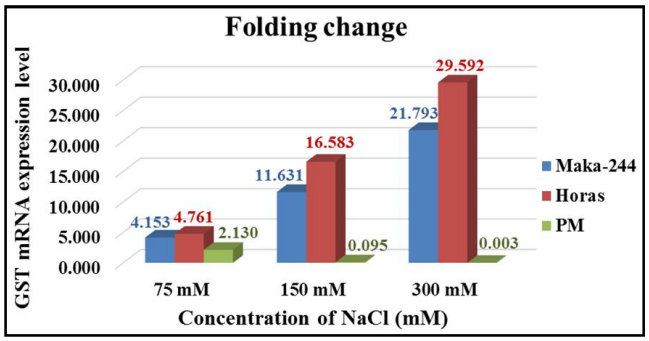

Fig. 4: The effect of the different salt stress concentration (control, 75, 150 and 300 mM NaCl) on the expression level of *glutathione S-transferase* (GST) gene using qRT-PCR analysis of the sorghum cultivars (Maka-244, Horas and PM).

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

Cultivars	NaCl(mM) Treatment	Parameter	
		Ψs (MPa)	OA
Maka-244	0	-1.44	
	75	-2.50	1.07
	150	-6.22	4.78
	300	-8.87	7.44
Horas	0	-2.13	
	75	-4.04	1.91
	150	-6.44	4.31
	300	-9.62	7.48
Giza -420	0	-2.78	
	75	-4.14	1.36
	150	-5.48	2.70
	300	-7.63	4.86
PM	0	-1.78	
	75	-3.72	1.93
	150	-4.66	2.87
	300	-3.02	1.24
Special-85	0	-2.48	
	75	-3.12	0.64
	150	-5.18	2.70
	300	-5.08	2.60
Special-90	0	-1.46	
	75	-2.45	0.99
	150	-4.46	3.00
	300	-4.09	2.63

75, 150 and 300 mM NaCl, respectively.

Discussion

Salt stress is a complex environmental limitation inhibits the growth rate and production of plants because it produces toxic salt ions that directly destroy the plant cells particularly in seedling stage. Therefore, in the present study the salinity tolerance among six sorghum cultivars was evaluated in order to improve crop yields in salt-affected lands. The results indicated that, the FW, DW, shoot and root length and RWC of salt treated plants were decreased with increasing NaCl concentration. The cultivars Maka-244 and Horas were more tolerant than other cultivars at 300mM NaCl. Reduction of these parameters might be due to inhibition of leaf initiation and expansion as well as internode growth and by expedite leaf abscission (Qu *et al.,* 2012). This finding are agreement with the results of the previous studies that proves the harmful effects of salinity on seedling stage and even in case of salt-tolerant plants (Roy *et al.,* 2018; Sarker *et al.,* 2019; Sun *et al.,* 2019 and Yang *et al.,* 2020).

Leaf relative water content is an important indicator of water status in plants; it reflects the balance between water supply to the leaf tissue and transpiration rate. Soltys-Kalina *et al.,* (2016) reported that the plants treated with salt stress suffer from reduction in leaf relative water content leading to overall growth tardiness. The 15-day salt treatment decreased the leaf water content of the six cultivars compared to thir control. Meanwhile, under saline stress, the plants enhanced ionic and osmotic balance and increased compatible solute concentration of cells to maintain the water potential gradients needed to ensure continued absorption of water during the stage of salinity (Alharby *et al.,* 2019). In this experiment, osmotic potential was declined with increasing in concentration of salt stress. In contrast, osmotic adjustment was increased in all treated plants and was greater in Maka-244 and Horas cultivars and lowest in PM cultivar similarly, decrease of osmotic potential and an increase in osmotic adjustment during salt stress have been reported by Turner *et al.,* (2007) in chickpea., Pilon *et al.,* (2018) in cotton and Miranda *et al.,* (2020) in citrus.

Proline is a multi-functional amino acid like maintaining cell membrane safety, stabilizing protein structure and nutrient storage and providing osmoprotectant against environmental stresses (Ghaffari *et al.,* 2019). Hasanuzzaman *et al.,* (2014), Kibria *et al.,* (2016) and Zali and Ehsanzadeh (2018) demonstrated that the biosynthesis of proline in plant tissues is linked with energy storage to reduce the adverse effects of environmental stresses. Hence, any up-regulation in proline contents within the plant can improve stress tolerance which may lead to favor the osmatic adjustments of plant tissues under stress. Therefore, the capacity of proline to accumulate under stress conditions could be a beneficial indicator of plants tolerance (Dien *et al.,* 2019). According to the results obtained ,the concentration of

proline was increased to fivefold with raising salinization level in Maka-244 and Horas cultivars and was raised twofold with increasing the concentration to 150 mM NaCl while was decreased with increasing the concentration to 300 mM NaCl in Giza -420 cultivar. The proline concentration were almost undetectable in other cultivars with raising salinization level. This change in proline concentration in sorghum cultivars might be correlated with its ability to adapt and tolerate to salt stress condition. Where the Maka-244 and Horas cultivars revealed highly tolerant and the Giza -420 cultivar demonstrated moderate tolerant, while PM, Special-85 and Special-90 were the sensitive cultivars. These results confirmed by the results represented in previous reports revealing that total free proline concentration in the leaves are higher in salt tolerant cultivars than in salt sensitive cultivars of tomato (Awaly *et al.,* 2020).

Quantitative PCR is the most efficient for detection gene expression levels due to its simplicity, sensitivity, accuracy and cost (Reddy *et al.,* 2016 and Abd-Rabo *et al.,* 2020 and Awaly *et al.,* 2020). Therefore, the study of salt responsive genes expression patterns under abiotic stress in different cultivars of sorghum will provide a good understanding of their genetic responses. In this work, the effect of salt stress on the expression levels of Betaine aldehyde dehydrogenase1and Glutathione S-transferase genes was detected. The results showed different expression levels of the both genes among the six sorghum cultivars. Betaine aldehyde dehydrogenase1 and Glutathione S-transferase genes were up-regulated in response to75, 150 and 300 mM NaCl treatments in the Maka-244 and Horas cultivars. However, these genes exhibit different expression level for PM cultivar, it was up-regulated at 75 mM NaCl and down- regulated at 150 and 300 mM NaCl concentrations. These findings could be a good indicator of the salt tolerance mechanism present in the studied sorghum cultivars. Determined of the expression levels of antioxidant genes lead to improve salt tolerance of agricultural economic crops (Xu *et al.,* 2015; Sui *et al.,* 2015 and Hadwan, 2018).

Conclusion

There was a difference in responses of different cultivars towards salt stress. NaCl produce a dramatic reduction in morphological and physiological parameters in PM and Special-90 cultivars, whereas, these parameters in Maka-244 and Horas were less affected. These results were harmonic with data of quantitative PCR analysis for target genes. In addition, increasing NaCl concentration resulted in a significant up-regulation of *Betaine aldehyde dehydrogenase1*and *Glutathione S-transferase* genes. Further, the expression level of both genes enhance that Maka-244 and Horas are salt-tolerant cultivars while, PM is salt-sensitive cultivar. These findings could be a good indicator of the salt tolerance mechanism present in the studied sorghum cultivars.

Acknowledgments

The authors express deep sense of gratitude to the staff members of the Genetic Engineering Research Center, Cairo University for all the support, assistance and constant encouragements to carry out this work.

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