Plant Archives Vol. 20, Supplement 2, 2020 pp. 1089-1093 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ASSESSMENT OF SALT TOLERANCE BETWEEN THE SELECTED WHEAT GENOTYPES AND LOCAL CULTIVARS BY USING MOLECULAR TECHNIQUE (PCR AND REAL TIME PCR)

Eman N. Ismail[1], Ibrahim I.H. Al-Masshadani[1] and Ali A. Al-Salihy[2]
[1]Biotechnology Research Center, Al-Nahrain University, Iraq
[2]Institute of Genetic Engineering and Biotechnology, University of Baghdad, Iraq

Abstract

The (*TaCTR1, TaCTR2* and *TaCTR3-1*) genes are a member of calreticulin (CRT) family in wheat, in the previous work, indicated that these genes enhanced tolerance to the salinity. The aimed of this study to detect the salt tolerant genes (*TaCTR1, TaCTR2*, *TaCTR3-1*) with estimation their expression under salinity conditions and Ca^{+2} ion treatments in some wheat selected genotypes and local cultivars. PCR and real-time PCR were used to realize the above aimed. The results of real-time PCR quantitative showed the bands of these genes appeared only in Furat, 4H and 7H (the selected genotypes) while these bands absent in Iraq and Orok (salt sensitive cultivars). Under all salinity levels (2, 8 and 16 ds/m), the values of expression of *TaCTR1, TaCTR2* and *TaCTR3-1* of genes have been enhanced only in selected genotypes, and these values increased at high salinity level (16 ds/m) as compared with (2 ds/m) and (8 ds/m). The selected genotypes differed between them in expression degree at all genes and salinity levels. Whilst there are no genes expression appeared in local cultivars. The results also showed that adding Ca^{+2} ions to the growth soils increased the values of expressions of all studied genes and genotypes especially at high salinity level. Therefore, according to these results of genes detection and expression there is improvement in these selected genotypes for salt tolerance through the plant breeding programs and by using Ca^{+2} ion as fertilizer.
***Keywords*:** Wheat, Salinity, Genotypes, Salt Tolerance, *TaCRT* gene, Real-time PCR, PCR.

Introduction

Using plant breeding technique to select genotypes of crops with high salt tolerance degree is one of important factor to overcome salinity problems. Programs were used to increase salt tolerance in wheat progeny, are crossing, exposure and selection. Some wheat genotypes were selected through these programs with high salt tolerance (Al-Mishhadani, 2012). On the other work, these genotypes were grown under salinity conditions in pots and field at different salinity levels with high vegetation growth and seed yield. Al-Mishhadani *et al*. (1999) reported that all the selected genotypes through the plant breeding programs were superior in growth and seed yield over the unselected plant and check cultivars. Also they reported that high progress in salt tolerance character was achieved in all wheat selected genotypes as compared with the original plants (unselected) through the exposure and selection program. On the other hand, the same results were reported by (Al-Mishhadani, 2010 and Al-Mishhadani *et al*., 2015). Al-Mishhadani (2012) indicated that good progress performed in F3 generations at one cycle of exposure and selection as compared with F2 plants (unselected).

Induce new cultivars or genotypes of wheat (high salt tolerance) requires creation new genetic source of salt tolerance with high genetic variation and will need more effective techniques to identify salt tolerance germaplasm (Al-Mishhadani, 2012). Applications of biotechnology technique are important achievement acquired to improve tolerance of salinity in wheat genotypes or varieties effectively, so to utilize soil affected salt (Al-Mishhadani, 2015). The salt tolerance of plant depends on salt tolerance mechanisms which expressed by responsible Genes of this characters (Al-Mishhadani *et al.*, 2014). The tolerance of salinity of plant controlled by genes, therefore detection these genes with understanding their mechanisms and function are the most Important aims in research today that concern with the improvement of salt tolerance in plant. Perversely, *TaSTK* and *TaNIP* genes involved in salt tolerance mechanisms in wheat with high expression under salinity condition were detected in some selected wheat genotypes (Deijila, Furrat, N3, 1H, 2H, Iraq and tamooze) (AL-Mishhadani *et al*., 2016). On the other hand, other salt tolerant genes (*TaGSK1* and *TaSC*) were detected in some selected genotypes of wheat with high gene expression under salinity conditions (Ismail *et al*., 2014; Majeed *et al.*, 2014). Also Gao *et al.* (2010) identified and cloned *TaNIP* gene in wheat mutant RH8706+49 under salinity conditions by using Real-time-PCR.

