Iraqi Journal of Biotechnology, 2017, Vol. 16, No. 4, 69-78

InVitro: Detection *TaSOS1* gene in four Iraq genotypes of Bread Wheat under different salt stress levels.

Ali A. AL-Salihy [1] , Mundher Kh. Jabbar [2]

[1] Institute of Genetic engineering and Biotechnology , Baghdad University
[2] College of Agriculture , Al-Qassim green University

Received: August 13, 2017 / **Accepted**: October 10, 2017

Abstract: In this study, Four Iraqi genotypes of Bread Wheat (Iraq, 2H,3H and Hussein) were used to detection (*TaSOS1)* gene (a transmembrane Na^+/H^+ antiporter) under salt stress with different concentrations of NaCl (4,8,12 and 16 $ds.m^{-1}$) by using PCR technique. The results showed that Iraq cultivar was sensitive to salinity, It gave less values in all trait studies. 2H and Hussein genotypes were salt tolerance, It showed in most trait studies (Callus relative growth , Calli water content , Proliferation efficiency of dry weight, Regeneration frequency , shoot length and K+/Na+) , 3H genotypes was salt tolerance but less degree from 2H and Hussein. The detection on gene by using PCR technique , Gene was shown in three genotypes (2H,3H and Hussein) but it was not found in Iraq . So this gene was found important to salt tolerance .

Key words : *TaSOS1* , 2H , 3H , K^+/Na^+ , Bread Wheat , Gene detection .

Corresponding author : should be addressed (Email: Mastermundher@gmail.com).

Introduction

Salinity is an important and serious problem for agricultural production in many regions. Salinity can increase rapidly in soil and adapting to this increase is challenging for plants (16 , 17).

According to the UN Food and Agricultural Organization (13), over 800 million ha of land world-wide (which accounts for only 6% of the total land area of the world) are influenced by saline soil. Salinity affects approximately 32 million ha of the 1,500 million ha farmed in dryland agriculture. Of the 230 million ha of irrigated land, 45 million ha are salt affected (13).

High salinity interferes with plant growth and development and can also lead to physiological drought conditions and ion toxicity (28). In order to study the mechanism of Plant response to the salinity needs to understand the physiology and molecular biology of salinity tolerance , *InVitro* experiments are one of important methods used in checking , detection and identify efficiency genotypes of salt stress tolerance .

In Many studies used Growth Callus and regeneration as means to study it effect factor, it is given indictors very important for field plant , it is necessary to study all effects from any stress on plant cells .

Effect of salt stress in growth callus was differentiated among different Wheat genotypes depending on salt

tolerance degree (15) studied effect of NaCl salt added in callus growth of two genotypes found differentiations in response among genotypes. Callus regenerated percentage differed between genotypes . Another study , one genotype gave high Callus regenerated percentage with no differences under salt stress (4) .

In study of calli and shoots under 11 NaCl concentration ,the genotypes differed in relative fresh weight in high level between 0 to 5.76 % , all genotypes gave regeneration under all concentrations with difference in percentage of regeneration and number of shoot per callus (23).

The mechanisms of salt tolerance are controlled by factors run in manner of aggregation , These factors are known (genes) , There are many genes responsible for tolerance of salinity , *SOS1* is one of important genes in tolerance of salinity , *Triticum aestivum* L. *SOS1* is encoded putative plasma membrane Na+/H+ antiporter Protein (It is also known as Salt overly sensitive1) , Function of protein is the exclusion of Na+ from cells and instead of it is K+ . Na+ is ported in receptors instead of H+but this protein will be preventing this porter , some studies noted found K+ in receptors with high expression of *SOS1* gene under salinity conditions and others exclusion Na+ outside cells (1) .

SOS1 plays a crucial role in sodium exclusion from root epidermal cells under salinity (6) noted for *TaSOS-1*, a plasma membrane Na+/H+ antiporter, more transcripts accumulated in the roots and leaf sheaths, The role of *SOS1* in controlling ion homeostasis has been shown through a combination of biochemical , genetic and physiology-ical analyses (20,26) .

