African Journal of Biotechnology Vol. 10(27), pp. 5327-5336, 15 June, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1917
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Isolation and drought-tolerant function analysis of *ZmPti1-1*, a homologue to *Pti1*, from maize (*Zea mays* L.)

Zhiliang Li[1,2,3], Mingdi Bian[2], Zhongyi Wu[2], Xiuhai Zhang[2], Qing Yang[1], and Conglin Huang[2]*

[1]College of Life Science, Nanjing Agricultural University, Nanjing, 210095, China.
[2]Beijing Agro-Biotechnology Research Center, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China.
[3]Department of Biology, Handan College, Handan, Hebei 056005, China.

Accepted 4 March, 2011

Pto-interacting 1(*Pti1*) has been well established to play important roles in plant disease and salt response, but its potential roles in the response to drought stress is unknown. In this study, the *Pti1*-like gene named as *ZmPti1-1* was cloned from maize, sequence analysis showed that *ZmPti1-1* encodes a polypeptide of 363 amino acids with predicted molecular mass of 39.0 kDa and an isoelectric point of 8.14. *ZmPt1-1* is dramatically induced by abscisic acid (ABA) and mannitol (data not shown). In order to analyze the further drought tolerant functions, *ZmPti1-1* was over-expressed in *Arabidopsis*. Under drought stress, compared with wild type, survival rate of the three transgenic lines, which was 70, 76 and 87%, respectively, was significantly higher than that of wild type which was 29%; there were lower water loss, lower cell membrane damage, higher relative water content, higher total soluble sugars, higher proline content and higher yield for transgenic plants. Based on the present knowledge, this is the first report that over-expression of *Pti1*-like gene improved drought tolerance in plants.

Key words: *ZmPti1-1*, transgenic *Arabidopsis*, drought tolerance.

INTRODUCTION

Drought is the major environmental stress affecting plant growth and productivity (Yamaguchi-Shinozaki and Shinozaki, 2006). Drought reduces plant productivity by inhibiting plant growth and photosynthesis (Shou et al., 2004). Plant responds to drought stress by activating a number of signal pathways which enable them to defend or adjust against the stress (Pandey, 2008). Drought stress signal transduction consists of ionic and osmotic homeostasis signal pathways, detoxification (damage control and repair) response pathways and pathways for growth regulation (Zhu, 2002). In these events, reversible protein phosphorylation catalyzed by protein kinases and protein phosphatases plays an important role (Yang et al., 1997).

The tomato gene Pto-interacting 1 (*Pti1*) encoding a serine/threonine kinase is phosphorylated by Pto; over-expression of tomato Pti1 in tobacco resulted in an accelerated hypersensitive response when the plants were challenged with the tobacco pathogen *Pseudomonas syringae* tabaci expressing *avrPto* (Zhou et al., 1995). Several Pto-interacting (Pti) proteins were identified to act in Pto mediated signal transduction in tomato, including protein kinase Pti1 and three transcription factors (Pti4/5/6) (Tian et al., 2004).

Some tomato *Pti1*-like kinase genes are cloned from maize (Herrmann et al., 2006; Zou et al., 2006). Zou et al. (2006) cloned and characterized a maize *Pti1* like kinase gene *ZmPti1*; according to its deduced amino acid

*Corresponding author. E-mail: conglinhuang@hotmail.com. Tel: 86-10-51503801; 86-13910285037. Fax: 86-10-51503980.

Abbreviations: ***Pti1,*** Pto-interacting 1; **ABA,** abscisic acid; **EST,** expressed sequence tag; **PCR,** polymerase chain reaction; **RT,** reverse transcription; **WT,** wild type; **FW,** fresh weight; **DW,** dried weight; **MDA,** malondialdehyde; **TW,** turgid weight; **RWC,** relative water content.

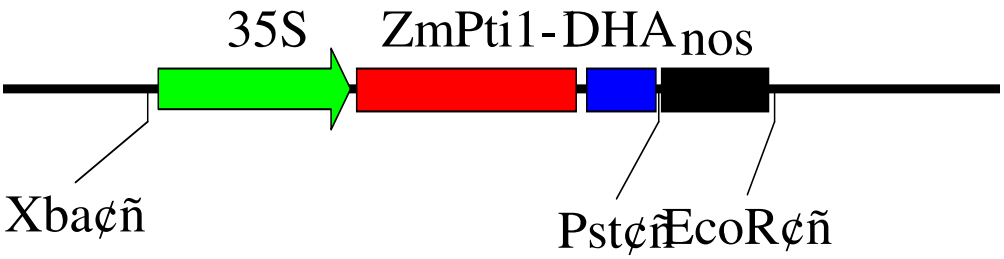

Figure 1. Part region of expression vector pGreen0029-ZmPti1-1.

sequence, it maybe Ser/Thr/Tyr protein kinase. Over-expression of *ZmPti1* increases salt tolerance of *Arabidopsis* (Zou et al., 2010). Herrmann et al. (2006) cloned and characterized four *Pti1* like kinase genes from maize, *ZmPti1a* encodes a functional serine/threonine kinase specific to pollen and is associated with plasma membrane regions responsible for callose deposition; *ZmPti1b* is likely to be involved in maize pathogen responses. Compared to *ZmPti1a* and *ZmPti1b*, *ZmPti1c* and *ZmPti1d* is expressed at low levels but in all sporophytic tissue. Only transcripts of *ZmPti1c* could also be detected in mature and germinated pollen.

In our research, a new maize *Pti1*-like kinase was cloned and designated as *ZmPti1-1* (GenBank accession no. EF158035). According to its deduced amino acid sequence, it maybe Ser/Thr kinase. *ZmPt1-1* was dramatically increased upon a wide range of abiotic stresses, such as abscisic acid (ABA), mannitol, salt stress and 4℃ treatment (data not shown). The result indicated that, maize *ZmPti1-1* may be responsible for abiotic stresses. However, a precise role of *Pti1* in plant drought tolerance has remained unclear. In this study, the *ZmPti1-1* gene was cloned and introduced into *Arabidopsis* by the floral dip method (Clough and Bent, 1998), drought tolerance of transgenic *Arabidopsis* plants was investigated further. The results showed that *ZmPti1-1* play an important role in drought adaptation of *Arabidopsis*.

