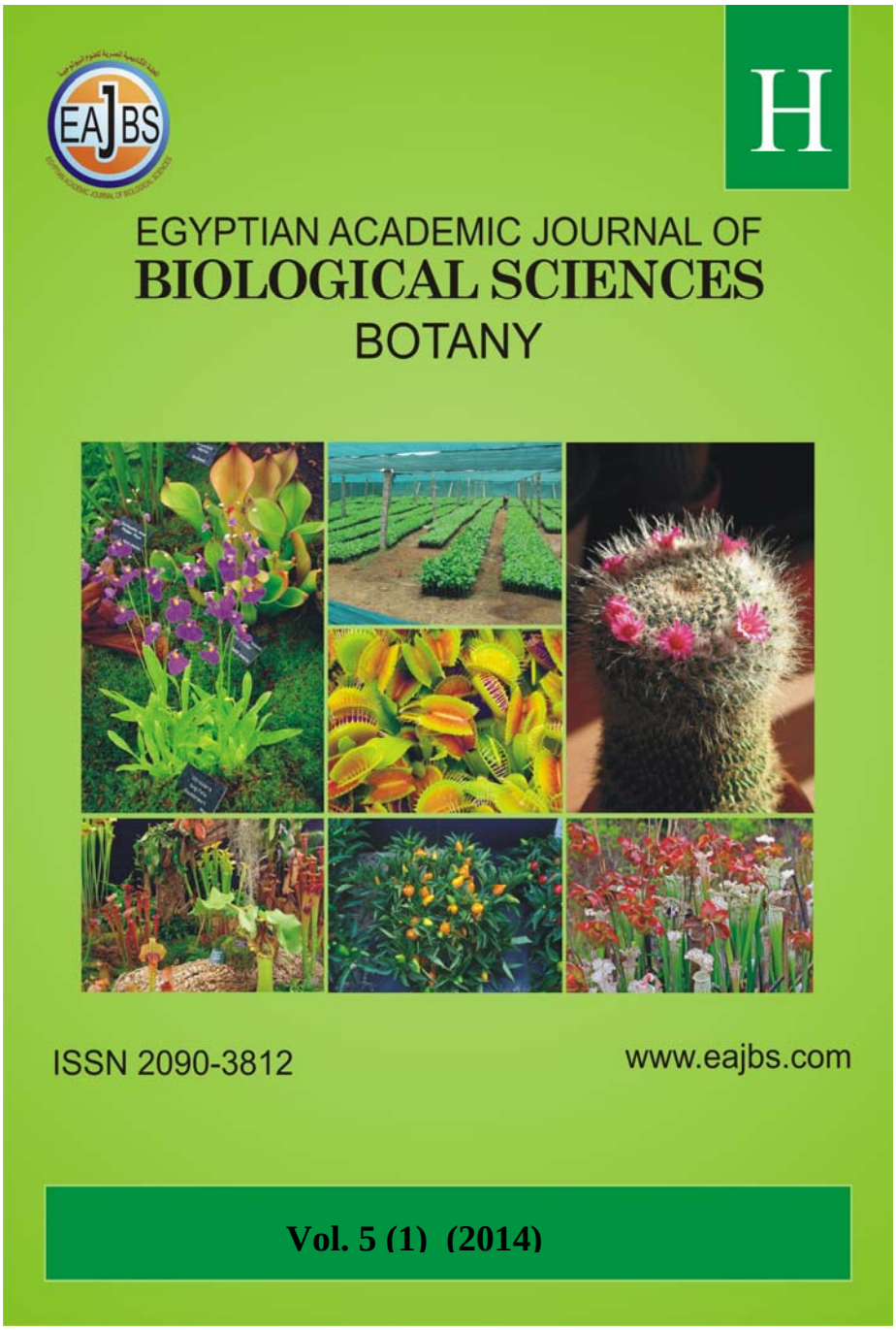


Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences ,Department of Entomology ,Faculty of Sciences Ain Shams University .

