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DETECTION OF *TASOS2, TASOS3, TASST* GENES AND ITS EXPRESSIONS IN SEVERAL IRAQI SELECTED GENOTYPES OF BREAD WHEAT *TRITICUM AESTIVUM* L. UNDER DIFFERENT SALT STRESS LEVELS

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Abstract

Three genes responsible for salttolerance in the plants (*TaSOS2, TaSOS3* and *TaSST*) were detected and their genetic expression were estimated in three Iraqi genotypes of bread wheat *Triticum aestivum* L. selected for salinity tolerance through breeding programs (2H, 4H, Furat) compared to the salt-sensitive variety (Iraq) under different concentrations of salinity (2, 8 and 16 ds/m). The results of the analysis of qualitative PCR reaction and Real Time PCR showed that the length of the bands of the three genes was (156, 176, 113 bp) respectively, which appeared only in the selected genotypes and were absent in the salt-sensitive variety. Genetic expression of genes was positively correlated with salinity, where the highest gene expression was at the highest salinity concentration (16ds/m). Gene expression values were close in the selected genotypes, but no genetic expression was observed for the sensitive cultivar.

Key words: Bread wheat *Triticum aestivum* L., *TaSOS2, TaSOS3, TaSST*, Gene detection.

Introduction

Salinity is one of the major abiotic stresses that limits the growth and production of plants in general (Zhu, 2001) and wheat in particular, which is a staple food for human beings, especially in central and southern of Iraq (Al-Mishhadani *et al.,* 2015), which results in yield reduction of about 88% in bread wheat under high salinity irrigation (Jafari-Shabestari*et al.,*1995 and Genc *et al.,* 2019), therefore researchers aim to develop new genotypes of high productivity and tolerant to stress conditions by identifying and exploring the plant salt tolerance genes and its functions (Chaves *et al.,* 2002) to overcome the problem of salinity and enables the plant to complete its life cycle in environment with high concentrations of salinity and thus secure the food source for humans (Li *et al.,* 2016).

Bread wheat possesses many genes that enablesit to grow in a wide range of salinity, but these genes are foundin varying degrees in the genotypes (Agarwal and Jha, 2010). Numerous studies have revealed genes responsible for salt tolerancethat control the plant's response to salinity, such as SOS pathway (salt overly sensitive protein signaling family) (Zhu, 2000; Ji *et al*., 2013), which consists of SOS1 Na^+/H^+, SOS2 the central component which represent a wide family of protein kinase (Hrabak *et al.*, 2003) and SOS3 a calcium-binding proteins (Zhu, 2002). SOS2-SOS3 complex controls the plasma membrane Na^+/H^+ antiporter SOS1, which is important for Na^+ exclusion andion balance and salt tolerance in plant (Shi *et al*., 2002; Zhu, 2016). SOS pathway is activated when an increase in the concentration of calcium ions (induced by increased sodium ions under salt stress conditions) that binds to SOS3-calcium binding protein which in turn interacts physically with FISL motif in the regulatory region at the C-terminal of SOS2 and activates serine / threonine protein kinase SOS2 activity, which eventuallyactivates SOS1 (Munns, 2005) and thus the sodium ions are excluded to the surface of the root and the transport of sodium ions from the root to the stem are organized and thus maintains the required percentage of K^+/ Na^+ in the leaves (Sathee *et al*., 2015).

A new gene named *Triticum aestivum* Salt Stress protein (*TaSST*). Li *et al*., (2016) reported that the

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expression of this gene was induced by salt, ABA, PEG and that *TaSST* could improve salt tolerance in *Arabidopsis*-over-expressed *TaSST* under salt stress conditions and the physiological indicators of transgenic *Arabidopsis*were better compared to wild-type plants.

The objective of this study is to identify the genes responsible for salt tolerance *TaSOS2, TaSOS3, TaSST* and to estimate their genetic expression in some selected wheat genotypes for salt tolerance through breeding programs 2H, 4H, Furatcompared to the salt-sensitive local cultivar Iraq under different concentrations of salinity.