MATERIALS AND METHODS

Cloning of *ZmPti1-1* cDNA

The sequence of At2g47060 was used as the query probe to search homologues in maize expressed sequence tag (EST) TUGs database. A highly homologous maize EST contig (Zm-tu- 03-08-11, 14960) was obtained for analysis of motifs/domain in ScanProsite in PlantsP database (Gribskov et al., 2001). According to the EST contig sequence, two primers, 5'-CTT CCT ACC CGC TTC C-3'(forward primer) and 5'-CAT CGA CGA GTC ATA TAC CTT-3' (reverse primer; synthesized at Sunbiotech, China) were used to amplify DNA encoding ZmPti1-1 by polymerase chain reaction (PCR). The first strand cDNA was obtained by reverse transcription (RT) using M-MLV reverse transcriptase (Promega, USA) in a 20 µl reaction volume with 5 µg total RNA prepared from maize roots treated with 250 mM NaCl for 24 h. RNA was isolated using TRIzol Reagent (Invitrogen, USA). The RT-PCR product was cloned into pGEM-T vector (Promega) and sequenced (Sangon, China).

Plant material, transformation and growth condition

Full length cDNA of *ZmPti1-1* in vector pGEM-T was amplified by PCR with adding *Bgl*II and *Sma*I sites into 5' and 3' primers, respectively and confirmed by sequencing, then was subcloned into the *Bam*H I/*Stu*I site of the vector pUC18. The expression vector pGreen0029, which contained kanamycin resistance gene, was digested with *Xba* I and *Eco*R I to release short fragment. Then, the fragment *35S-C4DDPK-ZmPti1-1-DHA-His6-nos* in vector pUC18 was subcloned into the *Xba* I and *Eco*R I site of the vector pGreen0029 to construct *ZmPti1-1* gene expression vector pGreen0029-ZmPti1-1 (Supplemental Figure S1) according to the standard mole-cular protocol (Sambrook and Russell, 2001).

pGreen0029-ZmPti1-1 vector was introduced into *Agrobacterium tumefaciens* strain GV3101 which was then, used to transform *Arabidopsis* (ecotype Columbia) using the floral dip method. T_0 seeds were screened by 50 $mg \cdot l^{-1}$ kanamycin medium. Resistant seedlings were transferred to soil in the plastic pots and grown for seeds. T_3 homozygous seeds or seedlings were used for molecular identification and drought tolerance assay. The pot-grown wild type and transgenic *Arabidopsis* plants were placed in growth chambers with the condition of 20℃ temperature, 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light, 16/8 h day/night cycle and 60% relative humidity.

Molecular characterization of the *ZmPti1-1* gene in transgenic *Arabidopsis*

PCR assays were performed with specific primers for the *ZmPti1-1* gene: P1, 5′- CAC AGA TCT ATG TCG TGC TTT GCG TGC -3′; P2, 5′- TAT CCC GGG TGA CCC AGC ATG ATC -3′. For RNA Gel-blot analysis, total RNA was extracted from 4-week-old seedlings of *Arabidopsis* with use of Trizol reagent (TIANGEN, Beijing, China). Total RNA of 10 µg was electrophoresed on 1.2% (w/v) agarose gel containing 5% (v/v) formaldehyde and transferred to Hybond-NY+ nylon transfer membrane (Millipore Corporation Bedford MA 01730, USA). The membrane was hybridized with a DIG-labeled *ZmPti1-1* cDNA specific probe at 42℃ for 16 to 18 h. The hybridized membrane was washed and detected according to the protocol of Dig nucleic acid detection kit (DIG High prime DNA labeling and detection starter kit II, Roche Corporation, Basel, Switzerland). The ethidium bromide-stained ribosomal RNA was used as loading control. Images were acquired by scanning the membranes with a LAS-4000 image reader (FUJIFILM Corporation, Japan).

Drought treatment of *Arabidopsis* seedling

Seeds of *Arabidopsis* wild type (WT) and T3 homozygous

```
  1 MSCFACCGDE DTQVPDTRAQ YPGHHPARAD AYRPSDQPPK GPQPVKMQPI AVPAIPVDEI
 61 REVTKGFGDE ALIGEGSFGR VYLGVLRNGR SAAVKKLDSN KQPDQEFLAQ VSMVSRLKHE
121 NVVELLGYCA DGTLRVLAYE FATMGSLHDM LRGRKGVKGA QPGPVLSWSQ RVKIAVGAAK
181 GLEYLHEKAQ PHIIHRDIKS SNVLLFDDDV AKIADFDLSN QAPDMAARLH STRVLGTFGY
241 HAPEYAMTGQ LSSKSDVYSF GVVLLELLTG RKPVDHTLPR GQQSLVTWAT PRLSEDKVRQ
301 CVDSRLGGDY PPKAVAKFAA VAALCVQYEA DFRPNMSIVV KALQPLLNAH ARATNPGDHA
361 GS
```

Figure 2. ZmPti1-1 amino acid sequence deduced from cloned *ZmPti1-1*. The underlined amino acids are protein kinase domain. ATP-binding region signature is shown in bold type. Serine/ threonine protein kinases domain is shown in bold and italic type. Four sequences in frames are potential N-myristoylation sites.

transgenic plants were germinated and grown in 7 cm plastic pots filled with 1:1 vermiculite: nutrient soil mixture with constant watering. 14-days old seedlings were hold without watering for 9 days. After that, all the pots were re-watered simultaneously and the plant re-growth was scored 5 days later.

Quantification of water-loss in *ZmPti1-1* transgenic *Arabidopsis* plants under drought stress

0.5 g fully expanded leaves from 25-days old wild type and *35S* :: *ZmPti1-1* T3 plants were detached and weighed immediately (FW_0). Then, the leaf discs were incubated on Petri dishes in the dark at room temperature. The leaves fresh weight (FW) was measured at 1 h interval to determine the rate of water loss. Twelve hours later, the leaf discs were dried in an oven at 75℃ for 72 h and weighed (DW). The rate of water loss was calculated from the equation:

Water loss (%) = (FW_0 -FW)/ (FW_0-DW) ×100%

At least three replicates per line were used. Statistical differences were determined using Student's t-test.

