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ORIGINAL PAPER

Characterization of the *ZmCK1* Gene Encoding a Calcium-Dependent Protein Kinase Responsive to Multiple Abiotic Stresses in Maize

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Abstract Calcium-dependent protein kinases (CDPKs) play a vital regulatory role in abiotic stress responses in plants. We isolated the *ZmCK1* gene encoding a CDPK from maize seedlings. The predicted ZmCK1 protein contains a typical Ser/Thr protein kinase domain and four EF-hand calcium-binding motifs in its N-terminal and C-terminal halves, respectively. The catalytic and regulatory domains were linked by a well-conserved junction domain. A ZmCK1::hGFP fusion protein was found to localize into the cytoplasma and nucleus upon introduction into *Arabidopsis* mesophyll protoplasts. *ZmCK1* transcription was highly activated by salt and cold, and moderately by drought and exogenous ABA in maize seedling. Isolation of the *ZmCK1* promoter revealed some *cis*-acting elements responding to stresses. Overexpression of *ZmCK1* improved drought, salt, and cold stress tolerance in transgenic *Arabidopsis* plants. Our results suggested that *ZmCK1* produces a functional kinase that may play a regulatory role in abiotic stress response.

Keywords CDPK · Induction kinetics · Abiotic stress · Transgenic plants · Maize

Abbreviations

ABA	Abscisic acid
CBL	Calcineurin B-like proteins
CDPK	Calcium-dependent protein kinases
CaM	Calmodulin
MAPKs	Mitogen-activated protein kinases
RLKs	Receptor-like kinases
Q-RT-PCR	Real-time quantitative RT-PCR

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Introduction

On exposure to continuously changing stress surroundings, including drought, salt, and cold, plants can perceive stress stimuli and produce various biochemical and physiological responses to acquire stress tolerance (Xu et al. 2008a; Prabu et al. 2011; Min et al. 2012). Protein kinases play vital roles in perceiving and transmitting external stimuli (Xu et al. 2009b). Based on substrate specificity, all identified protein kinases were divided into three major classes: Ser/Thr protein kinases, tyrosine protein kinases, and dual specificity for Ser/Thr/Tyr protein kinases (Hanks et al. 1988; Lindberg et al. 1992; Xu et al. 2006). Protein kinases induced by environmental stresses mainly include calcium-dependent protein kinases (CDPKs), mitogen-activated protein kinases (MAPKs), receptor-like kinases (RLKs), ribosomal protein kinases, or transcription regulation proteins (Ludwig et al. 2004).

In plants, changes in cytosolic-free calcium concentration are apparent during transduction of abiotic stimuli including extreme temperatures, hyperosmotic, and oxidative stresses (Rudd and Franklin-Tong 2001; Sanders et al. 2002). Calcium is the second messenger coupling physiological responses to external and developmental signals (Reddy and Reddy 2004). Changes in cytosolic-free calcium level are sensed by a specific set of proteins named calcium sensors. It has been suggested that CDPKs are calcium sensors unique to plant cells as they have not been found in members of other phylogenetic branches, including fungi, insects, and mammals (Sanders et al. 2002). The CDPK protein has four well-defined domains: N-terminal variable region, Ser/Thr kinase catalytic domain,

autoregulatory/autoinhibitory domain, and calmodulin-like domain (Harmon 2003). The calmodulin-like domain generally contains four highly conserved calcium-binding EF-hand motif which is the predominant calcium sensor (Reddy and Reddy 2004). The binding of calcium to the calmodulin-like domain can cause structural change in the autoinhibitory domain and exposure of the active site of the kinase (Christodoulou et al. 2004). The activated CDPKs participate in different signal transduction pathways through phosphorylation of specific substrates (Hernandez et al. 2004).

Accumulating evidence indicate that CDPKs mediate stress signaling pathways (Ludwig et al.2005; Romeis et al. 2000). Expression profiling indicated that *LeCPK2* expressed predominantly in flowers and responded divergently to heat and cold stress. Mechanical wounding and phytohormones including ethylene, methyl jasmonate, and salicylic acid also arouse the expression of *LeCPK2* (Chang et al. 2009). In soybean (*Glycine soja*), *GsCBRLK* transcripts were induced by drought, cold, and salinity stresses. Overexpression of *GsCBRLK* resulted in enhanced plant tolerance to high salinity and increased the expression pattern of a number of stress gene markers in transgenic *Arabidopsis* (Yang et al. 2010). Overexpression of the rice *OsCDPK7* provides cold, salt, and drought tolerance for the transgenic rice plants, demonstrating the potential of CDPK engineering to generate stress tolerance-enhanced crops (Saijo et al. 2000, 2001). Therefore, CDPKs mediate multiple abiotic stress responses, resulting in improved tolerance to multiple stresses in transgenic plants.

The sequenced genome of maize paved the way for identifying some candidate genes for abiotic stress adaption in major cultivable crop by functional genomics strategy (Zheng et al. 2012). Through transgenic methods, these candidate genes were transferred into plants for the improvement of plant resistance to abiotic stresses (Xu et al. 2009a, 2011). Therefore, finding the key genes that play a pivotal function in plants' response to abiotic stresses is the first task in the pipeline of biotechnology engineering.

