Available online at www.sciencedirect.com
SCIENCE DIRECT®
Plant Science 165 (2003) 1369–1375
PLANT SCIENCE
www.elsevier.com/locate/plantsci

Isolation and characterization of *TaGSK1* involved in wheat salt tolerance

Gui Ping Chen [a,c,1], Wen Shi Ma [a,1], Zhan Jing Huang [a], Tao Xu [a], Yong Biao Xue [b,2], Yin Zhu Shen [a,*]

[a] *College of Life Science, Hebei Normal University, Shijiazhuang 050016, PR China*
[b] *Institute of Development Biology, The Chinese Academy of Science, Beijing 100080, PR China*
[c] *Biology and Science Technology Department, Tangshan Teachers College, Tangshan 063000, PR China*

Received 30 May 2003; received in revised form 30 July 2003; accepted 30 July 2003

Abstract

cDNA-amplified fragment length polymorphism (AFLP) was used to analyze differentially expressed genes in wheat RH8706-49, a salt-stress resistant line (SR) and H8706-34, a salt-stress sensitive line (SS) with or without NaCl stress. A large number of gene fragments related to salt-stress were found. Among them, a full-length cDNA encoding glycogen synthase kinase–shaggy kinase (*TaGSK1*) was subsequently cloned from SR leaves. The corresponding amino acid sequence was deduced and the primary and secondary structures of the protein predicted, that it has a N-terminus outside transmembrane region with a Serine/Threonine (Ser/Thr) protein kinase catalytic domain. The molecular weight of mature peptide is 43.5 kDa and the isoelectric point is 8.66. Northern blot analysis showed that *TaGSK1* was induced by NaCl stress, and expressed more strongly in SR than in SS. These results suggest that *TaGSK1* is involved in wheat response to salt-stress as a part of the signal transduction component.

Keywords: Wheat; cDNA-AFLP; Salt-stress; Mutant; *TaGSK1*; Transmembrane protein

1. Introduction

In recent years, accumulated results from model organism, such as *Saccharomyces cerevisiae*, *Arabidopsis thaliana* and rice are paving the way for understanding the molecular mechanism of salt tolerance. Many studies have focused on salt-stressed protein [1], accumulation of the organic matter [2], distribution of the Na^+ in the plants [3], and regulation of the gene expression by salt-stress conditions, etc. [4]. More recently, significant progress has been obtained in revealing salt-stress signal pathway. For example, several kinds of protein kinases were found involved in response to the NaCl and to high osmotic stress, such as MAPK kinase in yeast [5,6]. Of those signal pathway established for NaCl-stress, SOS system is more recent, which is proposed by Zhu through screening *A. thaliana* salt sensitive mutants [7]. Up to date, however the molecular mechanism of salt tolerance of wheat remains unclear. One possible reason is lack of proper materials with similar genetic background but with difference in salt tolerance.

In a previous study, we obtained one mutant by culturing the wheat anther callus that was mutated with EMS. The offsprings segregated and showed different salt-response. Among them, RH8706-49 (salt-stress resistant, SR) and H8706-34 (salt-stress sensitive, SS) are very different. The distinguishable properties of each mutant was stablly inherited for 12 generations. In this study, we used a RNA finger printing technique cDNA-amplified fragment length polymorphism (AFLP) to analyse genes that are differentially expressed in both SR and SS with or without salt-stress. The results showed that only 11.9% fragments were different among the four samples. We further cloned a cDNA encoding glycogen synthase kinase–shaggy kinase (*TaGSK1*) from SR leaves. Its expression was induced by NaCl stress and it expressed more strongly in SR than in SS as indicated in Northern blot, suggesting it may be involved in signal transduction of salt-stress in wheat.

* Corresponding author. Tel.: +86-311-6268230; fax: +86-311-5828784.
E-mail address: shenyinzhu@hotmail.com (Y.Z. Shen).
[1] The first and second author contributed equally to this paper.
[2] Co-corresponding author.

0168-9452/$ – see front matter
doi:10.1016/S0168-9452(03)00365-0

2. Materials and methods

2.1. Plant materials

Triticum aestivum L RH8706-49 and H8706-34 were derived from single seed which was obtained by culturing F_1 anther of punong 3665 and hannong 3039, inducing mutation with EMS, and screening through the salt tolerance ability [8], with salt tolerance index of RH8706-49 (SR) $\geq$ 1.3 and H8706-34 $\leq$ 0.5.