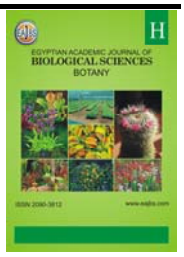
The Botany Journal publishes original research papers and reviews from any botanical discipline or from directly allied fields in ecology, behavioral biology, physiology, biochemistry, development, genetics, systematic, morphology, evolution, control of herbs, arachnids, and general botany..

www.eajbs.eg.net

Received : 28/11/2014 Accepted :24/12/2014

Egypt. Acad. J. Biolog. Sci., 5(1): 105 – 113 (2014)

Egyptian Academic Jour ofnal Biological Sciences
H. Botany
ISSN 2090-3812
www.eajbs.eg.net



Determination of *TaSC* salt tolerance gene expression in selected wheat under different salt stresses

Majeed, D. M.[1], Shwkat, M. S.[2] and Sabbah, M. A. [3]

1- Biotechnology research center, Alnahrain University, Baghdad, Iraq
2- Biotechnology Department, College of Science/ Baghdad University
3- Forensic DNA Center, Alnahrain University, Baghdad, Iraq

ABSTRACT

In previous studies *TaSC* gene was reported to selectively express in salt tolerance wheat. This work was conducted to reveal the *TaSC* gene expression in Iraqi selected wheat genotypes (*Triticum asetivium*) (Dijilla, Furat and N3) and local cultivar (Tamoose) under different salinity levels (0, 10, 16 and 20) ds/m, using real-time polymers chain reaction. RNA was extracted from leaves and roots then converted to cDNA which amplified with pairs of primers and probe. The results indicated that the selected genotypes (Dijilla, Furat and N3) were more salt tolerance than the local cultivar (Tamooze) at germination stage under salinity conditions. The results of the amplification of the *TaSC* gene showed that there is a single clear band with 100 bp was appeared only in selected genotypes, while it was absent in local cultivar and negative control. Also the results of real time polymerase chain reaction amplification showed that there are differences in *TaSC* gene expression among the selected genotypes, whilst there is no gene expression in the local cultivar under all salinity levels. The threshold cycle (C_T) values indicated that the selected genotypes differed significantly in their gene amount, compared with local cultivar and the quantity of the *TaSC* gene recorded for Dijilla, Furat and N3 genotypes were (1.909×10^{15}, 1.45×10^{15} and 1.279×10^{15}) respectively which leaded to cause significant differences between them in their gene expressions. The results reflected that *TaSC* gene is present in selected genotypes of wheat Dijilla, Furat and N3 and absent in local cultivar (Tamooze). In conclusion, the *TaSC* gene can be used as a genetic marker for the wheat genotypes under study.

Keywords: *TaSC* gene; wheat; real-time PCR; salt tolerance

INTRODUCTION

Wheat is an important crop in the world and considered as a source of carbohydrates, proteins and lipids (Braun, *et al*. 2010). The environmental stresses such as salt, cold and drought have negative effects on growth and production of bread wheat (*Triticum asetivium*). Salinity is one of the major stresses that reduce the growth and productions of crops through the effecting on germination, development

Received : 28/11/2014 Accepted :24/12/2014

of plants at molecular and physiological levels, reduce yield and final death of crops (Rhoades and Loveday, 1990). In fact several genes controlling the response to salinity stress that interacts with environmental condition, so the genetic variation can be demonstrated indirectly by measuring the growth or yield (Allen, *et al.* 1994). Munns (2005) reported that genes could increase salt tolerance divided to three major functional groups: (1) genes control the salt uptake; (2) genes could make plant grow in saline soil (3) genes that control osmotic functions.

One of the genes that involved in ions uptake in wheat is *TaGSK1* that induced expression under salt conditions (Chen, *et al.* 2003) and the gene *HKT1,* which its expression in wheat indicate its function in high-affinity K^+ and transport Na^+ (Rus, *et al.* 2001). Badawi *et al.* (2007) reported that *CBF* genes involved in regulation the growth under salinity stress. Several genes in higher plants are involved in osmotic adjustment. Huang *et al.* (2012) identified a novel gene (*TaSC*) in wheat for salt tolerance that involved in osmotic regulation. This gene is up regulated by ABA and NaCl which promoter responds to ABA and NaCl that activate the CDPK pathway and enhance the expression of downstream salt tolerance genes involved in the CDPK pathway (Sanders, *et al.* 2002; Huang, *et al.* 2012). Also the *TaSC* gene functions are enhancing the accumulation of proline and increasing K^+/Na^+ ratio, all this responses are important to plant growth and improving salt tolerance (Huang, *et al.* 2012). The aim of this work is to determine the *TaSC* gene expression in some Iraqi wheat genotypes under salt stress.

