Biochemical and Biophysical Research Communications 441 (2013) 630–636

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

A novel calcium-dependent protein kinase gene from *Populus euphratica*, confers both drought and cold stress tolerance

Jinhuan Chen, Bin Xue, Xinli Xia *, Weilun Yin *

National Engineering Laboratory for Tree Breeding, No. 35, Qinghua East Road, Beijing 100083, China
College of Biological Sciences and Technology, Beijing Forestry University, No. 35, Qinghua East Road, Beijing 100083, China

ARTICLE INFO

Article history:
Received 3 October 2013
Available online 26 October 2013

Keywords:
CDPK
Drought
Freezing
Populus euphratica
Stomatal aperture

ABSTRACT

Populus species are the most important timber trees over the Northern hemisphere. Most of them are cold- and drought-sensitive except the *Populus euphratica* Oliv. Here, a calcium-dependent protein kinase (CDPK) gene cloned from *P. euphratica*, designated as *PeCPK10*, was rapidly induced by salt, cold, and drought stresses. The protein encoded by *PeCPK10* was localized within the nucleus and cytosol, which may be important for its specific regulation in cellular functions. To elucidate the physiological functions of *PeCPK10*, we generated transgenic *Arabidopsis* plants overexpressing *PeCPK10*. The results showed that *PeCPK10*-transgenic lines experienced better growth than vector control plants when treated with drought. Stronger abscisic acid-induced promotion of stomatal closing has been showed in transgenic lines. Particularly, overexpression of *PeCPK10* showed enhanced freezing tolerance. Constitutive expression of *PeCPK10* enhanced the expression of several abscisic acid-responsive genes and multiple abiotic stress-responsive genes such as *RD29B* and *COR15A*. Accordingly, a positive regulator responsive to cold and drought stresses in *P. euphratica* is proposed.

1. Introduction

Populus are valued for the production of paper, veneer and plywood and they are the main potential species for biofuel production because of their fast growth [1,2]. Despite the high productivity, poplars are considered as the most sensitive trees to water deficit [3]. To sustain the extension of poplar cultivation in the future when worsening of droughts was predicted, more drought resistant hybrids are required [4]. Identification of key genes involved in drought response may help to select and generate genotypes with an improved ability to cope with drought in the future.

Calcium has emerged as an essential second messenger that mediates responses to developmental and stress stimuli in plants [5]. Specific calcium signatures may be recognized by different sensor proteins. Several families of Ca^{2+} sensors have been identified in higher plants: calmodulins (CaMs) and CaM-like proteins, calcineurin B-like (CBL) proteins, and calcium-dependent protein kinases (CDPK) [6–8]. Of these, CDPKs are a family of monomeric proteins, containing an N-terminal variable domain, a kinase domain, an autoinhibitory domain, and a CaM-like domain with four "EF-hand" motifs [9]. Upon binding Ca^{2+} to the CaM-like domain, they could relay the signaling to their downstream targets [7].

CDPKs are found in a wide range of vascular and nonvascular plants as well as in green algae and certain protozoa, suggesting their potential importance in plant cells. Analyses of *Arabidopsis* (*Arabidopsis thaliana*) and rice (*Oryza sativa*) genome sequences have led to the identification of multigene families of these CDPKs [10,11]. Some CDPKs are expressed ubiquitously, whereas others are present in specific tissues or their expression is regulated by different stimuli [12]. Different CDPKs reside in different subcellular locations, such as the cytosol, nucleus, plasma membrane, endoplasmic reticulum, and mitochondrial outer membrane, indicating potentially diverse functions [7]. Although there have been studies demonstrated various stress response of *CDPKs* [13,14], few studies have focused this family in trees, even in the tree model species poplar.

Populus euphratica, the only poplar species naturally distributed at the edge of barren deserts or semi-barren deserts, is characterized by its remarkable tolerance to freezing, soil salinity and drought [15]. Thus, *P. euphratica* is considered an ideal species to study the molecular mechanisms of the trees. Dozens of abiotic stress responsive genes have been identified in *P. euphratica*, but few calcium-dependent protein kinases have been characterized

* Corresponding authors at: College of Biological Sciences and Technology, Beijing Forestry University, No. 35, Qinghua East Road, Beijing 100083, China. Fax: +86 010 62336400.
E-mail addresses: xiaxl@bjfu.edu.cn (X. Xia), yinwl@bjfu.edu.cn (W. Yin).