There are very few studies on *SOS1* gene in bread wheat , So this study was conducted *InVitro* to study effect of salinity and detection *SOS1* gene .

Materials and Methods

This experiment was conducted in tissue culture lab. / Institute of Genetic Engineering and Biotechnology – University of Baghdad through 2016-2017 to study effect of salt stress in growth , chemo-biology and physio-logical traits of callus and regeneration.

Genotypes

Four Iraqi genotypes of Bread wheat (*Triticum aestivum* L) are G1= Iraq (salt-sensitive) , G2=2H, G3=3H and G4= Hussein (unknown to salt stress tolerant) were used .

Preparation of explain used

In Laminar air flow hood , seed soaked in ethanol 70% with starrier for 1 minute . Then seeds washed in distilled sterile water three time , Seed transfer to tubes have hypochlorite Sodium 5% + 1 drop tween 20 for 20 minutes with starrier , then seeds washed in distilled sterile water 3-5 minute three times with starrier (21) . Seeds soaked in distill sterile water for 12-24 hours , finally, embryos were directly isolated from seeds .

Callus induction media

For callus induction , Media were used in one liter of water which included 4.91 $g.L^{-1}$ M.S , 30 g sucrose , 3 mg 2,4-D +0.5 mg kinetin and 7 g agar (Hardening media) , pH set in 5.7 and autoclaved in 121°C with 1 bar

pressure for 15 minutes. (27) . Culture embryos were incubated in dark at 22 ± 2 ◦C (2). Embryos were left from 2-4 weeks to callus initiation and then transfer directly to stress media.

Reculture callus in stress media

For salt stress test , NaCl were added four concentrations (4,8,12 and 16 $ds.m^{-1}$) of media included 5 mg Benzaladinen + 1 mg 2,4-D and M.S media (4.91 $g.L^{-1}$ +30 g sucrose + 7 g agar with set pH on 5.7) .

Regeneration calluses were incubated in 16-h light/8-h dark photoperiod and 60 ± 10% relative humidity, calluses were left 4-6 weeks for regeneration then traits were measured.

Parameters evaluation

The studied parameters were calculated for each genotype, using the following formulae:

- Callus relative growth (CRG)= [(FFW (final fresh weight)-IFF (initial fresh weight)] / IFF (8)
- Calli water content: CWC (%) = 100 x (CFW (Callus Fresh Weight) – CDW (Callus Dry Weight)) / CFW. CDW (8)
- Proliferation efficiency for Dry weight : (PED)= [(FFW (final fresh weight)-IFF (initial fresh weight)] /FFW
- Regeneration fequency = (number of regenerated calli / total number of calli) x 100.
- Shoot length = measured in mm at end experiment.

Measurement of K+/Na+

Grilled dried models were taken 0.1 g dry weight and digested in 2.5 ml H_2SO_4 concentrate with 1.5 ml Perchloric acid , mixture was heated for half an hour with continuous stirring, the elements were measured in Flame photometer (14).

DNA extraction and PCR:

Total genomic DNA was extracted according to the standard procedure of. (12) with some modifications. About 0.1g of fresh and healthy callus was selected randomly from each genotype, ground to a fine powder using liquid nitrogen. Five milliliters of hot (60ºC) cetyltrimethylammonium bromide (CTAB) extraction buffer (2% CTAB, 1.4 M NaCl, 0.1 M Tris-HCl pH 8, 20 mM EDTA and 0.2% β-mercaptoethanol) were added, mixed well , and incubated at 60ºC in a water bath shaker. After 60 min of incubation with gentle swirling, the resulting cells were lysed and extracted with an equal volume of chloroform/isoamyl alcohol (24: 1 v/v). The cells lysate was then centrifuged at 4000 g، 20ºC for 15 min. The aqueous phase was transferred into another tube where precipitation occurred by adding of 2 ml of isopropanol. The precipitate was then collected by centrifugation (10000 g, 20ºC، and 10 min). Pellets were washed with 70% ethanol, dried and dissolved overnight at 4ºC in 1 ml of tris EDTA (TE) buffer (10 mM Tris – HCl pH 8.0, 1 mM EDTA). After purification The amplification reac- tions were carried out in a lineGeneK thermal cycler with initial denaturing of 94 °C for 2 min, followed by 40 cycles of ° 94C for 10 s, annealing temperature (Ta) of each of the primer pairs for 15 and 30 s of extension at 72 °C. After 40cycles, the specificity of the amplifications was checked based on melting curves

resulted by heating the amplicons from 50 to 95 °C. All amplification reactions were repeated twice under identical conditions, in addition to a negative control and five standard samples . the resultant DNA was quantified and its integrity was determined after agarose gel electrophoresis as previously described(7).

