

## DETECTION OF *TaST* SALT TOLERANCE GENE IN THREE SELECTED WHEAT GENOTYPES FOR SALINITY STRESS UNDER SALINITY CONDITIONS

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### ABSTRACT

The aim of this study is detection of salt tolerant gene (*TaST*) selected wheat genotypes for salt tolerance (1H, 2H, 3H) through plant breeding program as compared with local cultivars (sensitive) under salinity condition. Quantitative reverse transcription-PCR (q-RT-PCR) was used to detect *TaST* salt tolerant gene in these selected genotypes. Also chlorophyll and organic compounds contents were estimated in the upper leaves of the selected genotypes and local cultivars. Results of the PCR showed that the gene band of *TaST* appeared only in the selected genotypes with length 175bp at both salinity conditions, while this band absent in the local cultivars, (sensitive) (Iraq and latefyi), also at both salinity conditions. All the selected genotypes had the same band size (175bp). Chlorophyll content in the upper leaves of selected genotypes increased at salinity treatment, while it decreased in the leaves of local cultivars at this treatment as compared with non-saline conditions. The results also, showed that the selected genotypes had the highest organic compounds in their leaves, especially under salinity condition. It can be concluding that the selected genotypes (1H, 2H, and 3H) were more salt tolerance than local cultivars (Iraq, latefyi).

Key word: - wheat genotypes, salt tolerance gene, *TaST* gene, salinity.

مجيد

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الكشف عن الجين المتحمل للملوحة *TaST* في ثلاث تراكيب وراثية من الحنطة المنتخبة لتحمل الملوحة تحت ظروف الشد

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المستخلص

تهدف هذه الدراسة لتشخيص جين تحمل الملوحة (*TaST*) في بعض التراكيب الوراثية المنتخبة من الحنطة (1H, 2H, 3H) لصفة تحمل الملوحة من خلال برامج التربية وتحسين النبات بالمقارنة مع الاصناف المحلية الحساسة للملوحة (عراق ولطيفية) تحت ظروف الملوحة. استعملت تقنية PCR لتشخيص جين تحمل الملوحة (*TaST*) للتراكيب الوراثية المنتخبة والاصناف المحلية. بالاضافة الى تقدير محتوى الكلوروفيل والمركبات العضوية في الاوراق العلوية في تلك التراكيب الوراثية المنتخبة والاصناف المحلية. تشير نتائج PCR ظهور حزمة واحدة للجين *TaST* ذات وزن جزيئي 175bp فقط في التراكيب الوراثية المنتخبة وغيابه في الاصناف المحلية الحساسة للملوحة في التركيزين 2 و 16 ديسي سمنز م<sup>-1</sup>. لوحظ ايضا زيادة محتوى الكلوروفيل في الاوراق العلوية للتراكيب الوراثية المنتخبة تحت ظروف الملوحة بينما انخفضت في اوراق الاصناف المحلية بالمقارنة مع الترب العادية كذلك تشير النتائج ان التراكيب الوراثية تحتوي على نسبة عالية من المركبات العضوية في الاوراق خصوصا تحت ظروف الملوحة العالية. يمكن الاستنتاج بان التراكيب الوراثية المنتخبة (1H, 2H, 3H) اكثر تحملا للملوحة من الاصناف المحلية (عراق ولطيفية).

كلمات مفتاحية: الحنطة، جين تحمل الملوحة، *TaST*، الملوحة

## INTRODUCTION

Bread wheat (*Triticum aestivum* L.) considered as the most important crop in the world and Iraq. It has a high nutritional value and large grain yield. Salinity is one of the main factors that reduce plant growth and production and leads to crop failure. Crops cultivation in high concentrations of salt are subjected to the adverse effects of salt during all growth process, resulting in a lower production (18). There are efforts to induce salt tolerant cultivars or genotypes from wheat to overcome salinity problem and sustain the increasing in grain yield production in agriculture land under salinity condition (4) and also reducing the spread of secondary salinity (17). Increasing salt tolerance in plant requires new genetic source of this character powerful and molecular techniques for detecting salt tolerance genes in new material useful for determining the salt tolerance degree. Salt tolerance in plant depends on physiological mechanisms controlled by genes which gave expression under salinity conditions (2, 5). Plants have evolved several salt tolerance mechanisms to reduce the adverse effect of salinity (16), this mean that there are different genes for controlling these mechanisms. During the last decade, numbers of salt – responsive genes have been isolated and characterized (18). New visions were appeared to try determining genes that important role in plant salinity tolerance (19). Molecular techniques have been used to detect the salt –responsive genes in the some selected cultivars and genotypes of wheat, which selected for salt tolerance through plant breeding programs, these genes are *TaSC* and *TaSTK*. They detected in Dijilla, Furat cultivars and N3 genotypes (3, 15) and *TaGSK1* also was detected in Dijilla, Furat cultivars and 2H genotype (14). The gene *TaNIP* was detected in some other selected genotypes (1H, 2H and 3H) (2). The objective of this study is to detect a new salt tolerant gene (*TaST*) in selected wheat genotypes for salt tolerance and also to study some physiological characters such as chlorophyll and organic compounds content.