The aims of the current study are identifying the salt tolerance gene *TaCTR1*, *TaCTR2*, *TaCTR3*-1 and estimation them expression under different salinity levels in some wheat genotypes (Furrat, 4H and 7H) which selected through plant breeding and improvement programs for salt tolerance as compared with the two local wheat cultivars as salt sensitive (Orok and Iraq).

Material and Methods

In this study three genotypes of wheat and two local varieties were used for detection salt tolerant genes and estimation their expression under salinity condition. These genotypes were selected through plant breeding programs for salt tolerant. The selected genotypes which used were (furrat, 4H, 7H) and local cultivars were (Iraq and orok). Seeds of these genotypes and varieties were grown at all growth stages in prepared soils salinity (2, 8, and 16) ds/m under controlled condition in plastic house. At potting stage some leaves were taken for molecular studies. RNA extract and synthesis of cDNA according to the (Ismail *et al*., 2014). *TaCTR1, TaCTR2*, *TaCTR3-1* and *α-Tubulin* gene amplified and Gene expression examined by SYBR green real-time RT-PCR according to the Xiang *et al*. (2015). (Table 1)

Table 1: Primers used for amplification of target gene and reference gene for cDNA sequence

Primer Name	Type	5' - 3' Sequence primer
α-Tubulin	Forward	ATCTCCAACTCCACCAGTGTCG
α-Tubulin	Reverse	TCATCGCCCTCATCACCGTC
TaCRT1	Forward	TCTGATGACGAGAAGCACGATGAGC
TaCRT1	Reverse	GAGACAACTAATAAATCCTGGCAGCGG
TaCRT2	Forward	GGATGATGAGGAAGATGGTGAATGGAC
TaCRT2	Reverse	CAGGCTGTCAAAAGCGTAGATGTAAGG
TaCRT3-1	Forward	TTACAAGGACAGATACAAAAGACGCAACAG
TaCRT3-1	Reverse	TCCCTCACACGAGACAAGAAACACTTC

Results

Gene detection

The result of gene detection at cDNA showed that all the studied genes (*TaCTR1, TaCTR2* and *TaCTR3-1*) were appeared in all selected wheat genotypes for salt tolerance, while they absent in local cultivars (sensitive for salinity) (fig1). The molecular weight of these genes' bands was (110,179,129) bp respectively whilst, the bands of gene of α-*Tubulin* were appeared in all selected genotypes and local cultivars with molecular weight (218 bp).

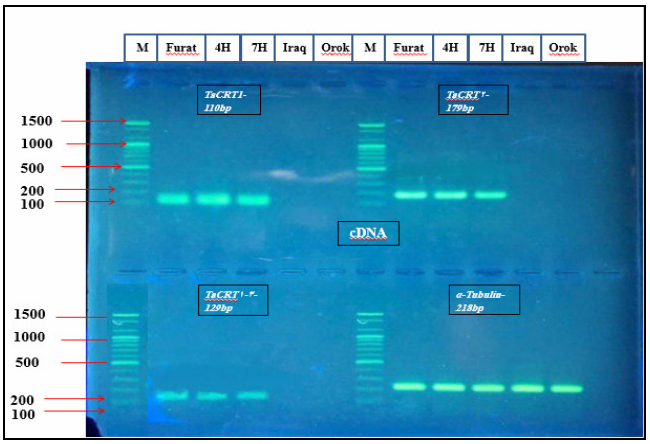

Fig. 1 : Red safe stained electrophoresis of agarose (1.5 %) of PCR product (*TaCTR1, TaCTR2* , *TaCTR3-1* and *α-Tubulin)* for wheat cultivars. M: Marker (100bp).

