ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 8 (2): 281-284, 2009
ISSN 1682-296X
© 2009 Asian Network for Scientific Information

Evaluation of TaGSK1 Gene Expression in Selected Wheat Genotypes as Salinity Marker Assisted Selection

[1]S. Bahrami, [2]M. Solouki, [2]B. Siasar and [2]A. Ghanbari
[1]Agricultural Biotechnology Institute, University of Zabol, Iran
[2]Faculty of Agricultural, University of Zabol, Iran

Abstract: In order to determine the level of TaGSK1 gene expression in 9 selected wheat genotypes an experiment was carried out in Agricultural Biotechnology Institute, University of Zabol. Seeds of wheat cultured on solid MS medium plate under positive and control condition and RNA was extracted for each genotype and treatment. Relative gene expression using cDNA as template was done in Real Time PCR. The results of real time PCR showed that the Bam genotype has maximum level expression of TaGSK1 gene between 9 genotypes. Minimum expression was found in ER-Salt-85-17 line originated from Tehran Province. TaGSK1 gene expression between genotypes is varied from 39% in ER-Salt-85-17 until 71% in Bam genotypes.

Key words: TaGSK1, real time PCR, relative gene expression

INTRODUCTION

Earth is a salty planet, with most of water containing about 30 g of sodium chloride per liter. This salt solution has affected and continues to affect, the land on which crops are, or might be, grown. Although, the amount of salt affected land (9003106 ha) is imprecisely known, its extent is sufficient to pose a threat to agriculture (Flowers and Yeo, 1995; Munns, 2002). Most plants and certainly most crop plants, will not grow in high concentrations of salt: only halophytes survive in concentrations of sodium chloride higher than about 400 mM. Consequently, salinity is a threat to food supply.

Salt stress, resulting from high concentrations of NaCl causes hyperosmotic stress, imbalance in the cellular ion concentration and general toxicity that adversely affects plant development. Numerous studies have been conducted to delineate the cellular changes that occur upon exposure to osmotic stress (Walton, 1980; Bohnert *et al*., 1995; Niu *et al*., 1995).

It is also very likely that protein kinases and protein phosphatases are involved in signal transduction in plants. In fact, many genes encoding protein kinases have been shown to be induced under high NaCl conditions and under exogenous ABA treatment (Anderberg and Walker-Simmons, 1992; Hwang and Goodman, 1995; Jonak *et al*., 1996; Mizoguchi *et al*., 1996). It has been suggested that protein kinases may be involved in osmotic stress signal transduction. Recently, the genes at the ABA-insensitive 1 and 2 loci of *Arabidopsis* have been cloned and shown to encode homologs of phosphatases (Leung *et al*., 1994, 1997; Meyer *et al*., 1994). However, in most cases the evidence for the involvement of protein kinases in the NaCl stress or osmotic stress signal pathway is rather circumstantial and to our knowledge no *in vivo* substrates for the kinases have been isolated. Also, the *in vivo* targets of the phosphatases remain to be defined.

Originally, GSK3 was identified as a kinase that phosphorylates glycogen synthase (Embi *et al*., 1980) but it has been recently shown that GSK3 is involved in developmental processes such as cell fate determination in *Drosophila melanogaster* (Ruel *et al*., 1993), *Xenopus* (Dominguez *et al*., 1995) and Dictyostelium (Harwood *et al*., 1995). Mutation in the MCK1 gene showed a cold-sensitive phenotype, a temperature-sensitive phenotype and loss of chromosomes during growth on benomyl. Based on these observations it has been suggested that MCK1 plays an important role in the regulation of kinetochore activity and entry into meiosis. In plants many genes encoding homolog of GSK3 have been identified by virtue of the conservation of the primary structure, i.e., the amino acid sequence and in many cases it has been suggested that they are involved in developmental processes (Bianchi *et al*., 1993, 1994).

In this study, we evaluated rate of GSK gene expression using real time PCR method between 9 selected wheat (*Triticum aestivum* L.) lines as well as in order to determinate salinity resistance, germination test under salinity treatments was carried out for these selected wheat lines.

Corresponding Author: Shahram Bahrami, Agricultural Biotechnology Institute, University of Zabol, Postal Code 65421236-587, Iran Tel: 0098 912338 9388 Fax: 0098 542222 6762

MATERIALS AND METHODS

Seed materials: Nine wheat lines tested were obtained from Zabol Agricultural Research, Sistan and Baloochestan, Iran (Table 1). Before the experiment, seeds were surface sterilized in 1% sodium hypochlorite solution for 5 min, then rinsed with sterilized water and air-dried.

Salt treatments: For each wheat line 10 seed were placed in glassware containing 50 mL of solid MS medium with 25 g L^{-1} sucrose without any growth regulators. Each treatment per genotype was replicated thrice. The glassware was placed on a growth chambers (Wisees) for 15 day under the growth conditions described above.