## MATERIALS AND METHODS

**Selected Genotypes Growth :** This study was carried out using three selected wheat

genotypes for salt tolerance which developed through plant breeding programs and two local cultivars were used to detect the salt tolerant gene (*TaST*) under salinity conditions. Seeds of selected genotypes (1H, 2H and 3H) and local cultivars (Iraq, latefyi) were planted in prepared soil salinity at two levels (2, 16 ds/m) under plastic house condition; five seeds for each pot and for each genotype and cultivars were sown. Three replications for each treatment and the plants grow under salinity conditions for 60 days from the sowing date. Then leaves sample were taken for RNA extraction.

### RNA Isolation and cDNA Synthesis

Total RNA were isolated using Geneaid total RNA purification mini kit (Taiwan) according to the manufacturer's instructions. Isolated RNA was treated with RNase-free DNase-I (Biobasic, Canada) for 20 min at 37°C, DNase-I was inactivated at 65°C for 10 min. The integrity of the RNA was verified after separation by 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 reverse transcription system (Bioneer, Korea) with an oligo-dT<sub>15</sub> primer. The reaction solution was used as templates for reverse transcriptase polymerase chain reaction (RT-PCR).

***TaST* Gene Amplification :** *TaST* (target gene) cDNA were amplified using specific primers shows in Table 1. 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, 40 cycles at 95°C for 1 min, 58°C for 45s and 72°C for 1 min and one final extension cycle at 72°C for 10 min.

**Table 1. Primers used for amplification of *TaST* sequences**

Primer	Sequences5'-3'	Reference
Forward	CGCAGGCCGTCGTCATG	(13)
Reverse	GACTGATCCTGCCAGCAAACAC	

### Estimation of organic compounds

**Ash (carbon) percentage:** Ash percentage was estimated according to the AOAC (9). Five gram of the plant were taken and burn in the oven (Muffle Furnace) at 500 C<sup>0</sup> until the plant samples changed to the white ash, then the sample was weighted and the percentage of the ash was calculated.

### Total lipids percentage

This percentage was estimated according to the AACC (1) by using soxhlet. 200ml of the petroleum ether were added the soxhlet and the thumble which contained 10g the dried plant material was put in the soxhlet. After 8h from the extraction, the solvent was evaporated by using rotary evaporator at 45C<sup>0</sup>. Then the total lipids were weighted and its percentage was calculated.

### Crude protein percentage

Nitrogen percentage in the plant sample was measured by using microkjeldahl (12). The percentage of crude protein was calculated as Following

$$\% \text{ crude protein} = \% \text{ Nitrogen} \times 6.25$$

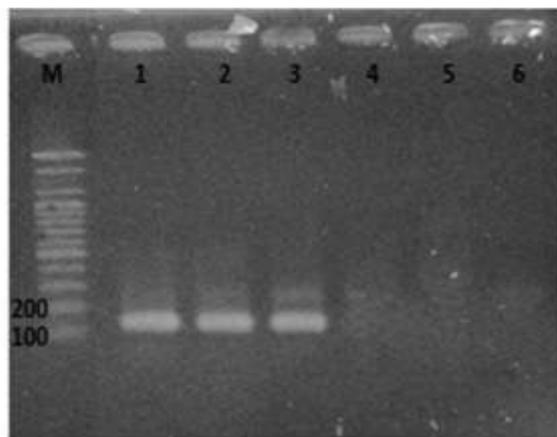
**Chlorophyll content:** The Chlorophyll content in the upper leaves was measured by using Chlorophyll meter Spad meter.

### RESULTS AND DISCUSSION

Salt stress is one of the main factors that reduce plant growth and production and lead to the crop failure; therefore, inducing salt – tolerant genotypes or cultivars are of great significance for overcoming salt problems and increase the yield production under salinity conditions.

