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Research Article

Drought and salinity stress response in wheat: physiological and *TaNAC* gene expression analysis in contrasting Egyptian wheat genotypes

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Abstract Drought and salinity are significant stressors for crop plants, including wheat. The relationship between physiological mechanisms and gene expression is important for stress tolerance. NAC transcription factors (TFs) play vital roles in abiotic stress. In this study, we assessed the expression of four *TaNAC* genes with some physiological traits of nine Egyptian wheat genotypes under different concentrations of PEG and NaCl. All the physiological traits that we assessed declined under both stress conditions in all genotypes. In addition, all the genes that we measured were induced under both stress conditions in young leaves. Shandaweel 1, Bani Seuf 7, Sakha 95, and Misr 2 genotypes showed higher gene expression and were linked with a better genotypic performance in physiological traits under both stress conditions. In addition, we found an association between the expression of NAC genes and physiological traits. Overall, NAC genes may act as beneficial markers for selecting for genotypic tolerance to these stress conditions in wheat.

Keywords Wheat, Gene expression, NAC transcription factor, RWC, Drought, Salinity

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Introduction

Wheat (*Triticum aestivum* L.) have a considerable role between crop species grown as staple food sources. Nakashima et al. 2011 stated that abiotic stresses, such as salinity and drought, influence the productivity and growth of plants, including crops. Producing higher-yielding genotypes with good technological quality that are resistant or tolerant to a broad range of biotic and abiotic stresses is a very important target. Tolerance to stress condition defined as an ability of plants to grow in low water potential and in this way, high relative water content (RWC) is one of tolerance mechanisms to stress conditions (Sinclair and Ludlow, 1985). In this regard, (Schonfeld et al. 1988) and (Ghogdi et al. 2012) revealed that RWC decreased by increasing of the drought and salinity levels and it was higher in tolerant wheat cultivars than sensitive ones. Many important leaf traits such as emergence, elongation, expansion, leaf enlargement and stomatal opening are important characteristics that influence plant water relations. Leaf area plasticity is an important strategy to regulate the water use in crops. (Franco et al. 1997) and (Ghaderi et al. 2015) found a reduction in the total leaf area under salinity and drought stress respectively. The lower leaf area ratio of tolerant cultivar is a beneficial morphological adaptation as the reduction in the photoassimilate per unit of plant material (LAR) reduces the water use (Richards 1992). In the same context, (Abbas et al. 2015) found higher leaf area ratio for salt sensitive cultivar than salt tolerant cultivar under drought and salinity stress. On the contrary, the sensitive cultivar to drought showed more decline in leaf emergence and leaf area (Ghaderi et al. 2015). Furthermore, leaf size along with leaf longevity were shrunk as a result of salinity stress (El-Hendawy et al. 2009). In addition, the width and length of wheat leaves reduced approximately by 20 ~ 30% when were exposed to 120 mM NaCl (Hu et al. 2000). Transcription factors (TFs) are the main regulators of different genes

involved in the reduction and/or protection from the stress cellular damage (Agarwal and Jha 2010). TFs play important roles either activating or preventing target gene expression in plants (Puranik et al. 2012; Nakashima et al. 2012). NAC superfamily is one of the largest plant-specific TF families. Primarily, NAC domain originated from the ATAF1, ATAF2, and CUC2 genes in Arabidopsis and NAM (No Apical Meristem) gene in petunia, (Souer et al. 1996; Aida et al. 1997; 1999). (Yamaguchi- Shinozaki et al. 1992) described the first NAC gene was in Arabidopsis (Responsive To Dehydration 26). 117 and 151 genes in Arabidopsis and rice have been identified as NAC family members, respectively (Nuruzzaman et al. 2010). Moreover, NAC TFs Clearly showed a very important role in plant's responses to biotic stress (Kim et al. 2007) and abiotic stresses (Ozhuner et al. 2013). In Arabidopsis *AtNAC2* has many roles in responding to salinity stress (He et al. 2005). Moreover, under low oxygen conditions *At-NAC102* is involved in seed germination regulation (Christianson et al. 2009). In rice, overexpression of *OsNAC5*, *OsNAC9*, and *OsNAC10* increase drought tolerance (Jeong et al. 2010 and 2013; Redillas et al. 2012). Meanwhile, *OsNAC6* and *OsNAC045* overexpression in rice enhances tolerances to drought, salt stresses (Nakashima et al. 2007; Zheng et al. 2009). Furthermore, the expression of *OsNAC78* was specifically induced in a drought tolerant line (Moumeni et al. 2011). Similar observation was noticed from groundnut plants transformed with *MuNAC4* gene of horse gram (Pandurangaiah et al. 2014). In cotton, expression patterns of six NAC members were induced by abiotic stresses (Meng et al. 2009). Expression of *ZmSNAC1* in maize was regulated by many abiotic stresses (Lu et al. 2012). In soybean, Overexpression of *GmNAC20* and *GmNAC11* ameliorate salt tolerance in transgenic Arabidopsis (Hao et al. 2011). However, (Quach et al. 2014) reported that the overexpression of soybean gene *GmNAC004* acts during moderate drought. In wheat, (Xia et al. 2010a and 2010b) revealed that TaNAC8 involved in wheat defense responses to both abiotic and biotic stresses. Also, (Mao et al. 2012), (Huang et al. 2015) and (Xue et al. 2006) reported that TaNAC2, TaNAC29 and TaNAC69 upregulated by various abiotic stresses. On other hand, (Mao et al. 2012 and 2014). showed that overexpression of TaNAC2 and TaNAC67 in transgenic arabidopsis enhanced tolerances to drought, salt and freezing stresses Investigating the molecular responses to drought and salinity stress and their association to physiological indicators may provide important information for evaluating different wheat genotypes under abiotic stresses. In this regard, The target of this study was to investigate the relationships between the expression levels of *TaNAC* genes and some physiological traits to drought and salinity tolerance in some Egyptian wheat genotypes.

Materials and Methods

Plant material

Nine Egyptian wheat cultivars (*Triticum astivum)*, (Giza 168, Misr 2, Shandaweel 1, Misr 1, Sids 12, Misr 3, Sakha 95, Bani Seuf 7 and Sohag 4) were chosen because of their different tolerance for drought and salinity stress.

Growth conditions and stress treatments

Seeds of each genotype disinfected by immersion in Ca $(ClO)_2$ solution containing 5% of chlorine, for 5 min. Seeds were then washed 3 times with sterilized distilled water. Seeds of each genotype were placed on the moist Whatman germination papers in petri dishes to provide appropriate moisture for seed germination. After 3 days germinated seeds were transferred in eight 50×20 mm plastic pot / genotype (10 seeds / pot) in three replications for every stress. All the pots containing sand, soil and peat (1:1:1). All the genotypes were grown under greenhouse conditions. The temperature was 25 ± 2°C, the relative humidity was 50% and a photoperiod of 14 h. Seedlings were watered daily with tap water for 3 weeks. Subsequently, drought and salinity stress treatments were imposed in the fourth week. Eight pots of each wheat genotype in three replications were treated with four treatments for every stress separately at the same time. Drought and salinity treatments were imposed by dissolving (0,5,15,25 % PEG 6000) or (0,50,150 and 250 mm NaCl) in distilled water. Solutions of PEG 6000 were prepared according to weight by volume (Bayoumi et al. 2008). After exposure to treatments for one week, leaves were directly frozen in liquid nitrogen and kept at -80°C for further analysis.

Physiological traits measurements

RWC was estimated in control and stressed seedlings. Fully expanded leaves were excised and fresh weight (FW) was directly recorded; thereafter leaves were soaked for four hours in distilled water at room temperature under a constant light, and the turgid weight (TW) was recorded. After drying for 24 hours at 80°C total dry weight (DW) was recorded. RWC was calculated according to the equation of (Tambussi

Table 1 Primer sequences used for real-time gene expression analysis in this study

	F	R	Reference
TaNAC2a	GGTAGTGCGGTGCTTCCAAT	TGAATGTTGTTGCTCGTCCC	Tang et al. 2012
TaNAC7	ATCGCCAAGCCACCCACAGG	GGAGGGGCCATTGGAGAAGC	Tang et al. 2012
TaNAC69-1	TGCCTCCCGAAAACCCA	TTGTTCACGTAGCCGTTGTTGT	Xue G. et al. 2011
TaNAC69-3	AACAATGGCTACGTGAACATCGA	AAACTGCCGCTGGACCTCTT	Xue G. et al. 2011
TaActin	CTTGTATGCCAGCGGTCGAACA	CTCATAATCAAGGGCCACGTA	Wang et al. 2013

et al. 2005): RWC (%) = [(FW - DW)/ (TW - DW)] x 100. Leaf Area (LA), Leaf length (LL) and Max Leaf wide (MLW) were recorded for the different studied genotypes / treatments with a portable leaf area meter LI-3000C.