Real-Time qPCR

The amplification of three salt tolerance genes (*TaCTR1, TaCTR2* and *TaCTR3-1*) were done by SYBR Real-time PCR technique to determine gene expression and CT values in salt tolerant genotypes (Furrate, 4H,7H) and local cultivars (Iraq, orok). The data obtained from of the Real-time PCR revealed that there were expressions of the three genes under salinity. There are differences in their expression among the salinity levels and selected genotypes depend on the amplification of the genes and CT values (Figs. 2, 3 and 4). Whilst the salt tolerance genes did not give any expression in local cultivars (Iraq, orok) at the same salinity condition, due to the absence of these genes in these cultivars. The results also showed that the degree of expression depend on salinity levels gene amplification, and CT value, therefore, there is differences between the selected genotypes in the curve of gene amplification and CT value which their determent the degree of gene expression. The results also revealed that the CT values were negative correlated with the concentration and expression of the genes. Generally, the CT values of each selected genotypes decreased with increasing salinity level, and increasing in the expression.

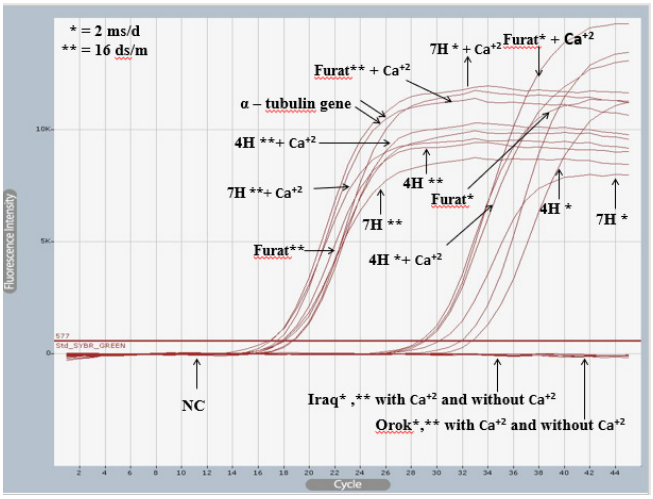

Fig. 2 : SYBR Real-time PCR amplification curves for *TaCTR1* gene and *α-Tubulin* gene of all wheat cultivars

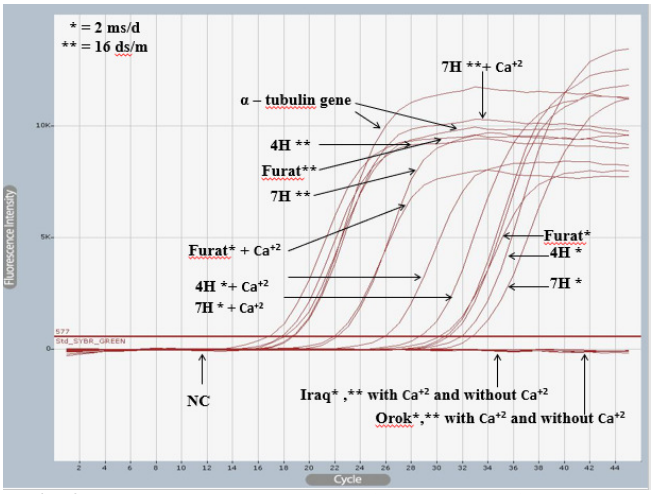

Fig. 3 : SYBR Real-time PCR amplification curves for *TaCTR2* gene and *α-Tubulin* gene of all wheat cultivars

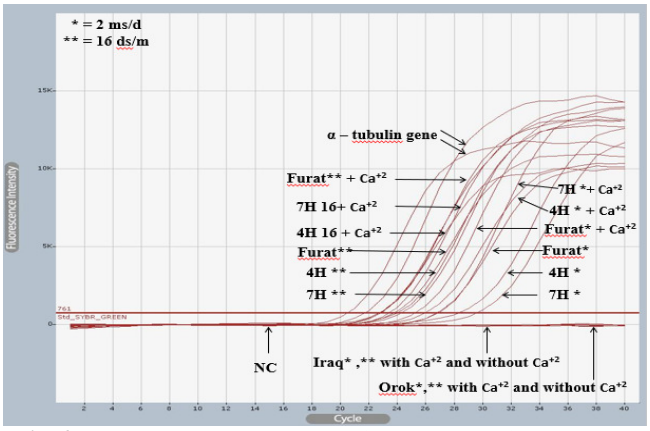

Fig. 4 : SYBR Real-time PCR amplification curves for *TaCTR3-1* gene and *α-Tubulin* gene of all wheat cultivars