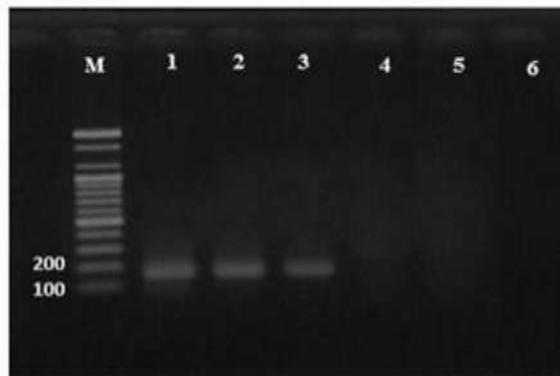
#### 3.1. Qualitative PCR reaction-Cdna

Gene detection technique was used to study the amplification of the *TaST* salt tolerant gene in three selected wheat genotypes and two local cultivars under saline and non-saline conditions. The results in Fig.1 show that the molecular weight of band of *TaST* gene was 175bp according to the DNA ladder. These results indicated that salt tolerant gene (*TaST*) was appeared only in the selected genotypes (1H, 2H and 3H) at non- saline conditions, while at the same condition the gene band was absent in both local cultivars (Iraq, latefyi) and negative control. The results of the amplification of the *TaST* gene under saline condition (16 ds/m) revealed that the gene band with 175 bp was appeared only in the selected genotypes (Fig.2), therefore at both conditions, the band of the *TaST* salt tolerant gene with 175 bp was appeared only in the selected genotypes and absent in the local cultivars (figs. 1, 2).



**Fig. 1. Ethidium bromide stained agarose electrophoresis (1.5 %) of PCR product (*TaST* gene) for wheat genotypes under non-saline condition. M: 100 bp DNA ladder, 1: 1H genotype, 2: 2H genotype, 3: 3H genotype, 4: Iraq cultivar, 5: latefyi cultivar, 6: Negative control**

This indicates that the salt tolerant gene (*TaST*) was found only in selected salt tolerant genotypes.



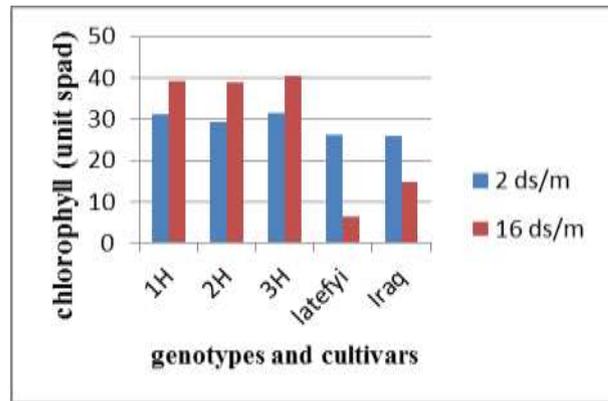
**Fig. 2. Ethidium bromide stained agarose electrophoresis (1.5 %) of PCR product (*TaST* gene) for wheat genotypes under saline condition. M: 100 bp DNA ladder, 1: 1H genotype, 2: 2H genotype, 3: 3H genotype, 4: Iraq cultivar, 5: latefyi cultivar, 6: Negative control**

Detection of salt tolerant genes in these selected wheat genotypes for salt tolerance and which used in this study and understanding their function have become the most urgent tasks in agricultural research today. During this study one of those genes that involved in salt tolerance in wheat (*TaST*) was in detected in these selected genotypes under both conditions, while it was absent in the local cultivars (sensitive) also under the both condition, this mean that *TaST* gene found only in the salt tolerant genotypes (selected

genotypes). Our results similar to those in the previous studies (13) whom they determine the relationship between *TaST* gene and salt tolerance, they performed expression pattern analysis in wheat mutants RH8706-49 and H8706-34 and they found that this gene is a salt – inducible gene.

**Chlorophyll content**

Chlorophyll content in the leaves is an important factor for determining the degree of plant response to the salinity. The results in the Fig. 3 summarize the chlorophyll content in the upper leaves of plant under salinity and non-salinity conditions. Those results were revealed that the chlorophyll content in the upper leaves of the local cultivars decreased to 6, 14 unit spad in latifyi and Iraq cultivars respectively under salinity condition (16 ds/m) as compared with 26, 26unit spad for the same cultivars respectively under the non-saline condition, while chlorophyll content increased under salinity condition to 39, 39, 41unit spad in the leaves, of the selected genotypes 1H, 2H and 3H respectively as compared with 31, 29, 32 unit spad for the same cultivars respectively under normal condition. The chlorophyll content under high salinity condition increased in the leaves of the selected genotypes, but it decreased in the leaves of local cultivars as compared with those of the non-saline condition (Fig. 3). The results also showed that the chlorophyll content of selected genotypes 1H, 2H and 3H was proximately similar (Fig.3), while the local cultivars Latefyi and Iraq significantly differed in their chlorophyll content under salinity condition. Under salinity condition the lowest content was 6 in the leaves of Latefyi cultivar.