Real time PCR analysis

Total RNA was isolated from plant material using Plant RNA reagent (Invitrogen, USA) according to the manufacturer's instructions. cDNA was synthesized using an oligo (dT20) primer from total RNA samples that were pre-treated with RNase- free DNase I (Xue and Loveridge, 2004) and purified through Qiagen RNeasy column (Qiagen, Australia). Transcript levels were quantified by real-time PCR with an ABI Prism 7900 sequence detection system (Applied Biosystems) using SYBR Green PCR Master Mix (Applied Biosystems) according to the manufacturer's instructions. *TaActin* gene used as internal reference genes for calculating relative transcript levels of genes of the interest in each comparative analysis. Sequences and names of primer pairs used are listed in the (Table 1). PCR was performed in three technical replicates. The reaction mixture was contained 2 µl of cDNA sample, 0.6 µl of each forward and reverse primer, 10 µl of SYBR Green and 6.8 µl of PCR grade water. PCR conditions include an initial cycle at 50°C for 2 min, one cycle at 95°C for 10 min, followed by 45 cycles at 95°C for 15 s and at 60°C for 1 min. The quantification of the relative transcript levels was performed using the comparative CT method (Livak and Schmittgen 2001).

Statistical analysis

Drought and salinity experiments were carried out in a random complete block design. All data were represented as ± (SD) of three replicates. One, two-way analysis of variance (ANOVA) was used to test the differences between the means of different variables. If there is significant tukey test was used to detect source of difference. For all statistical tests *P-values* < *0.05* was considered to be statistically significant. Data and statistical analysis were performed using Minitab version 18. The degree of correlation between studied genotypes (two genotypes as a model for tolerant group and another two genotypes as model for sensitive group for every stress) and all studied traits, transcript abundance for studied genes was calculated using Pearson correlation coefficient

Results

Physiological analyses

The stress, genotype and interaction between them had highly significant effects at *P-values* < *0.05* statistical level (Tables 2 and 3). As shown, in (Figs. 1 and 2) all the genotypes contrasting in their response to both stresses. Fig. 1 panels (a) to (d) revealed that Sakha 95 (95.15), Bani Seuf 7 (61.54) and Shandaweel 1 (32.40) were the highest RWC means under 5,15 and 25% of PEG respectively. Meanwhile, Giza 168 (73.75 & 25.57) and Misr 3 (15) had the lowest means of RWC under 5,15 and 25% of PEG. In addition, leaf area of Sakha 95, Bani Seuf 7 and Misr 2 genotypes were recorded the highest means (34.72, 22.05 and 5.27) under 5,15 and 25% of PEG respectively. Meanwhile, Giza 168 had lowest means (12.06 & 2.64) under 5 and 25% of PEG respectively and Sids 12 had lowest means under 15% PEG (10.64) for leaf area. Moreover, the highest means of leaf length under 5,15 and 25% of PEG were (63.87, 56 and 47.13) for Bani Seuf 7, Misr 3 and Bani Seuf 7 respectively. Notably, the lowest means under 5,15 and 25% of PEG were (23.10, 30.70 and 14.10) for Giza 168. For Max leaf wide, Bani Seuf 7 had the highest means under 5 and 15% PEG (1.15 and 1) respectively, while Shandaweel 1 had the highest means under 25% of PEG. Meanwhile, the lowest means under 5,15 and 25% of PEG were (0.60, 0.45 and 0.40) for Misr 3, Sakha 95 and Giza 168, respectively. On the other hand, (Fig. 2) panels (a) to (d) revealed that the mean analyses for most of studied genotypes and traits were decreased significantly under different concentrations of NaCl except Leaf area

Table 2 Analysis of Variance of Physiological traits versus genotypes, drought stress

	df	Leaf area		Leaf length		Leaf wide		RWC	
		F-Value	P-Value	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
Genotype	8	28.89	0.000	43.12	0.000	23.13	0.000	7.42	0.000
Drought	3	294.58	0.000	69.88	0.000	170.87	0.000	996.12	0.000
Genotype* Drought	24	14.66	0.000	15.68	0.000	17.42	0.000	5.80	0.000
Error	72								
Total	107								

P-Value at ($P < 0.05$) according to Tukey's test

Table 3 Analysis of Variance of Physiological traits versus genotypes, salinity stress

Source	df	Leaf area		Leaf length		Leaf wide		RWC	
		F-Value	P-Value	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
Genotype	8	27.64	0.000	21.25	0.000	1.21	0.304	4.05	0.001
Salinity	3	27.17	0.000	23.75	0.000	1.57	0.204	61.31	0.000
Genotype* Salinity	24	3.45	0.000	5.64	0.000	1.77	0.034	6.64	0.000
Error	72								
Total	107								

P-Value at ($P < 0.05$) according to Tukey's test

Table 4 Analysis of Variance of gene expression versus genotypes, drought stress

Source	df	*TaNAC2a*		*TaNAC7*		*TaNAC69-1*		*TaNAC69-3*	
		F-Value	P-Value	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
Genotype	8	122.12	0.000	14.78	0.000	39.53	0.000	13.93	0.000
Drought	3	123.38	0.000	196.3	0.000	313.94	0.000	53.84	0.000
Genotype* Drought	24	33.31	0.000	21.42	0.000	48.97	0.000	15.87	0.000
Error	72								
Total	107								

P-Value at ($P < 0.05$) according to Tukey's test

for (Bani Seuf 7, Misr 1 and Sids12), Leaf length for (Bani Seuf 7) and Max leaf wide for (Giza168, Misr 2, Shandaweel 1and Sids 12). The highest means under 50,150 and 250 mM NaCl for RWC were 97, 97 and 89.8 for Sohag 4, Bani Seuf 7 and Shandaweel 1 respectively. Meanwhile, Bani Seuf 7, Misr1 and Giza 168 had the lowest means 87.1, 62.1 and 50.9 under 50,150 and 250 mM NaCl respectively. Leaf area for genotypes Misr 2, Misr 2 and Sakha 95 were recorded the highest means 34.7, 26.3 and 27.7 under 50,150 and 250 mM NaCl respectively. Meanwhile, Sids 12 under 50 &150 mM NaCl (9.7,13.7) and Bani Seuf 7 under 250 mM NaCl (12.1) were the lowest means. In addition, the highest leaf length means under 50,150 and 250 mM NaCl were (51.1, 44.1 and 40.1) for Sohag 4, Misr2 and Misr 1 respectively, while the lowest means were (20.3 & 19.6) under 50 and 150 mM NaCl for Sids 12 and (21.4) under 250 mM NaCl for Giza 168. For Max leaf wide, Misr 2, Misr 2 and Bani Seuf 7 genotypes had the highest means (1.5, 1.2 and 1.3) under 50, 150 and 250 mM NaCl. Meanwhile, the lowest means under 50,150 and 250mM NaCl were (0.7, 0.6 and 0.6) for Sids 12, Misr1 and Misr 3, respectively.

Gene expression analyses

With a view to study the relationships between gene expression levels and physiological traits for the studied genotypes, four abiotic stress responsive genes, *TaNAC2a, TaNAC7, TaNAC69-1* and *TaNAC69-3* were selected to determinate their expression levels under drought and/or salinity conditions. Stress, genotype and interaction had highly significant effects at *P-values < 0.05* statistical level (Tables 4 and 5).

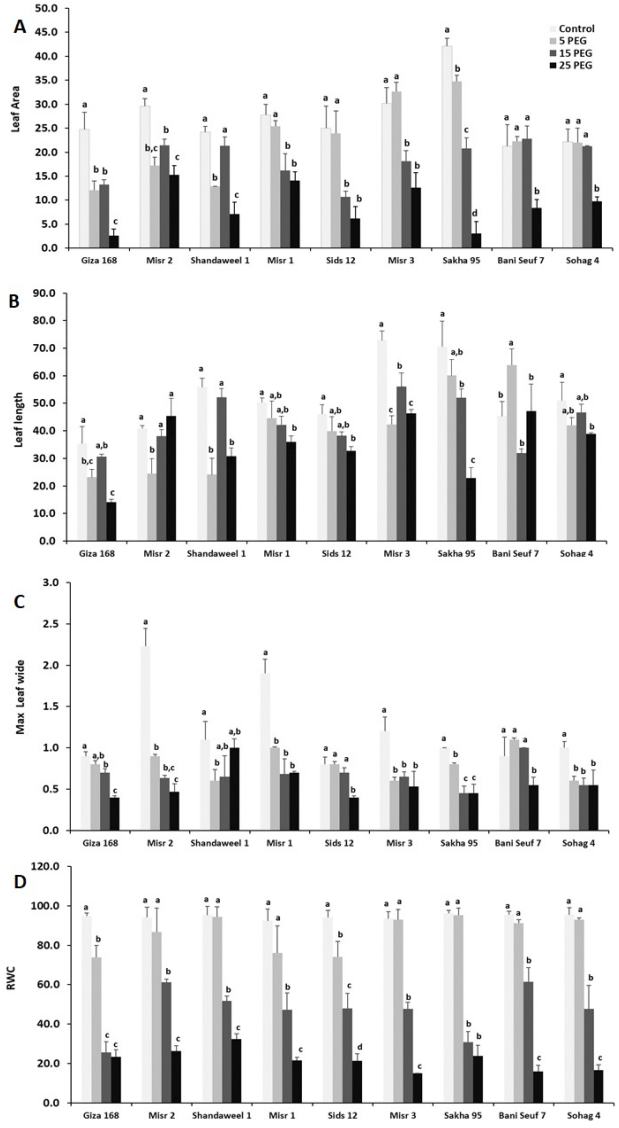

Fig. 1 Comparison of the physiological traits of the studied genotypes under different concentrations of PEG. (A) Leaf Area; (B) Leaf Length; (C) Max Leaf Width; (D) RWC. Bars represent standard deviation. Columns with different lower case letters indicate significant difference at $P < 0.05$ (Tukey test). All bars represent the mean of three replications