Estimation of genes expression

The result of the real-time PCR reaction that concern with the expression of the studied genes (*TaCTR1, TaCTR2* and *TaCTR3-1*) which responsible for salt tolerance in wheat plant were summarized in fig (5,6, and 7). These results revealed that the genes expression differed significantly with the increasing salinity level at each selected genotypes (furrat, 4H, 7H). In contrast, the genes in Iraq and orok cultivars did not give any expression at all salinity levels, due to the absence of these genes in those cultivars. The selected genotypes of wheat gave the highest values of genes expression at the highest salinity level (16 ds/m) as compared with the lower levels (2, 8 ds/m). The results also showed that adding Ca^{+2} ion (20 mM/L) to the salinized soils (2, 8, 16 ds/m) caused increasing in genes expression at all salinity levels, but the highest values of genes expression were at the height salinity level (16 ds/m). Therefore, the Ca^{+2} ion was more influence on expression of all studied genes at 16 ds/m compared with the 2 and 8 ds/m in each selected wheat genotype (fig. 5, 6 and7). There are differences between the selected genotypes in their gene expression at all studied genes. At the highest salinity level, furrat genotype gave the high gene expression at all the genes except *TaCTR3-1* gene. At this gene the 4H genotype gave the highest gene expression as compared with other genotypes (fig 7). Also, the results indicated that there are significant differences between the genes in their expression at each genotype and salinity level. *TaCTR1* gene gave the high expression in furrat genotype and the lowest expression in 4H genotype, while *TaCTR2* gave the highest expression in furrat, but the lowest was in 7H genotypes at the highest salinity level (fig 5 and 6). Whist the results in fig.7 showed that the *TaCTR3-1* gave the lowest expression in Furrat genotype and highest in 4H genotype.

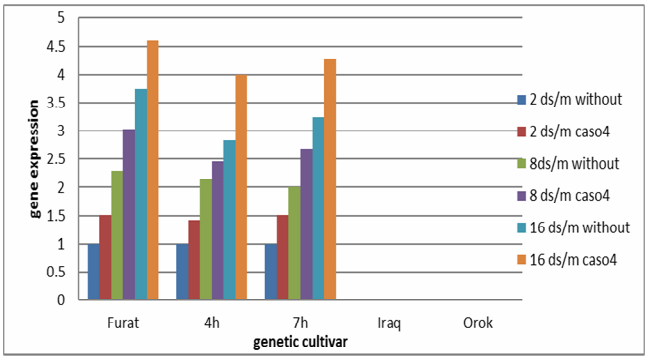

Fig. 5 : *TaCTR1* gene expression under different NaCl concentrations (2, 8 and 16 ds/m) and Ca^{+2} ion (20mM/L).

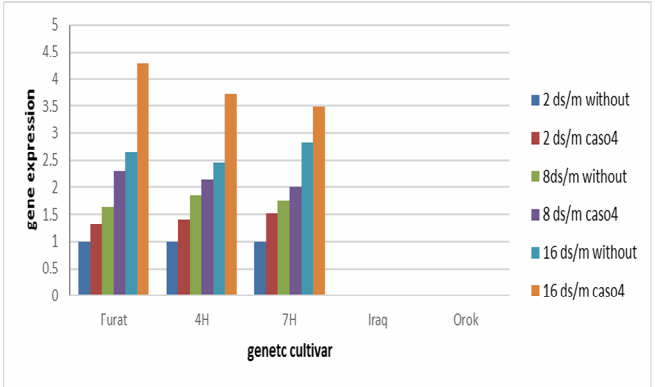

Fig. 6 : *TaCTR2* gene expression under different NaCl concentrations (2, 8 and 16 ds/m) and Ca^{+2} ion (20mM/L).