Primer design was carried out using Allele ID 7 software for the internal control, *TaSOS1* (AY326952) , SOS1-1F GCGTAAATGTGCGACACTCC , SOS1-1R TCATCTCATCTGACGGCACC , SOS1-2F AGCGTGTCGTATCCAAA G , SOS1-2R GTCGTCATCTTCTCC TACC.

Design and statistical analysis

Factorial Experiments in (CRD) Completely Randomized Design by using statistical analysis GenStat program (10) , The averages were Comparted by using Least Significant Differences at probability level (0.05) .

Results:

Callus relative growth:

After 4 weeks of growth callus under different salt stress levels , There were differentiations among genotypes , In S1 level all genotypes did not affect but in S2,S3 got clear reduction in G1 genotype , All genotypes had negative growth under S4 level Figure (1).

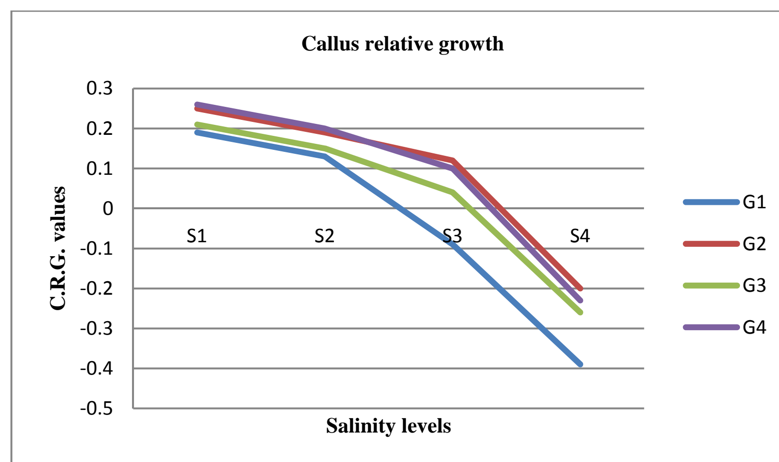


Figure (1): Callus relative growth of four genotypes (G1 : Iraq , G2 : 2H , G3 : 3H and G4 : Hussein) under four salinity levels (S1 : 4 *ds.m*$^{-1}$, S2 : 8 *ds.m*$^{-1}$, S3 : 12 *ds.m*$^{-1}$ and S4 : 16 *ds.m*$^{-1}$) in *In Vitro* experiment.

Calli water content

The CWC is percentage of increase water in cells , Any increase give indicator to loss in dry matter . The callus genotypes G2,G4 gave less water content under S1 salinity level with simple increased at S2,S3 level , All genotypes had significant increased in water content in different degrees in S4 level figure (2).