Seeds of the two materials were submerged in water for 24 h at 25 °C and then transferred to the light incubator at the time the root and the bud had emerged, and then cultured in fresh water under 8 h of light region each day. When the second leaves had grown, the plants were cultured in one-half Hoagland solution in the absence or presence of 1% NaCl for 72 h, then the second leaves were harvested and frozen in liquid nitrogen until used.

2.2. cDNA-AFLP

cDNA-AFLP was performed as described by Bachem et al. [9]. Total RNA from leaves was extracted from four different wheat samples (SR and SS with or without salt-stress) using a Qiagen Plant RNeasy Kit (Germany). First and second strand cDNA were synthesized using Superscript II RNase H^- reverse transcriptase (Gibco/BRL) and SMART cDNA Library Construction Kit (Clontech), respectively. After *PstI/MseI* digestion and adaptor ligation, the cDNA products were pre-amplified and diluted for cDNA-AFLP. Amplified products from different primer combinations were separated in a sequencing gel and exposed to X-ray film. Differentially expressed fragments cut from gel were cloned and sequenced. Homology search was done by the Blast program.

2.3. Isolation of a full-length TaGSK1 cDNA clone from RH8706-49 leaves

Among those specific expressed fragments in RH8706-49 leaves, a 181 bp clone had a significant similarity to *GSK*–shaggy kinase of maize. Based on its sequence, the 5′ and 3′ RACE (rapid amplification of cDNA ends) primers were designed as follows:

5′RACE: 5′-CCATGAGTTGAAGGGTGTGC-3′;
5′-AAGCAGTGGTATCAACGCAGAGT-3′;
3′RACE: 5′-GAGAATCTCAAACTCCTGGG-3′;
5′-ATTCTAGAGGCCGAGGCGGCCGACATG-d-$(T)_{30}$ $N_{-1}N_{-3}$-3′N = A, G, C or T; N_{-1} = A, G or C;
5′RACE and 3′RACE was performed to isolate the full-length cDNA sequence of *TaGSK1*.

The specific primers were designed according to the 3′ and 5′ terminus regions and synthesized by Beijing SBS Biotechnology Corporation.

5′-cDNA primer;
5′-CGTCTAGAGTTGGTGTGGTGCGTCCTTCC-3′;

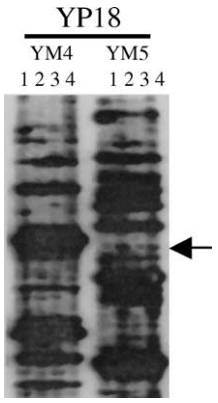

Fig. 1. Analysis of mRNA in wheat leaf used by cDNA-AFLP. Lane1-4 indicate that SR-0, SR-1, SS-0, SS-1. The primers are combinations of YP18 and YM4, AYM5. The arrow indicates the amplified fragment of *TaGSK1*.

3′-cDNA primer;
5′-CGCCCGGGGGGGGCCTCGATCCATGAAC-3′.

A 1.6 kb fragment was amplified using pfu polymerase from Shanghai Sangon Corporation and RH8706-49(SR) double-stranded cDNA as template by common PCR method. The fragment was ligated into the multiple cloning region of pGEM-T vector. The recombinant-$P^{D\text{-}T}23$ was obtained after transformed into *E. coli* DH5α competent cells and sequenced by TaKaRa Biotechnology (Dalian) Corporation.

