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ORIGINAL ARTICLE

Investigation of Ta GSK gene expression in Tritipyrum genotypes as Salinity Marker assisted Selection

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ABSTRACT
In order to determine TaGSK gene expression levels in 12 selected tritipyrum genotypes, a comparison between genotypes were performed under control and stress conditions in Agricultural Biotechnology Institute, University of Zabol. Three replicates for each genotype was considered and then specimens under stress were subjected NaCl 200 mM. RNA was extracted from leaf samples. cDNA was designed using reverse transcription. 18S rRNA gene expression was used as internal control gene in Real Time PCR to normalize TaGSK1 gene expression levels. Results showed St/b genotype had significant differences between level of TaGSK1 gene expression under conditions of salinity and control. Ta GSK gene expression between genotypes is varied from 12.5 fold in St/b until 1.04 fold in La/b genotypes.
***Key Words**: TaGSK1 gene, Gene expression, Salt stress, Tritipyrum and Real Time PCR*

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INTRODUCTION

The effects of saline soils on plant growth have been a focus of research for nearly 100 years because salt stress is a major stress limiting crop productivity [1]. Mechanisms of salt injury and salt tolerance of whole plant have been studied extensively, studies at the cellular, organellar, and molecular levels are still limited [2].

In plant, many genes encoding homologues of shaggy/ GSK-3 have been identified. Indicating that it is a multigene family in plants [3, 4]. The proteins encoded by these genes belong to the shaggy/GSK-3 subfamily of protein kinase. Genetic and biochemical studies revealed that they are involved in multiple functions in plants including hormone signaling, development, and stress responses [5]. In Arabidopsis, a shaggy/GSK-3 like gene AtGSK1 can complement yeast salt stress-sensitive mutants and is induced by NaCl an exogenous abscisic acid (ABA), suggesting its role in the osmotic stress response [6]. Moreover, the transgenic Arabidopsis over-expressing AtGSK1 enhanced the resistance to NaCl stress as well as the accumulation of anthocyanin [7]. In alfalfa, a shaggy/GSK-3 like gene WIG is activated by wounding. Also, it was demonstrated that WIG activations is a post-translation process [2].

Tritipyrum, a new salt tolerant amphiploid, is the third handmade cereal after Triticale and Tritordeum. Now days, wheat is a sample food for humans and it supplies more than 20 percent of calories for humans in the word [8, 9], so plant improvement for making new salt varieties is very important. One of these new salt tolerant varieties is Tritipyrum, that is derived from bread wheat *Thinopyrum bessarabicum*. Tritipyrum can survive in salt concentration around 250mmol NaCl. In this amphiploid, triats including salt tolerance, and perenniality, have made it a superior plant for saline soil and /or saline water [10, 11]. Therefore this plant could be a new opportunity for enhanced salt tolerance and studying its superior characteristics are necessary.

In this study, we investigate rate of GSK gene expression using real time PCR method between 12 Tritipyrum lines as well as in order to determinate salinity resistance, germination test under salinity treatments was carried out for these selected Tritipyrum lines.

MATERIALS AND METHODS

Plants materials and salt stress treatments: Seeds of 12 Tritipyrum lines (Table 1) were surface-sterilized for 5 min in 1% (W/V) sodium hypochlorite and then was washed in distilled water. Three replication for each genotype was considered and then specimens under stress were subjected NaCl 200M. After15 days leaves were harvested and stored in -80°C.

RNA extraction: The leaves were collected and immediately frozen in liquid nitrogen. The material was then ground to fine powder with a pre-cooled pestle and mortar under liquid nitrogen. total RNA was extracted from 100 mg of leaves meristem using the ISOI-RNA Lysis Reagent Manual (5PRIME) according to manual's protocol.

After extraction, to determine the total RNA concentration and purity, the absorbance of the isolate was measured at wave lengths of 260 and 280 nm using the bio photometer. The result indicated that the total RNA yield ranged with an average measured as OD 260/280 more than 1.90.The purified total RNA was stored at -20°C.

RT-PCR (Reverse Transcription polymerase chain reaction) reaction: First-strand cDNA was prepared from 100 ng of total RNA, using QuantiTect® Reverse Transcription Kit (QIAGEN) according to manual's protocol.