Materials and Methods

Cultivating the selected genotypes

Three genotypes selected for salinity tolerance through breeding programs (2H, 4H, Furat) and one local cultivar (salt-sensitive Iraq) were used to detect genes responsible for salinity tolerance (*TaSOS2, TaSOS3, TaSST*) and its expressions under saline conditions, 10 seeds per genotype and the local cultivar were cultivated in pots in prepared soil with different salt concentrations (2, 8, 16 ds/m) and three replicates for each treatment in a plastic house conditions. Plant leaves (samples) were taken after 45 days from the sowing date for RNA extraction.

RNA Isolation and cDNA synthesis

The RNA extraction was performed with the Zymo Research Quick-RNA Plant Miniprep Kit and according to the manufacturer instructions. RNA integrity was estimated by electrophoresis on 1.5% agarose gelstained with red safe nucleic acid stain.

cDNA synthesis done by Prime Script RT Master Mix (TaKaRa-Japan) from 500 ng of total RNA.

Amplification of *TaSOS2, TaSOS3, TaSST* genes

Target genes *TaSOS2, TaSOS3, TaSST* were amplified with housekeeping gene B-actin with cDNA using primers showed in table 1. The PCR reaction was carried out by Labnet Thermocycler (USA) at 95°C for 2 min and 40 cycles at 95°C for 20 sec, 55°C for 40 sec and 72°C for 1 min.

Gene expression (Real time PCR reaction)

SYBR Green real-time RT-PCR technique was used to determine the value of genes expression by using Accu Power® Green star qPCR PreMix (by following the manufacturer's protocol) and Exicycler real time PCR (Bioneer-Korea), the program in table 2 was carried out in this analysis.

QPCR results analysis

Data were analyzed using the (Livak and Schmittgen, 2001) method.

Folding = 2-(ΔΔCt)

Results

Qualitative PCR -cDNA

Table 1: Primers used in the search.

Primer	Type	Primer Sequence 5'- 3'
Beta-actin	Forward	TGCTATCCTTCGTTTGGACCTT
	Reverse	TCCGTGTCCTTGACGCCGAG
TaSST	Forward	GCCTACCAGAGGTGCGGCAC
	Reverse	TCCGTGTCCTTGACGCCGAG
TaSOS2	Forward	CATGCCCAGATTGGTTCTCT
	Reverse	TGACGTTTTCATCCTCACCA
TaSOS3	Forward	GGGACAGGCTACATCGAGAA
	Reverse	TTGACGAACTCCTCCCACTC

Table 2: Real Time PCR reaction conditions.

Step	Temperature	Time	No. of cycles
Pre - Denaturation	95°C	2 min	1
Denaturation	95°C	20 sec	40
Annealing	55°C	40 sec	
Extension	72°C	1 min	
Detection (Scan)	–		
Final Extension	72°C	10 min	1
Melting Curve	55-95°C		1

