Estimation of \((TaGSK1)\) gene expression related with salt tolerance in four bread wheat cultivars \((Triticum aestivum \, L.)\)

Ashwaq S. Abed, Duha M. Majeed, Eman N. Ismail, Abedaljasim M. Jasim Al-Jibouri

Biotechnology Research Center/ Al-Nahrain University, Baghdad 10072, Iraq.

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*Corresponding Author:*  
Duha M. Majeed  
duha_majeed@yahoo.com

**ABSTRACT**

Salinity stress and low water availability affect crop plants in agricultural systems, limiting the productivity of different crops because the mineral toxicity with deficiency frequently causing high osmotic stress as well as overlap toxic ions with main metabolic processes. The aim of this work was to study \((TaGSK1)\) gene expression in some selected cultivars of bread wheat (Furat and Dijila) which selected for salt tolerance through plant breeding programs, while (Lateffyia and Tamooze-2) are Iraqi local cultivars, using real-time PCR TaqMan gene expression assays. \((TaGSK1)\) gene expression was detected by agarose gel electrophoresis in Furat and Dijila cultivars with amplicon products sizes \((189\, bp)\) product while it was absent in (Lateffyia and Tamooze-2) cultivars under both of salinity and non-salinity stress. Real time PCR quantification showed that rising of salt stress levels led to increasing the gene expression, so the highest expression values of \((TaGSK1)\) gene were \((8.491 \times 10^{15} \text{ and } 8.379 \times 10^{15})\) respectively in Furat and Dijila cultivars at high salt stress level \((20\, ds/m)\).

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Numerous studies suggested that salinity tolerance controlled by many genes i.e., \(AKT1\), \(AKT2\), \(KAT1\) and \(HKTI\) genes (Munns, 2005), \(TaSC\) genes (Majeed et al., 2014), \(TaGSK1\) genes (Fakheri et al., 2015). Mizoguchi et al. (1996) proved that many genes which respond to high water stress, encoding protein kinases. Originally, protein kinases is involved in the NaCl stress or osmotic stress signal transduction pathway in wheat, so \(TaGSK1\)gene used as salt tolerance marker in wheat (Bahrami, 1996). Chen et al. (2003) suggested that \(Triticum \, aestivum\) glycogen

**1. INTRODUCTION**

Wheat \(Triticum \, aestivum\) are the second most produced food among the cereal crops and consider as major crop in the world and it is known as king of cereals (Braun et al., 2010). The environmental stresses such as cold, salt and drought considr as an important factors affect on the activities of whole wheat bread \((Triticum \, aestivum \, L.)\) such as high salinity which consider as factor limiting the molecular and physiological levels of germination and development of plants and lead to the death of crops (Rhoades and J. Loveday, 1990).
synthase kinase gene (TaGSK1) was found to be involved in NaCl stress tolerance in wheat under salinity; it's a salt inducible gene. "TaGSK1 gene contains a 703-bp-long intron in the 5' non coding region that significantly enhances gene expression" (He et al., 2012). Munns et al. (2012) reported that HKT1 gene encoding to Na\(^+\)-selective transporter in durum wheat, by exclusion of Na\(^+\) from root xylem vessels to leaf sheaths, reducing shoot accumulation. Biotechnology techniques are advanced using in plants to support the diagnostic purpose like DNA microarray (Kawaura et al., 2006), northern blot (Rahaie et al., 2011) and real time PCR (Al-Mashhadani et al., 2016) technologies have been employed to study expression profiles in wheat and other plant species. TaqMan real time PCR is one of molecular techniques using to detect or determine the genes expression. The aim of current study is to estimate the TaGSK1 gene expression using TaqMan real-time PCR method between four selected Iraqi wheat cultivars under salt stress.

2. MATERIALS AND METHODS

2.1 Plant material and germination test

Four cultivars of Triticum aestivum L. were used in this study: (Furat and Dijila) genotypes were selected for salt tolerance through plant breeding programs, while (Lateffya and Tamooze-2) are Iraqi local cultivars. All works were done in Biotechnology Research Center/Al-Nahrain University, Baghdad-Iraq. Seeds of the four wheat plants were washed with tap water for 30 min, immersed in 50% of sodium hypochlorite then treated with 2-3 drops of Tween 20 for 10 min; lastly these seed were rinsed once with 70% ethanol and several times with sterile distilled water. From each plant, five sterilized seeds were placed in culture bottle containing 15 ml of solidified MS medium (Murashige and Skoog, 1962) with each of 0 ds/m, 15 ds/m and 20 ds/m of salt concentrations (NaCl). Each treatment for wheat was replicated thrice. All cultures were performed under 16 h/day in photoperiod condition at 25±2 °C. Germination rates were recorded after 15 days and leaves were taken for next steps (Debergh and Maene, 1981).