Real-time PCR quantification: Sequences of primers used for Real Time PCR amplification of Ta GSK genes was 5´ -CAA ATC AAA GCT CAC CCA-3´ as forward and 5´ -AAA GAG GGG AGG AAG AAAA-3´ as reveres primer and the amplicon size was 198 base pair that it was accommodating for quantification PCR. Reactions for the real-time PCR using SYBR green detection consisted of 11.25 ul of Real Master Mix SYBR ROX (5PRIME, Germany), 270 nm forward and reverse primers, 100 ng of first-strand cDNA, synthesized as described above and Molecular Biology Grade Water to 25 ul. Thermal cycling condition was as follows: 2min incubation at 95°C for activation of the polymerase followed by 40 cycles of 15s denaturation at 95°C and 45s annealing and extension at 50°C.

Gene transcript quantification was performed on the RG-3000 Corbett Research using the Roto Gene 6.0 software. Experiments were conduct on the three biological replicates. Wheat 18S rRNA, amplified with primers 5-AGT TTG TTT GAT GGT ACG TG-3´ as forward and 5´ -TTT GAAA TGA TGC GTC GCC-3´ as revers primer was used as the internal standard in the experiment. Amplification specificity was checked with a heat dissociation protocol (melting curves in 60-95°C range), as a final step of the PCR. All primer pairs showed a single peak on the melting curve.

Data analysis: For QPCR (Quantitative PCR) data, relative expression for the Gene of Interest (GOI) was determined using ΔΔct method. The expression of target genes was relative to a control plant sample which was no exposed to salinity stress. Δct= ct (Target gene sample) - (Reference gene sample)

ΔΔct= Δct (treatment) - Δct (control)

RESULTS

After Real Time PCR, the Ta GSK gene expression data were normalized and results showed that there was significant difference between level of GSK gene expression under salinity and control in all lines except Cr/b and Ma/b Cr/b genotypes. The expression levels between genotypes are varied from 12.5 fold in St/b until 1.04 fold in La/b genotypes. A different pattern of Ta GSK transcript accumulation was found between 12 selected lines that were exposed on control and stress conditions. Ta GSK expression was induced by NaCl and the expression level and salt tolerance ability showed positivecorrelation. These results have accordance to other studies on this gene family in wheat and maize. Figure 1 and 2 shows that levels of Ta GSK gene expression after than St/b genotype, La/b (4B)(4D), Az/b, St/b Cr/b, Ma/b, Ka/b, Ka/b Cr/b genotypes have maximum level expression of Ta GSK gene. The result of analysis by Real Time PCR of Ta GSK expression under 0 and 200 mol m^{-3} salinity are presented in Fig 1and 2.

Table 1:Tritipyrum genotypes used in this study

Names of Lines	Proprietary codes of Lines
Macount/*Thinopyrum bessarabicum*	Ma/b
Stewaet/*Thinopyrum bessarabicum*	St/b
Creso/ *Thinopyrum bessarabicum*	Cr/b
Aziziah/*Thinopyrum bessarabicum*	Az/b
Karan/*Thinopyrum bessarabicum*	Ka/b
Longdon/*Thinopyrum bessarabicum*	La/b
Longdon(4B)4D/*Thinopyrum bessarabicum*	La(4B) 4D)/b
Karan/*Thinopyrum bessarabicum* Creso/ *Thinopyrum bessarabicum*	Ka/b Cr/b
Macount/*Thinopyrum bessarabicum*Creso/	

Thinopyrum bessarabicum Stewaet/*Thinopyrum bessarabicum*Creso/ *Thinopyrum bessarabicum* Chiness Spring/ *Thinopyrum bessarabicum* Chiness Spring/ 5Edition line bessarabicum	Ma/b Cr/b St/b Cr/b Cs/b Cs/5Eb