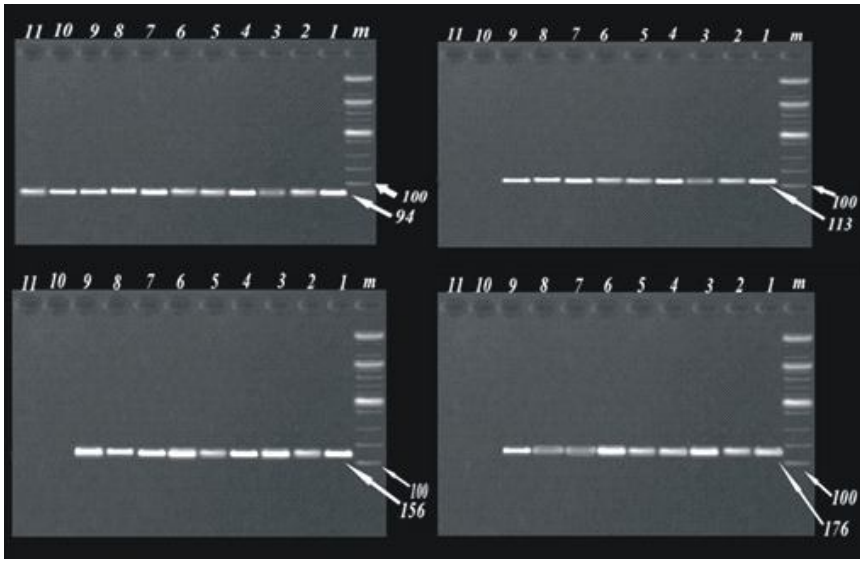

Fig. 1: PCR products of *B-actin*, *TaSST, TaSOS2, TaSOS3* genes on (1.5%) agarose gel stained with Red Safe dye of wheat, M =marker (100bp), (1,2,3) Furat, (4,5,6) 2H, (7,8,9) 4H, (10,11) Iraq, in (2,8,16 ds/m) respectively.

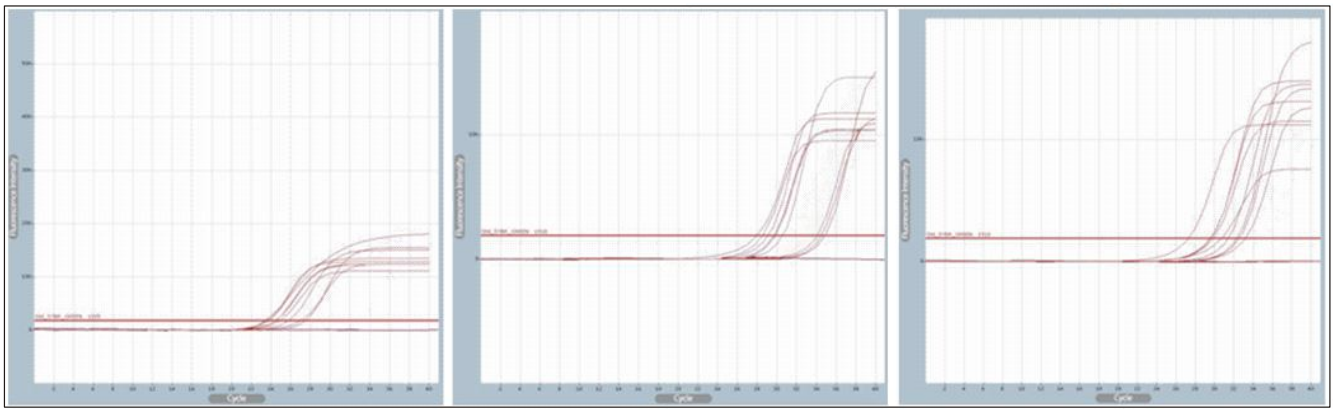

Fig. 2: Amplification curves of Real-Time PCR for *TaSST*, *TaSOS2*, *TaSOS3* genes of wheat (2H, 4H, Furat genotypes and Iraq cultivar)

The salt tolerant genes (*TaSST, TaSOS2, TaSOS3*) were amplified by conventional PCR and electrophoresis ed over the agarose gel with Red Safe nucleic acid staining solution and visualized under U.V. light. The results showed, fig. 1 that the band length of the *B-actin* was 94 which appeared in all the studied wheat genotypes and cultivar and the band length of the *TaSST* gene was 113bp and for the genes *TaSOS2, TaSOS3* was 156, 176 bp, respectively, which appeared only in the selected genotypes for salt tolerance, (2H, 4H, Furat), while these bands didn't appear in the salt-sensitive local cultivar (Iraq) under the same conditions.

Quantitative Real-Time PCR

Real time PCR provided a clear data about the estimation of the amount of the goal gene in a sample, which correlated with the threshold cycle (CT) fig. 2. The values of threshold cycle negatively correlated with the gene expression of each salt tolerant gene (*TaSOS2, TaSOS3, TaSST*) in each selected genotype (2H, 4H, Furat). The results revealed that (CT) values decreased with increasing in salinity concentration. This provide a clear proof that the amount of these genes are positively correlated with salinity levels. In the mean while these genes didn't appear in salt-sensitive cultivar Iraq and didn't show any gene expression.