2.2 RNA extraction and cDNA synthesis

Total RNA was extracted and purified from the fresh leaves using total RNA mini kit (Geneaid) through following procedure: To get purified RNA, the extraction was treated with RNase-free DNasel (Biobasic, Canada) at 20°C for 30 min., DNasel was inactivated for 10 min at 65°C. Qualitative estimation of RNA was assessed by agarose gel electrophoresis method on a 1% (w/v) agarose gel. Reverse Transcription System (Bioneer, Korea) was used to synthesize first-strand cDNA from 500 ng of total RNA with an oligo-dT15 primer. Reaction solution was utilized as template for Reverse transcription Polymarase chain Reaction (RT-PCR) for the whole wheat plants.

2.3 TaGSK1 gene amplification

The target gene (TaGSK1) was amplified to cDNA using primers are listed in Table (1). Polymerase chain reaction (PCR) was initiated using the cDNA template with hot-start method on Thermocycler (Labnet, USA). The PCR reaction was carried out at 95°C for 5 min, 40 cycles at 95°C for 1 min, 60°C for 45 s and 72°C for 1 min, then final extension 72°C for 10 min (Ismail et al., 2014). PCR products were analyzed by agarose gel electrophoresis on a 1% (w/v) agarose gel.

2.4 Expression analysis by quantitative (TaqMan) real-time RT-PCR

The expression of TaGSK1 gene was subjected using TaqMan real-time PCR using Exicycler real time PCR (Bioneer, Korea).
Sybr green analysis was done at 60-95°C to optimize primers for the next TaqMan step. A single step RT-PCR was performed using premix RT-PCR qPCR kit (Bioneer, Korea), following manufacturers protocol. Thermal cycling profile consist of initial denaturation at 95°C for 5 min; 40 cycles at 95°C for 1 min; 60°C for 45 s and 72 °C for 1 min.

Rats were divided randomly into two groups; group 1: control group (n=8), group 2: fluoxetine-exposed rats in which rats were orally administrated with 10 mg/kg body weight/day (n=8).

The amplified fragment was determine using 1% agarose gel electrophoresis containing 0.5% ethidium bromide. Each sample was run in triplicate. To get estimation of standard curve (discuss briefly): serial dilution (1:10) for cDNA were done, determination the concentration and purity for each diluted cDNA, then online Software was used to convert DNA concentration to log copy number, (http://cels.uri.edu/gsc/cndna.html). The gene expression quantity was determined by the following equation (VanGuilder, et al. 2008):

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

### 3 RESULTS AND DISCUSSION

### 3.1 Germination test

As show in Table (2), control treatment (0 ds/m) have 100% of germination rate for all cultivars. 15 and 20 ds/m salinized treatments caused a apparent decrease in the percentage of germination for (Lateffyia and Tamooze-2) cultivars, reached to (0%), while the percentage of germination for (Furat and Dijila) recorded (100% and 94%) respectively, at 20 ds/m.

So the yield data indicat that (Furat and Dijila) cultivars are potentially more salinity stress tolerant than (Lateffyia and Tamooze-2) cultivars.