**Fig. 3. chlorophyll content in the upper leaves of the selected genotypes and local cultivars of wheat under salinity conditions** Hung *et al* (13) used *TaST* gene to transform Arabidopsis plants to test the effect of this gene on the salt tolerance of transgenic Arabidopsis under saline conditions, the seeds germination and chlorophyll content in these plant were significantly better than those in the wild-type control. Therefore, these results indicating that the *TaST* gene can increase the salt tolerance of plants. This result is conformed the results of this paper, which showed that *TaST* gene found only in salt tolerant selected genotypes and absent in the sensitive cultivars (Figs. 1, 2).

**Organic compound**

The organic compounds (Carbone, lipids, protein) in the leaves of selected genotypes and local cultivars were summarized in table 2. The ash, lipids and protein of the selected genotypes 1H increased to 12.87%, 5.54% and 2.98% respectively under salinity condition compared with those under normal condition 9.97%, 0.89% and 1.17% respectively, similar results was obtained in genotypes 2H and 3H (table 2). While in the local cultivars Latifyi and Iraq the percentage of ash, lipids and protein were not reported because the plants were died under salinity conditions.

**Table 2. Organic compounds contents in the leaves of the selected genotypes and local Iraqi cultivars of wheat under salinity conditions.**

salinity ds/m	1H			2H			3H			Latefyi			Iraq		
	Ash %	lip %	pro %	Ash %	lip %	pro %	Ash %	lip %	pro %	Ash %	lip %	pro %	Ash %	lip %	Pro %
2 ds/m	9.97	0.89	1.17	10.26	1.03	1.42	8.99	1.26	1.18	7.09	0.34	1.01	7.98	0.20	0.92
16 ds/m	12.87	5.54	2.98	14.03	4.86	3.01	14.01	4.29	2.55	*	*	*	*	*	*

\* Missing values because death of plant due to salinity

The increased in organic compounds percentage under salinity condition similar to (20) who reported that soluble carbohydrates and proteins accumulated in plants as a response to salinity which played a major role in adjustment of osmotic potential and plants salt tolerance. Huang *et al* (13) also investigated that the *TaST* gene induced some salt tolerance mechanism; the results showed that the *TaST* gene can significantly reduce the Na<sup>+</sup> concentration in the intra-cellular, and increase K<sup>+</sup> content and maintain a high K<sup>+</sup>/Na<sup>+</sup> ratio in the intra-cellular of transgenic Arabidopsis plants. Previously, Munns (17) reported that the K<sup>+</sup>/Na<sup>+</sup> ratio is an important indicator of plant salt tolerance. Therefore, the salt tolerance of plant increased with increasing the K<sup>+</sup>/Na<sup>+</sup> ratio in plant, especially in the upper leaves (6). Also Cuin *et al* (11) other researchers reported that K<sup>+</sup>/Na<sup>+</sup> ratio is an important index of plant salt tolerance. The results of transgenic plants and the Wild- type controls (13) showed that the *TaST* gene was significantly increased the proline content and osmotic pressure in transgenic plants as compared with the control. Increasing the osmotic pressure was correlated with the high accumulation of soluble sugar in these transgenic plants. However, Chyzykova and Palladina (10) showed that accumulation of soluble sugar also increase the plant cells osmotic pressure. Also the *TaST* gene increased the intracellular Ca<sup>2+</sup> content in transgenic plants. Then the *TaST* gene is the best indicator for salt tolerance in plant. The results of this study showed that the *TaST* gene found only in the selected genotypes (1H, 2H, 3H) under saline conditions (Fig.1, 2). In the previous study, Al-Mishhadani *et al* (2) showed that the *TaNIP* gene found only in these selected genotypes with length of 189 bp, while this gene absent in salt sensitive cultivar (Iraq) and gave high gene expression at high salinity level (16 ds/m). Therefore, according to these results, these genotypes are considered as a high salt tolerant genotypes, which improved through plant breeding programs genotypes, this conclusion is agreed with the previous studies, which they reported that there is a strong development in salt tolerance obtained in these selected genotypes through plant breeding program (2,

5, 7, 8). Under high salinity condition, the selected genotypes were more salt tolerance than the local cultivars, may be due to they contained high organic compounds in their upper leaves (Table 2). This means that there is highest correlation between the contents of organic compounds in the leaves and salt tolerance degree, because these compounds would be essential for osmotic pressure adjustment in plant cells and also for allowing the turgor maintenance of cells that would otherwise dehydrate (21). Also, they reported that these compounds could stabilize the membrane proteins and so maintain growth at high salinity levels. Generally, the conclusions of these results showed that the salt tolerant gene band with size 175 bp was found only in the selected salt tolerant genotypes. Salt tolerance of these selected genotypes was more correlated with high contents of chlorophyll and organic compounds in the upper leaves under high salinity condition.

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