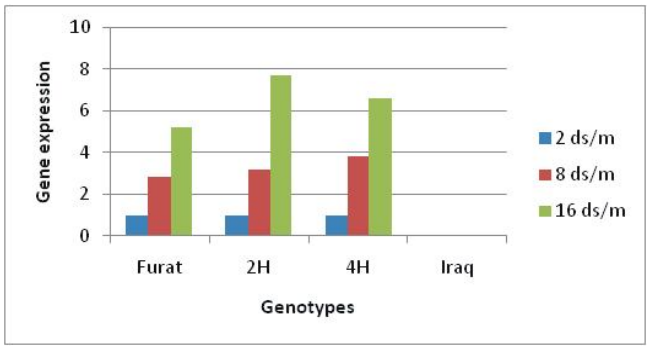

Fig. 3: Effect of salinity on the genetic expression of the *TaSST* gene in leaves of studiedgenotypes and cultivars of wheat.

The values of the gene expression shown in the fig. 3, 4, 5 showed a significant increase, especially in the third saline level (16 ds/m) compared to the first and second saline levels and for all the studied genotypes and for all genes. Salinity stimulated the studied genes to give a high gene expression, where the gene expression at the third saline level (16 ds/m) was the highest compared to the gene expression in the first saline levels (2 ds/m) and second (8 ds/m) respectively. The results indicated that the *TaSST* gene gave the highest value of gene expression at the (16 ds/m) in the selected genotype 2H compared to the two genotypes Furat and 4H, fig. 3. For the *TaSOS2* gene, the highest expression was expressed

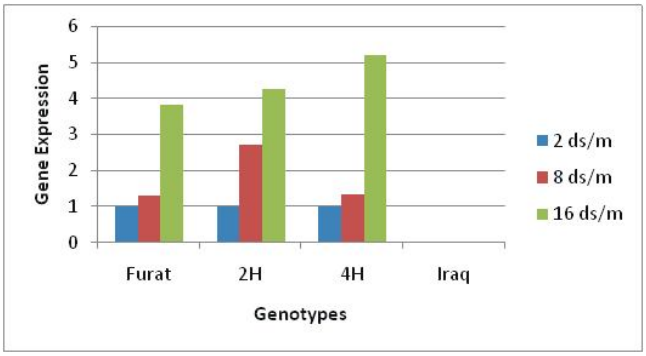

Fig. 4: Effect of salinity on the genetic expression of the *TaSOS2* gene in leaves of studiedgenotypes and cultivars of wheat.

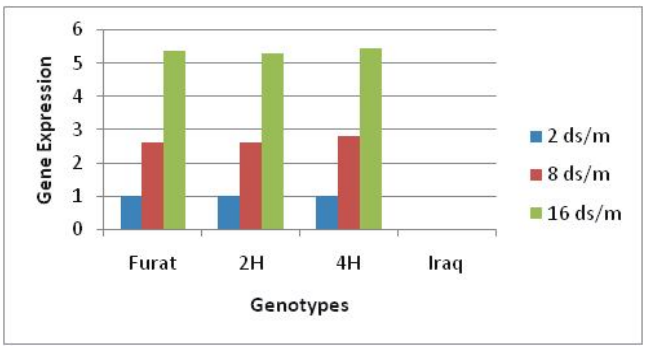

Fig. 5: Effect of salinity on the genetic expression of the *TaSOS3* gene in leaves of studiedgenotypes and cultivars of wheat.

in the selected genotype 4H at the (16 ds/m), fig. 4. *TaSOS3* gene gave the highest value of gene expression in the 4H genotype, at the same time the values were very close to 2H and Furat genotypes, fig. 5. These results revealed that the *TaSST* gene gave the highest value of gene expression compared to the other studied genes *TaSOS2* and *TaSOS3* and for all studied genotypes and at all saline levels, especially at the third saline level, followed by the *TaSOS3* gene, then the *TaSOS2* gene which gave the lowest gene expression values.