In maize (*Zea mays*), an important food and feed crop in the world, only a few CDPK transcription factors have been described. In this study, we report the cloning and characterization of the *ZmCK1* gene. We present a detailed expression analysis showing the involvement of this gene in the abiotic stress response. The subcellular localization and *cis*-acting elements in the promoter of *ZmCK1* were also investigated. Importantly, overexpression of the *ZmCK1* gene improved drought, salt, and cold tolerance in transgenic *Arabidopsis* plants. These evidences indicate that *ZmCK1* encode for a stress-related protein kinase.

Materials and Methods

Plant Materials and Stress Treatments

Seeds of miaze (X178) were sown in soil at 25 °C under growth chamber conditions. After germination, seedlings were grown in the growth chamber under 250 μmol m^{-2} s^{-1} illumination, with a 14-h photoperiod; the 10-day-old seedlings were employed in different treatments. Seedlings were exposed to air on filter paper in a growth chamber (25 °C, 50–60 % relative humidity, continuous light) for induction of rapid dehydration stress conditions, or placed in a 4 °C chamber for cold stress. To mimic salinity and for ABA treatments, seedlings were transferred into solutions containing 2 % salt (NaCl) and 200 μM ABA, respectively. Materials were collected at 0, 1, 2, 5, 12, or 24 h after treatments. Harvested leaves were dropped immediately into liquid nitrogen and stored at −80 °C for RNA extraction.

Isolation of Entire cDNA and Genomic Sequence

In order to isolate the genes encoding CDPKs from maize, rice *OsCDPK7* (Saijo et al. 2000) was used as a query to search the expressed sequence tag (EST) database of maize (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/Blast/index.cgi). EST sequences were obtained and further systematic phylogenetic analyses of those sequences were carried out on the basis of homology of *OsCDPK7* (Saijo et al. 2000). Using the reverse transcription PCR (RT-PCR) method, several full-length cDNA sequences were isolated from total RNA of maize cv. X178. In order to study the characteristics and functions of a member of the CDPK family, *ZmCK1* was chosen for further analyses (GenBank no. FJ805744). To obtain the genomic sequence of *ZmCK1*, the specific primer set of 5′-GTCGTTTGATGGGCAACGCAT-3′ and 5′-CGTGTTAAGTTTCAGAATGCACCAGGTG-3′ were designed according to the *ZmCK1* cDNA sequences. Genomic DNA was isolated from young leaves using the CTAB method. The PCR reactions were performed using high-fidelity Pyrobest polymerase (TaKaRa, Dalian, China) under the following conditions: 95 °C for 5 min, 35 cycles of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 3 min, and a final extension at 72 °C for 10 min. The PCR products were recovered, cloned into the pMD18-T vector (TaKaRa, Dalian, China), and sequenced. On the basis of maize genome sequences, the 5′-flanking region was amplified using the specific primer set of 5′-CGACCATGGATTCTCAGGC-3′) and 5′-GCTGTGCTGGTACTTGGATC-3′. The methods used to clone the 5′-flanking region were the same as the one above.

Quantitative Q-RT-PCR

Total RNA was extracted from leaves of plants under the different treatments using TRIzol reagents according to the

manufacturer's protocol (Tiangen, Beijing, China) and then digested with DNaseI (TaKaRa, Dalian, China) at 37 °C for 30 min to remove potential genomic DNA contamination. Quality and concentration of total RNA was examined using ethidium bromide (EB)-stained agarose gel electrophoresis and spectrophotometric analysis, respectively. The first-strand cDNA was synthesized using the PrimeScript® RT reagent Kit (Perfect Real Time) (TaKaRa, Dalian, China) following the manufacturer's instructions. The real-time quantitative RT-PCR (Q-RT-PCR) was conducted using the ABI Prism 7000 system (Applied Biosystems, USA) according to the modified protocol (Livak and Schmittgen 2001). The actin gene, was used as an internal reference. The transcript analysis of the *ZmCK1* gene was performed using gene-specific primers (5′-TGCAGCTGATATCGACAAC-3′ and 5′-CCACAAGCTGTACATCGTG-3′), which were located in the 3′-terminal region of the gene. Q-RT-PCR was performed according to the instructions of RealMasterMix (SYBR Green) (Tiangen, Beijing, China). The amplification program was 95 °C for 20 s, 60 °C for 30 s, and 68 °C for 30 s, repeated for 40 cycles. Validation experiments were performed to demonstrate that amplification efficiency of the *ZmCK1*-specific primers were approximately equal to the amplification efficiency of the endogenous reference primers. Quantification of the target gene expression was carried out with comparative CT method (Livak and Schmittgen 2001). Average CT values for the target gene from at least three PCRs were normalized to average CT values for actin from the same cDNA preparations and analyzed using Microsoft Excel. The relative expression of *ZmCK1* indicated the increasing fold of the gene expression over the control (0 h). Designing for all the PCR primers was performed with software Primer 5.0.

Subcellular Localization Analysis

To confirm the subcellular localization of the *ZmCK1* protein, the ORF of the *ZmCK1* cDNA was amplified using the high-fidelity Pyrobest DNA polymerase (TaKaRa, Dalian, China) and the PCR product was cloned into the N terminus of the *hGFP* gene under control of the 2× CaMV35S promoter. Subcellular localization of *ZmCK1::hGFP* was assessed with transient expression after PEG-calcium transfection in *Arabidopsis* mesophyll protoplasts (Yoo et al. 2007). Subcellular localization of the *ZmCK1::hGFP* fusion protein and *hGFP* control in *Arabidopsis* mesophyll protoplasts were monitored using a confocal microscope (Leica Microsystem, Heidelberg, Germany) 18 h after PEG-calcium transfection.