Measurement of electrolyte leakage and malondialdehyde (MDA) levels

Membrane damage was assayed by measuring ion leakage and MDA from leaf discs. Measurement of electrolyte leakage and malondialdehyde levels was conducted at 0, 3, 7, 14 days, respectively, after drought treatment.

To measure ion leakage ratio as relative electric conductivity parameter, 0.1 g leaves were removed from different T3 transgenic plants, rinsed briefly with deionized water and immediately placed into a tube with 20 ml of deionized water. Conductivity EC_1 was measured using an electroconductivity meter (DDS-307, Shanghai, China) after the tubes were placed at 4℃ overnight. Then, the samples were heated at 100℃ for 20 min and conductivity EC_2 was measured again. Ion leakage ratio was expressed as (EC_1/EC_2) ×100%. MDA was determined by a color reaction with thiobarbituric acid (Heath and Packer, 1968; Zhao et al., 1994).

Quantification of relative water content (RWC)

The leaf discs were punched out from healthy and fully expanded leaves of T3 transgenic plants and the fresh weight (FW) of leaf discs was immediately recorded. After soaking them in deionized water at 4℃ overnight, their turgid weight (TW) was determined. Then, they were dried in an oven at 75℃ for 72 h and weighed (DW). RWC was calculated from the equation of Gaxiola et al. (2001):

RWC (%) = (FW-DW)/(TW- DW) × 100 %

Measurement of total soluble sugars and proline content

Measurement of total soluble sugars and proline content was conducted at 0, 3, 7, 14 days, respectively, after drought treatment. Total soluble sugars of T3 *Arabidopsis* leaves were extracted in boiling water for 30 min and determined by anthrone reagent using glucose as the standard (Yemm and Willis, 1954). Proline was detected as shown previously by Bates et al. (1973).

Quantification of plant yield under drought treatment

The T3 transgenic lines and WT plants were cultivated under drought stress or normal condition. After treatment, the stated plants were harvested for seed weight measurement.

RESULTS

Isolation and structure analysis of *ZmPti1-1*

To confirm whether the EST contig (Zm-tu-03-08-11, 14960) is a full-length gene, we analyzed the sequence in http://au.expasy.org/tools/dna.html. Analysis results showed that, there is an in-frame stop codon TAG upstream from the first initiation codon ATG at the 5'-end and there is a poly (A) structure downstream from the in-frame stops codon TGA at the 3'-end (data not shown). These indicate that, it is a full-length gene. To get basic information about the encoded protein, the RT-PCR product was sequenced. Analysis based on the sequencing result indicated that the deduced protein, ZmPti1-1, contains 363 amino acids with an estimated molecular mass of 39.0 kDa and an isoelectric point of 8.14.

Using the PlantsP program, four potential N-myristoylation sites GVLRNG, GVKGAQ, GAAKGL and GQLSSK and a protein kinase ATP-binding region signature IGEGSFGRVYLGVLRNGRSAAVKK were found in *ZmPti1-1*. Additionally, a serine/threonine protein kinases active-site signature IIHRDIKSSNVLL was also identified in *ZmPti1-1* (Figure 1a). This feature is similar to those

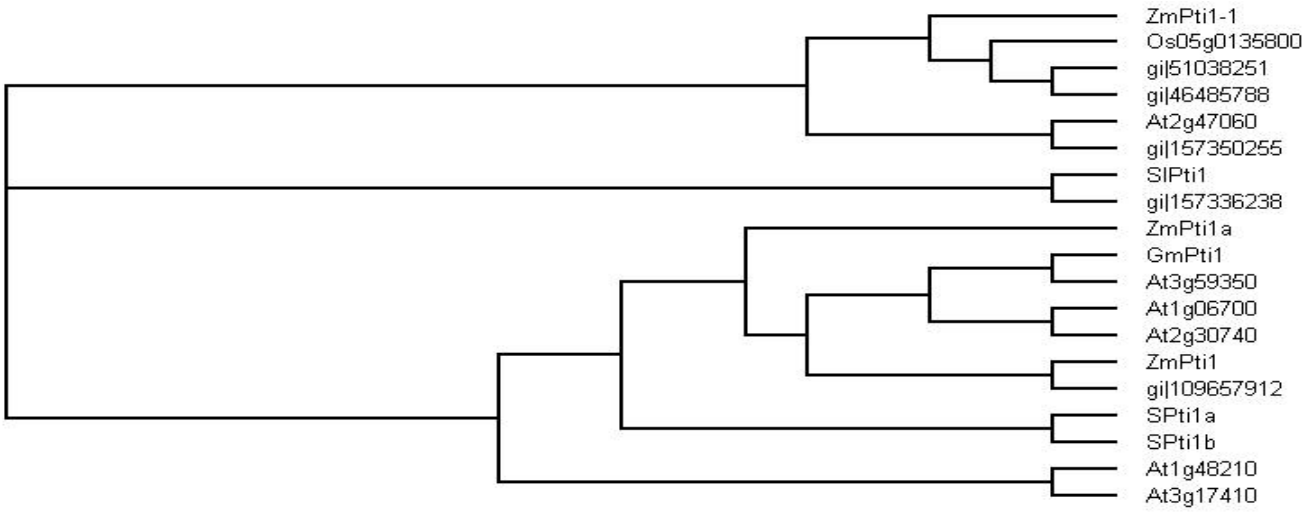

Figure 3. Phylogenetic analysis based on an alignment of maize *ZmPti1-1* with those close sequences from plant in database. These *Pti1s* are putative *Pti1* from Arabidopsis (AAN12919, AAM10114, ABD85164, AAK17158, AAM20245, AAK96830), putative *Pti1* from rice ((NP001054576, AAT94054, AAS98413), putative *Pti1* from maize (AAT57905, AY708048, ABG36852), putative *Pti1* from *Vitis vinifera* (CAO040193, CAO070923), soybean *GmPti1*, tomato *Pti1*, soybean *sPti1a* and *sPti1b*.