2.4. RNA expression analysis

Total RNA were prepared as described [10] above and 35 μg total RNA from each sample was subjected to 1% formaldehyde denatured agarose gel electrophoresis. After electrophoresis, the RNA was blotted on to Hybond N^+ nylon membrane (Amersham Pharmacia, USA) with 20× SSC. After pre-hybridization, the hybridization, probe preparation and membrane washing were according to the manufacturer's manual (Amersham Pharmacia, USA). The membrane was exposed to X-ray film according

Table 1
Analysis of the differentially expressed fragments from cDNA-AFLP

Primer combination	SNI	SIN	SAS	SIR	SIS	Sub-total
P14M4	36	4	2	1	0	43
P18M2	50	1	4	0	0	55
P12M8	40	3	3	2	1	49
P20M5	55	2	2	1	1	61
P18M5	45	2	3	0	2	52
P7M7	48	0	2	0	1	51
Total	274	12	16	4	5	311
Percentage	88.10	3.86	5.14	1.29	1.61	100

Note: SNI: salt-stress non-inducible, SIN: salt-stress inducible, SAS: salt-stress suppressible, SIR: salt-stress inducible in SR, SIS: salt-stress inducible in SS.

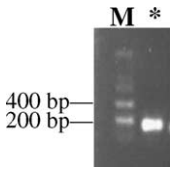

Fig. 2. Second amplification product of TaGSK1 fragment. M: molecular weight markers, (*) lane indicates the fragment of the *TaGSK1*.

to the manufacturer's manual (Fuji Photo Film Co., Japan).

2.5. Structure of TaGSK1 protein

The amino acid sequence of *TaGSK1* was deduced and the primary and secondary structures of the protein were analysed and contrasted by using DNAtools, Omiga, GenBank, iPSORT are softwares for Molecular Biology.

3. Results

3.1. cDNA-AFLP analysis of differentially expressed genes between the two wheat lines under normal and salt-stress conditions

To analyse genes related to salt tolerance in wheat, differentially gene expression pattern was compared between SR and SS under salt-stress and non-salt-stress by cDNA-AFLP approach (Fig. 1). A total of 90 pairs of primer combinations were used for selective amplification. To reveal the expression patterns, the displayed transcripts from randomly selected six pairs of primer combinations were analyzed (Table 1). The results showed that 88.10% fragments were expressed in all the four samples, they belong to salt-stress non inducible (SNI), only 11.9% fragments were different from each other, of which 3.86% fragments were salt-stress inducible (SIN), and 5.14% fragments were salt-stress suppressible (SAS) in both SR and SS, indicating involvement of these genes in salt-stress. These changes do not have any relationship with the genetic background of these materials. In addition to that, 1.29% fragments were specifically induced in SR (salt-stress inducible in SR SIR), while 1.61% specially in SS (salt-stress inducible in SS SIS), representing the genes which are salt-stress related to the genetic background.

To further characterize the differentially expressed genes, 68 fragments were cloned, of which 35 clones were sequenced. BLAST search in EMBL database indicated that 24 sequences (68.6%) showed no similarity to known genes, and 11 sequences (31.4%) were highly homologous to known genes. A SR specific fragment (see Figs. 1 and 2) showed high similarity with maize *GSK*–shaggy kinase gene and was named *TaGSK1(Triticum aestivum* L. glycogen synthase kinase). Possible relationship between *TaGSK1* and salt tolerance in wheat was further investigated because a previous study showed that *GSK* is related to NaCl stress in *A. thaliana* [11].

3.2. RNA expression analysis of TaGSK1

To verify the expression pattern of *TaGSK1* observed after cDNA-AFLP analysis, Northern-blot analysis was