Arabidopsis Transformation and Stress Treatment

To construct an expression vector for *Arabidopsis*, the full-length *ZmCK1* cDNA was ligated into the modified vector pBI121 under the control of the CaMV35S promoter. Columbia (Col-0) ecotype *Arabidopsis* plants were transformed using the vacuum infiltration method. Transformants were selected on MS medium containing 50 μg mL^{-1} kanamycin. T2 generation plants were used for further analysis.

Arabidopsis plants of two independent lines grown for 4 weeks at normal growing conditions were subjected to stress treatment. Sixty plants of each transgenic line and the wild type were used for each treatment and the whole experiment was repeated three times. For dehydration stress treatment, plants were grown at normal growing conditions without watering for 2 weeks and then rewatered at normal growing conditions. For cold stress treatment, transgenic and wild-type *Arabidopsis* were exposed to −6 °C for 12 h and then returned to normal growing conditions for 2 weeks (Xu et al. 2007).

Root growth was used as an indicator of seedling response to salt stress. For root growth assays, germinated *Arabidopsis* seeds were transferred to MS medium plates with 0 and 100 mM NaCl, respectively. Seedling root lengths were measured 10 days after salt treatment was initiated (Xu et al. 2008b).

Results

Isolation and Characterization of *ZmCK1* cDNA

Using the RT-PCR method, full-length cDNA sequence of the *ZmCK1* gene, a homologous gene of *OsCDPK7*, was isolated from maize. The cDNA sequences of *ZmCK1* comprised a 1,671 bp ORF encoding a 61.2 kDa protein with p*I*5.84. A sequence alignment using DNAMAN (version 6.0.3; Lynnon BioSoft, Vaudreuil, Canada) indicated that the deduced protein ZmCK1 shared 90 % and 76 % identity with OsCDPK7 (Saijo et al. 2000) and AtCDPK5 (NP_195257), respectively (Fig. 1). Further analysis showed that the deduced amino acid sequences of *ZmCK1* contain eleven typically canonical subdomains of protein kinases, and four EF-hand calcium-binding motifs in its N-terminal and C-terminal halves, respectively. The catalytic and regulatory domains were linked by a well-conserved junction domain (Fig. 1). Also, ZmCK1 contain a MGNACGG sequence in the N terminus of the protein for a predicted N-myristoylation signal (PSORT: http://psort.hgc.jp/) that potentially acted as subcellular localization signal (Fig. 1). In addition, the fragment of approximately 4,600 bp was isolated from genomic DNA using PCR primers corresponding to the 5′ and 3′ ends of *ZmCK1* cDNA. Sequence alignment of the cDNA with its genomic counterpart revealed six introns (Fig. 2).