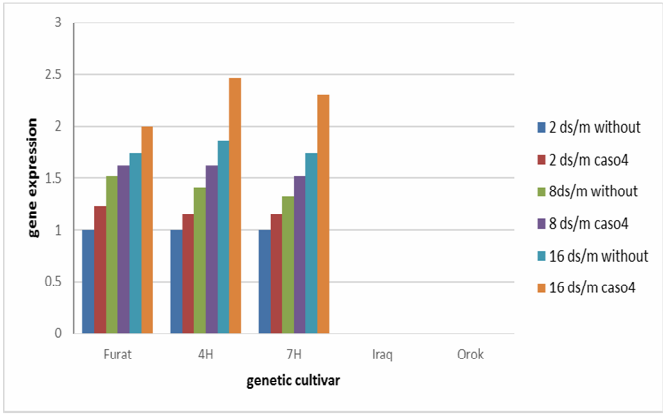

Fig. 7 : *TaCTR3-1* gene expression under different NaCl concentrations (2, 8 and 16 ds/m) and Ca^{+2} ion (20mM/L).

Discussion

In the fact that the selected genotypes of wheat (4H and 7H) and furrat cultivar used in this study were derived from plant breeding and improvement programs and after six cycles of screening and selection under salinity condition (30 ds/m) through plant breeding and improvement programs. The seeds and seedling F2-F7 were exposed to the salinity (30 ds/m drainage water), the aims of these programs are to selected salt tolerant wheat genotypes with high salt tolerance to utilize the salinity affected soil. The results of previous studies revealed that there is a improvement in salt tolerance acquired in most selected genotypes included (furrat, 4H,7H) through these programs of plant genetics and breeding (AL-Mishhadani, 2012; AL-Mishhadani *et al*., 2014; AL-Mishhadani *et al*., 2015). Increasing the salt tolerance in these genotypes (furrat, 4H, 7H) may be because of the segregation of high salt tolerance genes in generations plants during the cycles of screening and selection (AL-Mishhadani *et al*., 2016). The salt tolerances of selected plants depend on the type and quantities of salt tolerant genes which isolated during cycles of screening and selection (Munns, 2005). Therefore, there is high correlation between salt tolerance and genes which segregated in plants through generations.

Molecular studies were used to the selection of salt tolerant genes (*TaCTR1, TaCTR2* and *TaCTR3-1*) and estimation their expression in these selected genotypes (furrat, 4H, 7H) of wheat under salinity conditions as compared with the local cultivars (Iraq and Orok). These genes were detected only in selected genotypes and cultivar and not found in local cultivars (Fig. 1). This reflected that these salt tolerant genes found only in salt tolerant genotypes (furrat, 4H, 7H) genotypes but they did not found in sensitive cultivars (Iraq and Orok). Also these genes gave expression under all salinity level, but this expression highly increased at high salinity level (16ds/m) as compared with 2 and 8 ds/m only in selected genotypes. These results indicated that these salt tolerant genes and their gene expression found in the selected genotypes only and absent in sensitive cultivars which play an important of role in salt tolerance of these genotypes (Furrat, 4H, 7H). This conclusion supported the previous results. Which revealed that all selected genotypes have high tolerance to the salinity, especially furrat cultivar and 4H genotype (Al-Mishhadani 2012, Al-Mishhadani *et al*., 2014). Therefore, there is high correlation between expressions of (*TaCTR1, TaCTR2* and *TaCTR3-1*) genes and

salt tolerance of these selected genotypes. According to the values of expression of these genes under different salinity levels. Salt tolerance of these genotypes increased with increasing salinity level.

Previously, some salt tolerant genes (*TaNIP, TaSC* and *TaGSK1)* were detected and estimated their gene expression in some selected genotypes of were under salinity. The result of these studies indicated that these genes were found only in selected genotypes and gave expression which increased with increasing salinity level. These results agreed with the results which reported by (Ismal *et al*., 2014; Majeed *et al*., 2014; Al-Mishhadani, 2015; Al-Mishhadani *et al*., 2016). Therefore these results reported that the salt tolerance of the selected genotypes of wheat more correlated with the presence of these salt tolerant genes and gave high expression at high salinity level. By contrast, these genes did not found in sensitive cultivars (local cultivars). So, the salt tolerance in plant depends on type and the quantities of salt tolerant genes and their expression degree. The salt tolerance in plant is more correlated with salt tolerant mechanisms which controlled by salt tolerant genes such as *TaCTR1, TaCTR2*, *TaCTR3-1* and other genes. Therefore, the effect of these mechanisms depends on kind of gene and the degree of its expression. Japing wang *et al*. (2017) reported that the *TaCTR-D* genes exhibited different expression patterns in wheat seeding grown under environmental stresses and these genes displayed more tolerances to salinity, drought, cold and other abiotic stresses at two growth stages (seed germination and seedling). Also, they found that *TaCTR1-D* genes function importantly in plant responses to stress and marker-assistant selection in wheat breeding. However, these genes expressed happened in all meristematic and mature cells (Nardi *et al*., 2006). Anther handwork evidenced that endogenous basal expression levels of CRT mRNA and protein are up regulated in response to environmental stress, exogenous abscisic acid (ABA) treatment, and water stress (Kim *et al*., 2013). Generally, these genes gave tolerance at different environmental stress. *TaCRT1*showed enhanced tolerance to salt and drought stresses. However, the transgenic line was showed decreased water loss but higher sensitivity to exogenous abscisic acid (ABA) as compared with type (col-o) (Yang Xiang *et al*., 2018). Also they reported that *TaCTR1*gene may be able to use in improvement the drought tolerance in crop.