RNA isolation and first strand cDNA synthesis: Total RNA was extracted from 0.2 g of leaves meristem using RNasy Total RNA Isolation Kit (QIAGEN) according to manual's protocol. RNA was treated by RNase-free DNaseI (Ambion, Inc., Austin, TX, USA) for removal of genomic DNA before first-trand synthesis. First-strand cDNA was prepared from 120 ng of total RNA, using universal Oligo$(dT)_{15}$ primer and 200 units of SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA), at 42°C for 1 h in a 20 mL reaction volume.

Real-time Polymerase Chain Reaction (QPCR): Gene quantification was performed using real time PCR (RG-3000 Corbett Research). Sequence-specific primers were designed based on sequences reported target genes. Each reaction was performed in 5 mL of 1:100 (v/v) dilution of the first-strand cDNA, synthesized as described above, in a total reaction volume of 25 mL using SYBR Green PCR Master Mix (Applied Biosystems) and 270 nM of each forward and reverse primer. Sequences of primers used for Real Time PCR and amplification of TaGSK1 genes was 5`-CCATGAGTTGA AGGGTGTGC-3` as forward and 5`-AAGCAGTGGTATCA ACGCAGAGT-3` as reverse primer and the amplicon size was 181 base pair that it was accommodating for quantification PCR. Reaction conditions for thermal cycling were: 54°C for 2 min, 95°C for 7 min, followed by 35 cycles of 95°C for 15 sec and 63°C for 1 min. The Real Time PCR experiments were conducted on the three biological replicates. The wheat ubiquatin gene was used as a internal control gene to normalize expression of the Genes of Interest (GOI). 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 and a single band of the expected size was observed using agarose gel electrophoresis.

Table 1: Wheat genotypes used in this study

Name of Lines	Origin
ER-salt-81-14	Esfahan
ER-Salt-85-15	Esfahan
ER-Salt-85-19	Tehran
ER-Salt-85-12	Sistan and Baloochestan
ER-Salt-85-17	Tehran
Kavir	Kerman
Mahdavi	Fars
Bam	Kerman
Akbari	Sistan and Baloochestan

Data analysis: For QPCR data, relative expression for the GOI was determined using ΔΔCt method (Livak and Schmittgen, 2001). The expression of the GOI was relative to a control plant sample which was no exposed to salinity stress. Kavir line was chosen and termed the calibrator.

$$\Delta Ct_{(sample)} = Ct_{(GOI\ sample)} - Ct_{(Refrence\ gene\ sample)}$$

$$\Delta\Delta Ct = \Delta Ct_{(sample)} - \Delta Ct_{(calibrator)}$$

$$\text{Relative expression} = 2^{-\Delta\Delta Ct}$$

RESULTS AND DISCUSSION

A different pattern of TaGSK1 transcript accumulation was found between 9 selected lines that were exposed on control and stress conditions. The expression level for three lines including ER-Salt-81-14, ER-Salt-85-12 and Mahdavi lines were at least 50% higher than other six genotypes (Fig. 1). There was significant difference between level of GSK gene expression under salinity and control in all lines except Bam and Akbari genotypes. TaGSK1 expression was induced by NaCl and the expression level and salt tolerance ability showed positive correlation.

It is possible that TaGSK1 may be involved in NaCl stress signal transduction pathway in wheat. The higher salt tolerance ability of SR (salt resistance) may be related to the higher expression of the TaGSK1 in SR than in SS (Salt Sensitive) under salt-stress. Further research on the mechanism of TaGSK1 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

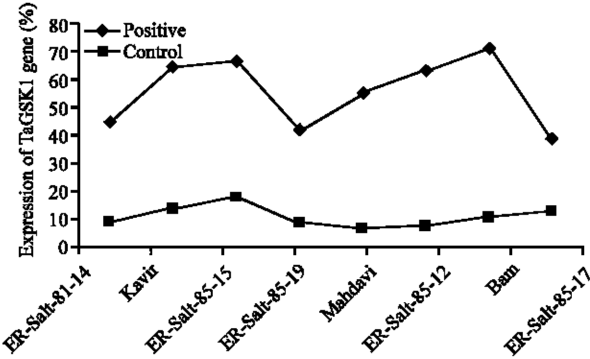

Fig.1: Percentage of TaGSK1 gene expression using mRNA from shoot meristem of 9 wheat accession belonged to various region of Iran under two conditions

transduction pathway and serine/threonine protein kinases.

Unpublished study showed that ER-Salt-85-19 and ER-Salt-85-12 are sufferer genotypes under salt region in Iran. By and large, excepted three genotypes, post-transcriptional gene regulation were demonstrated by TaGSK1 gene in selected genotypes.