0006-291X/$ - see front matter Crown Copyright © 2013 Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.10.103

in this species. Our data will provide valuable information for further investigation on the unique abiotic stress adaption of *P. euphratica* via CDPK signaling.

2. Materials and methods

2.1. Plant material and stress treatments

Two-year-old *P. euphratica* plantlets from the Inner Mongolia autonomous region of China were subjected to different abiotic stress conditions. Briefly, plants were removed from field plots and placed on filter paper at 25 °C, which simulated dehydration conditions. Salt stress was initiated by watering young plants with 300 mM NaCl. Cold treatment was conducted by transferring the young trees to a growth chamber set at 4 °C under a 16 h light/8 h dark cycle. ABA treatment was conducted by spraying leaves of the young plants with a 200 μM ABA solution. For all treatments, leaves were collected at different time points and immediately frozen in liquid nitrogen.

2.2. PeCPK10 isolation

Total RNA was extracted from 2-year-old *P. euphratica* using the CTAB method and then treated with an RNAase-free DNAase I (Promega) to eliminate genomic DNA contamination [16]. After evaluation using an Agilent 2100 Bioanalyzer, approximately 500 ng of total RNA was subjected to a reverse transcription reaction (total volume of 20 μl) using a First Strand cDNA Synthesis Kit (D6110A; TaKaRa), following the manufacturer's protocol. The full-length cDNA was isolated by PCR using the primers Pcpkf and PcpkR. The PCR products were cloned into pMD18-T for sequencing. The primers used in this paper are listed in Appendix S1 in Supporting Information.

2.3. Subcellular localization studies

For subcellular localization analysis, the full-length coding region of *PeCPK10* (without the termination codon) was inserted upstream and in-frame with the green fluorescent protein (*GFP*) gene. The fusion construct under the control of the cauliflower mosaic virus (CaMV) 35S promoter was introduced into onion epidermal cells by the particle bombardment method using the PDS-1000 System (Bio-Rad) at 1100 psi helium pressure. A 35S-GFP vector was also introduced as a control. GFP expression was observed by a fluorescence microscope (Leica) after incubation for 24 h at 22 °C [17].

2.4. Arabidopsis transformation

Arabidopsis [ecotype Columbia-0 (Col-0)] was used for transformation. To construct the plasmids for transformation, the full-length *PeCPK10* cDNA was inserted into the *BamH*I site of the pBI121 vector under the control of the CaMV35S promoter. The construct was introduced into *Agrobacterium tumefaciens* strain GV3101. *Arabidopsis* used for transformation were grown in 9-cm pots filled with customized soil mixture under cool-white light (16 h light/8 h dark, 18,500 lux, 22 °C, 70% relative humidity) until they bloomed. Plants were then transformed using the floral-dip method [18]. The PBI121vector was also transformed as a control. The seeds of T_0 transformants were screened on half-strength Murashige–Skoog (1/2 MS) agar medium supplemented with 80 mg/L kanamycin, and the surviving plants were further confirmed using PCR method. Two representative T_3 homozygous lines (L1 and L5) with different expression levels of *PeCPK10* were chosen for detailed analyses.

2.5. Freezing tolerance analysis

Sterilized seeds of T_3 homozygous lines germinated and grown on 1/2 MS agar medium for 10 days were transferred to mixed soil and maintained with sufficient watering until they were 3 weeks old. For freezing stress treatment, 36 seedlings of each transgenic line and 36 vector control seedlings in each of three independent experiments were exposed to −4 /−8 °C for 8 h under dark conditions and then removed to normal growth conditions. After 7 d, the surviving plants were counted and the photographs were taken.

Proline was measured immediately after freezing treatment on the fourth to seventh rosette leaves according to Bates et al. [19].