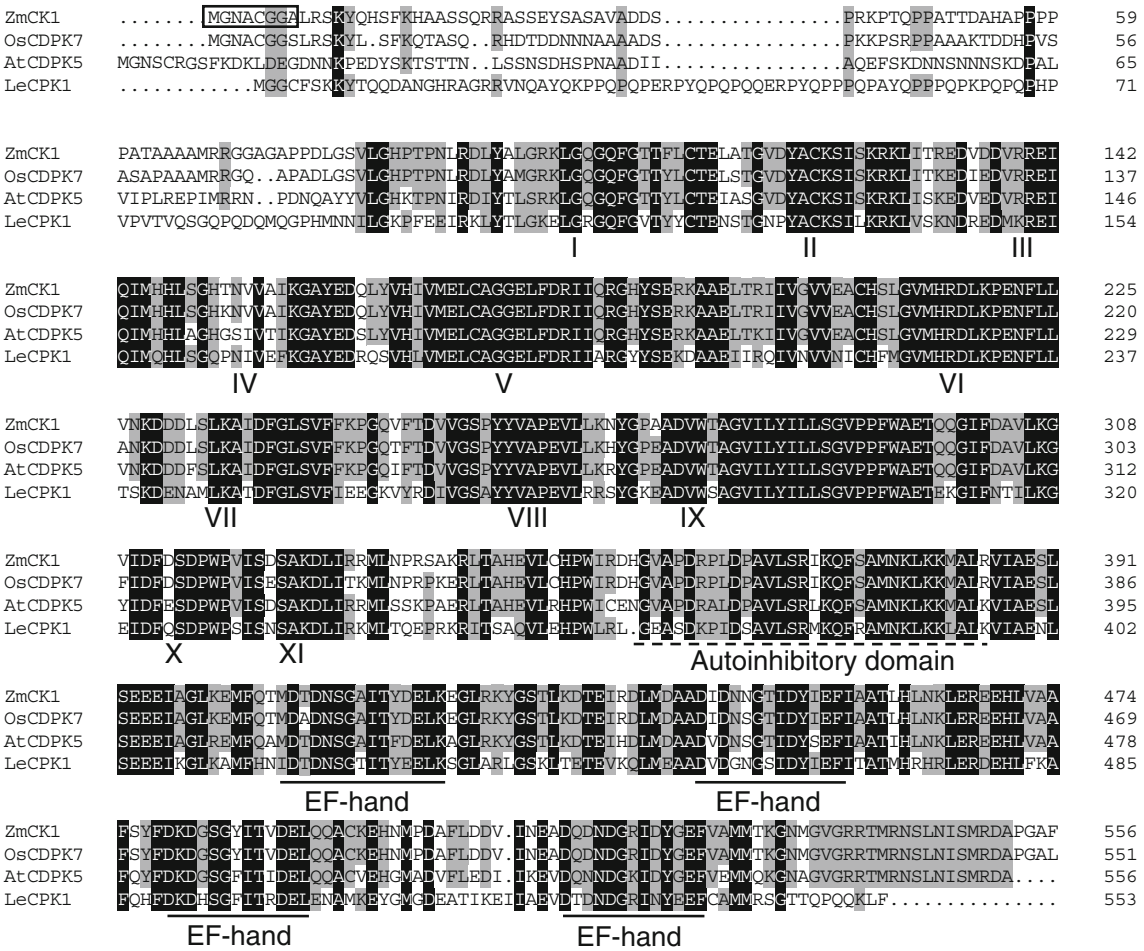

Fig. 1 Alignment of ZmCK1 with other closely related CDPK proteins. Amino acid sequence alignment of ZmCK1 and CDPK from rice OsCDPK7 (Saijo et al. 2000), *Arabidopsis* AtCDPK5 (NP_195257), and tomato LeCPK1 (Rutschmann et al. 2002). A predicted N-myristoylation signal (PSORT: http://psort.hgc.jp/) is *boxed*. *Roman numbers* designate the position of 11 kinase subdomains. The autoinhibitory domain and EF-hand motifs are also indicated. Consensus sequences (100 % and 75 %) are exhibited in *black and grey shading*

Subcellular Localization of ZmCK1 Protein

A MGNACGG sequence for a putative N-myristoylation signal in the N terminus of a protein was commonly expected to localize on plasma membrane (Xu et al. 2006). To investigate the biological activity of the putative N-myristoylation signal, the *ZmCK1* cDNA sequence was fused to the C terminus of the *hGFP* reporter gene and subcloned into an expression vector under the control of the CaMV 35S promoter. This construct was transferred into *Arabidopsis* mesophyll protoplasts to investigate intracellular localization. The ZmCK1::hGFP fusion protein was observed to be mainly localized into the cytoplasma and nucleus, and the control hGFP was uniformly distributed throughout the onion epidermal cell (Fig. 3).

ZmCK1 is Induced by Multiple Stresses

To quantitatively determine the expression pattern of the *ZmCK1* gene, we used Quantitative Q-RT-PCR. Total RNA was extracted from maize seedlings subjected to various abiotic stress conditions, such as drought, salt, cold,

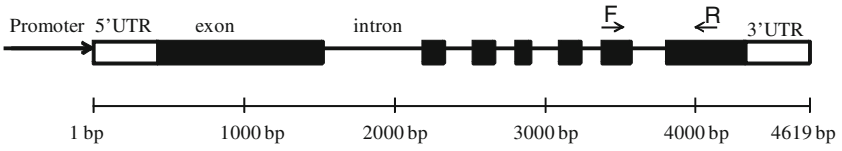

Fig. 2 Schematic diagram of *ZmCK1*. Exons and introns are indicated by *black boxes* and *single lines*, respectively. *White boxes* represent the untranslated regions (UTRs). The length of *ZmCK1* gene can be estimated using the scale at the bottom. The position of the RT-qPCR primers is indicated by arrow plot and F/R

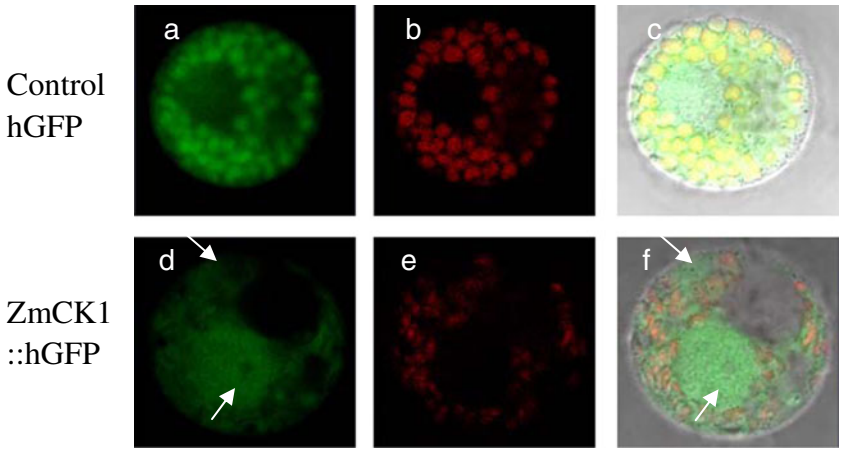

Fig. 3 Subcellular localization of the ZmCK1 protein in *Arabidopsis* mesophyll protoplasts. The fusion construct for *ZmCK1::hGFP* and the control plasmid hGFP were introduced into *Arabidopsis* mesophyll protoplasts by PEG-calcium transfection, respectively. The transformed *Arabidopsis* mesophyll protoplasts were cultured at 25 °C for 18 h and observed under a confocal microscope. Photographs were taken in the dark field for green fluorescence (**a** and **d**), in the dark field for exciting light (**b** and **e**), and in combination for morphology of the cells (**c** and **f**)

and exogenous ABA. Quantitative Q-RT-PCR showed that *ZmCK1* transcripts accumulated greatly in seedlings in response to salt and cold stresses (Fig. 4). Under cold conditions, *ZmCK1* mRNA rapidly accumulated and reached its maximum at 5 h after treatment, and last out to 12 h (Fig. 4c). Under salt treatment, the expression pattern of *ZmCK1* was similar to that with dehydration stress treatment, but the maximum time of salt-induced transcription was later (24 h) than with dehydration stress treatment (Fig. 4b). Compared with drought and ABA, salt and cold have greater impact on ZmCK1 transcript (Fig. 4a and d).