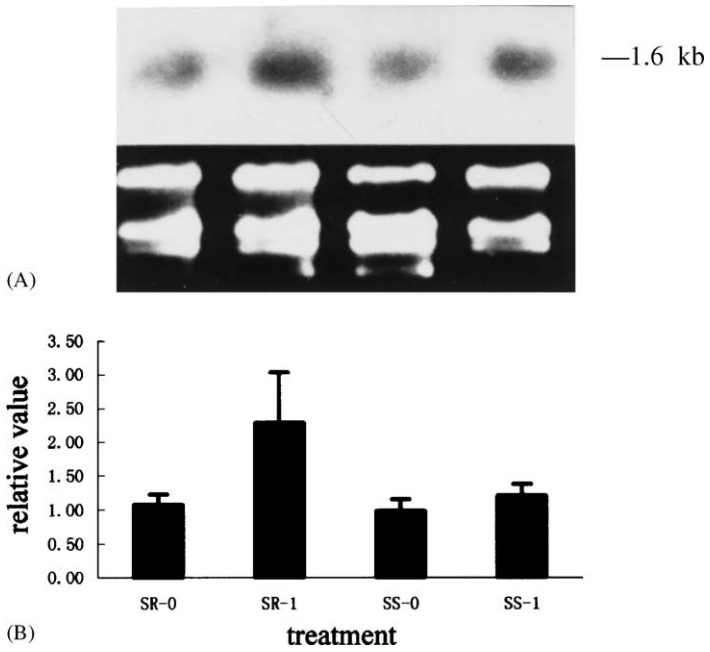

Fig. 3. Northern blot and relative expression the wheat *Ta-GSK1*. (A) Northern-blot SR-0: salt resistant-0% NaCl; SR-1: salt resistant-1% NaCl; SS-0: salt-sensitive-0%; SS-1: salt-sensitive-1%. (B) Relative expression in the four sample. Control of loading (1 μg) has been dye with ethidium bromide staining. The analysis was completed through computer scanning system.