### 3.2 Quantitative real-time RT-PCR of the TaGSK1 gene

To determine the salt tolerance and estimated the expression of TaGSK1 gene in selected wheat genotypes and local cultivars, total RNA was extracted from leaves. Gel electrophoresis was done to assess the quality of the RNA (Figure 1). Conventional PCR results showed the appearance of single and clear band about 189 bp in line with 100 bp DNA ladder in salt tolerance cultivars, and disappeared in local cultivars (Figure 2). From
the above results we observed that $TaGSK1$ gene was found only in the salt tolerant cultivars, while it was absent in the salt sensitive cultivars. The amplification of diluted cDNA was done to drawing the standard curve (Figure 3) which was performed depending on the CT values and Log copy number (Table 3), the slope was $(-3.439)$, $R^2=(0.973)$, $b=(75.28)$ and $E=95.336\%$ which means that the efficiency of the amplification reaction was duplicated every cycle (Vaerman, et al. 2004). TaqMan real time PCR analysis illustrated that salt treatments had a evident effects on the expression of $TaGSK1$gene in T. aestivum. As show in Table (4) and Figure (4), Furat and Dijila cultivares gave the highest expression, reached to $(8.491 \times 10^{15}$ and $8.379 \times 10^{15}$) respectively at the highest salinity level $(20 \text{ ds/m})$, while the control treatments recorded $(1.716 \times 10^{12}$ and $1.520 \times 10^{12}$) respectively, whereas Lateffya and Tamooze-2 did not showed any expression of $TaGSK1$gene under the same conditions. These results corresponded with Bahrami et al. (2009) and Fakheri et al. (2015), they reported that $TaGSK1$ gene expression was not detectable under control treatment, also pointed that this gene controls some tolerance mechanisms, support salt tolerance genotypes to growth normally and consider as a marker for salt tolerance. Chen et al. (2003) revealed that the $TaGSK1$ gene is one of serine-threonine protein kinase that gave the highest gene expression under salinity conditions. Guan et al. (2011) proved that $TaGSK1$ gene of Triticum aestivum play an important role under high salinity conditions. Maas et al. (1996) and He et al. (2012) whom they found that expression of $TaGSK1$ response to salinity and regulation the osmotic pressure through increasing proline concentration and increasing $K^+/Na^+$ ratio in the upper leaves. Previously, another study, Majeed et al. (2014) was carried out on (Furat and Dijila) cultivars illustrated that TaSC salt tolerance gene expression was detected in these cultivars, while there was no gene expression in the other local cultivars.

**Table (2): Germination rate (%) of wheat cultivars Triticum aestivum L. under salinity conditions after 15 days.**

| Cultivars | Salinity level | Mean |
|-----------|----------------|------|
|           | 0 (ds/m) | 15 (ds/m) | 20 (ds/m) |
| Furat     | 100%     | 100%      | 100%      | 100% |
| Dijila    | 100%     | 95%       | 88%       | 94%  |
| Lateffya  | 100%     | 12%       | 0         | 37%  |
| Tamooze-2 | 100%     | 14%       | 0         | 38%  |
| Mean      | 100%     | 55%       | 47%       |      |

**Figure (1):** Gel electrophoresis of total RNA in four wheat cultivars. Lanes: M=ladder, F=Furat, D=Dijila, T=Tamooze-2, L= Lateffya.

**Figure (2):** Gel electrophoresis of conventional PCR products for $TaGSK1$ genes in four wheat cultivars. Lanes: M=ladder, F=Furat, D=Dijila, L=Lateffya, T=Tamooze-2.
Figure (3): Standard curve shows slope and R² values to determine the efficiency of PCR reaction for TaGSK1 gene amplification.

Figure (4): Amplification curves in real-time PCR obtained from cDNA of wheat cultivars. Lanes: F=Furat, D=Dijila, L=Lateffyia, T=Tamooze-2

Table (3): CT values and number of copies of the DNA template of the TaGSK1 gene

| CT values | Log copy number |
|-----------|-----------------|
| 20.42     | 15.633          |
| 25.86     | 14.397          |
| 29.36     | 13.794          |
| 31.97     | 12.602          |
| 35.66     | 11.678          |
| 36.66     | 10.913          |

Table (4): TaGSK1 gene expression values vs. salt utilized salt concentrations using Quantitative real-time RT-PCR.

| Cultivars  | Salt concentration (ds/m) | Gene expression     |
|------------|----------------------------|---------------------|
| Furat      | 0                          | 1.716 x 10¹²        |
|            | 15                         | 4.022 x 10¹⁴        |
|            | 20                         | 8.491 x 10¹⁵        |
|            | 0                          | 1.520 x 10¹²        |
| Dijlah     | 15                         | 3.650 x 10¹⁴        |
|            | 20                         | 8.379 x 10¹⁵        |
| Lateffyia  | 0                          | -----               |
|            | 15                         | -----               |
|            | 20                         | -----               |
| Tamooze-2  | 15                         | -----               |
|            | 20                         | -----               |

4 CONCLUSION

Generally, from our data we have concluded that environmental condition play an important role for rise gene expression, we have also demonstrated that some genes which related with salinity stress in wheat crop, have correlation with genetic genotype, TaGSK1 gene is one of these genes, for this reason TaGSK1 gene was be used as marker for salt tolerance.

To understand the mechanisms of salt stress tolerance in wheat, it is necessary to identify the most genes which involved in the main mechanisms.

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