The results of real-time PCR showed that Ca^{+2} ions increased the values of the genes (*TaCTR1, TaCTR2* and *TaCTR3-1*) expression under salinity conditions, particularly at high salinity level 16 ds/m. Ca^{+2} ions is very important in growth under salinity conditions, especially at high levels of salinity. Ca^{+2} ions increased growth of plant through increasing number of tillers and leaves, and improve the physiological processes in life cells. Also, the Ca^{+2} ion is very important for reduction the toxic of salinity ions in the cytoplasm of the cells, because there is competition between Na^{+} and Ca^{+2} ions through ion uptake. Therefore, increasing Ca^{+2} ions in the growth soil under salinity condition caused reduction in salt ions uptake. Increasing the expression of salt tolerant genes by adding Ca^{+2} ions to the growth soil is very important factor for increasing salt tolerance in plant. Increasing gene expression by Ca^{+2} ions through regulate the gene expression (Poovaiah and Reddy, 1987). On the other hand, observed changes in signal-induced changes in gene expression, and some of these changes are mediated by Ca^{+2} (Hu *et al*., 2004; McAinsh and Pittman, 2009). Some studies demonstrated that elevated levels of Ca^{+2} modulate gene expression (Kaplan *et al*., 2006). Regulate gene expression either directly or indirectly through Ca^{+2} sensors, first , activated of Ca^{+2} sensors can bind to Cis-elements in the promoters of specific genes and repress or induce their expression, the second, activated Ca^{+2} sensors can bind to DNA binding proteins and activate or repression of gene expression (Reddy *et al*., 2011).

The conclusions of this study are the salt tolerant genes (*TaCTR1, TaCTR2* and *TaCTR3-1*) are found only in the selected genotypes with gave high expression under high salinity level, and adding Ca^{+2} ions to the salinized soil improved the expression of these genes especially at high salt level.

References

Al-Mishhadani, I.H.I. (2012). Breeding and Selection of Some Lines of Bread Wheat for Salt Tolerance. Journal of Agriculture Science and Technology B; 12(8B): 934.

Al-Mishhadani, I.H.I. (2010). Response of some newly developed salt- tolerant genotypes of wheat to naturally salinized soil. The 5th Int. Conf. for Develop. And the Env. In the Arab world. 21-23.

Al-Mishhadani, I.H.I.; Ismail, E.N.; Jaddoa, K.A.; Majeed, D.M. and Mohammed, O.A. (2015). Estimation of the Interaction Effect Between Salinity and Growth Regulators on Salt Tolerance of Two Bread Wheat Cultivars. International Journal of Applied Agricultural Sciences. 1(4): 95-101.

Al-Mishhadani, I.H.I. (2012). Breeding and Selection of Some Lines of Bread Wheat for Salt Tolerance. Journal of Agriculture Science and Technology B; 12(8B): 934.

Al-Mishhadani (2015). Estimation of salt tolerance degree in some selected wheat genotypes by using detection of salt tolerant gene (TaSTK) and its expression under salinity condition. International Journal of Applied Agricultural Sciences. 1(2): 31-35.

Al-Mishhadani, I.I.H.; Abdula, K.H.; Ismail, 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 Technology B 4(2):150-156.