MATERIALS AND METHODS

Collection and Planting of seeds

Seeds of selected genotypes of wheat (Dijilla, Furat and N3) and locale cultivar (Tamoose) were planted in prepared soil salinity levels with 5 seed for each genotype under plastic house in Biotechnology Research Center /Al- Nahrain University in Baghdad, Iraq and irrigated with 100 ml of normal water. The germination percentage was taken after four weeks.

RNA isolation and cDNA synthesis

Total RNA was isolated from the leaves after two weeks from the sowing date using (Geneaid @ Total RNA Mini Kit Plant) kit. Isolated RNA was treated with RNase-free DNase I (Biobasic, Canada) for 30 min at 20°C, DNase I was inactivated at 65°C for 10 min. The quality of RNA was assessed by agarose gel electrophoresis on a 1.5% agarose gel containing 0.5% (v/v) ethidium bromide. First-strand cDNA was synthesized from 500 ng of total RNA using AccuPower® RocketScript™ RT PreMix Kit provided by Bioneer-Korea with an oligo-dT_{15} primer. The reaction solution was used as templates for reverse transcriptase polymerase chain reaction (RT-PCR). The quality of cDNA was assessed by Mini-PROTEAN® TGX™ Precast Gel was provided by Bio-RAD.

TaSC gene amplification

TaSC (target gene) cDNA were amplified using specific primers in Table (1) which designed by Primer Express 3.0 software according to the sequence of the gene *TaSC* in National Center for Biotechnology Information (NCBI). Polymerase chain reaction was initiated with hot start method using the cDNA template on Labnet Thermo cycler (USA). The PCR reaction was carried out at 95°C for 5 min and 40 cycles at 95°C for 1 min, 58°C for 45 s and 72°C for 1 min. PCR products were analyzed by agarose gel electrophoresis on a 1.5% agarose gel containing 0.5% (v/v) ethidium bromide.

Table 1: specific primers used for *TaSC* amplification

Primer name	Sequence
SC 1 F	5' CACTACCTGCCCGGGATCTT 3'
SC 1 R	5' CATCCCCATAGGACGAGTAGTAGTC 3'

TaSC gene Quantitation by Taqman Real-Time PCR

The expression of *TaSC* gene was examined by Taqman real-time PCR according to the method of DualStar qPCR PreMix Kit provided by Bioneer-Korea using Exicycler real time PCR (Bioneer, Korea). Specific probe were used with sequence (FAM5' CGCGCTCATGTTC 3'BHQ). The thermal cycling profile consisted of pre-denaturation at 95°C for 5 min and 40 cycles at 95°C for 30 sec and 58°C for 45 sec. Quantitation of relative expression was determined by absolute method (Livak and Schmittgen, 2001). For estimation of standard curve, online software was used to convert DNA concentration to log copy number (http://www.uri.edu/research/gsc/resources/cndna.html).

Statistical Analysis:

The values of the investigated parameters were given in terms of mean ± standard error, and differences between means were assessed by analysis of variance (ANOVA) using SAS computer program version 7.5. Differences in results were considered significant at probability value equal or less than 0.001(SAS, 2004).

RESULTS

The results in Table (2) showed that there are significant differences among the salinity levels in their effect on the germination percentage of the selected genotypes of wheat. Salinity level 20 ds/m gave the lowest value of the germination percentage (58.32) compared with other salinity levels. The selected genotypes (Dijilla, Furat and N3) were differed significantly with the local cultivar (Tamooze) but there are no significant differences between the selected genotypes in their germination under salinity conditions.

Total RNA was isolated, genomic DNA digested with DNase I and the integrity were estimated by agarose electrophoresis (Fig. 1-A). After staining with ethidium bromide and visualized under UV light, the results of conventional PCR showed the appearance of a single and clear band about (100 bp) according to the 100 bp DNA ladder in selected wheat genotypes, while this band was absent in both local cultivar and the negative control (Fig. 1-B).