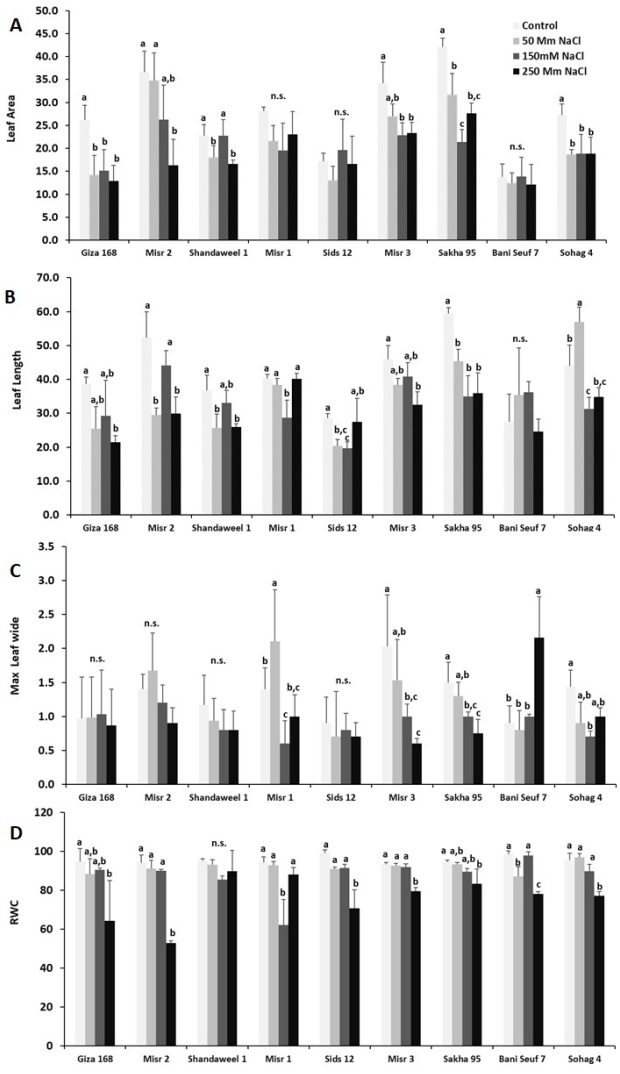

Fig. 2 Comparison of the physiological traits of the studied genotypes under different concentrations of NaCl. (A) Leaf Area; (B) Leaf Length; (C) Max Leaf Width; (D) RWC. Bars represent standard deviation. Columns with different lower case letters indicate significant difference at $P < 0.05$ (Tukey test). All bars represent the mean of three replications

Table 5 Analysis of Variance of gene expression versus genotypes, salinity stress

Source	df	*TaNAC2a*		*TaNAC7*		*TaNAC69-1*		*TaNAC69-3*	
		F-Value	P-Value	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
Genotype	8	25.13	0.000	21.78	0.000	56.96	0.000	46.86	0.000
Salinity	3	221.81	0.000	190.73	0.000	77.07	0.000	95.35	0.000
Genotype* Salinity	24	25.42	0.000	17.40	0.000	23.38	0.000	15.05	0.000
Error	72								
Total	107								

P-Value at ($P < 0.05$) according to Tukey's test

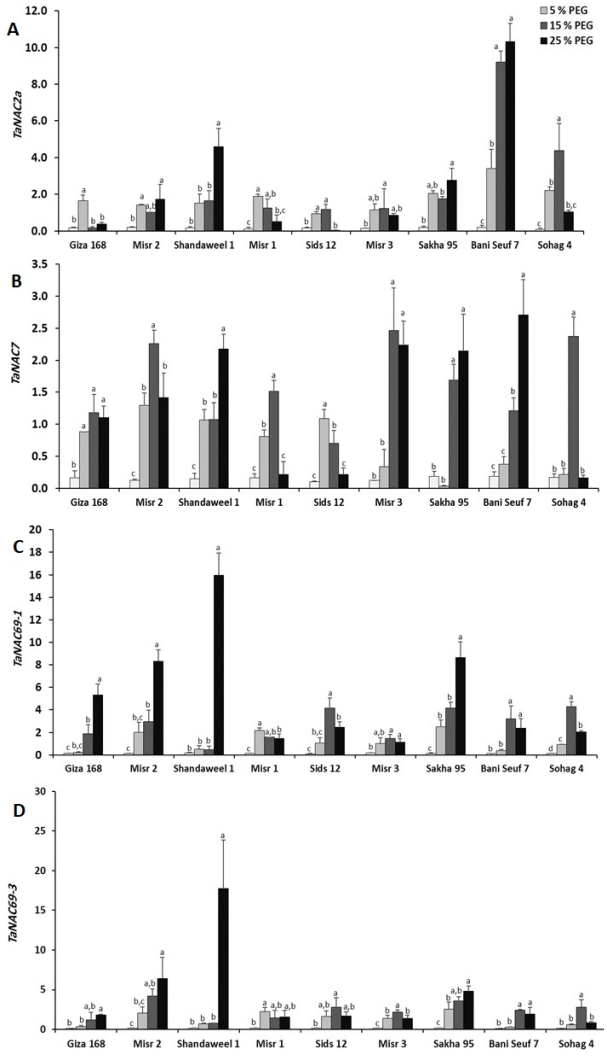

Fig. 3 Gene expression of (A) *TaNAC2a*, (B) *TaNAC7*, (C) *TaNAC69-1*, and (D) *TaNAC69-3*. Data represent the mean fold change in relative expression versus the control sample, normalized to the reference gene, *TaActin*. Genotype expression under different concentrations of PEG was measured in three replications. Bars represent standard deviation. Columns with different lower case letters indicate significant difference at $P < 0.05$ (Tukey test)

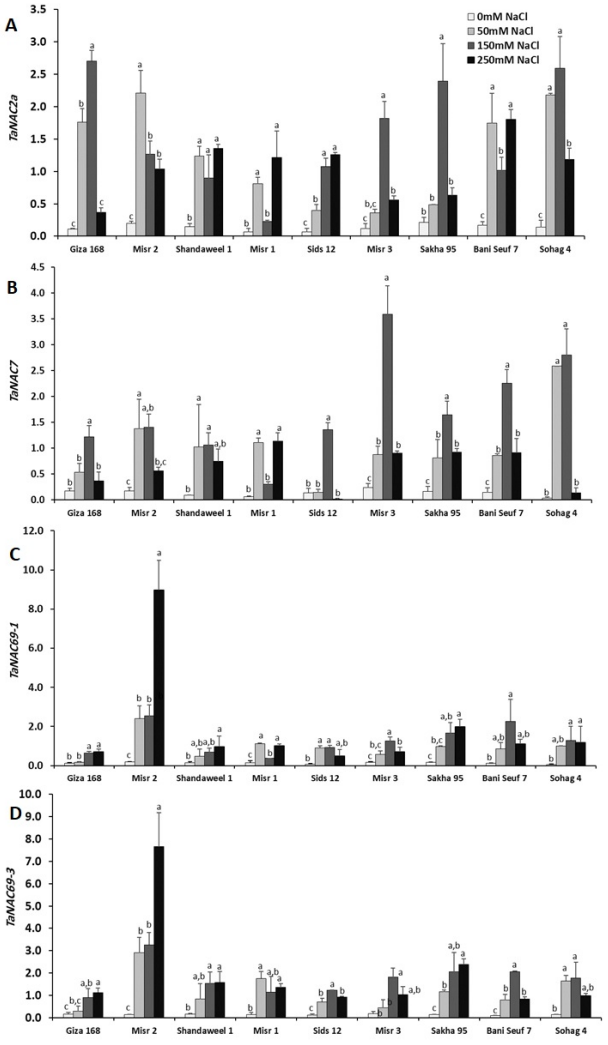

Fig. 4 Gene expression of (A) *TaNAC2a*, (B) *TaNAC7*, (C) *TaNAC69-1*, and (D) *TaNAC69-3*. Data represent the mean fold change in relative expression compared with the control samples, normalized to the reference gene, *TaActin*. Genotype expression under different concentrations of NaCl was measured in three replications. Bars represent standard deviation. Columns with different lower case letters indicate significant difference at $P < 0.05$ (Tukey test)