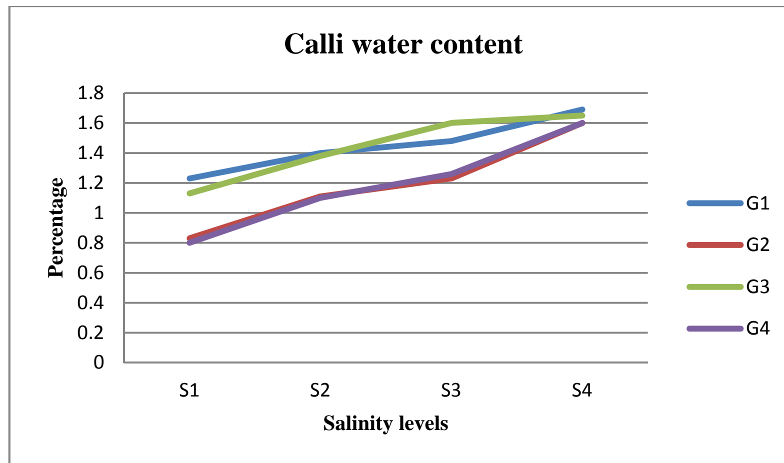


Figure (2): Calli water content (%) of four genotypes (G1 : Iraq , G2 : 2H , G3 : 3H and G4 : Hussein) under four salinity levels (S1 : 4 *ds.m*$^{-1}$, S2 : 8 *ds.m*$^{-1}$, S3 : 12 *ds.m*$^{-1}$ and S4 : 16 *ds.m*$^{-1}$) in *In Vitro* experiment

Proliferation efficiency of dry weight (P.E.)

This trait refers to quantity increased in dry matter in callus . All genotypes gave significant P.E. in S1,S2 with superiority to G2,G4 . In S3 level G1 was only decreased but all genotypes gave low values in S4 level figure (3).

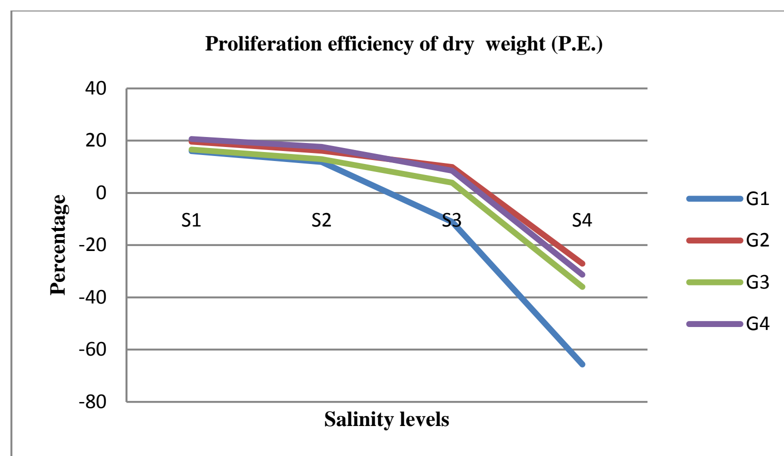


Figure (3) : Proliferation efficiency (%) of four genotypes (G1 : Iraq , G2 : 2H , G3 : 3H and G4 : Hussein) under four salinity levels (S1 : 4 *ds.m*$^{-1}$, S2 : 8 *ds.m*$^{-1}$, S3 : 12 *ds.m*$^{-1}$ and S4 : 16 *ds.m*$^{-1}$) in *In Vitro* experiment .

Regeneration frequency %(R.F.)

In figure (4), it indicate effect of salinity on shoots regenerated from callus . In S1 level genotypes G2,G3 and G4 gave more than 70% R.F., G2, G4 gave between 60-70% R.F. in S2 level Figure(1) but in S3 level gave between 40-50% R.F., All genotypes had negative regeneration under S4 level.

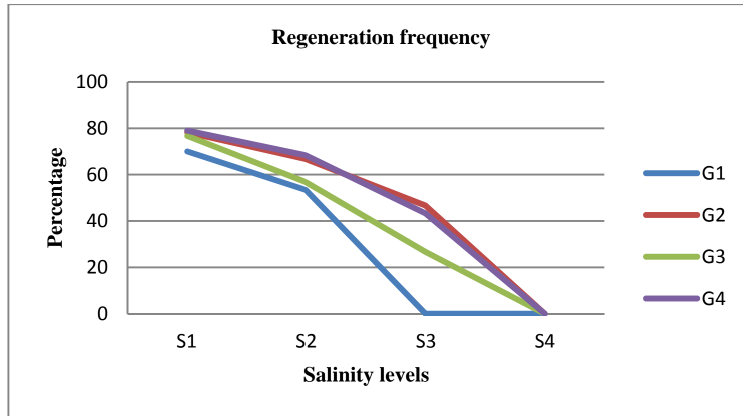
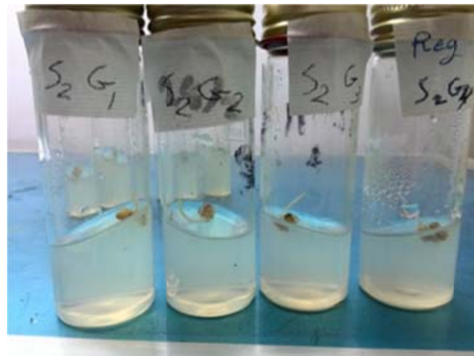