Table (2): Germination percentage of genotypes and local cultivar under salinity conditions

Genotype	Salinity level (ds/m)				Mean
	0	10	16	20	
	Germination %				
Dijilla	100	100	86.7	86.7	93.35
N3	100	100	66.7	73.3	85
Furat	100	93.3	86.7	73.3	88.33
Tamoose	93.3	60	30	0	45.83
Mean	98.33	88.33	67.53	58.32	

L.S.D at ≤ 0.01 Genotype= 21.1 Levels=21.1 Levels X Genotype= 10.6

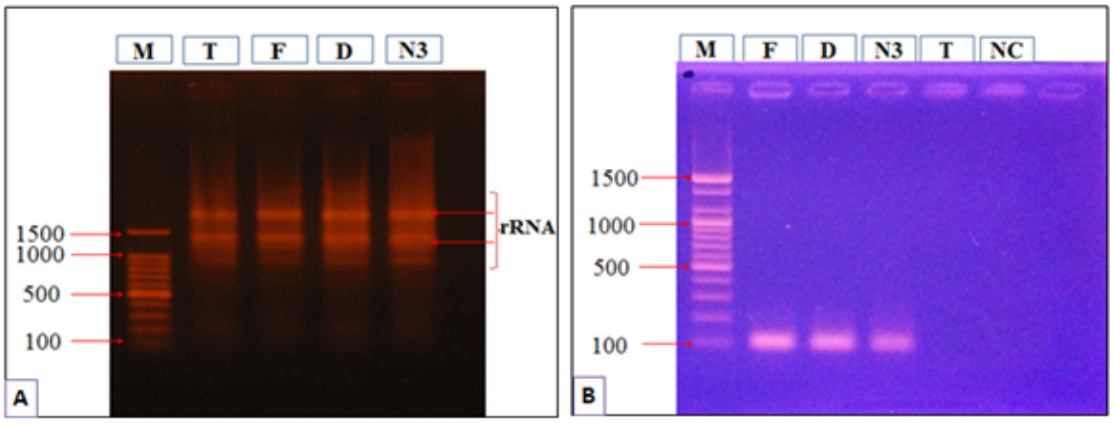


Fig. 1: A- Gel electrophoresis of total RNA of leaves for wheat varieties by 1% agarose gel (1X TBE, 75 v/cm, 1.15 hr) and visualized under UV light after staining with ethidium bromide. Lanes: M= ladder, T = Tamoose cultivar, F = Furat genotype, D= Dijila genotype, N= N3 genotype. B- Gel electrophoresis of PCR products for *TaSC* gene in selected wheat genotypes and local cultivar by 1% agarose gel (1X TBE, 75 v/cm, 1.15 hr) and visualized under UV light after staining with ethidium bromide. Lanes: M= ladder, F = Furat genotype, D= Dijilla genotype, N= N3 genotype, T = Tamoose cultivar.

The amplification of the gene *TaSC* was done by Taqman real-time PCR in order to estimate the gene expression in the selected wheat genotypes (Dijilla, Furat and N3) and the local cultivar (Tamooze) (Figs. 2 & 3).The result of the analysis of the real-time PCR showed that there is gene expression (*TaSC)* under salinity condition, and there are differences in these expression among the salt levels which increased with increasing salt levels. The selected wheat genotypes (Dijilla, Furat and N3) gave the highest gene expression at 16 ds/m and 20 ds/m, while the local cultivar (Tamooze) didn't show any gene expression under the same conditions, due to the absence of the gene *TaSC* in this cultivar, while the selected genotypes have this gene (*TaSC*) which gave gene expression under high salinity conditions.