ZmCK1 Promoter Contains Diverse Stress-Responsive Elements

To further investigate the mechanism responsible for regulation of *ZmCK1* gene expression, we cloned a 1.35-kb promoter region upstream of the *ZmCK1* ATG start codon from maize genomic DNA. Putative *cis*-acting elements in the promoter region were sought using plant *cis*-acting regulatory element databases, PLACE (http://www.dna.affrc.go.jp/PLACE/) and PlantCARE (http://intra.psb.ugent.be:8080/PlantCARE/). Several distinct regulatory motifs, homologous to *cis*-acting elements involved in responses to drought and

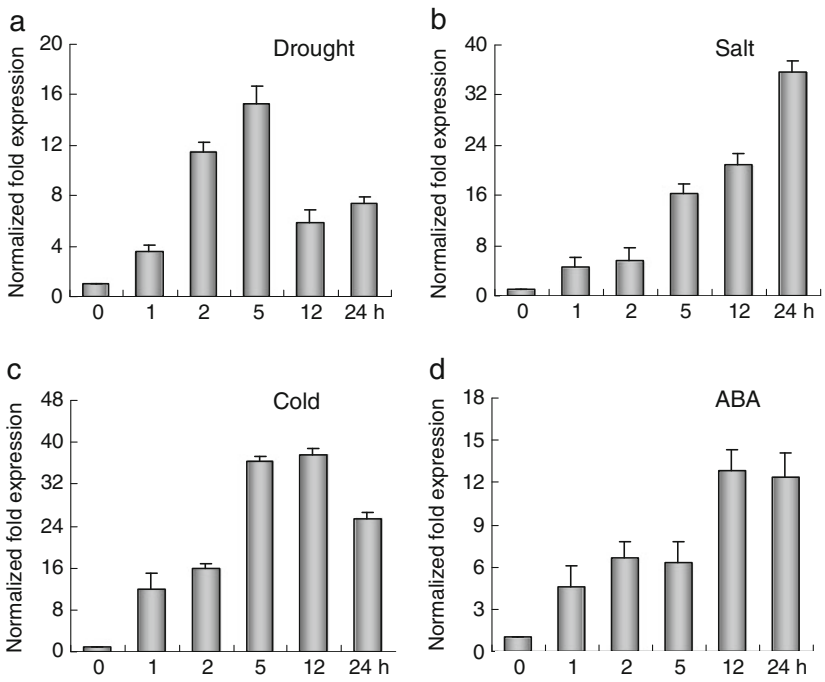

Fig. 4 RT-qPCR assessment of relative level of expression of the maize *ZmCK1* under different abiotic stress conditions, including drought (**a**), NaCl (**b**), cold (**c**), and ABA (**d**). Total RNA was isolated from leaves of 3 maize seedlings. The actin gene was used as an internal reference