2.6. Drought tolerance analysis

Long-term water loss analysis was performed firstly. Wild-type and transgenic plants were grown on soil in the growth chamber under normal conditions for 3 weeks before treatment. Drought treatment was imposed simply by withdrawing irrigation until lethal effects were observed in all genotypes. The experiments were repeated three times to verify the results.

2.7. Water loss measurement

For a rapid water loss assay, leaves were detached from 4-week-old plants, weighed immediately on a piece of weighing paper, and then subjected to air-drying (the aerial relative humidity was 40%, and the temperature was between 22 and 23 °C). Weights were measured for 10 plants per line at the designated time points of 30, 60, 90, and 120 min. The percentage loss of fresh weight was calculated based on the initial fresh weight of the plants.

2.8. Stomatal aperture measurements

To measure the stomatal aperture, excised rosette leaves from 4-week-old *Arabidopsis* were floated on medium containing 5 mM KCl, 50 μM $CaCl_2$, and 10 mM MES–Tris (pH 6.15) for 2 h in the light to induce stomatal opening, followed by the treatment of 10 μM ABA [20]. The abaxial epidermis was peeled from the treated leaves, and stomata on the abaxial leaf surfaces were observed with microscope (Leica DMR) using ImageJ software. Twenty stomatal apertures were measured for each individual experiment.

2.9. Semi-quantitative RT-PCR

Semi-quantitative RT-PCR was carried out to determine the expression level of *PeCPK10* in transgenic *Arabidopsis*. PCR amplification (30 s at 95 °C, 30 s at 58 °C and 45 s at 72 °C) was performed for 25 cycles using gene-specific primers PsqF/PsqR. The expression level of the observed gene was compared to that of housekeeping gene *AtActin* (At5g09810), which was amplified by primers aActF/aActR. The final products were separated by 1% agarose gel electrophoresis.

2.10. Quantitative PCR analysis

Quantitative PCR (qPCR) was performed to determine the expression level of *PeCPK10* in *P. euphratica* and to validate the expression changes of marker genes in transgenic lines. The qPCR was conducted using a power SYBR Green PCR Kit (Applied Biosystems) in a MicroAmp™ 96-well plate with a StepOnePlus™ Real-Time PCR System (Applied Biosystems). The relative quantification value was calculated by the $2^{-\Delta\Delta C}{}_T$ method using *PeActin* (GenBank Accession No. EF148840) or *AtActin* as an internal control. The gene-specific primers used in the qPCR analysis were

Pqpk10F/Pqpk10R for *PeCPK10*, pActF/pActR for *PeActin*, and aQActF/aQActR for *AtActin*. Each PCR assay was conducted for three biological replicates, and for each replicate, three technological replicates were repeated. All the gene-specific primers used above are listed in Appendix S1 in Supporting Information.

2.11. Statistical analysis

The SPSS 16.0 statistical package was used for the statistical analysis. The significance was tested using the least significant difference (LSD) at the 5% level.

3. Results

3.1. Identification of the PeCPK10 gene from P. euphratica

A gene encoding a potential CDPK protein was cloned from salt-treated leaves of *P. euphratica* and designated as *PeCPK10* (GenBank Accession No. JX292958). The full-length cDNA of *PeCPK10*, spanning 1668 bp from ATG to the stop codon, encoded a protein of 555 amino acid residues. PeCPK10 shared typical characteristics of CDPKs: an N-terminal variable domain (NTV) with subdomains I-IX, a junction or pseudo-substrate domain, and a CaM-like domain (CLD) with four EF hands (Fig. S1). Overall, PeCPK10 was 81.26% identical to AtCPK10 and 80.54% identical to AtCPK30 at the amino-acid level. Clustering analysis using amino acid sequences of the entire open reading frame (ORF) also indicated that PeCPK10 was more closely related to AtCPK10 and AtCPK30 than to other CDPK members (Fig. S2).