These results have accordance to other studies on this gene family in whit and maize. Figure 1 shows that levels of TaGSK1 gene expression between genotypes are varied from 39% in ER-Salt-85-17 until 71% in Bam genotypes. The result of analysis by Real Time PCR of TaGSK1 expression under 0 and 200 mol m^{-3} salinity are presented in Fig. 1. Expression TaGSK1 gene was not detectable under control in 3 genotypes. Apparently, this may carry out for a mutation in sequences of TaGSK1 gene in these genotypes and hereupon the amplicon hasn't been produced.

ACKNOWLEDGMENTS

The authors would like to acknowledge Dr. Mostafa Heydari for supplying required instruments. This research was funded by Sistan Agricultural Biotechnology Institute, University of Zabol, Iran.

REFERENCES

Anderberg, R.J. and M.K. Walker-Simmons, 1992. Isolation of a wheat cDNA clone for an abscisic acid-inducible transcript with homology to protein kinases. Proc. Natl. Acad. Sci. USA., 89: 10183-10187.

Bianchi, M.W., S.E. Plyte, M. Kreis and J.R. Woodgett, 1993. A Saccharomyces cerevisiae protein-serine kinase related to mammalian glycogen synthase kinase-3 and the *Drosophila melanogaster* gene shaggy product. Gene, 134: 51-56.

Bianchi, M.W., D. Guivarch, M. Thomas, J.R. Woodgett and M. Kreis, 1994. *Arabidopsis* homologs of the shaggy and GSK-3 protein kinases: Molecular cloning and functional expression in *Escherichia coli*. Mol. Gen. Genet., 242: 337-345.

Bohnert, H.J., D.E. Nelson and R.G. Jensen, 1995. Adaptations to environmental stresses. Plant Cell, 7: 1099-1111.

Dominguez, I., K. Itoh and S.Y. Sokol, 1995. Role of glycogen synthase kinase 3 beta as a negative regulator of dorsoventral axis formation in *Xenopus embryos*. Proc. Natl. Acad. Sci. USA., 92: 8498-8502.

Embi, N., D.B. Rylatt and P. Cohen, 1980. Glycogen synthase kinase-3 from rabbit skeletal muscle: Separation from cyclic-AMP dependent protein kinase and phosphorylase kinase. Eur. J. Biochem., 107: 519-527.

Flowers, T.J. and A.R. Yeo, 1995. Breeding for salinity resistance in crop plants: Where next? Aust. J. Plant Physiol., 22: 875-884.

Harwood, A.J., S.E. Plyte, J. Woodgett, H. Strutt and R.R. Kay, 1995. Glycogen synthase kinase 3 regulates cell fate in *Dictyostelium*. Cell, 80: 139-148.

Hwang, I. and H.M. Goodman, 1995. An *Arabidopsis thaliana* rootspecific kinase homolog is induced by dehydration, ABA and NaCl. Plant J., 8: 37-43.

Jonak, C., S. Kiegerl, W. Ligterink, P.J. Barker, N.S. Huskisson and H. Hirt, 1996. Stress signaling in plants: A mitogen-activated protein kinase pathway is activated by cold and drought. Proc. Natl. Acad. Sci. USA., 93: 11274-11279.

Leung, J., M. Bouvier-Durand, P.C. Morris, D. Guerrier, F. Chefdor and J. Giraudat, 1994. *Arabidopsis* ABA response gene ABI1: Features of a calcium-modulated protein phosphatase. Science, 264: 1448-1452.

Leung, J., S. Merlot and J. Giraudat, 1997. The *Arabidopsis* ABSCISIC ACID-INSENSITIVE 2 (ABI2) and ABI1 genes encode homologous protein phosphatase 2C involved in abscisic acid signal transduction. Plant Cell, 9: 759-771.

Livak, K.J. and T.D. Schmittgen, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) method. Methods, 25: 402-408.

Meyer, K., M.P. Leube and E. Grill, 1994. A protein phosphatase 2C involved in ABA signal transduction in *Arabidopsis thaliana*. Science, 264: 1452-1455.

Mizoguchi, T., K. Irie, T. Hirayama, N. Hayashida and K. Yamaguchi-Shinozaki *et al*., 1996. A gene encoding a mitogen-activated protein kinase kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold and water stress in Arabidopsis Thaliana. Proc. Natl. Acad. Sci. USA., 93: 765-769.

Munns, R., 2002. Comparative physiology of salt and water stress. Plant Cell Environ., 25: 239-250.

Niu, X., R.A. Bressan, P.M. Hasegawa and J.M. Pardo, 1995. Ion homeostasis in NaCl stress environments. Plant Physiol., 109: 735-742.

Ruel, L., M. Bourouis, P. Heitzler, V. Pantesco and P. Simpson, 1993. Drosophila shaggy kinase and rat glycogen synthase kinase-3 have conserved activities and act downstream of Notch. Nature, 362: 557-560.

Walton, D.C., 1980. Biochemistry and physiology of abscisic acid. Annu. Rev. Plant Physiol., 31: 453-489.