performed. A 1.6 kb band was detected using 181 bp cDNA-AFLP fragment as a probe, The result also showed that the isolated cDNA clone representing a full-length copy of *TaGSK1* (AF525086). Northern analysis showed that its expression was induced both in SR and in SS (Fig. 3). Its expression in SR leaves under salt-stress was 2.14-fold higher than non salt-stress and in SS which was 1.23-fold higher, thus indicating that *TaGSK1* was highly expressed under salt-stress and the expression was higher in SR than in SS (Fig. 3).

```
   1 GTT GGT GTG GTG CGT CCT TCC TCG CGC TTT CAG AAC GAC ACG AGT ACT AGT GGT GAT GCC    60
  61 GAC CGA CTT CCG AAC GAG ATG GGC AAT ATG AGC ATA AGG GAT GAC AGG GAC CCT GAG GAT   120
                          1   M   G   N   M   S   I   R   D   D   R   D   P   E   D    14

 121 ATA GTA GTC AAC GGC AAT GGG ACG GAA CCA GGC CAT ATT ATA GTC ACA AGC ATT GAG GGA   180
  15 I   V   V   N   G   N   G   T   E   P   G   H   I   I   V   T   S   I   E   G    34

 181 AGA AAT GGG CAA GCA AAA CAG ACC ATT AGC TAC ATG GCT GAG CGT GTG GTT GGT AAT GGG   240
  35 R   N   G   Q   A   K   Q   T   I   S   Y   M   A   E   R   V   V   G   N   G    54

 241 TCA TTT GGA ACT GTT TTC CAG GCT AAG TGT CTT GAA ACT GGC GAG ACG GTG GCT ATA AAG   300
  55 S   F   G   T   V   F   Q   A   K   C   L   E   T   G   E   T   V   A   I   K    74

 301 AAG GTT CTT CAA GAC AAG AGA TAT AAG AAC CGT GAG CTG CAA ACG ATG CGA GTT CTT GAC   360
  75 K   V   L   Q   D   K   R   Y   K   N   R   E   L   Q   T   M   R   V   L   D    94

 361 CAC CCA AAT GTT GTG GCT TTA AAG CAT TGT TTT TTC TCA AAG ACT GAG AAA GAG GAG CTT   420
  95 H   P   N   V   V   A   L   K   H   C   F   F   S   K   T   E   K   E   E   L   114

 421 TAC CTC AAC CTG GTG CTT GAG TAT GTG CCG GAG ACT GCT CAT CGT GTC ATT AAG CAT TAT   480
 115 Y   L   N   L   V   L   E   Y   V   P   E   T   A   H   R   V   I   K   H   Y   134

 481 AAC AAG ATG AAC CAA CGC ATG CCA TTG ATA TAT GCA AAA CTG TAC ATG TAT CAG ATA TGT   540
 135 N   K   M   N   Q   R   M   P   L   I   Y   A   K   L   Y   M   Y   Q   I   C   154

 541 AGA TCT TTG GCA TAC ATT CAC AAC AGC ATT GGA GTA TGC CAC AGA GAC ATC AAG CCT CAA   600
 155 R   S   L   A   Y   I   H   N   S   I   G   V   C   H   R   D   I   K   P   Q   174

 601 AAT CTT CTG GTG AAT CCA CAT ACG CAC CAA TTG AAA TTA TGT GAC TTC GGA AGT GCG AAA   660
 175 N   L   L   V   N   P   H   T   H   Q   L   K   L   C   D   F   G   S   A   K   194

 661 GTG TTG GTA AAA GGA GAA CCA AAT ATT TCC TAT ATC TGT TCA AGG TAC TAT AGA GCC CCA   720
 195 V   L   V   K   G   E   P   N   I   S   Y   I   C   S   R   Y   Y   R   A   P   214

 721 GAG CTC ATA TTT GGT GCT ACT GAA TAC ACA ACG GCA ATT GAC GTT TGG TCT GCT GGC TGT   780
 215 E   L   I   F   G   A   T   E   Y   T   T   A   I   D   V   W   S   A   G   C   234

 781 GTT CTT GCT GAA CTC CTT CTA GGA CAG CCT ATA TTC CCT GGC GAC AGT GGT GTT GAT CAG   840
 235 V   L   A   E   L   L   L   G   Q   P   I   F   P   G   D   S   G   V   D   Q   254

 841 CTT GTT GAA ATC ATC AAG GTT TTA GGT ACC CCT ACA AGA GAA GAA ATT AAG TGC ATG AAT   900
 255 L   V   E   I   I   K   V   L   G   T   P   T   R   E   E   I   K   C   M   N   274

 901 CCA AAT TAT ACG GAG TTT AAA TTC CCA CAA ATC AAA GCT CAC CCA TGG CAC AAG ATC TTC   960
 275 P   N   Y   T   E   F   K   F   P   Q   I   K   A   H   P   W   H   K   I   F   294

 961 CAT AAA AGA ATG CCT GCT GAA GCA GTA GAT CTT GTC TCC AGA CTC TTG CAA TAT TCA CCA  1020

 295 H   K   R   M   P   A   E   A   V   D   L   V   S   R   L   L   Q   Y   S   P   314
1021 AGC CTG CGT TCA ACT GCT TTG GAA GCA TTA ATT CAT CCA TTC TTC GAT GAA CTC CGG GAC  1080

 315 S   L   R   S   T   A   L   E   A   L   I   H   P   F   F   D   E   L   R   D   334

1081 CCA AAC ACC CGT TTG CCG AAC GGC CGT TTT CTT CCT CCC CTC TTT AAC TTT AAG CCC CAT  1140
 335 P   N   T   R   L   P   N   G   R   F   L   P   P   L   F   N   F   K   P   H   354

1141 GAG TTG AAG GGT GTG CCG ATG GAC ATC CTG GTG AAG CTC ATC CCT GAA CAT GCT CGG AAG  1200
 355 E   L   K   G   V   P   M   D   I   L   V   K   L   I   P   E   H   A   R   K   374

1201 AAC TGT GCC TTT GTA GGA TGG TGA TCC GCC AGA CGG CTG CTT GAA GTT TAG TTC AGA ACA  1260
 375 N   C   A   F   V   G   W   *   381
1261 AAT CCA GTT GTT GTC TAC TAG AAA CCC CAG GAG TTT GAG ATT GTC TGC AGC CAC ACG GGA  1320
1321 TAT AGG CGA TGA CAC ATG TGA TTA TTA TTC CTT TTC TCG TCC GAG ACC TCG ATG CCA TGT  1380
1381 ATT CTT TCC CCC TAC TGC CGA TGT AAC AAA CCA CCC ATG ATA CTG TAA GTA GAT GAG AAG  1440
1441 TGT TTC GAC CGT TTT CCC CTG AGC TCA TGT GCT ATG CAA TGA AGG ATG CAC CCT ATG TAC  1500
1501 CGC CAA TAT TTG GTC CAG TAT TTG TTC ATG GAT CGA GGC CCC CAA AAA AAA AAA AAA AAA  1560
1561 AAA AAA AAA AAA A                                                              1573
```

Fig. 4. The nucleotide sequence and its deduced amino acid sequence of *TaGSK1* cDNA. M: prediction of a chloroplast transit peptide, V: protein kinase ATP-binding region signature.