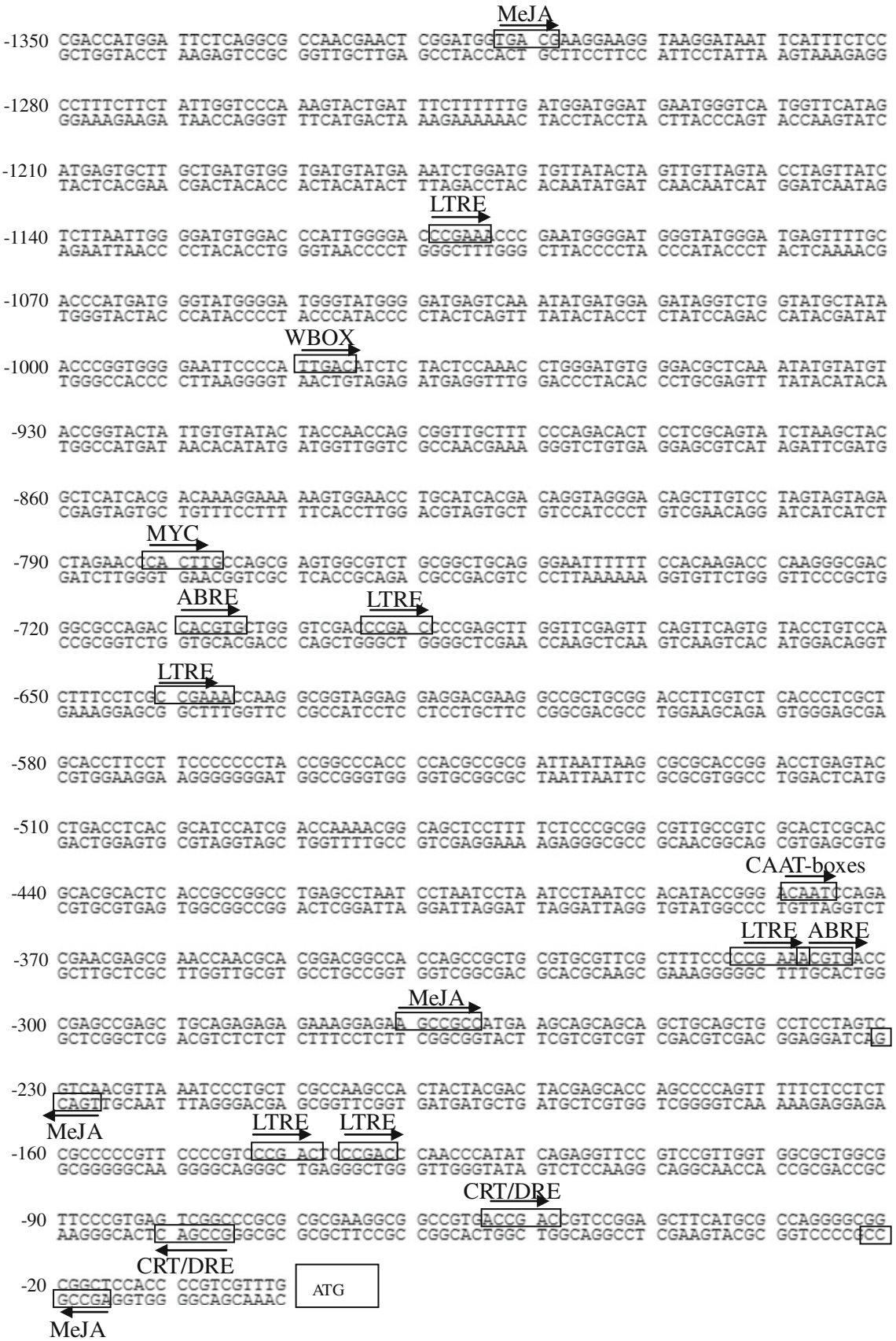

Fig. 5 Nucleotide sequence and putative *cis*-acting elements of the 5′-flanking region of *ZmCK1*. *Arrows* transcription and translation start sites. *Boxes* putative *cis*-acting elements

cold stresses, were identified (Fig. 5), including two CRT/DRE elements (A/GCCGAC) and five LTRE core sequences (CCGAC). Also, *cis*-acting elements involved in responses to plant hormones were found, including three TGACG motifs (TGACG) and one GCC box involved in the MeJA responsiveness, one WBOX (TTGAC) involved in the SA responsiveness, and two ABRE elements (CACGTG) and one MYC binding site (CACTTG) responsive to ABA and dehydration. In addition, there was one sequence resembling CAAT boxes (CAAT; a common *cis*-acting element and enhancer region).

Improved Survival Rate Under Drought and Cold Stresses and Promoted Root Growth Under Salt Stress

Overexpression of rice *OsCDPK7* gene confers drought, salt, and cold tolerance on rice plants (Saijo et al. 2000). To investigate whether *ZmCK1* could improve abiotic stress tolerance, the *ZmCK1* gene under the control of CaMV35S was transformed into *Arabidopsis* plants. Nineteen independent transgenic lines were obtained by kanamycin selection. Two lines (TL-8 and TL-13) with high expression levels of *ZmCK1* were chosen for further analysis.

Drought and cold stress tolerance tests were carried out using seedlings of *35S::ZmCK1* transgenic and wild-type *Arabidopsis* grown for 4 weeks at 22 °C. Under water-free conditions, almost all of wild-type plants were dead after 2 weeks, whereas 31.6–43.3 % of the transgenic plants survived. Similarly, after exposure to −6 °C for 12 h for cold treatment, more than half of wild-type plants were dead; on the contrary, 60–70.6 % of the transgenic plants survived (Fig. 6a).

For salt stress, germinated *Arabidopsis* seeds were planted on MS media plates supplemented with NaCl. As shown in Fig. 6b, there were no obvious differences in root length between the transgenic and wild type plants on normal MS media. However, when supplemented with NaCl, transgenic plants displayed strong tolerance to salt stress due to superior root growth (6.2–6.7 cm) compared with wild type plants (4.8 cm). These results indicated that *ZmCK1* enhanced drought, salt, and cold stress tolerance in transgenic *Arabidopsis* plants.

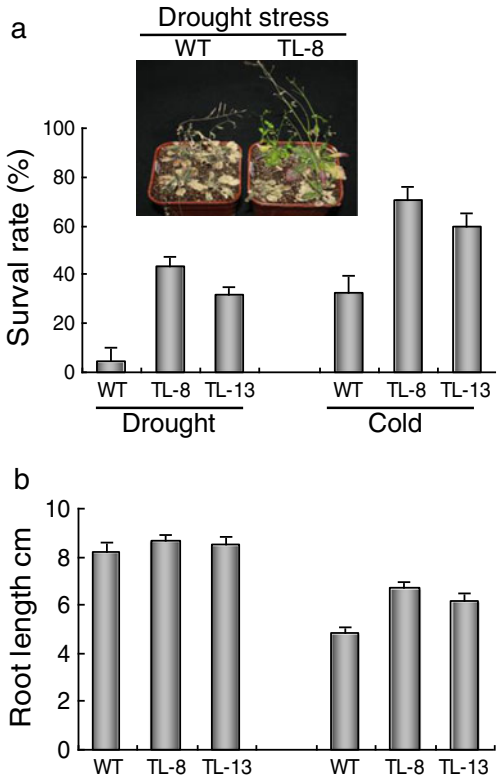

Fig. 6 Effect of *ZmCK1* expression on drought, cold, and salt tolerance in transgenic plants. Effect of *ZmCK1* expression on drought and cold tolerance in transgenic *Arabidopsis* plants. **a** Survival rates of WT and *CaMV35S::ZmCK1* transgenic *Arabidopsis* lines (TL-5 and TL-7) were estimated after drought and cold stress treatment. Stress treatments were applied to transgenic *Arabidopsis* and WT 3-week-old plants under common conditions. Results are averages of three replicates ± SD. **b** Root growth of *Arabidopsis* plants under salt (NaCl) stress treatment. Three-day-old seedlings of three transgenic lines and wild-type *Arabidopsis* were planted on MS media with added NaCl. One week later, the root lengths of the seedlings were measured. The results are averages of three replicates ± SD. *WT* wild-type plants

