STUDY OF *TaSOS4* GENE AND YIELD , CHEMICAL TRAITS IN SOME BREAD WHEAT GENOTYPES UNDER SALINITY CONDITIONS

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ABSETRACT

This experiment was carried out at greenhouse in Centre of Biotechnology Researches/ University of Nahrin using soil clay loam texture, soil was in pots closed to study the effect of different salinity levels on detection *TaSOS4* gene and yield traits in some bread wheat genotypes (*Triticum aestivum* L.).Wheat seeds were planted in Nov. 2016 with ten seeds in each pot. a factorial experiments within Randomized Complete Block Design with three replications was used and Four levels of salinity (4, 8 , 12 and 16 $ds.m^{-1}$) with four genotypes (G1:Iraq, G2: 2H ,G3: 3H and G4: Hussein). The result showed non-significant differences among genotypes under first salinity levels (4 $ds.m^{-1}$) in most traits. in second salinity level (8 $ds.m^{-1}$), G2,G4 gave variation on G1 over 10 in No. grains. $Spike^{-1}$, 5-10 in grain 1000weight , over 2% in percentage of protein and K^+/Na^+ ratio value over 7. in third salinity level (8 $ds.m^{-1}$), G2,G4 gave variation on G1 over 25 in No. grains. $Spike^{-1}$, over 12 in grain 1000weight, over 100% in plant grains yield , 2-3% in percentage of protein , over 5 in K^+/Na^+ ratio value . in forth salinity level (16 $ds.m^{-1}$), G2 gave variation on G1 over 25 in No. grains. $Spike^{-1}$, over 14 in grain 1000weight, over 100% in plant grains yield, over 3% in percentage of protein , over 5 in K^+/Na^+ ratio value. Molecular study showed *TaSOS4* gene was found in G2 ,G3 and G4 genotypes but it did not appear in G1 genotype. This indicator refers to differences among genotypes in salinity tolerance and sensitive to salinity in G1 genotype.

Key words: SOS4, gene detection, grains yield, wheat, genotypes , K^+/Na^+ ratio .
***Part of Ph.D Dissertation of third author.**

دراسة جين *TaSOS4* وصفات الحاصل والكيمياوية في بعض التراكيب الوراثية لحنطة الخبز تحت ظروف الاجهاد الملحي

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المستخلص

اجريت هذه الدراسة في بيت بلاستيكي في مركز بحوث التقانات الاحيائية/جامعة النهرين باستعمال تربة مزيجيه طينية وضعت في اصص مغلقة، بهدف دراسة تأثير مستويات مختلفة من الملوحة على الكشف لجين *TaSOS4* وصفات الحاصل ومكوناته في بعض التراكيب الوراثية لحنطة الخبز ، والتي زرعت بتاريخ 2016/11/15 بعشرة حبوب في كل اصيص، استعملت التجربة العاملية ضمن تصميم القطاعات الكاملة المعشاة وبثلاث مكررات تضمنت اربع مستويات ملوحة (4 ، 8 ، 12 ، 16 ديسي سيمنز. $م^{-1}$) مع اربع تراكيب وراثية (العراق ، 2H ، 3H ، الحسين). اظهرت النتائج عدم وجود فروقاً معنوية بين التراكيب الوراثية عند مستوى الملوحة الاول ، اعطت التراكيب الوراثية 2H والحسين في المستوى الثاني والثالث للملوحة تباين معنوي عن التركيب الوراثي العراق في كل من عدد الحبوب بالسنبلة ووزن الف حبة والنسبة المئوية للبروتين ونسبة البوتاسيوم الى الصوديوم ، في المستوى الرابع للملوحة التركيب الوراثي 2H اعطى تباين معنوي عن التركيب الوراثي العراق في كل من عدد الحبوب بالسنبلة ووزن الف حبة والنسبة المئوية للبروتين ونسبة البوتاسيوم الى الصوديوم . أظهرت الدراسة الجزيئية وجود الجين *TaSOS4* في التراكيب الوراثية 2H ، 3H والحسين وعدم ظهوره في صنف العراق .