Figure (4): Regeneration frequency(%) of four genotypes (G1 : Iraq , G2 : 2H , G3 : 3H and G4 : Hussein) under four salinity levels (S1 : 4 *ds.m*$^{-1}$, S2 : 8 *ds.m*$^{-1}$, S3 : 12 *ds.m*$^{-1}$ and S4 : 16 *ds.m*$^{-1}$) in *In Vitro* experiment.



Picture (1) : Shows regeneration calli in four genotypes (G1 : Iraq , G2 : 2H , G3 : 3H and G4 : Hussein) at salinity levels (S2 : 4 *ds.m*$^{-1}$) after 4 weeks

Shoot length (S.L.):

In figure (5), there were differentiations among genotypes in most salinity levels. The S.L. was between 40-50 mm of genotypes G2,G4 at S1 level and between 30-40 mm of the same genotypes at S2 level , At level S3 only G2 was given S.L. of 20 mm .

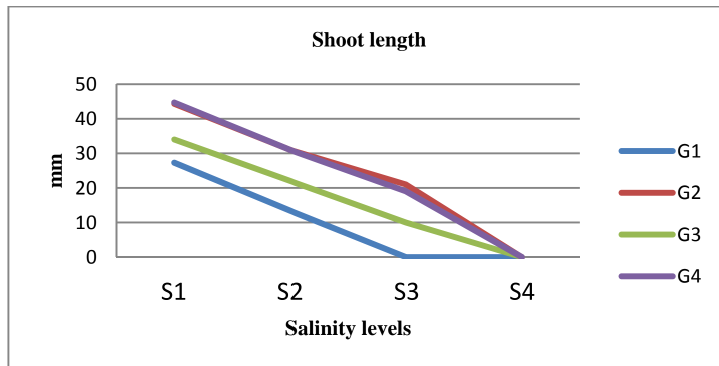
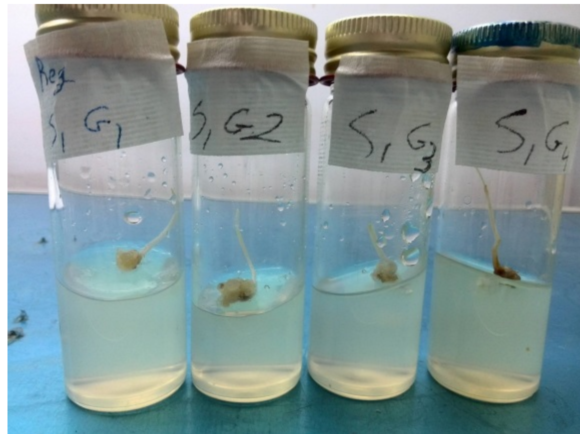


Figure (5) : Shoot length (mm) of four genotypes (G1 : Iraq , G2 : 2H , G3 : 3H and G4 : Hussein) under four salinity levels (S1 : 4 *ds.m*$^{-1}$, S2 : 8 *ds.m*$^{-1}$, S3 : 12 *ds.m*$^{-1}$ and S4 : 16 *ds.m*$^{-1}$) in *In Vitro* experiment.



Picture (1) : Shows shoot in four genotypes (G1 : Iraq , G2 : 2H , G3 : 3H and G4 : Hussein) at salinity levels (S1 : 4 *ds.m*$^{-1}$) after 4-6 weeks.

K+/Na+

This trait is giving indicator to role genes salinity tolerance, Genotypes G2,G4 gave values more than 12 in S1 level and values more than 8 in S2 level, G2,G4 gave values Between 4-6 in S3,S4 levels. So G2,G4 were the best in this trait figure (6).