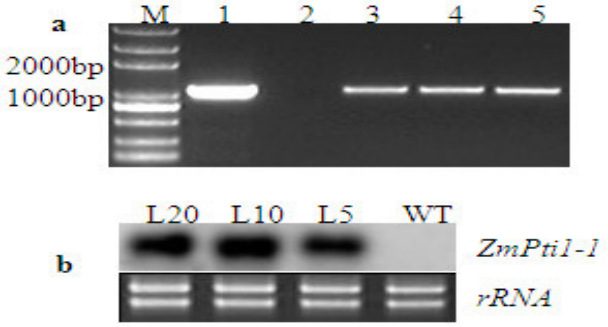

Figure 4. Molecular characterization of the *ZmPti1-1* gene in transgenic *Arabidopsis*. (a) PCR analysis of T3 transgenic plants. lane M, DNA marker; lane 1, PCR result of plasmid pGreen0029-35S-*ZmPti1-1*; lane 2, non-transformed control; lanes 3 to 5, PCR- positive plants:L5, L10, L20; (b) expression of independent transgenic plant lines of *Arabidopsis* by RNA gel-blot analysis. Each lane was loaded with 10 µg total RNA isolated from 4-week-old seedlings of T3 transgenic *Arabidopsis*. The RNA blot was hybridized with a DIG-labeled *ZmPti1-1* cDNA probe. Ethidium bromide-stained rRNA was used as a RNA-loading control.

of tomato *Pti1*, soybean *GmPti1* (Tian et al., 2004), *sPti1a* and *sPti1b* (Staswick, 2000). These indicate that *ZmPti1-1* may be a Ser/Thr protein kinase.

Phylogenetic analysis of the amino acid sequences of plant *Pti1s* or putative *Pti1* homologues revealed that, *ZmPti1-1* and putative rice *Pti1s* (GenBank accession no. NP_001054576; AAS98413) are grouped together with *Arabidopsis* putative *Pti1* (GenBank accession no. NP_001031552) which belong to dicot plants (Figure 1b). These analyses suggest that the *ZmPti1-1* may encode a functional homologue of *Pti1*.

Molecular characterization of *Arabidopsis* transgenic plants

After floral dip transformation, 38 individual kanamycin resistant lines were obtained from T_0 seeds. The kanamycin-resistant T_1 plants were transferred into pots. An initial PCR from T_1 plant DNA templates confirmed that, most kanamycin-resistant plants possess the transformed *ZmPti1-1* gene (data not shown). Only those PCR positive T_1 plants were allowed to set T_2 seeds. From T_2 seeds, plants were selected on kanamycin medium and grown in pots for T_3 seeds. Populations of T_3 seeds were established and were tested as homozygous transformants. The chosen homozygous transformants were used for PCR and RNA gel-blot analysis for further confirmation. Three T3 transgenic lines were randomly selected and the existence of the foreign gene in transgenic plants was confirmed by PCR amplification (Supplemental Figure S2a)..

Furthermore, RNA gel-blot analysis showed that *ZmPti1-1* was expressed at the higher levels in transgenic *Arabidopsis* than in the wild type (Supplemental Figure S2b). Our results demonstrated that ZmPti1-1 was integrated into Arabidopsis genome and expressed in transgenic Arabidopsis.

Higher survival rate and lower water loss of *ZmPti1-1* transgenic *Arabidopsis* plants under drought treatment

Fourteen-days old plants grown on soil were held without watering for 9 days, then re-watered again and grown under normal conditions for 5 days; transgenic plants

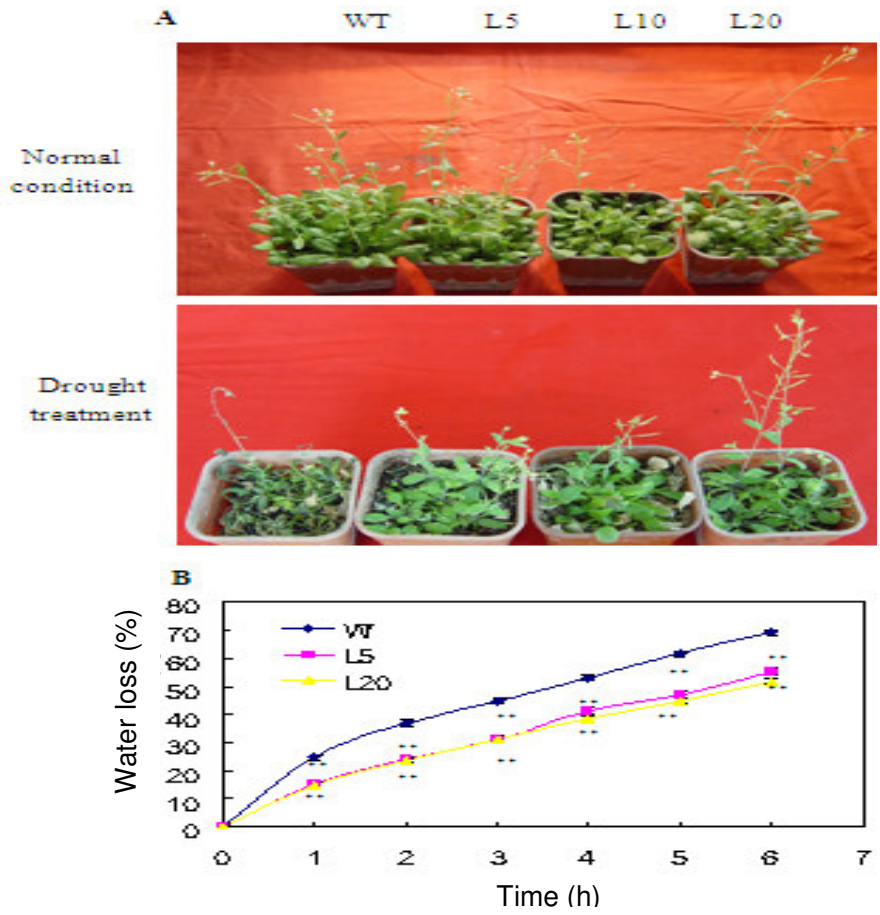

Figure 5. Effect of *ZmPti1-1* expression on drought tolerance in transgenic *Arabidopsis* plants. (a) Phenotype of *ZmPti1-1* transgenic plants under drought treatment: Top, wild-type (WT) and transgenic plants under normal condition; Bottom, 14-days old WT and transgenic plants were held without watering for 9d and then, re-watered for 5 days later photographs were taken; (b) water loss in wild-type and transgenic plants: Detached leaves from 25-days old plants grown on soil were incubated on a Petri dish and fresh weight (FW) was measured at the time intervals indicated, water loss was calculated from the decrease in FW compared with time zero. Error bars indicate ±SE (n = 3). * and **, significantly different from the WT at $P < 0.05$ and < 0.01, respectively, by Student's t-test.