الكلمات المفتاحية: SOS4، الكشف عن جين، حاصل الحبوب، الحنطة، تراكيب وراثية، نسبة البوتاسيوم الى الصوديوم.
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INTRODUCTION

Salinity is an important and serious problem for agricultural production in many regions. Salinity could be increase rapidly in soil and adapting to this increase is challenging for plants (16)(17) It considers soil salinity of the main problems of the plant, which directly affect the plant as the soil salinity caused major damage to the plant can be summarized as in the basic physiology of high salinity stress overlaps with each other as a high salt deposition in soil leads to a deposition of a low water potential zone in the soil , salinity causes ion-specific stresses resulting in an altered K^+/Na^+ ratio , it leads to a buildup of Na^+ and Cl^- concentrations in the cytosol, which can be ultimately detrimental to the cell , Higher concentrations of sodium ions are toxic to cell metabolism and can inhibit the activity of many essential enzymes, cell division and expansion, membrane disorganization, and osmotic imbalance, which finally can lead to growth inhibition (22). Higher concentrations of sodium ions can also lead to a reduction in photosynthesis and the production of reactive oxygen species (11) , High salinity can also injure cells in transpiring leaves, which leads to growth inhibition, The salt can concentrate in the old leaves then would be leaves died, which is crucial for the survival of a plant (15). In wheat, Baloch *et al.* (2014) found differences in plant height, no. branch/plant, biological yield, spike length , no. grains/spike and grain yield when studied five genotypes of Wheat under two effect of salinity , (LU-26 cultivar) was the best . Hamam and Negim (2014) noted at assess 16 genotypes of spring Wheat which planted in greenhouse and irrigated by salt water under 4 different levels NaCl found reduction in no. tillers.plant-1 , No. leaves.plant-1, leaf area in growth stage, no. grains.spike^{-1} , grains 1000 weight and grains yield , with K^+ concentration and K^+/Na^+ , the genotypes (Shakha 93 , HAAMA-14, Shakha8) could growth under high salt stress environments. Salt tolerance controlled plants in many mechanisms, this mechanism are organized by genetic factors(genes) run in manner of aggregation, more 8 genes are running plant to tolerance of salinity (production proteins and enzymes are responsible for management mechanisms cell) there are many genes responsible for tolerance of salinity (5), *SOS4* is one of important genes in tolerance of salinity in *Triticum aestivum* L. *SOS4* is encoded *SOS4* pyridoxal kinase (vitamin B6 kinase) enzyme (It is also known as salt overly sensitive4), it is known the active form of vitamin B6 and is involved as essential cofactor in amino acid biosynthetic pathways and a diversity of other enzymatic reactions, In plants, PLP is known as cofactor of various key enzymes which play a role in ethylene biosynthesis, chlorophyll, de novo sphingolipid biosynthesis and carbohydrate metabolism. Moreover, it have been shown to have antioxidant properties impeding the formation of reactive oxygen species (ROS) and are considered to be involved in stress response pathways, *TaSOS4* was showed appear with increase salinity in soils (23). So the study on molecular level of *SOS4* gene in Bread Wheat very important, this study was conducted *InVivo* to study effect of salinity on plant traits and detection *SOS4* gene.

MATERIALS AND METHODS

This experiment was conducted during winter 2016-2017 in Greenhouse of Biotechnology Researches Center – Univ-ersity of AL-Nahrin , to study effect of salt stress in growth , yield in four Iraqi genotypes of bread wheat and detection of *TaSOS4* gene.

Treatments experiment

In main plots were salinity levels in soil (mixture natural soil with salinity soil and measured continually) : S1 (4 $ds.m^{-1}$) , S2 (8 $ds.m^{-1}$) , S3 (12 $ds.m^{-1}$) and S4 (16 $ds.m^{-1}$) ; sub plots were the genotypes : G1 (Iraq) sensitive , G2 (2H), G3 (3H) and G4 (Hussein).

Experimental and statistical analysis

The experiment was carried out as a factorial experiment with in Randomized Complete Block Design with three replications . The results were analyzed by using (ANOVA) and means compared was by L.S.D. test under incorporeity level 5% (6) with S.A.S. program (21).

Experiment preparations

Soil was placed in pots , physical and chemical traits of the soil shows in Table 1 , Pots soil size was 5 kg with closed base to prevent salt washing , date of planting 15-11-2016 (1) . Added complex fertilizer (18:18:0) 400 kg.ha^{-1} in two dates with urea 120 kg.ha^{-1} (46% N) in

two dates (20) , fertilizer added by calculating pot area to limit fertilizer quantity in pot (4.5 g complex fertilizer / pot and 1.3 g urea / pot .