As it is evident, in (Fig. 3) panels (a) to (b) we observed that Bani Seuf 7 and Shandaweel 1 recorded the highest expression pattern and it increased by 3, 9 and 18 fold comparing with the untreated control under 5,15 and 25% PEG, respectively, while Sids 12 had the lowest expression under 15 & 25% of PEG and Sohag 4 under 5% PEG. Undoubtedly, in (Fig. 4) panels (a) to (b) Misr 2 genotype showed the highest expression pattern and it increased by 3, 3.5 and 9 fold compared with the untreated control under 50,150 and 250 mM NaCl, respectively. While, the expression of Sids 12 was the lowest under 50 & 250 mM NaCl and Misr 1 under 50 mM NaCl. Briefly, the relative expression of Shandaweel 1 and Misr2 was sharply increased and recorded the highest expression under 25% PEG and 250 mM NaCl respectively. Moreover, Sids 12 recorded the lowest expression under 25% PEG and 250 mM NaCl. As shown in (Figs. 3 and 4) no significant expression of any studied genes in control seedlings. The highest expression levels between all studied genes was under 25 % PEG and 250 mM NaCl except for *TaNAC2a* and *TaNAC7* under 150 mM NaCl. Under PEG stress the highest expression means were 10, 3, 16 and 18 times more than the control for *TaNAC2a*, *TaNAC7*, *TaNAC69-1* and *TaNAC69-3* respectively. Meanwhile, under NaCl the highest expression means were 3,3,9 and 8 times more

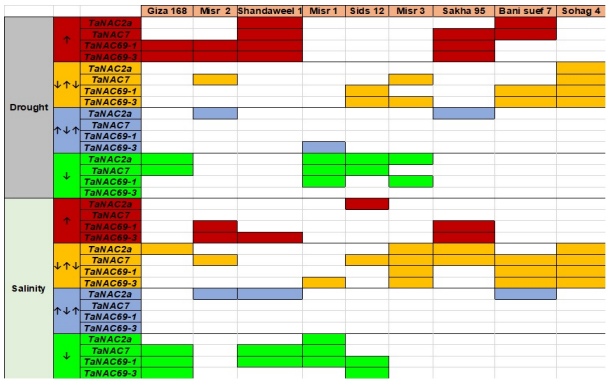

Fig. 5 Different expression patterns for all studied genes and genotypes. The first, second, third, and fourth patterns are colored with red, orange, blue, and green, respectively

than the control for *TaNAC2a*, *TaNAC7*, *TaNAC69-1* and *TaNAC69-3* respectively. In addition, under drought stress conditions all studied genes were expressed higher than under salinity stress for all the studied concentrations except *TaNAC7* was opposite. Notably, under both stresses the expression levels of *TaNAC69-1* and *TaNAC69-3* were very seems each other and had the highest fold change comparing with the control especially under the highest concentrations followed by the expression levels of *TaNAC2a*, *TaNAC7* under PEG stress and *TaNAC7*, *TaNAC2a* under NaCl stress. From (Fig. 5) we concluded that there are four different expression patterns of the studied genes /genotypes. First pattern includes all the genotypes that its level of mRNA was increased and the expression of all the studied genes upregulated under 5,15 and 25% of PEG or 50,150 and 250 mM of NaCl. In drought experiment expression of Shandaweel 1 for all the studied genes and Sakha 95 for all the studied genes except *TaNAC2a* belongs to this pattern of expression. In addition, in salinity experiment, expression of Misr 2 and Sakha 95 for *TaNAC69-1* and *TaNAC69-3* genes belongs to this pattern of expression. Second pattern includes all the genotypes that its level of mRNA was decreased after treated with 5% PEG or 50 mM NaCl and then increased after treated with 15% PEG or 150 mM NaCl and finally its expression decreased after treated with 25% PEG or 250 mM of NaCl. For example, in drought experiment expression, of Sohag 4 for all the studied genes belongs to this pattern of expression. Moreover, in salinity experiment, expression of Sohag 4 and Misr 3 for all the studied genes belongs to this pattern of expression. Third pattern includes all the genotypes that its level of mRNA was increased after treated with 5% PEG or 50 mM NaCl and then decreased after treated with 15% PEG or 150 mM NaCl and finally its expression increased after treated with 25% PEG or 250 mM of NaCl. In this regard, in drought experiment the expression of Misr 2 and Sakha 95 for just *TaNAC2a* gene are belongs for this pattern. In the same time, in salinity experiment, expression of Misr 2, Shandaweel 1 and Bani Seuf 7 for just *TaNAC2a* gene are belongs for this pattern. Finally, fourth pattern includes all the genotypes that its level of mRNA was decreased and the expression of all the studied genes downregulated under 5,15 and 25% of PEG or 50,150 and 250 mM of NaCl. In this context, under drought conditions, expression of Misr 1 for all studied genes except *TaNAC69-3* and expression of Misr 3 for *TaNAC69-1* and *TaNAC2a* are examples for this pattern. On other hand, in salinity experiment, expression of Giza 168 and Misr 1 for all the studied genes except *TaNAC2a* and *TaNAC69-3* respectively, are examples for this pattern.

Correlation analysis

In this study, an existent correlation was elucidated between studied genes expression and various abiotic stress responsive, physiological parameters for tolerant and sensitive genotypes. In (Fig. 6) (a) we observed that Shandaweel 1 represented high positive correlation with all studied genes under different concentrations of PEG and the highest correlation was with *TaNAC2a* (0.873). Meanwhile, (Fig. 6) (b) showed that Sids 12 had positive correlation with all studied genes and the highest correlation was with *TaNAC69-1* (0.502). On other hand, (Fig. 7) (a) revealed that Sakha 95 recorded high positive correlation with all studied genes under different concentrations of NaCl and the highest correlation was with *TaNAC69-1* (0.913). Moreover, Giza168 showed positive correlation with all studied genes and the highest correlation was with *TaNAC69-1* (0.959) (Fig. 7) (b). On other hand, in (Table 6) Bani Seuf 7 showed significant negative correlation among expression levels of all studied genes and physiological traits under drought stress. The highest correlation was between *TaNAC7*and RWC (-0.958). Moreover, the four studied genes had a positive correlation between each other, and the higher correlation was between *TaNAC69-1* and *TaNAC69-3* (0.942). Also, the correlation between all studied traits was positive. The highest correlation was between leaf area and leaf wide (0.922). On other hand, in (Table 7) Misr 2 showed significant negative correlation among the expression of all studied genes and physiological traits under salinity stress. The highest correlation was between *TaNAC69-1* and RWC (-0.945). Moreover, positive correlation was found between all studied genes and the highest correlation was between *TaNAC69-1* and *TaNAC69-3* (0.916). Also, the correlation between all studied traits were

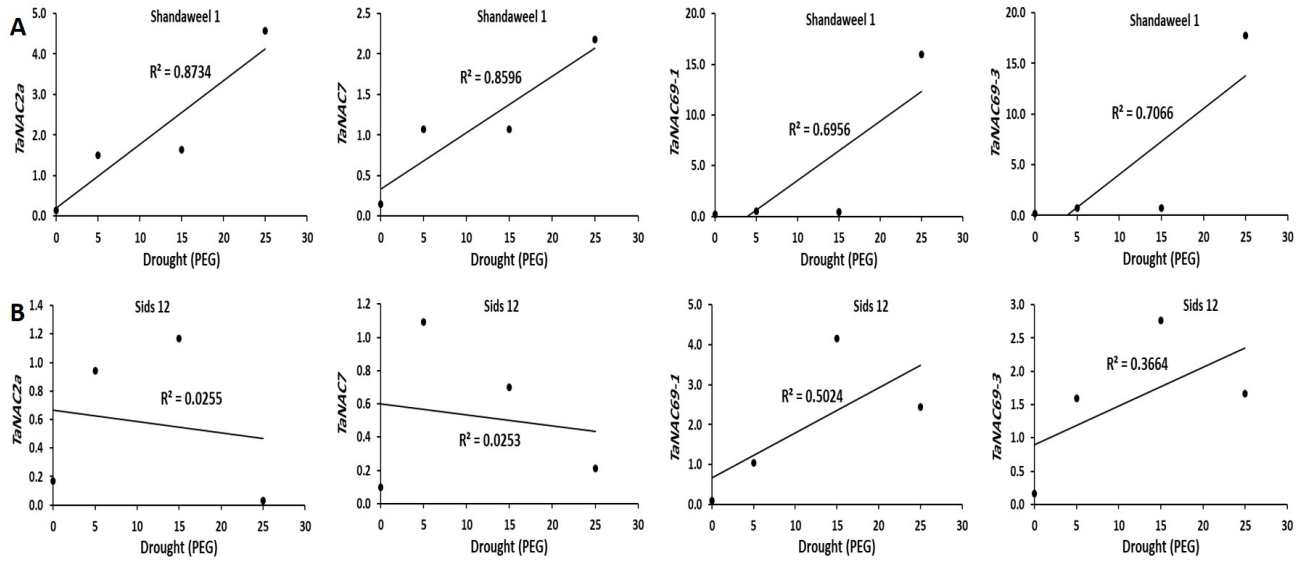

Fig. 6 Correlation coefficient analysis between the relative expression of studied genes and drought stress. (A) shandaweel 1 (tolerant genotype); (B) Sids 12 (sensitive genotype)