3.3. Isolation of TaGSK1 full-length cDNA clone and structure analysis of the putative protein

The full-length cDNA sequence of *TaGSK1* was obtained by 5′ and 3′ RACE. Further by designing specific primers, the full-length cDNA fragment of *TaGSK1* was obtained from RH8706-49 double-strand cDNA by PCR method and it was 1.573 kb long as confirmed through sequencing two times. The analysis of the sequence indicated that the third 'ATG' of the sequence was the valid beginning codon and

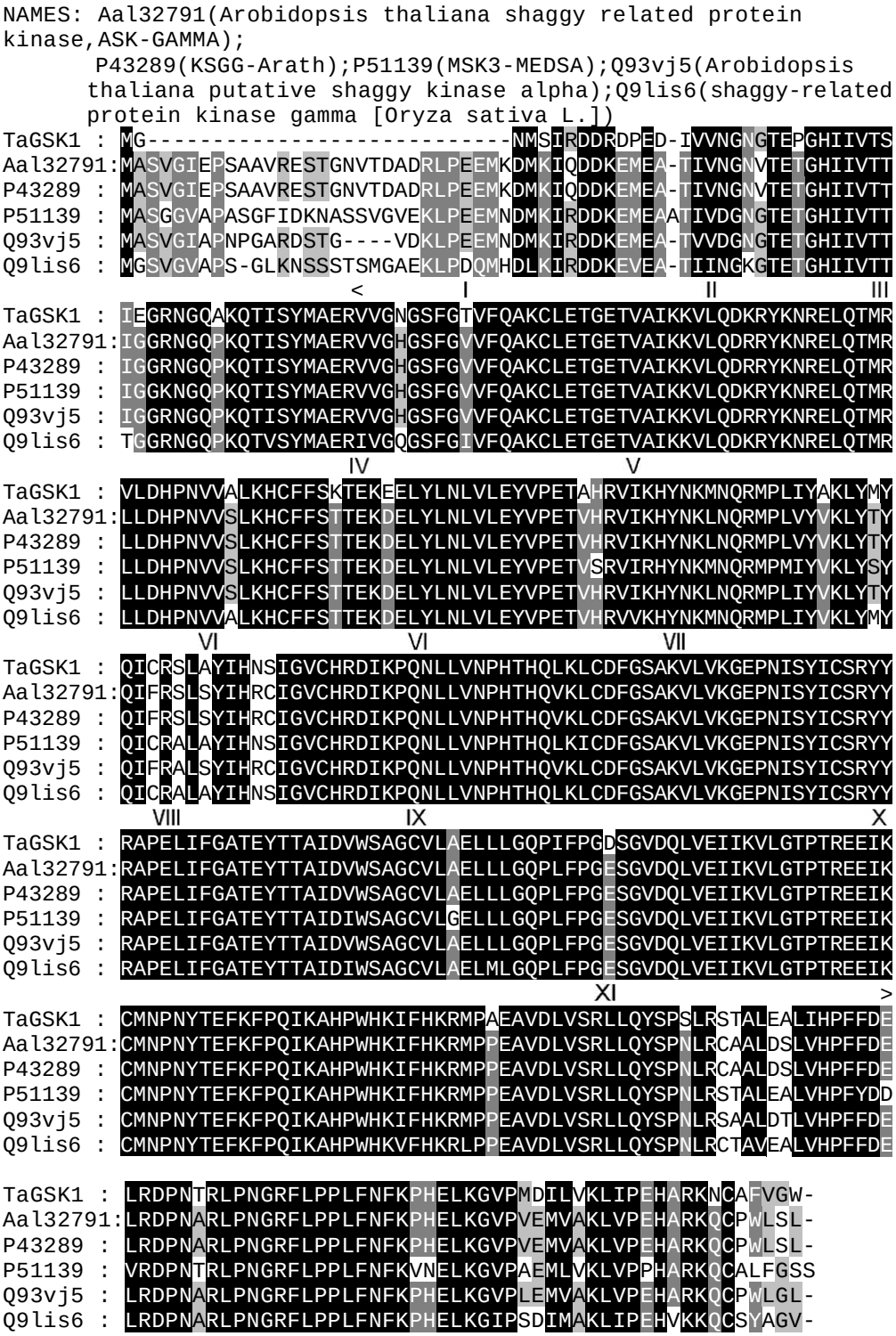

Fig. 5. Alignment of amino acid sequences of *TaGSK1* and other plants *GSK*. ⟨·⟩: The 285 residues-long protein kinase catalytic domain. The 11 catalytic subdomains (Hanks 1991) are indicated by roman numerals.