3.2. PeCPK10 is induced by multiple abiotic stresses

Expression of *PeCPK10* was investigated in *P. euphratica* leaves subjected to different treatments, including high NaCl concentration, drought, cold, and ABA. The qPCR analyses showed that expression of *PeCPK10* was enhanced after dehydration, high-salt and especially cold treatments. Under both cold and high-salt treatments, *PeCPK10* expression was induced rapidly, peaking at 6 and 3 h, respectively. Under the dehydration treatment, the expression level of *PeCPK10* became stronger within 12 h. However, the mRNA level of *PeCPK10* was not significantly altered by exogenous ABA application, given a fold-change cutoff of 2 (Fig. 1A).

3.3. PeCPK10 is localized in the nucleus and cytoplasm

Elucidation of PeCPK10's subcellular localization was particularly important because distinct subcellular locations provide the potential for specific regulation in various cellular functions [21]. Thus, we fused *PeCPK10* to the *GFP* gene and addressed its localization. The PeCPK10-coding region was fused to GFP under the control of 35S promoter. The fusion plasmid (35S-PeCPK10-GFP) and the control vector (35S-GFP) were introduced into onion cells by particle bombardment. The untransformed onion cells served as

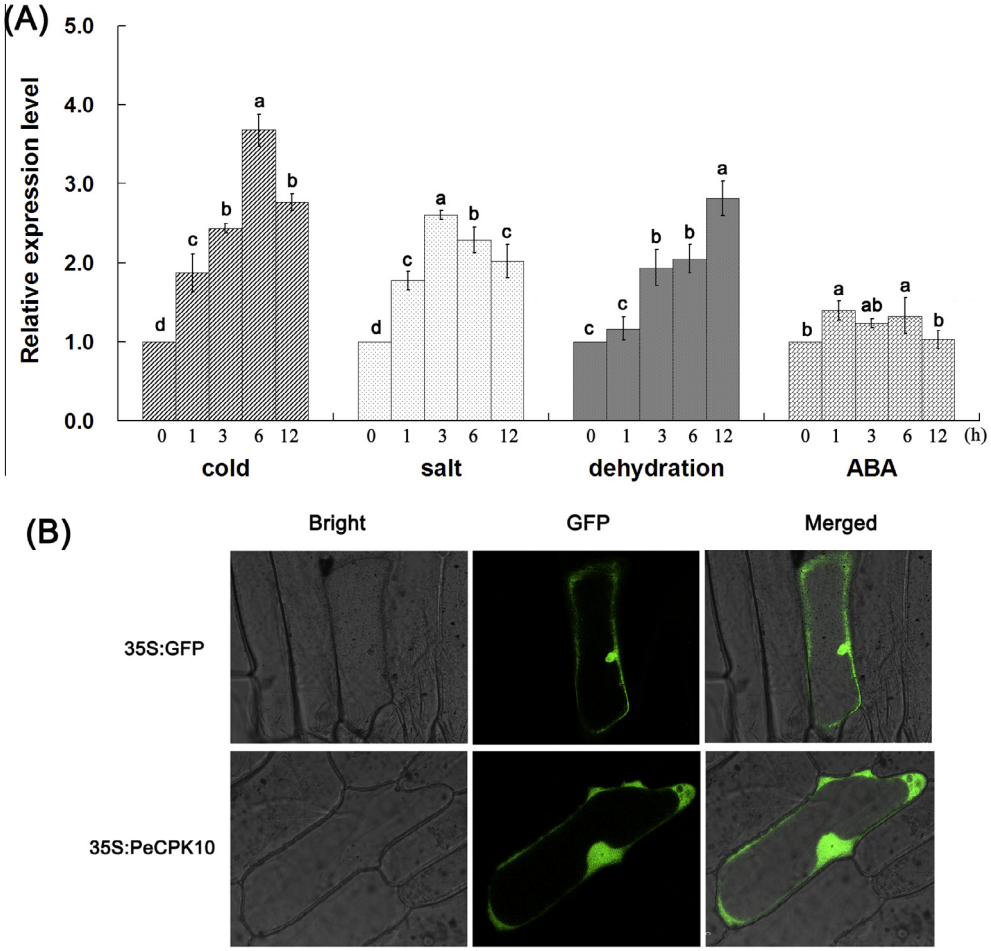