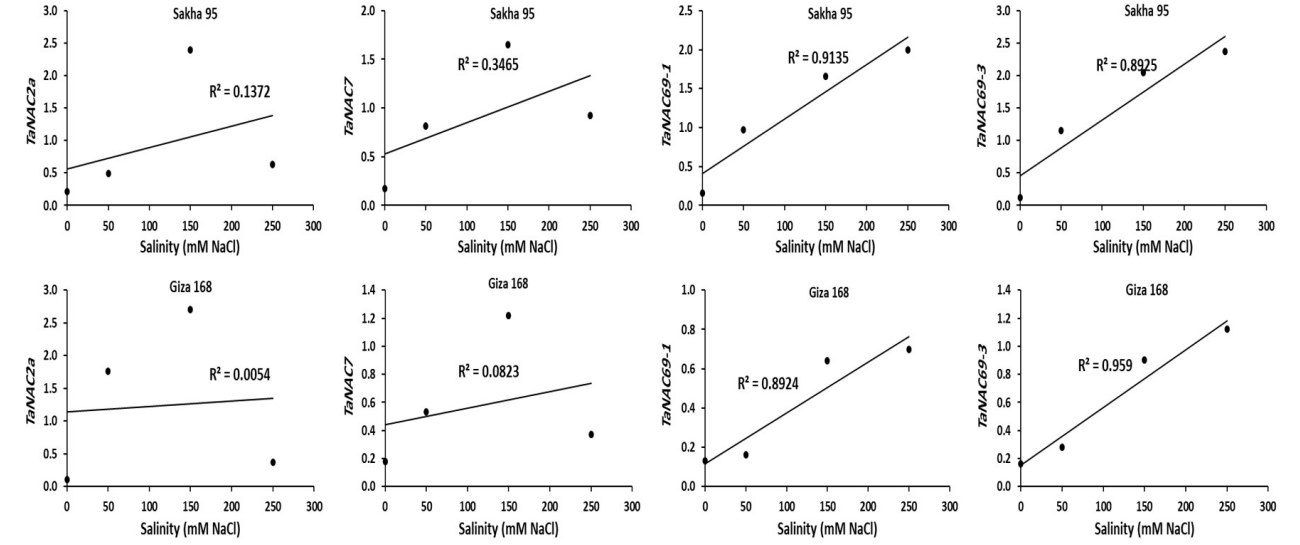

Fig. 7 Correlation coefficient analysis between the relative expression of studied genes and salinity stress. (A) Shakha 95 (tolerant genotype); (B) Giza 168 (sensitive genotype)

positive. The highest correlation was between leaf area and RWC (0.800).

Discussion

Plants undergo variety of changes from physiological adaptation to gene expression after exposure to abiotic stress, (Shinozaki et al. 2007). To react with these abiotic stresses, plants have developed many strategies enabling them to integrate activities at the whole-plant level. These strategies may involve avoidance and/or development of tolerance mechanisms. According to (Dencic et al. 2000), wheat is paid special attention due to its morphological traits during drought and salinity stress, including leaf (shape, expansion, area, size, senescence and waxiness). In this study, four leaf physiological parameters (RWC, LA, LL and MLW) were estimated for stressed and unstressed seedlings. A noticeable significant decline in all studied physiological traits under both stresses for most of the studied genotypes as shown in (Figs. 1 and 2). Accordingly, we classified the studied genotypes into three groups based on their stress tolerance ability. Under drought stress (Bani Seuf 7, Shandaweel 1 and Sakha 95) were tolerant

Table 6 Correlation coefficients analysis between relative expression of studied genes and physiological traits of Bani suef 7 genotype under drought stress condition

	TaNAC2a	*TaNAC7*	*TaNAC69-1*	*TaNAC69-3*	Leaf Area	Leaf Length	Leaf wide
TaNAC7	0.850						
	0.000						
TaNAC69-1	0.822	0.664					
	0.001	0.019					
TaNAC69-3	0.889	0.741	0.942				
	0.000	0.006	0.000				
Leaf Area	-0.513	-0.825	-0.236	-0.298			
	0.088	0.001	0.460	0.346			
Leaf Length	-0.434	-0.211	-0.551	-0.601	0.094		
	0.159	0.510	0.063	0.039	0.771		
Leaf wide	-0.443	-0.766	-0.310	-0.357	0.922	0.101	
	0.149	0.004	0.326	0.255	0.000	0.755	
RWC	-0.873	-0.958	-0.636	-0.711	0.808	0.282	0.752
	0.000	0.000	0.026	0.010	0.001	0.374	0.005

Every cell represents values of (Pearson correlation & P-Value at ($P < 0.05$)

Table 7 Correlation coefficients analysis between relative expression of studied genes and physiological traits of Misr2 genotype under salinity stress condition

	TaNAC2a	*TaNAC7*	*TaNAC69-1*	*TaNAC69-3*	Leaf Area	Leaf Length	Leaf wide
TaNAC7	0.803						
	0.002						
TaNAC69-1	0.119	0.042					
	0.713	0.897					
TaNAC69-3	0.262	0.130	0.916				
	0.410	0.687	0.000				
Leaf Area	-0.004	-0.011	-0.836	-0.775			
	0.989	0.972	0.001	0.003			
Leaf Length	-0.672	-0.336	-0.647	-0.628	0.411		
	0.017	0.286	0.023	0.029	0.185		
Leaf wide	-0.256	-0.193	-0.612	-0.504	0.779	0.236	
	0.423	0.548	0.035	0.095	0.003	0.461	
RWC	-0.041	-0.257	-0.945	-0.887	0.800	0.511	0.572
	0.898	0.420	0.000	0.000	0.002	0.090	0.052

Every cell represents values of (Pearson correlation & P-Value at ($P < 0.05$)

genotypes, (Misr 2, Sohag 4 and Misr 1) were moderate genotypes, while (Giza 168, Misr 3 and Sids 12) were sensitive genotypes. In this regard, (Mickky et al. 2017) studied some morphological traits on wheat seedlings after exposing wheat seedlings to PEG treatments and Sids 13 seemed to be the most tolerant variety followed by Masr 1, Masr 2, Gimmaza 9, Gimmaza 11, Sids 12, Sakha 93, Sakha 94, and Giza 186 and finally came Shandawel 1 with the maximum sensitivity. On the other hand, our results showed that under salinity stress, the tolerant genotypes included (Misr 2, Bani Seuf 7 and Sohag 4), the moderate genotypes included (Shandaweel 1and Sakha 95), and the sensitive genotypes included (Sids 12, Giza 168, Misr 1 and Misr 3). In this context, (Hamam et al. 2014) found that Giza 168, Sids 12 were more moderated to salinity at early growth stages. Interestingly, we found some genotypes