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 (8)

DNA extraction and PCR

Total genomic DNA was extracted according to the standard procedure of. (4) with some modifications by Hamorabi kit (product by the Institute of Genetic Engineering and Biotechnology – University of Baghdad). Type of PCR was Thermocycler PCR , the PCR program was remembered from (7), Primer design was showed in Table 2 .

Parameters evaluation

Studied traits were :== No. grains.spike^{-1} , weight of 1000 grain (g) , plant grains yield (g) , percentage of protein , potassium/ sodium ratio.

Table 1. Physical and chemical characters of the soil

Property	values	unit
Soil separators		
Sand	178	g.kg^{-1}
Silts	479	g.kg^{-1}
Clay	333	g.kg^{-1}
Type soil :		
Mixed Clay		
Virtual density	1.32	Mica Gram
pH	7.19	
Humidity	55	%
Organic matter	5.2	g.kg^{-1}

Table 2. Parameters of primers

Parameters of *TaSOS4* gene	Forward primer	Reverse primer
Primer design	CTGACCAG CTTTGCTT GCCT	GGCGGTTGT TCCTCTGTT CT
Length (bp)	20	20
GC (%)	55	55
Start transcription site	4	741
Stop transcription site	23	722
Temp.	61.18	60.25
Self-complementarity	4.00	2.00
Self 3´ completmentarity	1.00	0.00

RESULTS AND DISCUSSION

No. grains. Spike^{-1} : There was significant differences between genotypes G4 and G1 less 10 grains with treatment (4 *ds.m*$^{-1}$) but It was increased over 10 grains with treatment (8 *ds.m*$^{-1}$) , in treatments (12 *ds.m*$^{-1}$) and (16 *ds.m*$^{-1}$) were the variation between G2,G4 and G1 over 25 grains (Figure 1) .

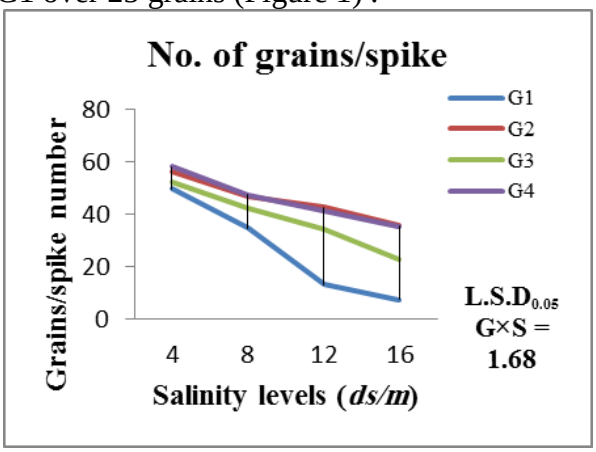


Figure 1. Shows interactions among four levels of salinity (4 , 8 , 12 and 16 *ds.m*$^{-1}$) with four genotypes G1, G2, G3 and G4.

Weight of 1000 grain (g)

All the genotypes gave variation in weight of 1000 grain less than (5 g) between higher value and lower value with treatment (4 *ds.m*$^{-1}$) but within (8 *ds.m*$^{-1}$) became 5-10 g , The variation increased between G2 and G1 in (12 *ds.m*$^{-1}$) over 12 g and (16 *ds.m*$^{-1}$) over 14 g (Figure 2) .

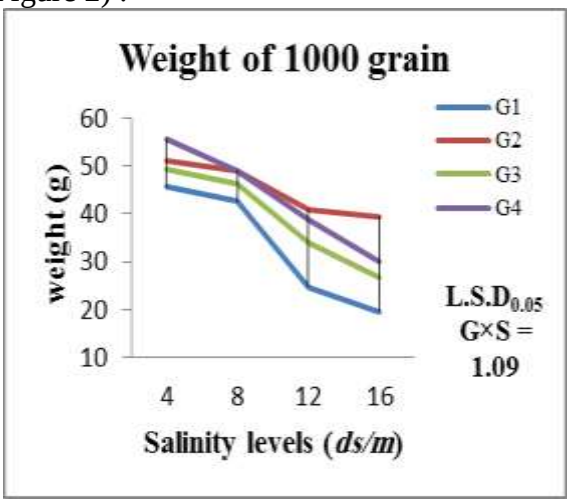


Figure 2. shows interactions among four levels of salinity (4 , 8 , 12 and 16 *ds.m*$^{-1}$) with four genotypes G1 , G2 , G3 : 3H and G4.