Al-Mishhadani, I.I.H.; Zakariya, B.F.; Ismail, E.N. and Wisam, M.D. (2015). Detection for salt tolerance character in two selected genotypes of wheat. International Journal of Biology, 7(1): 54- 60.

Al-Mashhadani, I.I.H.; Majeed, D.M.; Ismail, E.N. and Kadhim, M.S. (2016). Detection of Salt Tolerant Gene (*TaNIP*) and its expression in three selected wheat genotypes through plant breeding programs under salinity conditions. International Journal of Applied Agricultural Sciences, 2(1): 12-16.

Ismail, E.N.; Ali, N.A. and Sabbah, M.A. (2014). Determination of gene expression of salt tolerant gene TaGSK1 in wheat cultivars. Egypt. Acad. J. Biolog. Sci. 5(1): 85-92.

Majeed, D.M.; Shwkat, M.S. and Sabbah, M.A. (2014). Determination of *TaSC* salt tolerance gene expression in selected wheat under different salt stresses. Egypt. Acad. J.Biolog. Sci. 5(1): 105-113.

Gao, Z.; He, X. and Zhao, B. (2010). Overexpressing a putative aquaporin gene from wheat, *TaNIP*, enhances

salt tolerance in transgenic *Arabidopsis*. Plant Cell Physiol.; 51(5): 767–775.

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. (2005). Gene and salt tolerance: bringing them together. New Phytologist; 167: 645-663.

Wang, J.; Li, R.; Mao, X. and Jing, R. (2017). Functional Analysis and Marker Development of TaCRT-D Gene in Common Wheat (*Triticum aestivum* L.). Plant science, volume 8, article 1557.

Nardi, M.C.; Feron, R.; Navazio, L.; Mariani, P.; Pierson, E.; Wolters-Arts, M. (2006). Expression and localization of calreticulin in tobacco anthers and pollen tubes. Planta, 223: 1263–1271.

Kim, J.H.; Nguyen, N.H.; Nguyen, N.T.; Hong, S.W. and Lee, H. (2013). Loss of all three calreticulins, CRT1, CRT2 and CRT3, causes enhanced sensitivity to water stress in Arabidopsis. Plant Cell Rep., 32: 1843–1853.

Xiang, Y.; Du, C.; Jia, H.; Lei, l.; Min song, M.; Yun Wang, Y. and Ma, Z. (2018). A wheat calreticulin gene (*tacrt1*) contributes to drought tolerance in transgenic *Arabidopsis. Pak. J. Bot*., 50(1): 19-28.

McAinsh, M.R. and Pittman, J.K. (2009). Shaping the calcium signature. New Phytol. 181: 275–294.

Poovaiah, B.W. and Reddy, A.S.N. (1987). Calcium messenger system in plants. CRC Crit. Rev. Plant Sci., 6: 47–103.

Hu, X.Y.; Neill, S.J.; Cai, W.M. and Tang, Z.C. (2004). Induction of defence gene expression by oligogalacturonic acid requires increases in both cytosolic calcium and hydrogen peroxide in Arabidopsis thaliana. Cell Res. 14: 234–240

Kaplan, B.; Davydov, O.; Knight, H.; Galon, Y.; Knight, M.R.; Fluhr, R. and Fromm, H. (2006). Rapid transcriptome changes induced by cytosolic Ca2+ transients reveal ABRE-related sequences as Ca^{2+}-responsive cis elements in Arabidopsis. Plant Cell, 18: 2733–2748.

Reddy, A.S.; Ali, G.S.; Celesnik, H. and Day, I.S. (2011). Coping with Stresses: Roles of Calcium- and Calcium/Calmodulin-Regulated Gene Expression. The Plant Cell, 23: 2010–2032.

Hassan, I.S.; Attiya, H.J.; Shamma, A.M and Abdullah, K.H. (1999). Tolerance of some wheat genotypes (*Triticum aestivum* L.) to various levels of salinity. IPA J. of Agric. Res. Vol 9: No.1.

Xiang, Y.; Hai, Y.L.; Song, M.; Wang, Y.; Xu, W.; Wu, L.; Wang, H. and Ma, Z. (2015). Over expression of a *Triticum aestivum* Calreticulin gene (TaCRT1) Improves Salinity Tolerance in Tobacco. plos one doi:10.1371/journal.pone.0140591