Fig. 1. Expression patterns of *PeCPK10*. (A) Quantitative PCR analysis of *PeCPK10* in response to various stress treatments. Relative expression was calculated using *PeActin* as an internal reference. The expression level in untreated leaves was assigned a value of 1. Values are means of three independent biological replicates ± SE. The different letters on the top of the columns indicate significantly differences at $P \leqslant 0.05$. (B) Subcellular localization of PeCPK10 as revealed by GFP fusion proteins. Confocal images of GFP fluorescence in onion epidermal cell transformed with 35S::GFP or 35S::PeCPK10::GFP.

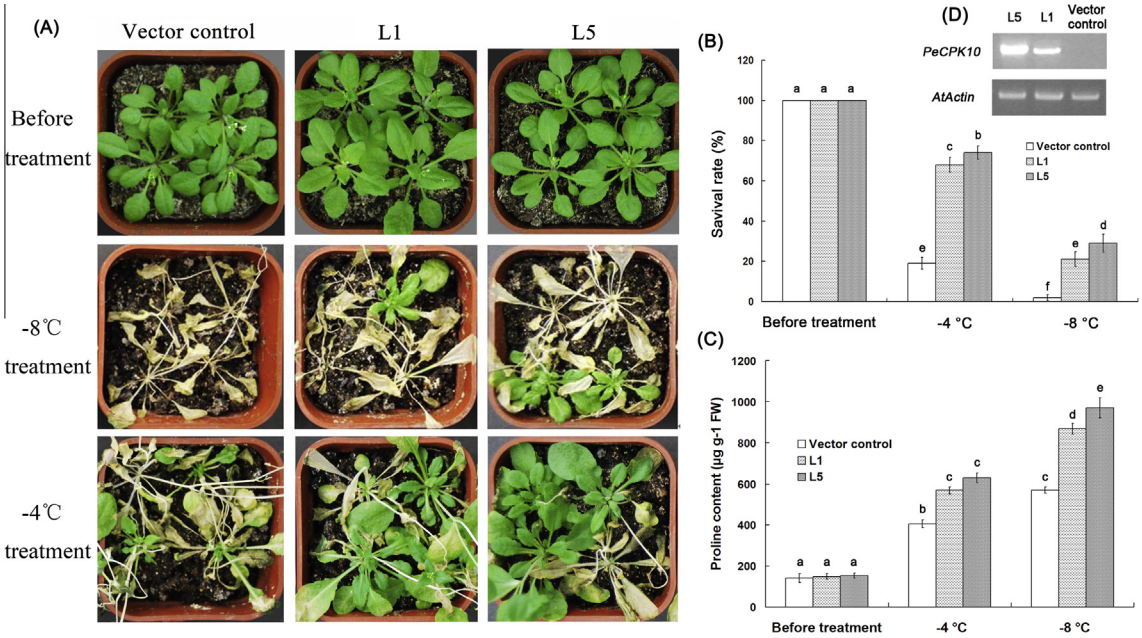

Fig. 2. Performance of vector control and 35S-*PeCPK10* transgenic lines (L1 and L5) under freezing stress. (A) 3-week-old T_3 plants were exposed to −4 °C or −8 °C for 8 h and then returned to normal growth conditions. Photographs were taken after recovery for 7 d. (B) Survival rate of the transgenic plants under freezing stress. (C) Proline levels in the vector control and transgenic lines before or after freezing treatment conditions. (D) Expression of *PeCPK10* in the vector control or transgenic *Arabidopsis* lines. Each data point in (B) and (C) is the average of three independent experiments. Each bar represents the means ± SE. The different letters on the top of the columns indicate significantly differences at $P \leqslant 0.05$.

a control. GFP expression was examined by fluorescence microscopy after 24 h of incubation. As shown in Fig. 1B, PeCPK10-GFP exhibited a nuclear and cytosolic distribution pattern similar to those of free GFP, demonstrating that PeCPK10 was a soluble enzyme with the potential to target the nucleus.