Discussion

SOS pathway is a regulatory mechanism found in monocotyledonous and dicotyledonous plants (Ismail *et al*., 2014), SOS1 activated by SOS2/SOS3 efflux Na^+ from the cytosol to the apoplast of the root tissue and also contributes to the transfer of Na^+ from the leaves to the phloem and then to the root to be thrown out of the plant (Munns and Tester, 2008; Zhu *et al*., 2016) and thus contributes to ion homoeostasis and salt tolerance in glycophytes plants such as *Arabidopsis* (Sathee *et al*., 2015). According to Sathee *et al*., (2015) physiological analysis showed that the salt tolerant genotype Karchia 65 had excluded Na^+ effectively from leaves and maintained a low percentage of Na^+ level in all of the plant parts, also *SOS2* gene expression was the highest in the leaves of the tolerant genotype Karchia 65 and that the gene expression was induced in the roots of this genotype under salinity conditions compared to control, whilst *SOS2* expression of the salt-sensitive genotype did not induce by salinity and that the genetic expression was the lowest in it compared to the tolerant one and this maybe has helped maintain the efficiency of *SOS1* under salinity conditions in the tolerant genotype, Similarly the salt tolerant gene *SOS2* was detected and gave high expression only in salt tolerant genotypes (2H, 4H and Furat).

SOS3 gene is necessary to activate *SOS2* (Halfter, 2000). Sathee *et al.,* (2015) reported that Gene expression of *SOS3* was high in both leaves and roots of the salt-tolerant genotype Karchia 65 (induced by the high concentration of salinity) but it was very low in the salt-sensitive genotype HD2687 which may be the reason that this genotype is sensitive to salinity, these results are consistent with the high genetic expression of the selected salt-tolerant genotypes 2H, 4H and Furat derived from plant breeding programs in order to improve wheat to tolerate salinity to some extent which in previous studies indicated that there is a big improvement in salt tolerance (Al-Mishhadani, 2012 and Al-Mishhadani *et al*., 2014). Yang *et al*., (2009) revealed that transgenic plants over expressing *SOS3* exhibited an increase in salt tolerance compared to control plants. Ye *et al*., (2013) stated that Plants that are lacking *SOS3* are sensitive to salt stress and this consistent with the result of the salt-sensitive cultivar Iraq, So the genetic expression of *SOS2/SOS3* was positively correlated with salt-tolerance in wheat (Sathee *et al.,* 2015).

In a previous study Li *et al*., (2016) revealed that *TaSST* gene is one of the genes that controls the plant's response to salinity by the osmotic pressure regulation mechanism by increasing the content of proline and soluble sugars in the transgenic *Arabidopsis* and thus raising the osmotic pressure inside this plant cells. It was also revealed that the content of MDA in *TaSST* transgenic *Arabidopsis* decreased significantly compared to the control and consequently decreased the damage extent of the membrane system of transgenic plants, Moreover K^+/Na^+ ratio, Ca^{+2} and chlorophyll contents were higher compared with WT plants under salt stress. qPCR analysis of *SOS2, SOS3* expressions were highly induced in *TaSST*- over expressing *Arabidopsis* compared with WT plants. *TaSST* expression in salt-tolerant wheat mutant RH8706-49 was induced after NaCl treatment and gave a high value compared to control and that the *TaSST* could obviously improve salt and drought tolerance of transgenic *Arabidopsis* through ABA signal transduction pathway, this is similar to the results of Iraqi genotypes chosen from breeding programs used in this research, as *TaSST* was identified and gave a high expression only in the selected genotypes and was absent in the local cultivar and this reflects that this gene plays an important role in salt-tolerance in the selected genotypes and then this conclusion supports the results of previous studies which confirmed that these selected genotypes have a high tolerance for salinity (Al-Mishhadani *et al*., 2015), whose tolerance to salinity is due to the segregation of genes responsible of salt-tolerance in them during screening and selection cycles in breeding programs. These genes have shown expression when growing under salinity conditions (Al-Mashhadani *et al*., 2016).

In general, these results concluded that the salt tolerant genes *TaSOS2, TaSOS3, TaSST* were found only in the selected genotypes, and gave a high expression under high salinity condition 16 ds/m.

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