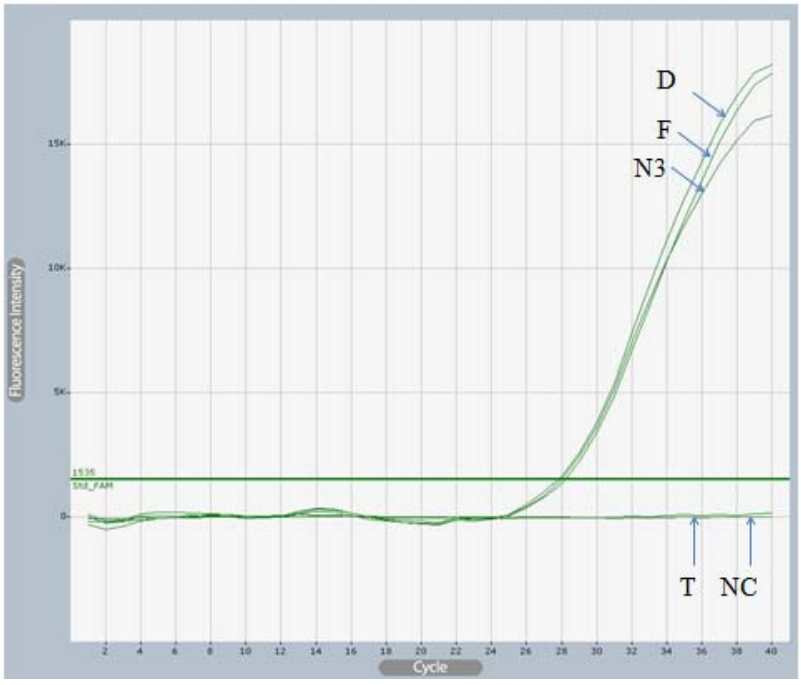


Fig. 2: Amplification curves in real-time PCR obtained from cDNA of wheat varieties at 10 ds/m. Lanes: F = Furat genotype, D= Dijilla genotype, N= N3 genotype, T = Tamoose cultivar, NC = negative control.

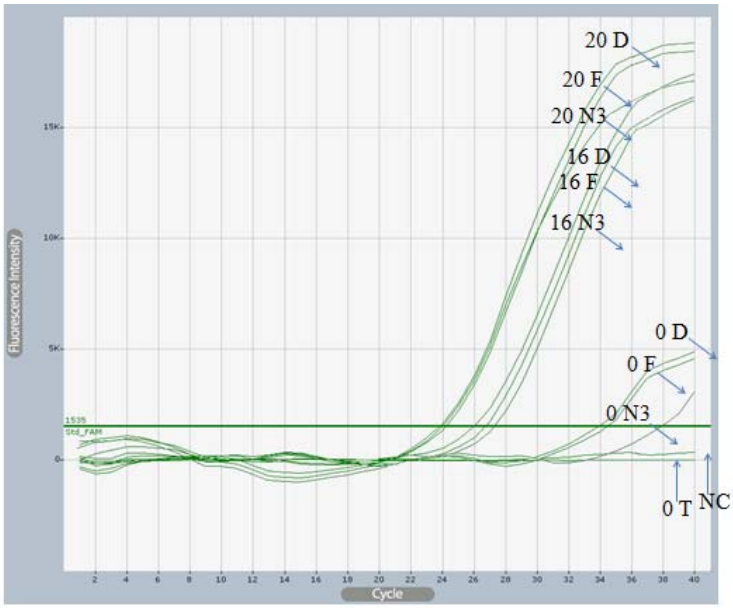


Fig. 3: Amplification curves in real-time PCR obtained from cDNA of wheat varieties at 20, 16, 0 ds/m. Lanes: F = Furat genotype, D= Dijilla genotype, N= N3 genotype, T = Tamoose cultivar, NC = negative control.

Real-time PCR can provide clear information about the determination of the amount of a certain gene in the sample, which correlated with the threshold cycle (C_T). The average of C_T values for each salinity level was shown in Table (3). The results revealed that these values were negative in correlation with the amount of *TaSC* gene. Also the results in the Table (3) explain that the C_T values of each selected genotypes (Furat, Dijilla and N3) decreased with the increasing of salinity levels, and this prove that the amount of the *TaSC* gene increases with the salinity levels, while the C_T values of the local cultivar (Tamooze) was zero and this mean that there isn't any amount of the above gene in this local cultivar.

Table 3: C_T values averages of *TaSC genes* Taqman real time PCR amplification.

Cultivars	Salt concentration ds/m	Average C_T value of *TaSC* gene
Furat	0	33.50 ± 0.76 A
	10	30.23 ± 0.54 B
	16	26.64 ± 0.36 C
	20	21.59 ± 0.14 D
Dijilla	0	33.42 ± 0.56 A
	10	30.30 ± 0.49 B
	16	27.2 ± 0.22 C
	20	21.18 ± 0.13 D
N3	0	33.44 ± 0.89 A
	10	30.45 ± 0.80 B
	16	27.14 ± 0.67 C
	20	21.78 ± 0.54 D
Tamooze 2	0	-----
	10	-----
	16	-----
	20	-----
Negative control		-----

Series dilutions of cDNA were done and then amplified in real-time PCR. C_T values were calculated and used with Log copy number to perform standard curve. Standard curves provide slope, efficiency reaction, correction factor (R^2) and y-intercept (b). The efficiency (E) reaction of qPCR was calculated by the flowing equation $E= 10^{-1/slope} -1$ (Livac and Schmittgen, 2001).