that could be able to tolerate both drought and salinity at the same time such as (Bani Seuf 7) and other genotypes can be sensitive to drought and salinity as (Sids 12, Giza 168, Misr 3). Moreover, we observed that the tolerant genotypes under both stresses had the highest means for all studied traits suggested that these genotypes have avoided osmotic stress resulted from both stresses. Furthermore, the sensitive genotypes recorded the lowest means for all studied traits, which may indicate that those genotypes showed a lower capacity to accord with stress conditions. In the same context, (Eftekhari et al. 2017; Kamoshita et al. 2000; Schonfeld et al. 1988) revealed that RWC for tolerant cultivar retain major amount of water than the non-tolerant and RWC reduced significantly under drought stress conditions. Under drought conditions, the decreasing in RWC fundamentally related with the capacity of more tolerant genotypes to better absorb soil, water and to prevent water loss through stomata (Keyvan et al. 2010). In the same direction, the decrease in RWC under salinity conditions in wheat genotypes was reported by (Ghogdi et al. 2012; Farooq and Azam 2006; Ouhaddach et al. 2018; Sairam et al. 2002). Similarly, our results are in agreement with previous data by (Rizza et al.2004; Rucker 1995 et al.; Dalirie et al. 2010) and (Franco et al. 1997; Ouhaddach et al. 2018) that showed low increase in leaf area under drought and salinity stress respectively. Overall, these results suggest that RWC is a relevant tool for screening drought tolerance. These findings were in accordance with the previous results by (Teulat et al. 2003). Moreover, (Chaves et al. 2009) stated that plants adapted to drought and salinity by inhibition of leaf growth, as a consequence, leaf area reduced that allows plants to cut water losses by lowering transpiration and delaying the onset of more severe stress. On other hand, (Anjum et al. 2016) reported that any abiotic stress decrease leaf size. (Passioura et al. 1996; Shao et al. 2008) confirmed that leaf extension, leaf size and longevity can be limited under water stress respectively. Meanwhile, (Hu and Schmidhalter 2000 and 2001; Neumann 1993) concluded that under salt stress, leaf length, leaf width and leaf extensibility were decreased. On the contrary, (Lonbani and Arzani 2011) stated that wheat flag leaf length and area increased while the flag leaf width did not change under drought stress. Notably, the highest reduction percentage under both stresses was under 25% PEG or 250 mM NaCl. These findings were in accordance with (Nayer et al. 2012) for leaf growth and RWC under salinity stress. In addition, the reduction percentage for all studied traits under drought stress was higher than under salinity stress except in leaf length parameter which gave opposite results. This proposed that ater flow and bio-synthetic activity in the growing tissues might be more inhibited by drought more than by salinity. Consequently, using the above mentioned physiological parameters were very promising for screening drought and salt tolerant wheat genotypes. In wheat, many NAC genes have been isolated, and various NACs displayed various expression patterns or played different roles in response to environmental stimuli (Xia et al. 2010a and b; Baloglu et al. 2012). In this research, we focused fundamentally on evaluating leaves expression because (He et al. 2005; Mitsuda et al. 2007) confirmed that this tissue specifically expressed TFs that played critical roles in plant development and growth. We found that *TaNAC* genes were induced simultaneously by two stress treatments in young leaves, which was consistent with the results of NACs in Arabidopsis, rice and soybean (Fujita et al. 2004; Hu et al. 2006; Nakashima et al. 2007; Hu et al. 2008; Tran et al. 2009). In accordance with these results (Tang et al. 2012) revealed that *TaNAC2a*, *TaNAC7* and *TaNAC4a* had a higher expression level in leaves than in stems and roots under dehydration and salinity stresses. Moreover, senescent leaves had higher expression of *TaNAC6, TaNAC13* and *TaNAC29* than in young leaves under NaCl, PEG6000, treatments, proposed that their functions might be correlated with leaf senescence (Huang et al. 2015). Our results showed that the relative expression for studied genes was higher under drought stress than salinity stress in all the studied concentrations except *TaNAC7* showing an opposite expression tendency under both stresses, so this requires further scrutiny and investigations (Figs. 3 and 4). This indicated that drought-tolerant wheat genotypes counter the damage caused by drought stress more effectively. Consequently, According to gene expression results, we divided all studied genotypes into three groups. The first group includes tolerant genotypes (Bani Seuf 7, Shandaweel 1, Sakha 95 and Misr2) and (Misr 2, Sakha95 and Bani Seuf 7) under drought and salinity stress, respectively. The Second group includes moderate genotypes (Sohag4 and Misr1) and (Bani Seuf 7, Sohag4, Misr3, Shandaweel 1and Sids 12) under drought and salinity stresses, respectively. The third group includes sensitive genotypes (Sids12, Giza168 and Misr3) and (Giza168 and Misr 1) for drought and salinity stress, respectively. Moreover, our results showed that, *TaNAC69-1* and *TaNAC69-3* genes had the highest expression fold change vs. controls. These results were congruence in line with (Xue et al. 2006 and 2011) under drought and salinity stresses. Also, the expression of *TaNAC69-1* and *TaNAC69-3* were they look very similar in both

stresses. This similarity comes from the amino acid sequence alignment of the three *TaNAC69* genes share amino acids with identity >95%. (Xue et al. 2006). Because of sharp expression increase of Shandaweel 1 and Bani Seuf 7 in all Studied genes except *TaNAC7* and the expression of Misr 2 for *TaNAC69-1* and *TaNAC69-3* under NaCl stress we confirmed the role of these genes in the protective mechanisms activated by plants in response to drought and salinity stresses. Simultaneously, our results considered Giza 168 as sensitive genotype, but interestingly the expression of *TaNAC69-1* and *TaNAC69-3* were upregulated, which suggesting that the transcription levels of these genes do not correlated with adaptation response of the plant, but it reflected the failure of sensitive cultivars to cope the stress conditions. These results were in agreement with (Yousfi et al. 2016). From the results shown in (Fig. 5) we concluded high variability in the expression of NAC gene family in the studied genotypes, this confirming the high complication of the mechanisms that regulate the expression of these genes. Each gene might have a different function in the molecular plant response to salinity and drought stresses. This characteristic has also been stated by (Tang et al. 2012) whereas the mRNA levels of (*TaNAC7* & *TaNAC13*) and (*TaNAC4a* & *TaNAC6*) showed similar expressions under various stresses, while others showed different expression levels under various treatments, such as *TaNAC2a* under salt and dehydration treatments, *TaNTL5* and *TaNAC4a* under cold and drought treatments. Differently, (Zhou et al. 2008) showed that under high salinity there is a significant increase of mRNA levels of many dehydration-inducible NAC genes. Overall, from (Fig. 5) we suggested that all the genotypes in first, second and third patterns can be considered as tolerant and moderate tolerant to drought and salinity stresses. But the genotypes in fourth pattern considered to be sensitive genotypes reflecting the weakness of metabolic functioning of the plant. These findings in accordance with (Yousfi et al. 2016). In the present investigation, there were significant positive correlations at ($p<0.05$) between the expression levels of tolerant and sensitive genotypes under drought and salinity stresses (Figs. 6 and 7). The results confirm that the tolerant genotypes highly correlated with the high expression of studied genes under both stresses. In the same time, the sensitive genotypes represent low correlation with the gene expression. Contrary, (Niazi et al. 2014) revealed that no significant correlation between GSTF1 expression profiles at 50, 100, and 200 mM NaCl concentrations and 100 mM ABA treatment in the cultivars. Interestingly, in this paper we have analyzed the relationship between the *TaNAC* expression profiles and the physiological data (Tables 6 and 7). However, little is known about gene expression and its morphophysiological consequences during abiotic stress such as drought and salinity. Our results showed significant correlations between all the studied physiological traits and the expression levels. The highest correlation was between *TaNAC7* and RWC (-0.958) and between leaf area and wide (0.922) for Bani Suef 7 genotype under drought stress. Moreover, for Misr 2 genotype under salinity the highest correlation was between *TaNAC69-1* and RWC (-0.945) and between leaf area and RWC (0.800). In addition, the high gene expression similarity between *TaNAC69-1* and *TaNAC69-3* confirmed by significant positive correlation between each other.

Conclusions

This study examines the potential role of four *TaNAC* genes as markers of the genotypic performance in nine Egyptian bread wheat genotypes under drought and salinity stress by qPCR. Physiological traits including, RWC, Leaf Area, Leaf length and Max Leaf wide were analyzed. Overall, we observed significantly drastic decrease in all the studied physiological traits during both stresses for all the genotypes. In addition, high differential expression patterns in all studied genes were noted in studied genotypes under both stresses. Importantly, according to our results we concluded that genotypes (Bani Seuf 7 & Shandawee l) and (Giza 168 & Sids 12) were tolerant and sensitive genotypes under PEG stress respectively. Moreover, (Misr 2 & Bani Seuf 7) and (Giza 168 & Misr 1) were tolerant and sensitive genotypes under NaCl stress respectively. Also, the relative gene expression showed significant correlation with studied physiological parameters. Collectively, our results support that *TaNAC* genes involved in diverse roles during stress regulation and wheat development and can be transferred in high-yield wheat genotypes to enhance drought and salinity tolerance. So, it can be said that selected genes of NAC TF described in this paper have a role in gene expression regulation in leaves under drought and salinity stress and can be transferred in high-yield wheat genotypes to enhance drought and salinity tolerance. Such observations make them good indicators of different behavior within the genotypes identified as ‘resistant’ and ‘sensitive’. Moreover, same conclusion for the studied physiological traits of the different genotypes.

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References

Abbas Z K, Mobin M (2015) Comparative growth and physiological responses of two wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance to salinity and cyclic drought stress. Archives of Agronomy and Soil Science, DOI: 10.1080/03650340.2015.108355

Agarwal PK, and Jha B (2010) Transcription factors in plants and ABA dependent and independent abiotic stress signaling. Biol. Plant. 54:201-212. doi: 10.1007/s10535-010-0038-7

Aida M, Ishida T, Fukaki H, Fujisawa H and Tasaka M (1997) Genes involved in organ separation in Arabidopsis: an analysis of the cupshaped cotyledon mutant. Plant Cell 9:841-857.