Discussion

Plants use different signaling pathways to acclimate to changing environmental conditions. CDPK and MAPK pathways are known to be involved in signaling of abiotic and biotic stress in animal, yeast, and plant cells (Ludwig et al. 2005). Both pathways are activated in response to the same stimuli leading to the question of a potential crosstalk between those pathways (Wurzinger et al. 2011). Early studies of CDPKs involved in the biotic stress response in tobacco indicated a crosstalk of CDPK and MAPK activities, whereas a recent study in *Arabidopsis* revealed that CDPKs and MAPKs act differentially in innate immune signaling and showed no direct crosstalk between CDPK and MAPK activities (Wurzinger et al. 2011). Fast changes in the concentration of free calcium are among the first responses to many stress situations. Calcium signals are decoded by CDPKs that are widely used to adapt the cellular metabolism to a changing environment. In the present study, we cloned a CDPK gene, *ZmCK1*, from maize. Sequence alignment showed that ZmCK1 was highly identical to OsCDPK7 from rice. Also, ZmCK1 contain a MGNACGG sequence in the N terminus of the protein. However, subcellular localization analysis suggests that ZmCK1 could not localize on the plasma membrane although it has a putative N-myristoylation signal in the N terminus. These suggest that nuclear activation of ZmCK1 protein is probably needed by modification to localize on the plasma membrane (Min et al. 2012).

CDPKs are believed to be involved in ABA signaling, and several members of the *Arabidopsis* CDPK superfamily have been identified as positive ABA signaling regulators. For example, *GsCBRLK* transcripts were induced by exogenous ABA. Overexpression of *GsCBRLK* resulted in enhanced plant tolerance to ABA and increased the expression pattern of a number of stress gene markers in response to ABA in transgenic *Arabidopsis* (Yang et al. 2010). These results identify GsCBRLK as a molecular link between the stress- and ABA-induced calcium/calmodulin signal and gene expression in plant cells. In this study, *ZmCK1* is positively regulated by exogenous ABA. Interestingly, some CDPKs negatively regulate ABA signaling. CPK12 interacted with, phosphorylated and stimulated a type 2C protein phosphatase ABI2, and phosphorylated two ABA-responsive transcription factors (ABF1 and ABF4) in vitro. *Arabidopsis* CPK12-RNAi lines showed ABA hypersensitivity in seed germination and postgermination growth, and altered expression of a set of ABA-responsive genes (Zhao et al. 2011). *Arabidopsis CPK12* is a negative ABA-signaling regulator, suggesting that different members of the CDPK family may constitute a regulation loop by functioning positively and negatively in ABA signal transduction (Zhao et al. 2011).

It was reported that the rice *OsCDPK7* gene improved tolerance to drought, salt, and cold stresses in transgenic plants (Saijo et al. 2000). As an ortholog of *OsCDPK7*, *ZmCK1* may play a role in resistance to abiotic stresses. The *ZmCK1* transcript level was elevated by multiple stresses (Fig. 4). Additionally, *cis*-acting elements related to drought, cold stresses, and ABA responsiveness were also found in the promoter. Therefore, *ZmCK1* should be involved in multiple stress responses. A functional analysis of *ZmCK1* in stress tolerance was carried out using transgenic overexpressing lines of *Arabidopsis*. The transgenic overexpression plants were observed to have more tolerance to salt/drought/cold stress than wild-type (Fig. 6). These findings suggested that *ZmCK1* produces a functional kinase that may play a regulatory role in abiotic stress responses.

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References

Chang WJ, Su HS, Li WJ, Zhang ZL (2009) Expression profiling of a novel calcium-dependent protein kinase gene, *LeCPK2*, from tomato (*Solanum lycopersicum*) under heat and pathogen-related hormones. Biosci Biotechnol Biochem 73:2427–2431

Christodoulou J, Malmendal A, Harper JF, Chazin WJ (2004) Evidence for differing roles for each lobe of the calmodulin-like domain in a calcium-dependent protein kinase. J Biol Chem 279(28):29092–29100

Hanks SK, Quinn AM, Hunter T (1988) The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science 241:42–52

Harmon AC (2003) Calcium-regulated protein kinases of plants. Gravitational Space Biol Bull 16:83–90

Hernandez SC, Hardin SC, Clouse SD, Kieber JJ, Huber SC (2004) Identification of a new motif for CDPK phosphorylation in vitro that suggests ACC synthase may be a CDPK substrate. Arch Biochem Biophys 428:81–91

Lindberg RA, Quinn AM, Hunter T (1992) Dual-specificity protein kinases, will any hydroxyl do? Trends Biochem Sci 17:114–119