the longest open reading frame (ORF) encoded a protein of 381 amino acids with a 78 bp 5' non-encoding region and a 352 bp 3′ non-encoding region. The end codon-'TGA' was at a distance of 319 bp from the upper poly(A)-tail at 3′ (Fig. 4). The predicted protein has a molecular weight of 43.5 kDa with pI = 8.66. Comparison of the predicted amino acid sequence of *TaGSK1* with *GSK* sequences from other organisms is shown in Fig. 5. The alignment illustrated that the *GSK* gene conserved in these plants and inferred that GSK may play a key part in stress signal transduction [12].

Using RPS-BLAST software, the amino acid sequence including a Serine/Threonine protein kinase catalytic domain and protein kinase domain was analysed. There was a protein kinase ATP-binding region signature at the 51AA in the front of the protein kinase domain-VGNGSFGTVFQAKCLETGETVAIKK. Predicted by computer models for transmembrane topology, the secondary structure indicated it was a transmembrane protein with N-terminus outside. There were two transmembrane domains: one was a 22AA transmembrane domain from 41AA (outside) to 60AA (inside) and a 19AA domain from 223AA (inside) to 241AA (outside), the other was a 21AA domain from 45AA (inside) to 65AA (outside) and a 22AA domain from 226AA (outside) to 247AA (inside). Predicted by the method of Chou-Fasman, the secondary structure of *TaGSK1* may contain helix conformation 22.83%, sheet 41.73%, turn 31.24% and coil 4.20%. The amino acids composition of *TaGSK1* was neutral hydrophobic 43.6%, neutral hydrophilic 29.7%, charged acidic 16.3% and charged basic 10.5%.

4. Discussion

In this study, the differential gene expression pattern was compared between the two wheat mutant lines under salt-stress and non-salt-stress by cDNA-AFLP. The results showed that only a small number of the differentially expressed genes are likely involved in salt-stress in wheat. cDNA-AFLP analysis showed that about 15–20% genes are likely involved in water stress, similar to our results [13]. Homologous analysis of different fragments indicated that the fragments we isolated from the wheat represented a series of genes in wheat related to salt-stress. Further research on the genes will benefit to our understanding the mechanism of salt tolerance in SR.

Recently great progress has been made about the salt-stress signal transduction in plant. *TaGSK1* shows high similarity to shaggy kinase in maize. In yeast there are two *GSK3* homologs, Mck1p and Mds1p [14,15], Mutation at the *MCK1* gene result in a cold-sensitive and temperature-sensitive phenotype. Though functional complementation of the yeast calcineurin mutant strain DHT22-1a with a NaCl stress-sensitive phenotype, Piao et al. [11] have isolated the *A. thaliana* cDNA *AtGSK1*, which encodes a *GSK3*–shaggy kinase-like protein kinase. *AtGSK1* expression was induced by NaCl and the expression level and salt tolerance ability showed positive correlation. They supposed that *AtGSK1* may be involved in a calcineurin signal transduction pathway in plants. The *TaGSK1* we isolated from wheat is also induced by NaCl. It is possible that *TaGSK1* may be involved in NaCl stress signal transduction pathway in wheat. The higher salt tolerance ability of SR may be related to the higher expression of the *TaGSK1* in SR than in SS 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 previous studies, three key components involved in the MAPK pathway in response to NaCl and osmotic stress [16], were identifical to be Ca^{2+}/camodulin-dependent protein phosphatase signal transduction pathway [17], *SOS2* involved in the SOS signal transduction pathway [18], and Serine/Threonine protein kinases. *TaGSK1* is also a kind of serine/threonine protein kinase. In this study we also isolated three other fragments (SAS30, 88 and 100) encoding Serine/Threonine protein kinases like protein and interestingly we found their expression was suppressed by NaCl. So the function of the Ser/Thr protein kinase are comprehensive. How was the function of *GSK*–shaggy like kinase function, and the relationship between *GSK* and shaggy kinase and other pathway still need further studies especially in relation to salt-stress.