Aida M, Ishida T, and Tasaka M (1999) Shoot apical meristem and cotyledon formation during Arabidopsis embryogenesis: interaction among the CUP-SHAPED COTYLEDON and SHOOT MERISTEMLESS genes. Development 126:1563-1570

Anjum SA, Jian-hang N, Ran W, Jin-huan L, Mei-ru L, Ji-xuan S, Jun L, Zohaib A, San-gen W, Xue-feng Z (2016) Regulation mechanism of exogenous 5-aminolevulinic acid on growth and physiological characters of *Leymus chinensis* (Trin.) under high temperature stress. The Philippine Agricultural Scientist 99(3):253-259

Bayoumi TY, Eid MH and Metwali EM (2008) Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. African J. Biotechnol 7:2341-2352

Baloglu MC, Oz MT, Oktem HA, Yucel M (2012) Expression analysis of *TaNAC69-1* and *TtNAMB-2*, wheat NAC family transcription factor genes under abiotic stress conditions in durum wheat (*Triticum turgidum*). Plant Mol Biol Rep 30:1246-1252

Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. Annals of Botany 103:551-560

Christianson JA, Wilson IW, Llewellyn DJ, Dennis ES (2009) The low-oxygen induced NAC domain transcription factor *ANAC102* affects viability of Arabidopsis seeds following low-oxygen treatment. Plant Physiol 149:1724-1738

Dalirie, MS, Sharif, RS, Farzaneh, S (2010) Evaluation of yield, dry matter accumulation and leaf area index in wheat genotypes as affected by terminal drought stress. Not. Bot. Horti Agrobotanici Cluj-Napoca 38(1):182-186

Dencic S, Kastori R, Kobiljski B, and Duggan B (2000) Evaluation of grain yield and its components in wheat cultivars and landraces under near optimal and drought conditions, Euphytica 113(1):43-52

Eftekhari A, Baghizadeh A, Yaghoobi MM and Abdolshahi R (2017) Differences in the drought stress response of *DREB2* and CAT1 genes and evaluation of related physiological parameters in some bread wheat cultivars, Biotechnology & Biotechnological Equipment, 31(4):709-716, DOI: 10.1080/13102818.2017.1316214

El-Hendawy SE, Ruan Y, Hu YC, Schmidhalter U (2009) A comparison of screening criteria for salt tolerance in wheat under field and environment controlled conditions. J Agron Crop Sci 195:356-367

Farooq S and Azam F (2006) The Use of Cell Membrane Stability (CMS) Technique to Screen for Salt Tolerant Wheat Varieties. J. Plant Physiol 163:629-637

Franco JA, Fernández JA, Bañón S and González A (1997) Relationship between the effects of salinity on seedling growth and yield of six muskmelon cultivars. Hort Science 32, 642-644

Fujita M, Fujita Y, Maruyama K, Seki M, Hiratsu K, Ohme-Takagi M, Tran L SP, Yamaguchi-Shinozaki K, and Shinozaki K (2004) A dehydration-induced NAC protein, RD26, is involved in a novel ABA-dependent stress-signaling pathway. Plant J. 39:863-876

Ghaderi N, Normohammadi S, Javadi T (2015) Morpho-physiological Responses of Strawberry (Fragaria×ananassa) to Exogenous Salicylic Acid Application under Drought Stress. J. Agr. Sci. Tech. 17:167-178167

Ghogdi EA, Darbandi AI and Borzouei A (2012) Effects of salinity on some physiological traits in wheat (*Triticum aestivum* L.) cultivars. Ind. J. Sci. Technol 5(1):1906-1906

Jeong JS, Kim YS, Baek KH, Jung H, Ha SH, Do Choi Y, et al. (2010) Root-specific expression of *OsNAC10* improves drought tolerance and grain yield in rice under field drought conditions. Plant Physiol 153(1):185-97

Jeong JS, Kim YS, Redillas MC, Jang G, Jung H, Bang SW, et al. (2013) *OsNAC5* overexpression enlarges root diameter in rice plants leading to enhanced drought tolerance and increased grain yield in the field. Plant Biotechnol. J. 11(1):101-14

Hamama KA, Negim O (2014) Evaluation of wheat genotypes and some soil properties under saline water irrigation. Annals of Agricultural Science 59(2):165-176

Hao Y, Wei W, Song Q, Chen H, Zhang Y, et al. (2011) Soybean NAC transcription factors promote abiotic stress tolerance and lateral root formation in transgenic plants. Plant J. 68:302-313

He, XJ, Mu RL, Cao WH, Zhang ZG, Zhang JS, and Chen, SY (2005) *AtNAC2*, a transcription factor downstream of ethylene and auxin signaling pathways, is involved in salt stress response and lateral root development. Plant J. 44:903-916

Hu, H, Dai M, Yao J, Xiao B, Li X, Zhang Q, and Xiong L (2006) Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proc. Natl Acad. Sci. U S A. 103:12987-12992

Hu H, You J, Fang Y, Zhu X, Qi Z, and Xiong L (2008) Characterization of transcription factor gene *SNAC2* conferring cold and

salt tolerance in rice. Plant Mol. Biol. 67:169-181

Hu Y, Schmidhalter U (2000) Reduced cellular cross-sectional area in the leaf elongation zone of wheat causes a decrease in dry weight deposition under saline conditions. Aust. J. Plant Physiol. 28:165-170

Hu Y, Schmidhalter U (2001) Effects of salinity and macronutrient levels on micronutrients in wheat. J. Plant Nutr. 24:273-281

Hu Y, Camp KH, Schmidhalter U (2000) Kinetics and spatial distribution of leaf elongation of wheat (*Triticum aestivum* L.) under saline soil conditions. Int J Plant Sci. 161:575-582

Huang Q, Yan W, Bin L, Junli C, Mingjie C, Kexiu L, Guangxiao Y and Guangyuan H (2015) *TaNAC29*, a NAC transcription factor from wheat, enhances salt and drought tolerance in transgenic Arabidopsis. BMC Plant Biology. 15:268. DOI 10.1186/s12870-015-0644-9

Kamoshita A, Wade LJ, Yamauchi A (2000) Genotypic variation in response of rainfed lowland rice to drought and rewatering. III. Water extraction during drought period. Plant Production Science 3:189-196

Keyvan S (2010) The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars. J Anim Plant Sci. 8:1051-1060

Kim SY, Kim SG, Kim YS, Seo PJ, Bae M, Yoon HK, Park CM (2007) Exploring membrane-associated NAC transcription factors in Arabidopsis: implications for membrane biology in genome regulation. Nucleic Acids Res 35:203-213

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real time quantitative PCR and the 2ΔΔ C(T) Method. Methods 25(4):402-408

Lonbani M and Arzani A (2011) Morpho-physiological traits associated with terminal drought stress tolerance in triticale and wheat. Agronomy Research 9(1-2):315-329

Lu M, Sheng Y. Deng-Feng Z. Yun-Su S. Yan-Chun S. Tian-Yu W.Yu L (2012) A maize stress-responsive NAC transcription factor, *ZmSNAC1*, confers enhanced tolerance to dehydration in transgenic Arabidopsis. Plant Cell Rep 31:1701-1711

Meng C, Cai C, Zhang T, Guo W (2009) Characterization of six novel NAC genes and their responses to abiotic stresses in *Gossypium hirsutum* L. Plant Sci 176:352-359

Mao X, Zhang H, Qian X, Li A, Zhao G, Jing R (2012) *TaNAC2*, a NAC-type wheat transcription factor conferring enhanced multiple abiotic stress tolerances in Arabidopsis. J Exp Bot. 63(8):2933-46

Mao X, Chen S, Li A, Zhai C, Jing R (2014) Novel NAC transcription factor *TaNAC67* confers enhanced multi-abiotic stress tolerances in Arabidopsis. PLoS ONE. 9(1):e84359

Moumeni A, Satoh K, Kondoh H, Asano T, Hosaka A, Venuprasa R, Serraj R, Kumar A, Leung H, Kikuchi S (2011) Comparative analysis of root transcriptome profiles of two pairs of drought tolerant and susceptible rice near-isogenic lines under different drought stress. BMC Plant Biol 11:174

Mickky BM, Aldesuquy HS (2017) Impact of osmotic stress on seedling growth observations, membrane characteristics and antioxidant defense system of different wheat genotypes. Egyptian Journal of Basic and Applied Sciences, 4:1, 47-54, DOI: 10.1016/j.ejbas.2016.10.001

Mitsuda N, Iwase A, Yamamoto H, Yoshida M, Seki M, Shinozaki K, Ohme-Takagi M (2007) NAC transcription factors, NST1 and NST3 are key regulators of the formation of secondary walls in woody tissues of Arabidopsis. Plant Cell 19:270-280