Table 1. Survival rates of transgenic plants under drought condition[a].

***ZmPti1-1*-overexpressing lines**	**Survival[b]**	**Total[c]**	**Survival[d] (%)**
Wild-type	26	90	29
L5	63**	90	70**
L10	78**	90	87**
L20	68**	90	76**

[a]Fourteen days old soil-grown plants withheld water for 9 d, then re-watered and scored 5 d later; [b] No. of survival plants; [c] Total no. of plants used in each assay. [d] Percentage of survival plants. * and **, significantly different from the WT at $P < 0.05$ and < 0.01, respectively, by Student's t-test.

showed a stronger growth recovery phenotype than wild-type plants (Figure 2a). Only 29% of the wild-type plants survived this treatment. In contrast, more than 70% of *ZmPti1-1*-overexpressed plants survived (Table 1) which primarily showed that *ZmPti1-1*transgenic Arabidopsis increased drought tolerance.

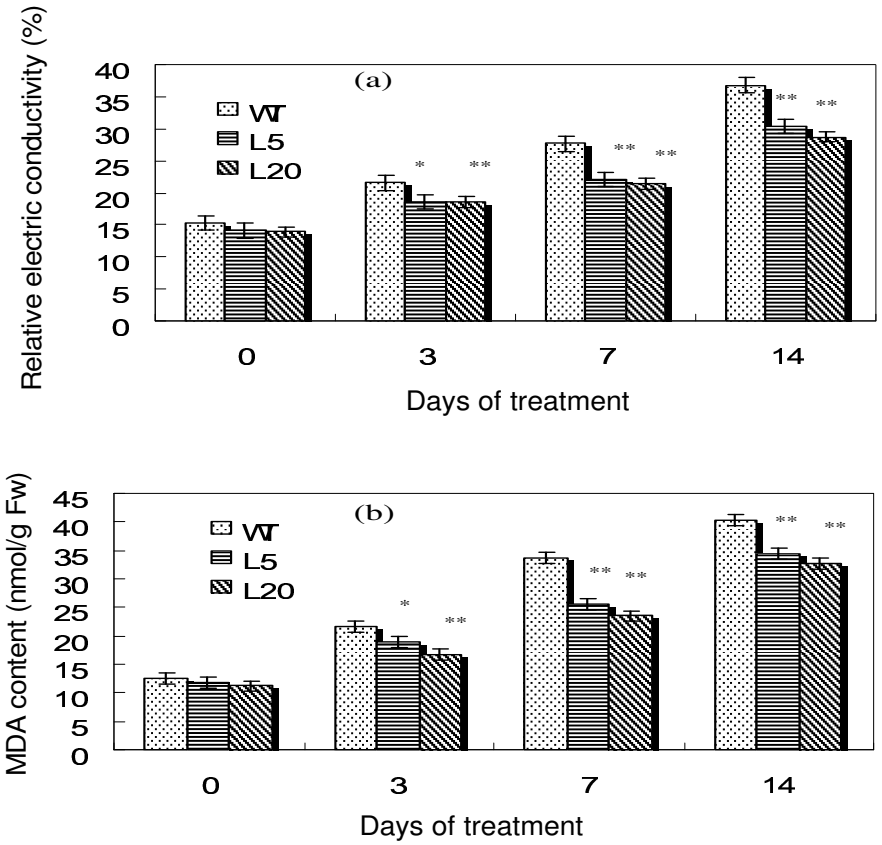

Figure 6. Cell membrane damage of transgenic Arabidopsis seedlings under drought stress. (a) Electrolyte leakage of leaf cells; (b) Malondialdehyde (MDA) content of the leaves. WT: wild type; Two transgenic lines: L5, L20. Error bars indicate ± SE (n = 3). * and **, Significantly different from the WT at $P < 0.05$ and < 0.01, respectively, by Student's t-test.

Compared with WT, water loss in detached leaves of transgenic plants was much lower. As shown in Figure 2b, after one hour of treatment, the water losses (%) in detached leaves of L5, L20 transgenic lines was 15.3 and 14.7%, respectively, and significantly lower than that of WT plants, which was 24.8%. On the sixth hour of treatment, the water loss in detached leaves of WT increased to about 69.1%, whereas, that of the transgenic lines L5, L20 was only 55.2 and 51.8%, respectively. These results further showed that over-expression of *ZmPti1-1* in *Arabidopsis* increased drought tolerance.

Lower cell membrane damage of *ZmPti1-1* transgenic seedlings under drought stress

Drought stress usually results in increased membrane damage of leaf cells. To study further the difference between transgenic and WT plants under drought stress, the electrolyte leakage (%) and MDA content of leaf cells from *Arabidopsis* seedlings were determined under drought stress for 0, 3, 7 and 14 days. As shown in Figure 3a,b, the leaf cell electrolyte leakage (%) and MDA content increase gradually with increasing drought treatment, but the damage to transgenic lines was much less than that to WT lines. For instance, after 3 days of drought treatment, the leaf cell electrolyte leakage (%) of WT was 21.5%, whereas, that of transgenic line L5 was only 18.5% and its MDA content was 18.9 nmol/g fresh weight, whereas, that of WT was 21.7 nmol/g fresh weight.

Under drought stress, compared to wild type, transgenic *ZmPti1-1* lines have lower cell membrane damage. These results indicate that over-expression of *ZmPti1-1* gene can decrease the injury of plants under drought stress.