Acknowledgements

We wish to thank Professor Ying Sun for her critical reading and correction. This project is supported by the National Natural Science Foudation of China (30070471) and the Natural Science Foundation of Hebei Province (301103).

References

[1] E.J. DeRocher, H.J. Bohnert, Development and environmental stress employ different mechanisms in the expression of a plant gene family, Plant Cell 5 (1993) 1611–1625.
[2] K.F. McCue, A.D. Hanson, Salt-inducible betaine aldehyde dehydrogenase from sugar beet: cDNA cloning and expression, Plant Mol. Biol. 18 (1992) 1–11.
[3] E. Blumwald, G.S. Aharon, M.P. Apse, Sodium transport in plant cells, Biochim. Biophys. Acta 1465 (2000) 140–151.
[4] J. Sheen, Ca^{2+}-dependent protein kinase and stress signal transduction in plant, Science 274 (1996) 1900–1902.
[5] T. Maeda, M. Takekawa, H. Saito, Activation of yeast PBS2 MAPKK by MAPKKKs or by binding of an SH3-containing osmosenser, Science 269 (1995) 554–558.
[6] S. Kiegerl, F. Cardinale, C. Siligan, A. Gross, E. Baudouin, A. Liwosz, et al., SIMKK, a mitogen-activated protein kinase (MAPK) kinase, is a specific activator of the salt-stress-induced MAPK, SIMK, Plant Cell 12 (2000) 2247–2258.

[7] J.K. Zhu, Genetic analysis of plant salt tolerance using Arabidopsis, Plant Physiol. 124 (2000) 941–948.
[8] Y.Z.H. Shen, Z.H.Y. Liu, C.F. He, et al., Rearches on variation of salt tolerance in induced calli of wheat and regeneration plants, Hereditas 19 (1997) 7–11.
[9] C. Bachem, R. van der Hoeven, S. de Bruijn, et al., Visualization of differential gene expression using a novel method of RNA fingerprinting based on AFLP: analysis of gene expression during potato tuber development, Plant J. 9 (1996) 745–753.
[10] Extraction Purification and Analysis of mRNA From Eukaryotic Cells Molecular Cloning, Cold spring Harbour Laboratory Press, 2002, Chapter 7, pp. 277–292.
[11] H.L. Piao, T.P. Kyeong, H.L. Jeong, G.K. Shin, B.J. Jing, H.K. Sung, H. Inhwan, An Arabidopsis *GSK3*/shaggy-like gene that complements yeast salt-stress-sensitive mutants in induced by NaCl and abscisic acid, Plant Physiol. 119 (1999) 1527–1533.
[12] Jonak, H. Hirt, Glycogen synthase kinase3/shaggy-like kinases in plants: an emerging family with novel functions. Trends Plants Sci. 7 (2002) 457–461.
[13] Y.F. Cheng, T.L. Pu, Y.B. Xue, C.H.L. Zhang, PcTGD, a highly expressed gene in stem, is related to water stress in reed (*Phragmites communis Trin*.), Chin. Sci. Bull. 46 (2001) 850–854.
[14] L. Neigeborn, A.P. Mitchell, The yeast *MCK1* gene encodes a protein kinase homolog that activates early meiotic gene expression, Genet. Dev. 5 (1991) 533–548.
[15] J.W. Puziss, T.A. Hardy, R.B. Johnson, P.J. Roach, P. Hieter, MDSI, a dosage suppressor of an Mck1 mutant, encodes a putative yeast homolog of glycogen synthase kinase 3, Mol. Cell Biol. 14 (1994) 831–839.
[16] M.J. Robinson, M.H. Cobb, Mitogen-activated protein kinase pathway, Curr. Opin. Cell Biol. 9 (1997) 180–186.
[17] J.M. Pardo, M.P. Reddy, S. Yang, et al., Stress signaling through Ca^{2+}/calmodulin-dependent promein phosphatase calcineurin mediates salt adaptation in plants, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 9681–9686.
[18] J. Liu, M. Ishitani, U. Halfter, C.S. Kim, J.K. Zhu, The *A. thaliana SOS2* gene encodes a protein kinase that is required for salt tolerance, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 3730–3734.