Nayer M K, R Heidari, N Abbaspour and F Rahmani (2012) Growth responses and aquaporin expression in grape genotypes under salinity. Iranian Journal of Plant Physiology 2(4):497-507

Nakashima, K, Tran L-SP, Van Nguyen, D, Fujita M, Maruyama K, Todaka D, Ito Y, Hayashi N, Shinozaki K, and Yamaguchi-Shinozaki K (2007) Functional analysis of a NAC-type transcription factor *OsNAC6* involved in abiotic and biotic stress-responsive gene expression in rice. Plant J. 51:617-630

Nakashima K, Takasaki H, Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2011) NAC transcription factors in plant abiotic stress responses. Biochim Biophys Acta 1819:97-103

Nakashima K, Takasaki H, Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2012) NAC transcription factors in plant abiotic stress responses. Biochim Biophys Acta. 1819(2):97-103

Neumann PM (1993) Rapid and reversible modifications of extension capacity of cell walls in elongating maize leaf tissues responding to root addition and removal of NaCl. Plant Cell Environ. 16:1107-1114

Niaz A, Amin R, Ali D (2014) GSTF1 Gene Expression Analysis in Cultivated Wheat Plants under Salinity and ABA Treatments. Molecular Biology Research Communications 3(1):9-19

Nuruzzaman M, Manimekalai R, Sharoni AM, Satoh K, Kondoh H, Ooka H, et al (2010) Genome-wide analysis of NAC transcription factor family in rice. Gene 465:30-44. doi: 10.1016/j.gene.2010.06.008

Ouhaddach M, H ElYacoubi, A Douaik, A Rochdi (2018) Morpho-Physiological and Biochemical Responses to Salt Stress in Wheat (*Triticum aestivum* L.) at the Heading Stage J. Mater. Environ. Sci. 9(6):1899-1907

Ozhuner E, Eldem V, Ipek A, Okay S, Sakcali S, et al. (2013) Boron stress responsive microRNAs and their targets in barley. PLoS One 8:e59543

Pandurangaiah M, Lokanadha R G, Sudhakarbabu O, Nareshkumar A, Kiranmai K, Lokesh U, Thapa G, Sudhakar C (2014) Overexpression of horsegram (*Macrotyloma uniflorum* Lam. Verdc.) NAC transcriptional factor (*MuNAC4*) in groundnut confers enhanced drought tolerance. Molecular Biotechnology 56:758-769

Passioura J B (1996) Drought and drought tolerance, "in Drought Tolerance in Higher Plants: Genetical, Physiological and Molecular Biological Analysis, E. Belhassen, Ed., pp. 3-12, Kluwer Academic, Dordrecht, The Netherlands

Puranik S, Sahu PP, Srivastava PS, Prasad M (2012) NAC proteins: regulation and role in stress tolerance. Trends Plant Sci. 17(6):369-81

Quach TN, Tran L-SP, Valliyodan B, Nguyen HT, Kumar R, et al. (2014) Functional Analysis of Water Stress-Responsive Soybean *GmNAC003* and *GmNAC004* Transcription Factors in Lateral Root Development in Arabidopsis. PLoS ONE 9(1):

e84886. doi: 10.1371/journal.pone.0084886

Rizza F, Badeck FW, Cattivelli L, Lidestri O, di Fonzo N, and Stanca AM (2004) Use of a water stress index to identify barley genotypes adapted to rainfed and irrigated conditions. Crop Science 44(6):2127-2137

Rucker KS, Kevin CK, Holbrook CC, and Hook JE (1995) Identification of peanut genotypes with improved drought avoidance traits. Peanut Science 22:14-18, 1995

Sairam, R. K., Rao, K. V. and Srivastava, G. C (2002) Differential Response of Wheat Genotypes to Long Term Salinity Stress in Relation to Oxidative Stress, Antioxidant Activity and Osmolyte Concentration. Plant Sci. 163:1037-1046

Schonfeld MA, Johnson RC, Carwer BF, Mornhinweg DW (1988) Water relations in winter wheat as drought resistance indicators. Crop. Sci. 28: 526-531

Shao HB, Chu LY, Jaleel CA, and Zhao CX (2008) Water-deficit stress-induced anatomical changes in higher plants. Comptes Rendus 331(3):215-225

Shinozaki K, Yamaguchi-Shinozaki K (2007) Gene networks involved in drought stress response and tolerance. J Exp Bot. 58:221-227

Souer, E, Van Houwelingen A, Kloos D, Mol J and Koes R (1996) The no apical meristem gene of petunia is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. Cell 85, 159-170

Sinclair TR and Ludlow MM (1985) Who taught plants thermodynamics? The unfulfilled potential of water potential. Aust. J. Plant Physiol. 12:213-217

Tambussi EA, Nogues S, Araus JL (2005) Ear of durum wheat under water stress: water relations and photosynthetic metabolism. Planta. 221:446-458

Tang Y, Liu M, Gao S, Zhang Z, Zhao X, Zhao C, et al. (2012) Molecular characterization of novel *TaNAC* genes in wheat and overexpression of *TaNAC2a* confers drought tolerance in tobacco. Physiol Plant 144(3):210-24

Teulat B, Zoumarou-Wallis N, Rotter B, Ben Salem M, Bahri H and This D (2003) QTL for relative water content in field-grown barley and their stability across Mediterranean environments. Theoretical and Applied Genetics 108:181-188

Tran LS, Quach TN, Guttikonda SK, Aldrich DL, Kumar R, Neelakandan A, Valliyodan B, Nguyen HT (2009) Molecular characterization of stress-inducible *GmNAC* genes in soybean. Mol Genet Genomics 281:647-664

Redillas MC, Jeong JS, Kim YS, Jung H, Bang SW, Choi YD, et al. (2012) The overexpression of *OsNAC9* alters the root architecture of rice plants enhancing drought resistance and grain yield under field conditions. Plant Biotechnol J. 10(7):792-805

Richards RA (1992) Increasing salinity tolerance of grain crops: is it worthwhile? Plant Soil 146:89-98

Wang C, Deng P, Chen L, Wang X, Ma H, Hu W, et al. (2013) A Wheat WRKY Transcription Factor TaWRKY10 Confers Tolerance to Multiple Abiotic Stresses in Transgenic Tobacco. PLoS ONE 8(6):e65120

Xia N, Zhang G, Sun Y, Zhu L, Xu L, et al. (2010a) *TaNAC8*, a novel NAC transcription factor gene in wheat, responds to stripe rust pathogen infection and abiotic stresses. Physiol Mol Plant Pathol 74:394-402

Xia N, Zhang G, Liu X, Deng L, Cai G, et al. (2010b) Characterization of a novel wheat NAC transcription factor gene involved in defense response against stripe rust pathogen infection and abiotic stresses. Mol Biol Rep 37:3703-3712

Xue G, Neil IB, McIntyre CL, Riding GA, Kemal Kazan K, et al. (2006) *TaNAC69* from the NAC superfamily of transcription factors is up-regulated by abiotic stresses in wheat and recognizes two consensus DNA-binding sequences. Funct Plant Biol 33:43-57

Xue G, Way H, Richardson T, Drenth J, Joyce P, et al. (2011) Overexpression of *TaNAC69* leads to enhanced transcript levels of stress up-regulated genes and dehydration tolerance in bread wheat. Mol Plant 4:697-712

Xue GP, Loveridge CW (2004) *HvDRF1* is involved in abscisic acid-mediated gene regulation in barley and produces two forms of AP2 transcriptional activators, interacting preferably with a CT-rich element. Plant J 37:326-339

Yamaguchi-Shinozaki K, Koizumi M, Urao S and Shinozaki K (1992) Molecular cloning and Characterization of 9 cDNAs for genes that are responsive to desiccation in Arabidopsis thaliana, sequence analysis of one cDNA clone that encodes a putative transmembrane channel protein. Plant Cell Physiol 33:217-224

Yousfi S, Marquez AJ, Betti M, Araus JL, Serret MD (2016) Gene expression and physiological responses to salinity and water stress of contrasting durum wheat genotypes. J Integr Plant Biol. 2016 (1):48-66. doi: 10.1111/jipb.12359

Zheng X, Chen B, Lu G, Han B (2009) Overexpression of a NAC transcription factor enhances rice drought and salt tolerance. Biochem Biophys Res Commun. 379(4):985-9

Zhou QY, Tian AG, Zou HF, Xie ZM, Lei G, Huang J, Wang CM, Wang HW, Zhang JS, Chen SY (2008) Soybean WRKY-type transcription factor genes, *GmWRKY13*, *GmWRKY21*, and *GmWRKY54*, confer differential tolerance to abiotic stresses in transgenic Arabidopsis plants. Plant Biotechnol J 6: 486-503