Higher RWC of transgenic plants under drought stress

RWC was considered as a parameter to assess the water status of plants. Before stress, the RWC (%) of trans-

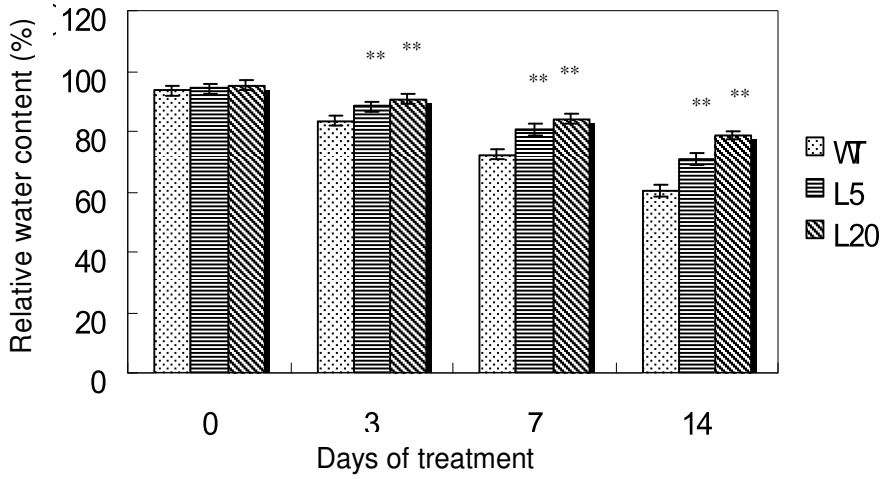

Figure 7. Relative water content (RWC) of transgenic plants under drought treatment. WT: wild type; Two transgenic lines: L5, L20. Error bars indicate ± SE (n = 3). * and **, significantly different from the WT at P < 0.05 and < 0.01, respectively, by Student's t-test.

genic plants L5, L20 was 94.1 and 95.3%, respectively, slightly higher than that of WT plants, which was 93.5% (Figure 4). Under drought stress, a higher water loss rate was observed in WT plants and the difference between transgenic and WT plants were significant. As shown in Figure 4, after 3 days of drought treatment, the RWC (%) of WT decreased to 83.6%, whereas, that of transgenic lines L5, L20 was 88.3 and 90.8%, respectively. These results indicate that over-expression of ZmPti1-1 gene can decrease the water loss of plants under drought stress. The higher RWC of transgenic *Arabidopsis* seedlings enabled them to maintain normal cell turgor under drought stress, which was beneficial for growth.

Higher total soluble sugar and proline content of transgenic seedlings under drought stress

To assess whether there were differences in the accumulation of total soluble sugars and proline between transgenic and WT seedlings, the total soluble sugar and proline contents of leaves were determined at different times of stress. The results are shown in Figure 5a, b. Before stress, the contents of total soluble sugars and proline were at similarly low levels in all seedlings. During drought stress, a significant difference in sugar content was observed between transgenic and WT seedlings (Figure 5a). With regard to proline, transgenic seedlings exhibited higher levels than WT seedlings (Figure 5b).

Under drought stress, transgenic ZmPti1-1 lines had higher total soluble sugar and proline content than wild type. These results suggested that over-expression of ZmPti1-1 gene can enhance drought stress tolerance of plants.

Higher yield of transgenic *Arabidopsis* plants under drought stress

Drought tolerance is a very important limited factor in terms of yield; seed weight is often the representative factor of yield. Two transgenic lines (L5 and L20) and WT plants were cultivated under drought stress or normal condition. As shown in Figure 6, under normal condition, seed weight of WT, L5, L20 plants was 3.53, 3.55, 3.56 g/100 plants, respectively, showing no difference. Under drought treatment, seed weight of L5, L20 plants was 0.316 and 0.338 g/100 plants, respectively, significantly higher than that of WT plants, which was 0.206 g/100 plants. These results showed, compared to wild type, transgenic *ZmPti1-1* lines had higher drought stress tolerance of plants.

DISCUSSION

Protein kinases are key elements involved in plant drought signal transduction. A number of studies demonstrate that over-expression of protein kinase genes can improve drought tolerance in *Arabidopsis* (Fujita et al., 2009; Osakabe et al., 2010; Umezawa et al., 2004; Xu et al., 2010), maize (Assem et al., 2009; Shou et al., 2004), rice (Ning et al., 2010; Ouyang et al., 2010; Xiang et al., 2007; Yang et al., 2008).

In this study, a maize cDNA designated as *ZmPti1-1* was cloned and characterized. Analysis of putative amino acid sequence in PlantsP database showed that, it contains one protein kinase ATP-binding region signature and four N-myristoylation sites. N-terminal myristoylation plays a vital role in membrane targeting and signal