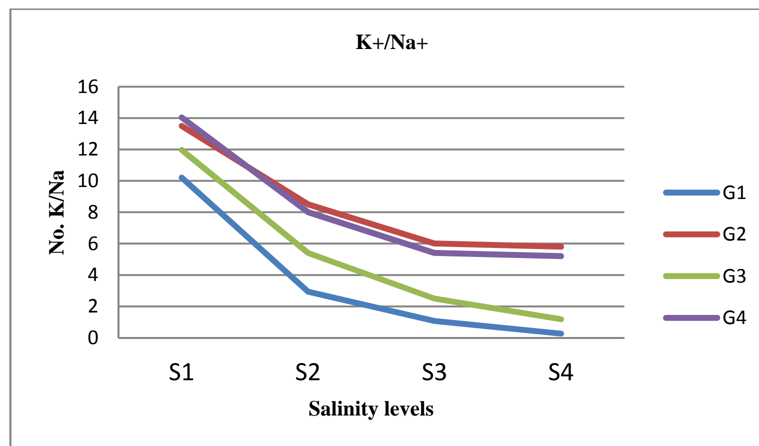
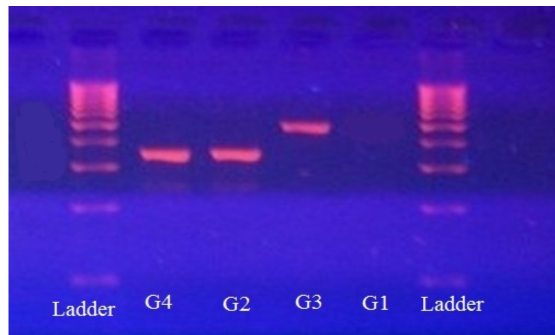


Figure (6) : Percent's K^{+}/Na^{+} of four genotypes (G1 : Iraq , G2 : 2H , G3 : 3H and G4 : Hussein) under four salinity levels (S1 : 4 *ds.m*$^{-1}$, S2 : 8 *ds.m*$^{-1}$, S3 : 12 *ds.m*$^{-1}$ and S4 : 16 *ds.m*$^{-1}$) in *In Vitro* experiment.

DNA detection (*TaSOS1*):

In the experiment use six primers were used for gene detection , but two primers gave bands of *TaSOS1* gene. Molecular study showed *TaSOS1* gene was found in all genotypes except G1. This indicator refers to genotypes efficiency to salinity tolerance especially G2,G4 and G3 in less degree.



Picture (3): Gel electrophoresis (1% agarose , 7 V/cm for 90 min) of *TaSOS1* in *Triticum aestivum L.* , first and last line : 50 bp DNA ladder , lines (2,3 and 4) positive results for *TaSOS1* gene with 135 amplicon.

Discussion:

Callus during the stress period did not affect in low salinity level in all genotypes (6) , when raised to high level (12 ds.m^{-1}) most genotypes were at significant level but G2,G4 gave superiority , It is clear in callus relative growth , Calli water content and Proliferation efficiency of dry weight . G1 gave clear negative growth with increased salinity in media (3) , So the callus genotypes are high tolerant characterized which resist to high salinity levels (5,19).

To select tolerance of genotypes *InVitro* , most study takes regeneration from callus . High tolerant genotypes have simple reduction in regeneration (4), G2,G4 had less reduced in regeneration frequency and shoot length until 12 ds.m^{-1} .

With the increase of Na+ , it had seen Accumulation inside cells and tissues (22) . K+ is the Alternative element to Na+ because two elements connect to the same porter in cell surface (25) , So any increase in K+ gave reduction in Na+ . The H.T.G. had high levels of percent K+/Na+ and less reduction with increased salinity levels. it had different among genotypes in K+/Na+ in this study , G2,G4 had gradual reduced but no differences among (S3) and (S4) levels especially G2 .

Early domestication of crop plants and plant breeding dramatically eroded the inherent allelic variation of many crop species. This has led to an increasing susceptibility of crop plants to environmental stresses, diseases and pests.