Grains yield . Plant^{-1} (g): The genotype G4 reverted variation in plant grains yield over (50 %) with G1 in treatment (4 *ds.m*$^{-1}$) , There are variation over 100% with increase salinity

to (12 , 16 *ds.m*$^{-1}$) was clearly among G2 with G1 which gave lowest (Figure 3).

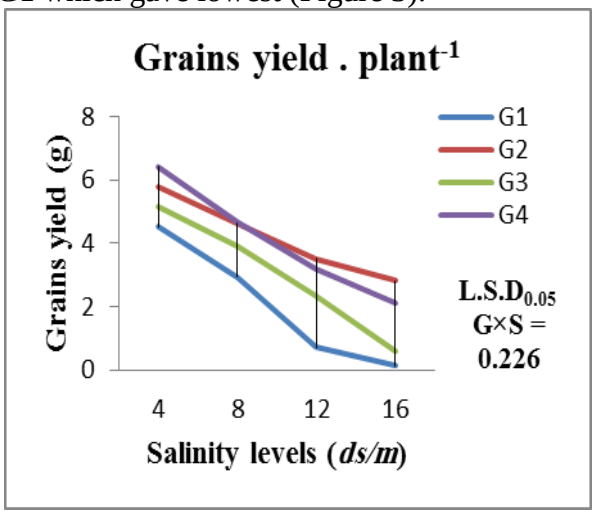


Figure 3 . Reveal interactions among four levels of salinity (4 , 8 , 12 and 16 *ds.m*$^{-1}$) with four genotypes G1, G2 , G3 and G4

Protein percentage (%)

All genotypes indicated variation under 2 % in treatment (4 *ds.m*$^{-1}$) but In treatment (8 *ds.m*$^{-1}$) and (12 *ds.m*$^{-1}$) was variation become over 2 % and under 3% , The variation arrived over 3% with treatment (16 *ds.m*$^{-1}$), especially between G2 and G1(Figure 4).

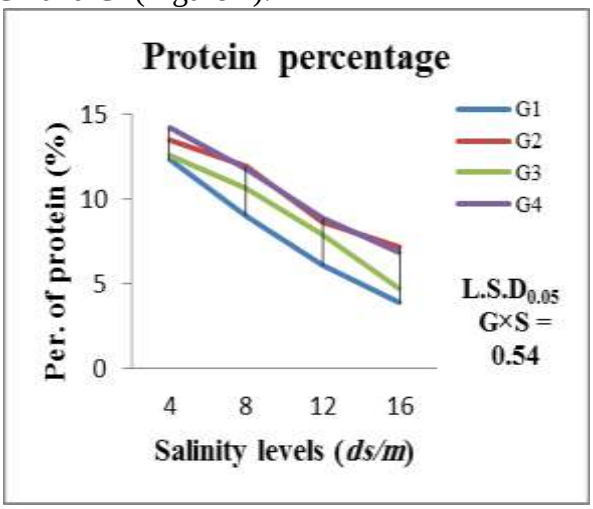


Figure 4 . Indicate interactions among four levels of salinity (4 , 8 , 12 and 16 *ds.m*$^{-1}$) with four genotypes G1, G2, G3 and G4 .

K^+/Na^+ ratio

The genotypes G2 ,G3 and G4 gave higher K/Na values over 10 with treatment (4 *ds.m*$^{-1}$), but genotypes G2 , G4 gave K/Na over 7 with treatment (8 *ds.m*$^{-1}$) , treatment (12 *ds.m*$^{-1}$) , (16 *ds.m*$^{-1}$) were with genotype G2 gave K/Na over 5 (Figure 5).