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402–408

Ludwig AA, Romeis T, Jones JD (2004) CDPK-mediated signaling pathways, specificity and cross-talk. J Exp Bot 55:181–188

Ludwig AA, Saitoh H, Felix G, Freymark G, Miersch O, Wasternack C, Boller T, Jones JD, Romeis T (2005) Ethylene-mediated cross-talk between calcium-dependent protein kinase and MAPK signaling controls stress responses in plants. Proc Natl Acad Sci U S A 102:10736–10741

Min DH, Zhang XH, Xu ZS, Zhao Y, Chen Y, Li LC, Chen M, Ma YZ (2012) Induction kinetics of a novel stress-related LEA gene in wheat. Plant Mol Biol Rep. doi:10.1007/s11105-012-0446-2

Prabu G, Kawar PG, Pagariya MC, Prasad DT (2011) Identification of water deficit stress upregulated genes in sugarcane. Plant Mol Biol Rep 29:291–304

Reddy VS, Reddy ASN (2004) Proteomics of calcium-signaling components in plants. Phytochemistry 65:1745–1776

Romeis T, Piedras P, Jones JD (2000) Resistance gene-dependent activation of a calcium-dependent protein kinase in the plant defense response. Plant Cell 12:803–816

Rudd JJ, Franklin-Tong VE (2001) Unravelling response-specificity in Ca2+ signaling pathways in plant cells. New Phytol 151:7–33

Rutschmann F, Stalder U, Piotrowski M, Oecking C, Schaller A (2002) LeCPK1, a calcium-dependent protein kinase from tomato. Plasma membrane targeting and biochemical characterization. Plant Physiol 129:156–168

Saijo Y, Hata S, Kyozuka J, Shimamoto K, Izui K (2000) Overexpression of a single Ca2+−dependent protein kinase confers both cold and salt/drought tolerance on rice plants. Plant J 23:319–327

Saijo Y, Kinoshita N, Ishiyama K, Hata S, Kyozuka J, Hayakawa T, Nakamura T, Shimamoto K, Yamaya T, Izui K (2001) A Ca2+ −dependent protein kinase that endows rice plants with cold- and salt-stress tolerance functions in vascular bundles. Plant Cell Physiol 42:1228–1233

Sanders D, Pelloux J, Brownlee C, Harper JF (2002) Calcium at the crossroads of signaling. Plant Cell 14:S401–S417

Wurzinger B, Mair A, Pfister B, Teige M (2011) Cross-talk of calcium-dependent protein kinase and MAP kinase signaling. Plant Signal Behav 6:8–12

Xu ZS, Ma YZ, Cheng XG, Cao LX, Li LC, Chen M (2006) Isolation and characterization of *GmSTY1*, a novel gene encoding a dual-specificity protein kinase in soybean (*Glycine max* L.). J Integr Plant Biol 48:857–866

Xu ZS, Xia LQ, Chen M, Cheng XG, Zhang RY, Li LC, Zhao YX, LuY NZY, Liu L, Qiu ZG, Ma YZ (2007) Isolation and molecular characterization of the *Triticum aestivum* L. ethylene-responsive factor 1 (*TaERF1*) that increases multiple stress tolerance. Plant Mol Biol 65:719–732

Xu ZS, Chen M, Li LC, Ma YZ (2008a) Functions of the ERF transcription factor family in plants. Botany 865:969–977

Xu ZS, Ni ZY, Liu L, Nie LN, Li LC, Chen M, Ma YZ (2008b) Characterization of the TaAIDFa gene encoding a CRT/DRE-binding factor responsive to drought, high-salt, and cold stress in wheat. Mol Genet Genomics 280:497–508

Xu ZS, Liu L, Ni ZY, Liu P, Chen M, Li LC, Chen YF, Ma YZ (2009a) W55a encodes a novel protein kinase that is involved in multiple stress responses. J Integr Plant Biol 51:58–66

Xu ZS, Xiong TF, Ni ZY, Chen XP, Chen M, Li LC, Gao DY, Yu XD, Liu P, Ma YZ (2009b) Isolation and identification of two genes encoding leucine-rich repeat (LRR) proteins differentially responsive to pathogen attack and salt stress in tobacco. Plant Sci 176:38–45

Xu ZS, Chen M, Li LC, Ma YZ (2011) Functions and application of the AP2/ERF transcription factor family in crop improvement. J Integr Plant Biol 53:570–585

Yang L, Ji W, Zhu Y, Gao P, Li Y, Cai H, Bai X, Guo D (2010) *GsCBRLK*, a calcium/calmodulin-binding receptor-like kinase, is a positive regulator of plant tolerance to salt and ABA stress. J Exp Bot 61:2519–2533

Yoo SD, Cho YH, Sheen J (2007) *Arabidopsis* mesophyll protoplasts: a versatile cell system for transient gene expression analysis. Nat Protoc 2:1565–1572

Zhao R, Sun HL, Mei C, Wang XJ, Yan L, Liu R, Zhang XF, Wang XF, Zhang DP (2011) The *Arabidopsis* Ca2+−dependent protein kinase CPK12 negatively regulates abscisic acid signaling in seed germination and post-germination growth. New Phytol 192:61–73

Zheng WJ, Xu ZS, Chen M, Li LC, Chai SC, Ma YZ (2012) Isolation and characterization of receptor-like protein kinase WELP1 in wheat. Afr J Microbiol Res 6:2410–2418