Also the results showed that the amount of *TaSC* gene is similar in all selected genotypes, which differed significantly with increasing salinity levels.

The concentration and purity of the diluted cDNA were (638.6 µg/ml – 2.838 µg/ml) and (1.7 -1.5) respectively. The amplification of diluted cDNA was done (Fig. 4). The standard curve (Fig. 5) was performed depending on the C_T values and Log copy number (Table 4) to determine the slope (-3.423), R^2 (0.967), b (73.49) and E =95.948% which means that the efficiency of the amplification reaction was duplicated every cycle (Vaerman, *et al.* 2004).

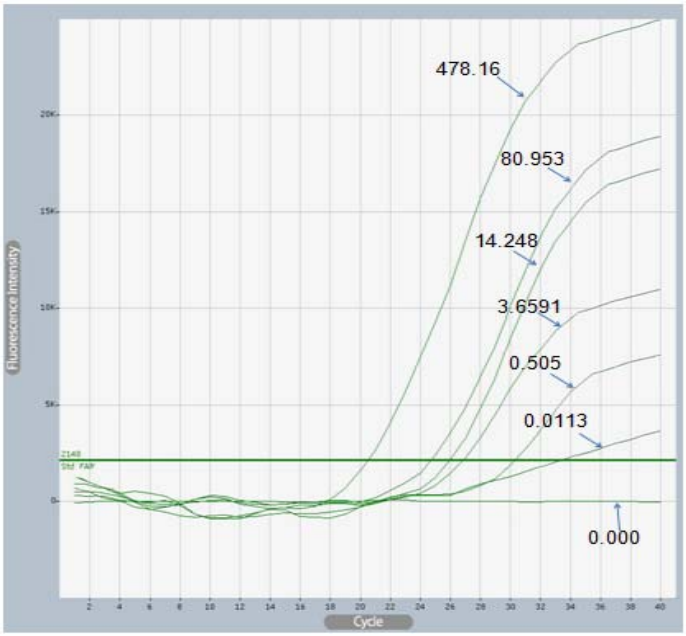


Fig. 4: Amplification curves in real-time PCR obtained from serial dilutions of target cDNA.

Table 4: C_T values and the number of copies of the DNA template of the gene *TaSC*

C_T values	Log copy number
19.42	15.647
21.56	14.875
25.86	14.12
28.88	13.53
30.01	12.67
35	11.02

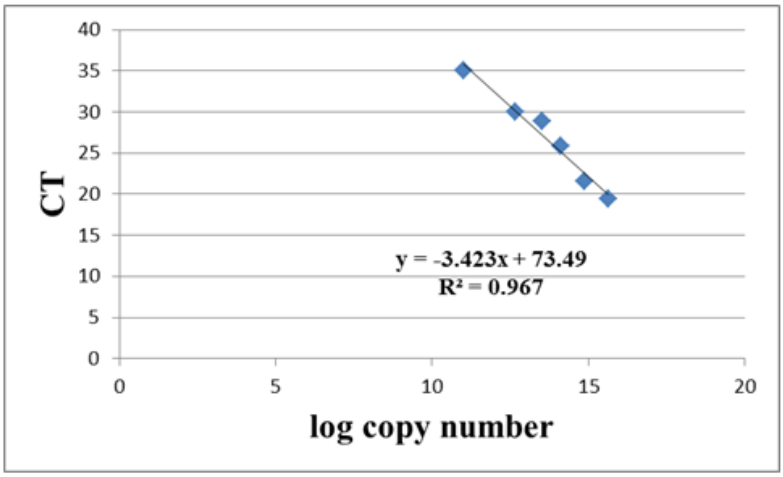


Fig. 5: Standard curve of *TaSC* gene dilutions. The slope, R^2 values and b value performed to calculate the efficiency of qPCR amplification reaction of *TaSC* gene.