Salt tolerance in cultivated wheat can be improved by using the allelic repertoire offered by wheat wild relatives. For this reason, the existing variability currently available in gene pools must first be characterized at physiological, morphological and genetic levels (24,18).

Transcript accumulation of the plasma membrane Na+/ H+ antiporter, *TaSOS1* , the existence of differences in the patterns of expression of *TaSOS1* between the three cultivars support the idea that Na+ membrane transporters may contribute to the observed differential stress tolerance. However, some recent findings have reported apparent correlation between leaf plasma membrane Na+/ H+ antiporter, *TaSOS1* and wheat cultivars salt tolerance (9). Thus, it appears that excluding Na+ and always sufficient to increase plant salt tolerance and that

other physiological traits should also be considered. (11) .

The genotypes were different in gene expression , G2,G4 gave high expression but G3 gave low expression with the increase of salinity levels (it is found in most traits) but G1 was sensitive to salinity (gene did not appear in detection).

Reference:

1. Agarwal, S.K.; Zhu, J.; Kim, K.; Agarwal, M.; Fu, X.; Huang, A. and Zhu, J.K. (2006). The plasma membrane Na +/H+ antiporter *SOS1* interacts with RCDl. and functions in oxidative stress tolerance in Arabidopsis. *Proc Natl Acad. Sci.* USA. 103:18816-18821.
2. Akhtar, N.; Sajjad, U.R.C.; Zubeda, C.M.H.M. and Mozammil, H. (2007). Callus induction and regeneration in wheat cultivars under sodium and calcium chlorides salt stress. *Sarhad J. Agric.,* 23(3):649-654.
3. Al Hattab, Z.N.; Hamdalla, M.Sh. and Mohammed, M.A. (2015). Salinity effect on wheat *Triticum aestivum* L. callus growth and development, *Int. J. of Multidisciplinary and Current research*, Vol.3 : 1185-1188.
4. Ayed-Slama, O.; Ayed, S. and Slam-Amara, H. (2015). Selection of Tolerant Lines to Salinity Derived from Durum Wheat (*Triticum durum* Desf.) in Vitro Culture. *Agri. Sci. J.* (6) :699-706.
5. Benderradji, L.; Faical, B.; Kamel, K.; Nadia, Y.; Abdelhamid, D.; Khaled, M. and Hamenna, B. (2012). Callus induction, proliferation, and plantlest regeneration of two bread wheat (*Triticum aestivum* L.) genotype under saline and heat stress conditions. *ISRN Agronomy*. 3 :1-8.
6. Benderradji, L.; Faical, B.; Amar, B.; Kamel, K.; Azaza, J.; Bouzerzour, H.; Khaled, M. and Hanin, M. (2011). Sodium transport in the seedlings of two bread wheat (*Triticum aestivum* L.) genotypes showing contrasting salt stress tolerance , *Aus. J. of crop Sci.*, 5(3) : 233-241 .
7. Biase, F.H.; Franco, M.M. ; Goulart, L.R. and Antunes, R.C. (2002). Protocol for extrac-tion of genomic DNA from swine solid tissues , *Genetics and Molecular Bio. J.*, 25(3) : 313-315.
8. Bouiamrine, H. and Diouri, M. (2012). Response of durum wheat (Triticum durum Desf.) callus culture to osmosis induced drought stress caused by polyethylene glycol (PEG). *Annals of Bio. Res. J.*, 3(9): 4555-4563.
9. Brini, F.; Amara, I.; Feki, K.; Hanin, M.; Khoudi, H. and Masmoudi, K. (2009). Physiolo-gical and Molecular analysis of seedlings of two Tunisian durum wheat (*Triticum turgidum* L. subsp. Durum [Desf.]) varieties showing contrasting tolerance to salt stress. *Acta. Physiol. Plant. J.* , 31: 145-154.
10. Compton, M.E. (1994). Statist-ical methods suitable for the analysis of plant tissue culture data. *Plant Cell Tiss. Org. Cult. J.* , 37: 217–242.
11. Cuin, T.A.; Tian, Y.; Betts, S.A.; Chalmandrier, R. and Shabala, S. (2009). Ionic relations and osmotic adjustment in durum and bread wheat under saline conditions. *Functional Plant Biology J.*, 36: 1110–1119.
12. D'Ovidio, R.; Tanzarella, O.A.; Lafiandra, D. and Porceddu, E. (1995). Identification of durum wheat cultivars with good and poor quality by PCR based markers, *CIHEAM J.* , Vol. 22 : 241-247.
13. FAO. (2014). "Declaration of the world Summit on Food Security." 8:30 am Retrieved 03 July.
14. Flowers, T. and Dalmond, D. (1992) Protein synthesis in halophytes: the influence of potassium, sodium and magnesium in vitro. Plant and Soil, 146: 153-161.
15. Javed, F. (2002). In vitro salt tolerance in wheat. III. Water relations in callus, *Int. J. Agri. Biol.,* 4(4):459-461 .
16. Munns, R. (2002). Comparative physiology of salt and water stress. Plant, *Cell & Environment J.*, 25: 239-250.
17. Munns, R. and Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology* 59: 651-681 .
18. Nevo, E. (2001). Evolution of genome-phenome diversity under environmental stress. *Proc Natl Acad. Sci. USA* 22, 98 (11): 6233–6240.
19. Omran, Z.S.; Muhammed, H.S.; Abd Al-Sahib, A.S. and Ibrahim, K.M. (2013). *In vitro* screening of three Iraqi Wheat (*Triticum aestivum* L.) genotypes for salt