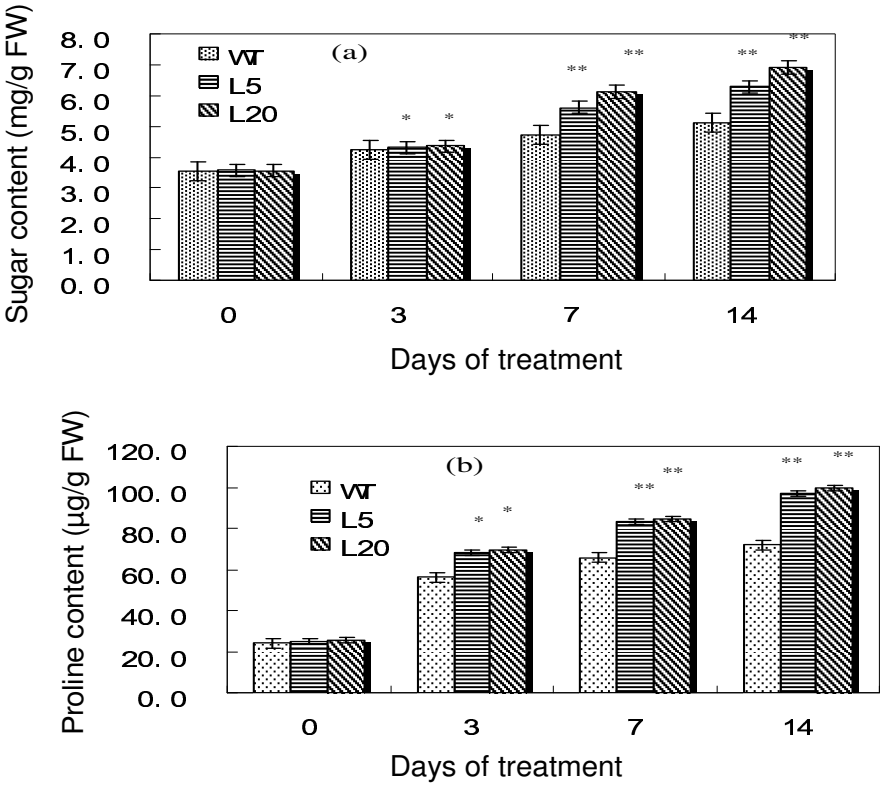

Figure 8. Changes in total soluble sugar (a) and proline (b) content in Arabidopsis seedlings under drought stress. WT: wild type; Two transgenic lines: L5, L20. Error bars indicate ± SE (n = 3). * and **, significantly different from the WT at P < 0.05 and < 0.01, respectively, by Student's t-test.

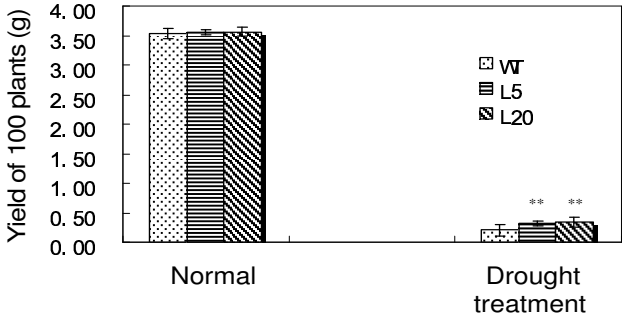

Figure 9. Seed weight of transgenic *Arabidopsis* plants under drought stress. WT: wild type; Two transgenic lines: L5, L20. Error bars indicate ±SE (n = 3). * and **, significantly different from the WT at *P* < 0.05 and < 0.01, respectively, by Student's *t* test.

transduction in plant responses to environmental stress and this modification is essential for protein function to mediate membrane association or protein-protein interaction (Podel and Gribskov, 2004; Ishitani et al., 2000). This strongly suggests that, *ZmPti1-1*, as a homologue to *Pti1*, may interact with other proteins and response to stress.

The phytohormone abscisic acid (ABA) plays important roles in the adaptation of plants to abiotic environmental stresses such as drought and high salinity (Uno et al., 2000). In this study, *ZmPt1-1* is dramatically induced by abscisic acid (ABA) and mannitol (data not shown). Our results suggest that *ZmPti1-1* may play roles in drought stress through the ABA signaling pathway. Drought may cause cellular damage and secondary stresses, such as osmotic and oxidative stress. The initial stress signals (e.g. osmotic and ionic effects, or temperature, membrane fluidity changes) trigger the downstream signaling process via protein kinases(e.g. *ZmPt1-1*) and transcription controls which activate stress-responsive mechanisms via transcriptional factors(e.g. CBF/ DREB, bZIP) to re-establish homeostasis and protect and repair damaged proteins and membranes. Thus, make plants of drought tolerance or resistance.

In this study, experiments were performed for the evaluation of drought tolerance of transgenic *ZmPti1-1* lines and WT plants. Our results primarily demonstrated that, the whole length of *ZmPti1-1* cDNA driven by CaMV 35S promoter was integrated into *Arabidopsis* genome and *ZmPti1-1* was expressed at the higher levels in transgenic *Arabidopsis* than in the wild type (Supplemental Figure S2).

Compared with WT, under normal growth conditions no

apparent phenotypic difference was noted in *ZmPti1-1* transgenic lines. However, significant differences in drought stress tolerance appeared by phenotypic change. The *ZmPti1-1* transgenic plants displayed an improved drought tolerance compared with the WT line (Figure 2a).. This was also characterized by the substantially increa-sed membrane integrity as determined by ion leakage and lipid peroxidation (MDA) (Figure 3). Transgenic plants retained more water and solutes content compared with the WT (Figure 2b, 4, 5). Over-expression of *ZmPti1-1* gene can improve drought tolerance in transgenic plants.

Plant yield is one useful trait for stress tolerance evaluation. Plants at the reproductive growth stage of the life cycle are particularly sensitive to abiotic stresses (Boyer, 1982). Drought reduces productivity by inhibiting plant growth and photosynthesis (Taiz and Zeiger, 1998). Here, we investigated the plant's response to drought stress and recovery from the stress by exposure to drought at the six true leaves stage for 14 days. The reproductive development of transgenic lines was less inhibited by drought stress compared with that of WT (Figure 2a) and the decrease of seed weight due to drought stress in transgenic lines was much less than that of WT (Figure 6). Therefore, the transgenic lines had higher yields than that of the WT lines after drought stress. These results showed that, *ZmPti1-1* may play an important role in drought resistance signal pathway. It is possible that the drought tolerance could be substantially improved by the manipulation of the *ZmPti1-1* gene expression in plants.

ACKNOWLEDGEMENTS

This study was financially supported by grants from China National Transgenic Biology Program (No.2009ZX-08003-009B), National Natural Science Foundation of China (No. 30400228, 30700433), Beijing Municipal Natural Science Foundation (No.5062012).

REFERENCES

Assem SK, Hussein EHA, Hussein HA, Basry M (2009). Genetic transformation of the *Nicotiana* protein kinase (NPK1) gene confers osmotic tolerance in Egyptian maize. Aust. J. Basic Appl. Sci. 3(2): 828-835.

Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water-stress studies. Plant Soil, 39: 205-207.

Boyer JS (1982). Plant productivity and environment. Science, 218: 443-448.

Clough SJ, Bent AF (1998). Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis* thaliana. Plant J. 16: 735-743.

Fujita Y, Nakashima K, Yoshida T, Katagiri T, Kidokoro S, Kanamori N, Umezawa T, Fujita M, Maruyama K, Ishiyama K, Kobayashi M, Nakasone S, Yamada K, Ito T, Shinozaki K, Yamaguchi-Shinozaki K (2009). Three SnRK2 protein kinases are the main positive regulators of abscisic acid signaling in response to water stress in *Arabidopsis*. Plant Cell Physiol. 50(12): 2123-2132.