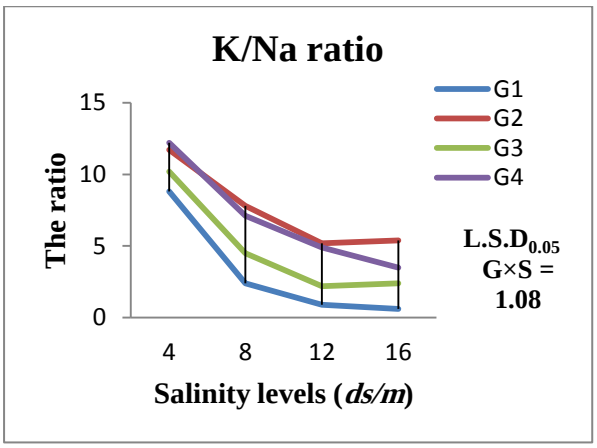
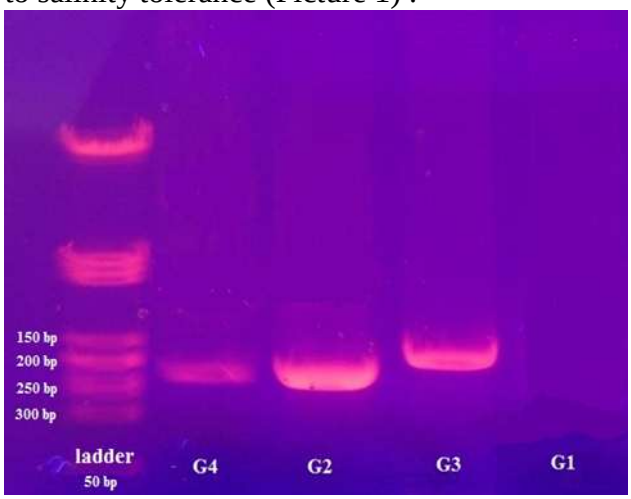


Figure 5 . Shows interactions among four levels of salinity (4, 8, 12 and 16 *ds.m*$^{-1}$) with four genotypes G1 , G2, G3 and G4

DNA detection (*TaSOS4*):

In this experiment used four primers for gene detection , but one primer gave bands of *TaSOS4* gene. Molecular study showed *TaSOS4* gene was found in G2 ,G3 and G4 genotypes but it did not appear in G1 genotype . This indicator refers to genotypes efficiency to salinity tolerance (Picture 1) .



Picture 1. Gel electrophoresis (1% agarose , 7 V/cm for 90 min) of *TaSOS4* in *Triticum aestivum L.* , first and last line : 50 bp DNA ladder, lines (2,3,4) positive results for *TaSOS4* gene.

Salinity is directly affected on yield and its components, some genotypes could be produced perfect number of grains.spike^{-1} and weight 1000 grain under salt stress by its ability in adaptation to the tolerance , this tolerance found through ability to tolerant cultivars on earliness of flowering and increase or keep fertility in flowers (10,3,12), This idea agree with the results of this study . Grain weight was found affected from many factors, salinity reduce from ability of plants in dry

matter accumulation because low biological events in leaves result small size and lowest number of leaves with effect chlorophyll content , that is reduced in photosynthesis process and active enzyme with stop some genes , all that has been mentioned is reflected on weight of 1000 grain (2) , but tolerant genotype was increased in composites transfer from factory to the store as way adaptation by increase gene expression to some tolerance salinity enzymes such as pyridoxal kinase which transcripted from *SOS4* gene (like in 2H genotype). Tolerance genotypes could begin normal grain yield under high salinity levels because its ability to adaptation by management all biochemical and physiological processes in plant to salt effect resistance (19). (such as 2H genotype in study). Amino acids are playing roles in salt stress tolerance, it is entry into enzymes and proteins composition, the accumulation of amino acids were reduced with increase salinity which causes low protein contents by effect on protein synthesis and amino acids production , same genotypes are tolerance high salinity by increase gene expression in same gene which responsible for low salt stress to allow protein building (18). It is stated that K/Na ratio play an important role in the measurement of salt tolerance, In our study significant decreases were observed in the K/Na ratio depending on increasing salt concentration. As a matter of fact the K/Na ratio decreased by % 48 in other applications in comparison with that of the control group this indicators refer to ability of cells and organs in tolerant genotypes to exclusion Na^+ ion, control of osmotic effect, also hormones management and Compartmentalization of Salt Elements (13) . *SOS4* is encoding the pyridoxal (PL) kinase enzyme, respectively, are the only known genes involved in the salvage pathway of pyridoxal 5'-phosphate (PLP) in plants , the *SOS4* mutant plants showed a distinct phenotype characterized by chlorosis and reduced plant size. so any increase in salinity is affected on growth and yield in plant, It is connected with appear salt tolerance genes such as *SOS* family genes, as well as hypersensitivity to sucrose in addition to, previously noted NaCl sensitivity in same genotypes, The *SOS4* showed increased activity of PDX3 as well as of the B6 de novo pathway enzyme PDX1. So this gene was involved in important mechanisms keep cells and divisions with growth and effect on plant yield and its composite (9) so if this gene and like others genes shall be appearing in any plant genotype, this refer to the genotype is tolerant with different degrees depend on gene expression .

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