The gene expression quantity was determined by the following equation:

$$\text{Gene expression (Quantity)} = 10^{[(C_T\text{-b})/\text{slop}]}$$

The results of this equation were summarized in Table (5) which revealed that the gene expression differs significantly with increasing salinity level at each genotype. The results showed that the selected wheat genotypes (Dijilla, Furat and N3) gave the highest value of the *TaSC* gene expression at the highest salt level (20 ds/m) as compared with the local cultivar Tamooze that have no gene expression of *TaSC* gene. However, the selected genotype Dijilla gave the highest gene expression value ($1.909X10^{15}$) than the other genotypes at salt concentration of 20 ds/min in comparison with Tamooze cultivar which show no expression at all salinity levels due to the absence of *TaSC* gene.

Table 5: *TaSC* gene expression values.

Cultivars	Salt concentration ds/m	Gene expression
Furat	0	$4.819 \times 10^{11} \pm 0.89$ A
	10	$4.345 \times 10^{12} \pm 0.45$ B
	16	$4.852 \times 10^{13} \pm 0.33$ C
	20	$1.45 \times 10^{15} \pm 0.15$ D
Dijila	0	$5.08 \times 10^{11} \pm 0.12$ A
	10	$4.149 \times 10^{12} \pm 0.58$ B
	16	$3.334 \times 10^{13} \pm 0.32$ C
	20	$1.909 \times 10^{15} \pm 0.83$ D
N3	0	$5.011 \times 10^{11} \pm 0.19$ A
	10	$3.749 \times 10^{12} \pm 0.34$ B
	16	$3.467 \times 10^{13} \pm 0.90$ C
	20	$1.279 \times 10^{15} \pm 0.46$ D
Tamooze 2	0	-----
	10	-----
	16	-----
	20	-----

DISCUSSION

From the above results we can conclude that the *TaSC* gene was found in the salt tolerant genotypes, while it was absent in the salt sensitive cultivar. Therefore, these results reflect that there is a high correlation between *TaSC* gene and salt tolerance of the selected genotypes, in comparison with salt sensitive cultivar (Tamooze). This result was similar with the result that reported by Haung *et al.* (2012), they showed that the gene *TaSC* was identified in salt tolerant wheat and gave high expression under salinity conditions.

From the above results, *TaSC* gene expression under salinity conditions in selected genotypes support wheat genotypes to growth normally because this gene is considered as salt tolerant gene according to the (Huang, *et al.* 2012) and can protect the plant from the negative effect of the salinity by some tolerance mechanisms that controlled by this gene. The results showed in Table (5) indicated that there were high gene expression of this gene under salinity conditions only in selected genotypes which gave the highest germination percentage at high salinity level (20 ds/m) (Table 2) compared with the local cultivar that gave the lowest germination percentage under the same conditions, and this may be due to the absence of *TaSC* gene and may be other genes in the local cultivar and there isn't any salt tolerance mechanism which help the plant to grow and produce well under salinity conditions.

Our results are agreed with Sanders *et al.* (2002) whom they found that the real-time PCR results showed that the *TaSC* gene is up-regulated by ABA and NaCl under stress condition which suggested that the promoter responded to ABA and salinity, also *TaSC* gene may be involved in CDPK pathway. From these results the increasing salt tolerance in these selected genotypes was associated with the gene expression of *TaSC* gene by regulation the osmotic pressure through increasing proline content in cells, activating CDPK pathway, increasing K^+/Na^+ ratio and increasing the chloroplast function (Huang, *et al.* 2012).

The salt tolerance of these selected genotypes was examined by Al-Mishhadani *et al.* (2014) and their results indicated that 5H (Furat), 6H (Dijilla) and 3H (N3) were more salt tolerance as compared with the local cultivars. On other hand, Fang-Fang *et al.* (2013) found that *TaSC* gene product has played an important role in the salt tolerance mechanism.

The gene expression of all selected genotypes increased with increasing salinity levels; in contrast Tamooze cultivar didn't have any gene expression at all salinity levels due to the absence of *TaSC* gene amplification.

The results obtained from this study (Table 5) indicated that there are high correlation between the salt levels and amount of *TaSC* gene because of the absent of C_T value in local cultivar. These results agreed with the result that reported by Huang *et al.* (2012) for the same gene, they reported that the gene expression degree increased with increasing salinity levels.