Gaxiola RA, Li J, Undurraga S, Dang LM, Allen GJ, Alper SL, Fink GR (2001). Drought- and salt-tolerant plants result from overexpression of the AVP1 H^+-pump. Proc. Natl. Acad. Sci. USA, 98: 11444-11449.

Gribskov M, Fana F, Harper J, Hope DA, Harmon AC, Smith DW, Tax FE, Zhang G (2001). Plants: a functional genomics database for plant phosphorylation. Nucleic Acids Res. 29: 111-113.

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplast.I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125: 189-198.

Herrmann MM, Pinto S, Kluth J, Wienand U, Lorbiecke R (2006). The PTI1-like kinase ZmPti1a from maize (*Zea mays* L.) co-localizes with callose at the plasma membrane of pollen and facilitates a competitive advantage to the male gametophyte. BMC Plant Biol. 6: p. 22.

Ishitani M, Liu J, Halfter U, Kim CS, Shi W, Zhu JK (2000). SOS_3 function in plant salt tolerance requires N-myristoylation and calcium binding. Plant Cell, 12: 1667-1677.

Ning J, Li XH, Hicks LM, Xiong LZ (2010). A raf-like MAPKKK gene DSM1 mediates drought resistance through reactive oxygen species scavenging in rice. Plant Physiol. 152: 876-890.

Osakabe Y, Mizuno S, Tanaka H, Maruyama K, Osakabe K, Todaka D, Fujita Y, Kobayashi M, Shinozaki K, Yamaguchi-Shinozaki K (2010). Overproduction of the membrane- bound receptor-like protein kinase 1, RPK1, enhances abiotic stress tolerance in *Arabidopsis*. J. Biol. Chem. 285(12): 9190-9201.

Ouyang SQ, Liu YF, Liu P, Lei G, He SJ, Ma B, Zhang WK, Zhang JS, Chen SY (2010). Receptor-like kinase OsSIK1 improves drought and salt stress tolerance in rice (*Oryza sativa*) plants. Plant J. 62(2): 316-329.

Pandey GK (2008). Emergence of a novel calcium signaling pathway in plants: CBL-CIPK signaling network. Physiol. Mol. Biol. Plants, 14: 51-68.

Podel S, Gribskov M (2004). Predicting N-terminal myristoylation sites in plant proteins. BMC Genomics, 5: 37-51.

Sambrook J, Russell DW (2001). Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, New York, pp. 27-99, 518-552.

Shou HX, Bordallo P, Wang K (2004). Expression of the *Nicotiana* protein kinase (NPK1) enhanced drought tolerance in transgenic maize. J. Exp. Bot. 55: 1013-1019.

Staswick P (2000). Two expressed soybean genes with high sequence identity to tomato Pti1 kinase lack autophosphorylation activity. Arch. Biochem. Biophys. 383: 233-237.

Taiz L, Zeiger E (1998). Stress physiology. In: Plant physiology, 2nd edn. Sinauer Associates Inc, Sunderland, pp. 725-757.

Tian AG, Luo GZ, Wang YJ, Zhang JS, Gai JY, Chen SY (2004). Isolation and characterization of a *Pti1* homologue from soybean. J. Exp. Bot. 396: 535-537.

Umezawa T, Yoshida R, Maruyama K, Yamaguchi-Shinozaki K, Shinozaki K (2004). SRK2C, a SNF1-related protein kinase 2, improves drought tolerance by controlling stress-responsive gene expression in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA, 101: 17306-17311.

Uno Y, Furihata T, Abe H, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K (2000). Arabidopsis basic leucine zipper transcription factors involved in an abscisic acid- dependent signal transduction pathway under drought and high-salinity conditions. Proc. Natl. Acad. Sci. U.S.A. 97:11632–11637.

Xiang Y, Huang Y, Xiong L (2007). Characterization of stress-responsive *CIPK* genes in rice for stress tolerance improvement. Plant Physiol. 144: 1416-1428.

Xu J, Tian YS, Peng RH, Xiong AS, Zhu B, Jin XF, Gao F, Fu XY, Hou XL, Yao QH (2010). AtCPK6, a functionally redundant and positive regulator involved in salt/drought stress tolerance in *Arabidopsis*. Planta, 231: 1251-1260.

Yamaguchi-Shinozaki K, Shinozaki K (2006). Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annu. Rev. Plant Biol. 57: 781- 803.

Yang W, Kong Z, Omo-Ikerodah E, Xu W, Li Q, Xue Y (2008). Calcineurin B-like interacting protein kinase OsCIPK23 functions in pollination and drought stress responses in rice (*Oryza sativa* L.). J. Genet. Genomics, 35: 531-543, S1-2.

Yang Y, Shah J, Klessig DF (1997). Signal perception and transduction in plant defense responses. Genes Dev. 11: 1621-1639.
Yemm EW, Willis AJ (1954). The estimation of carbohydrates in plant extracts by the anthrone. Biochem. J. 57: 508-514.
Zhao SJ, Xu CC, Zou Q, Meng QW (1994). Improvements of method for measurement of malondialdehyde in plant tissues. Plant Physiol. Commun. 30: 207-210 (in Chinese).
Zhou JM, Loh YT, Bressan RA, Martin GB (1995). The tomato gene *Pti1*encodes a serine/threonine kinase that is Phosphorylated by Pto and is involved in the hypersensitive response. Cell, 83: 925-935.
Zhu JK (2002). Salt and drought stress signal transduction in plants. Annu. Rev. Plant Biol. 53: 247-273.
Zou HW, Wu ZY, Yang Q, Zhang XH, Cao MQ, Jia WS, Huang CL, Xiao X (2006). Gene expression analyses of ZmPti1, encoding a maize Pti-like kinase, suggest a role in stress signaling. Plant Sci. 171: 99-105.
Zou HW, Wu ZY, Zhang XH, Wang YQ, Huang CL (2010). Over-expression of *ZmPti1*, a homologue to *Pti1*, increases salt tolerance of *Arabidopsis thaliana*. Afr. J. Biotechnol. 9: 656-662.