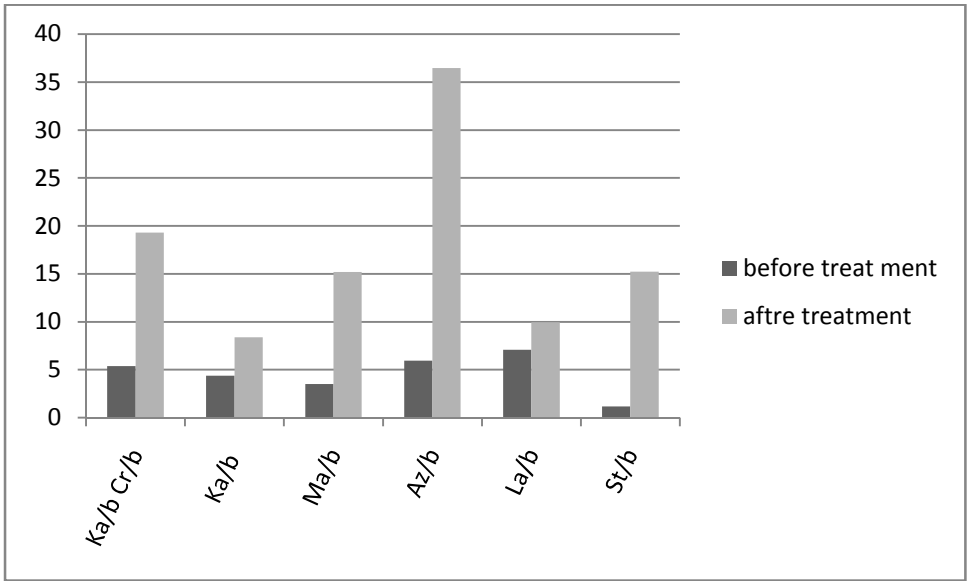

Fig 1: Expression changes rather than control sample after than normalization rather than reference gene in Ka/b Cr/b, Ka/b, Ma/b, Az/b, La/b, St/b genotypes

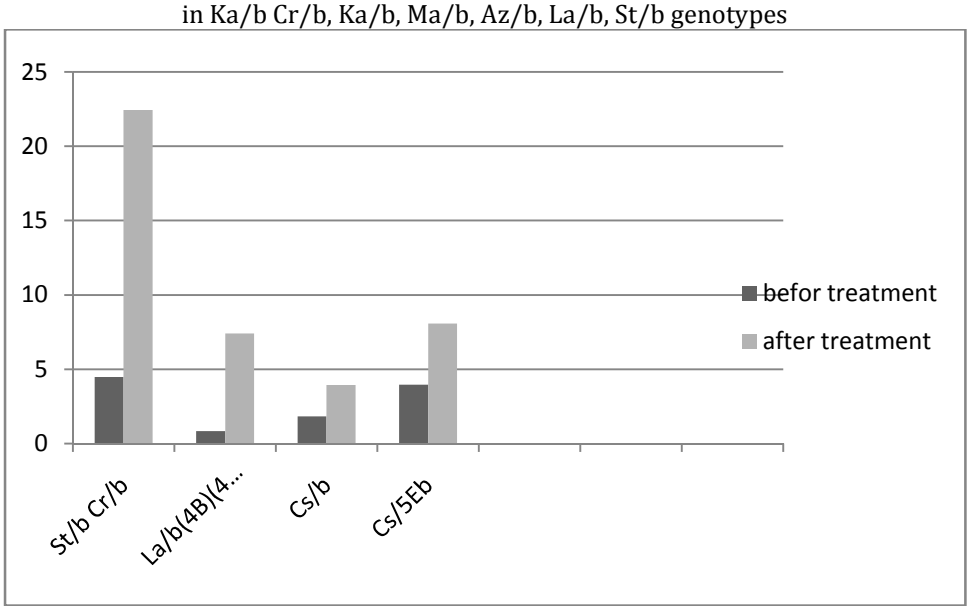

Fig 2: Expression changes rather than control sample after than normalization rather than reference gene in St/b Cr/b, La/b(4B)(4D), Cs/b, Cs/5Eb genotypes

DISSCUSSION

It is possible Ta GSK may be involved in NaCl stress signal transduction pathway in Tritipyrum. The higher salt tolerance ability of SR (Salt Resistance) may be related to the higher expression of Ta GSK in SR than in SS (Salt Sensitive) under salt-stress. Further research on the mechanism of Ta GSK involved in salt-stress signal transduction pathway is needed. Protein Kinases play an important role in the signal transduction pathway in eukaryotic organism. In earlier studies, three key components involved in the MAPK pathway in response to NaCl and osmotic stress were identical to be Ca^{2+} /camodulin-dependent protein phosphatase signal transduction pathway, SOS_2 involved in the SOS signal transduction pathway and serine /threonine protein kinases. St/b, La/b (4B)(4D), Az/b, St/d Cr/b, Ma/b, Ka/b genotype that

have maximum level expression of Ta GSK gene are sufferer genotypes under salt region. Expression Ta GSK gene was not detectable under control in 2 genotypes. Apparently, this may carry out for a mutation in sequences of Ta GSK gene in these genotypes and hereupon the amplicon hasn't been produced.

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