The result reported in Figs. (2, 3) showed that gene expression curve increased with increasing salinity levels only in selected genotypes while there is no gene expression in the local cultivar and negative control because of the gene expression in any cultivar at any salt level depends on the presence and the amount of the salt tolerance gene in the plant samples, therefore high gene expression in selected genotypes was more correlated with the amount of the gene. These results agreed with the result that reported by Huang *et al*. (2012) for the same gene, they reported that the gene expression degree increased with increasing salinity levels.

Generally, the conclusion of these results is the selected genotypes are more salt tolerant than the local cultivar. The salt tolerance of these selected genotypes is correlated with the gene expression of the *TaSC* gene which is increased with increasing salinity levels. Also the *TaSC* gene considered high salt tolerant gene because it gave high gene expression under high salinity condition (20 ds/m). So this gene can be used as a salt tolerance marker for our local wheat.

Attempt to detect salt tolerant genes in cultivars and genotypes of wheat are needed to develop wheat cultivation particularly in soil suffering from salinity. Using the application of molecular techniques in wheat improvement programs that save time, effort and money as well as high accuracy is recommended. The correlation between genetic variation in salt tolerance and salt tolerance mechanisms under different salinity levels is required more study and clarification.

REFERENCES

Allen, J. A.; Chambers, J. L. and Stine, M. (1994). Prospects for increasing the salt tolerance of forest trees: a review. *Tree Physiology.* 14 (7-8-9): 843-853.

Al-Mishhadani, I. I. H.; Abdula, K. H.; Isamil, E. N.; Thahre, Y. D. and Weab, I. A. (2014). Estimation of new wheat genotypes for salt tolerance which induced through plant breeding programs. Journal of Agricultural Science and Techbology B. 4(2):150-156.

Badawi, M.; Danyluk, J.; Boucho, B.; Houde, M. and Sarhan, F. (2007). The *CBF* gene family in hexaploid wheat and its relationship to the phylogenetic complexity of cereal *CBFs*. *Molecular Genetics and Genomics.* 277:533-554.

Braun, H. J.; Atlin, G. and Payne, T. (2010). Multilocation testing as a tool to identify plant response to global climate change. In: Climate change and crop production. Reynolds, C. R. P. (Ed.). CABI London UK.

Chen, G. P.; Ma, W. S.; Huang, Z. J.; Xu, T.; Xue, Y. B. and Shen, Y. Z. (2003). Isolation and characterization of TaGSK1 involved in wheat salt tolerance. *Plant Sci.* 165: 1369–1375.

Fang-Fang, L.; Xu, Y.; Xiao-Cui, W.; Xiao-Mei, L.; Rong-Chao, G. E. and Bao-Cun, Z. (2013). Fishing *TaSC* Interacting Protein in Wheat Using Split Ubiquitin Yeast Two Hybrid System. Acta Agronomica Sinica., 39(3): 423 - 430.

Huang, X.; Zhang, Y.; Jiao, B.; Chen, G.; Huang, S.; Guo, F.; Shen, Y.; Huang, Z. and Zhao, B. (2012). The wheat gene *TaSC* can increase the salt tolerance of transgenic *Arabidopsis*. Plant Cell Rep., 31: 339–347.

Livak, K. J. and Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta}C_T$ Method. Methods., 25: 402-408.

Munns, R. (2002). Comparative physiology of salt and water stress. Plant, Cell and Environment., 25: 239-250.

Munns, R. (2005). Genes and salt tolerance: bringing them together. New Phytol., 167: 645-663.

Rhoades, J. D. and Loveday, J. (1990). Salinity in Irrigated Agriculture. In: Irrigation of Agricultural Crops. Stewart, B. A. and Nielsen, D. R. (ed.) Agron. Monogr. 30. ASA, CSSA and SSSA, Madison, WI.

Rus, A.; Yokoi, S.; Sharkhuu, A.; Reddy, M.; Lee, B.; Matsumoto, T. K.; Koiwa, H.; Zhu, J-K.; Bressan, R. A. and Hasegawa, P. M. (2001). AtHKT1 is a salt tolerance determinant that controls Na+ entry into plant roots. Proceedings of the National Academy of Sciences, USA., 98: 14150–14155.

Sanders, D.; Pelloux, J. and Brownlee, C. (2002). Calcium at the crossroads of signaling. The Plant Cell., 14: 401–417.