tolerance . *Egypt J. Exp. Biol.*, 9(2) :193-197.

20. Qiu, Q.; Guo, Y.; Dietrich, M.A.; Schumaker, K.S. and Zhu, J.K. (2002). Regulation of *SOS1*, a plasma membrane Na+/H+ exchanger in Arabidopsis thaliana, by *SOS2* and *SOS3*. *Proc. Natl Acad. Sci. USA*, 99:8436-8441.
21. Rashid, U.; Ali, S.; Ali, G.M.; Ayub, N. and Masood, M.S. (2009). Establishment of an efficient callus induction and plant regeneration system in Pakistani wheat (Triticum aestivum L.) cultivars. *Electronic J. of Biotech.* 12(3):1-12.
22. Rajendran, K. (2012). Comp-onents of salinity tolerance in Wheat. degree of Doctor, School of Agriculture, Food and Wine Discipline of Plant Breeding and Genetics Australian Centre for Plant Functional Genomics.
23. Rattana, Kh. and Bunng, S. (2015). Differential Salinity tolerance in calli and shoots of four rice cultivars. *Asian J. of Crop Sci.* ,7(1) : 48-60.
24. Robinson, D.; Handley L.L.; Scrimgeour, C.M.; Gordon, D.C.; Forster, B.P. and Ellis, R.P. (2000) Using stable isotope natural abundances (delta 15 N and delta 13C) to integrate the stress responses of wild barley (*Hordeum spontaneum* C. Koch.) genotypes. *J. of Exp. Bot.* 51(342):41–50.
25. Shamaya, N.J. (2013). Genetic Studies of Salinity Tolerance in Wheat. degree of Doctor , School of Agriculture, Food and Wine Discipline of Plant Breeding and Genetics Australian Centre for Plant Functional Genomics .
26. Shi, H.; Quintero, F.J.; Pardo, J.M. and Zhu, J.K. (2002). The putative plasma membrane Na+ /H+ Antiporter SOS1 controls long-distance Na+ transport in plants , *The American plant cell J.*, 14 : 465-477.
27. Zale, J. M.; Barmony-Wier, H.; Kidwell, K.K. and Steber, C.M. (2004). Callus induction and plant regeneration from mature embryos of a diverse set of wheat genotypes, *Plant Cell, Tissue and Organ Culture J.* , Vol. 76 : 277-281 .
28. Zhu, J.K. (2002). Salt and drought stress signal transduction in plants. . *Annual Review of Plant Physiology*, 53: 247-273.