3.4. Overexpression of PeCPK10 enhances freezing tolerance in transgenic Arabidopsis

To elucidate the role of *PeCPK10* in freezing tolerance, 3-week-old T_3 plants were exposed to −4 °C or −8 °C for 8 h and then returned to normal growth conditions. Freezing tolerance was estimated as the percentage of plants surviving after 7 d of recovery. Results indicated that the vector control plants were dramatically affected by freezing treatment, while the transgenic plants were less affected (Fig. 2A). After −4 °C treatment, 66% of L1 plants and 72% of L5 plants survived, whereas only 19% of vector control plants survived. In the case of −8 °C treatment, 20% plants in line L1 and 28% plants in line L5 survived, but almost all vector control plants wilted completely (Fig. 2B). Proline content, measured immediately after the freezing treatments, increased in both control and *PeCPK10* transgenic plants, but significantly higher levels were detected in the *PeCPK10*-over-expressing plants as compared with the control plants (Fig. 2C). L5 accumulated higher levels of proline than L1, which may be related to higher expression level of *PeCPK10* in L5 (Fig. 2D), suggesting that proline might contribute to the phenotypic changes caused by *PeCPK10* overexpression under stress conditions. These results indicated that *PeCPK10* conferred freezing tolerance to the transgenic *Arabidopsis*.

3.5. Overexpression of PeCPK10 enhances drought tolerance by reducing water loss

To examine the drought responses of transgenic plants, irrigation was withheld from soil-grown plants until lethal effects were observed in the two genotypes. Under normal growth conditions, the transgenic lines and vector control plants showed a similar performance (Fig. 3A). After a 2-week exposure to drought, almost all *PeCPK10* transgenic plants continued to grow, whereas most of the vector control plants withered and became chlorotic (Fig. 3A). After 3 weeks of drought conditions, all of the vector control plants withered and died, whereas 50–75% of the transgenic plants survived (Fig. 3B). Water loss was also examined to evaluate plant performance under drought stress. Analyses of detached leaves showed that the *PeCPK10*-overexpressing plants lost water more slowly compared to the vector controls (Fig. 3C), indicating increased tolerance of transgenic plants to dehydration/drought stress. Taken together, these results suggested that *PeCPK10* positively regulated in plant responses to drought stress.

Stomatal apertures averaged 2.73 μm in L1, 2.28 μm in L5, and 3.92 μm in the vector controls in the absence of ABA treatment (Fig. 3D). When exposed to 10 μM exogenous ABA, the stomatal apertures of transgenic plants decreased by more than 50%, whereas that of vector controls were reduced by only 38% (Fig. 3E). As mentioned above, detached leaves of *PeCPK10*-overexpressing plants lost less water than the vector controls under dehydration conditions (Fig. 3C). Line L5 lost less water than line L1, which may have been due to stronger ABA-sensitivity in ABA-induced promotion of stomatal closure in L5.

3.6. Constitutive expression of PeCPK10 enhances the expression of stress/ABA-responsive genes in transgenic Arabidopsis seedlings

To reveal downstream genes of *PeCPK10*, twenty-eight stress-related genes were selected for qPCR analysis. Among these, 17 genes including *RD22* (*At5g25610*), *RD29B* (*At5g52300*), *LEA* (*At2g21490*), *COR15A* (*At2g42540*), *P5CS1* (*At2g39800*), *HSFA6A* (*At5g43840*), *RNS1* (*At2g02990*), *UBQ1* (*At3g52590*), *GRF5* (*At3g13960*), *MYB101* (*At2g32460*), *HAI1* (*At5g59220*), ABI5 (*At2g36270*), *NF-YC2* (*At1g56170*), *NF-YB7* (*At2g13570*), *RCI3* (*At1g05260*), and *LTP3/4* (*At5g59320/At5g59310*), were significantly up-regulated by an fold change >2.0 in transgenic lines under normal conditions (Fig. 4). These genes have been identified to be responsive to various abiotic stresses and/or ABA in *Arabidopsis*. *RD22*, *RD29B*, *COR15A*, *HAI1*,

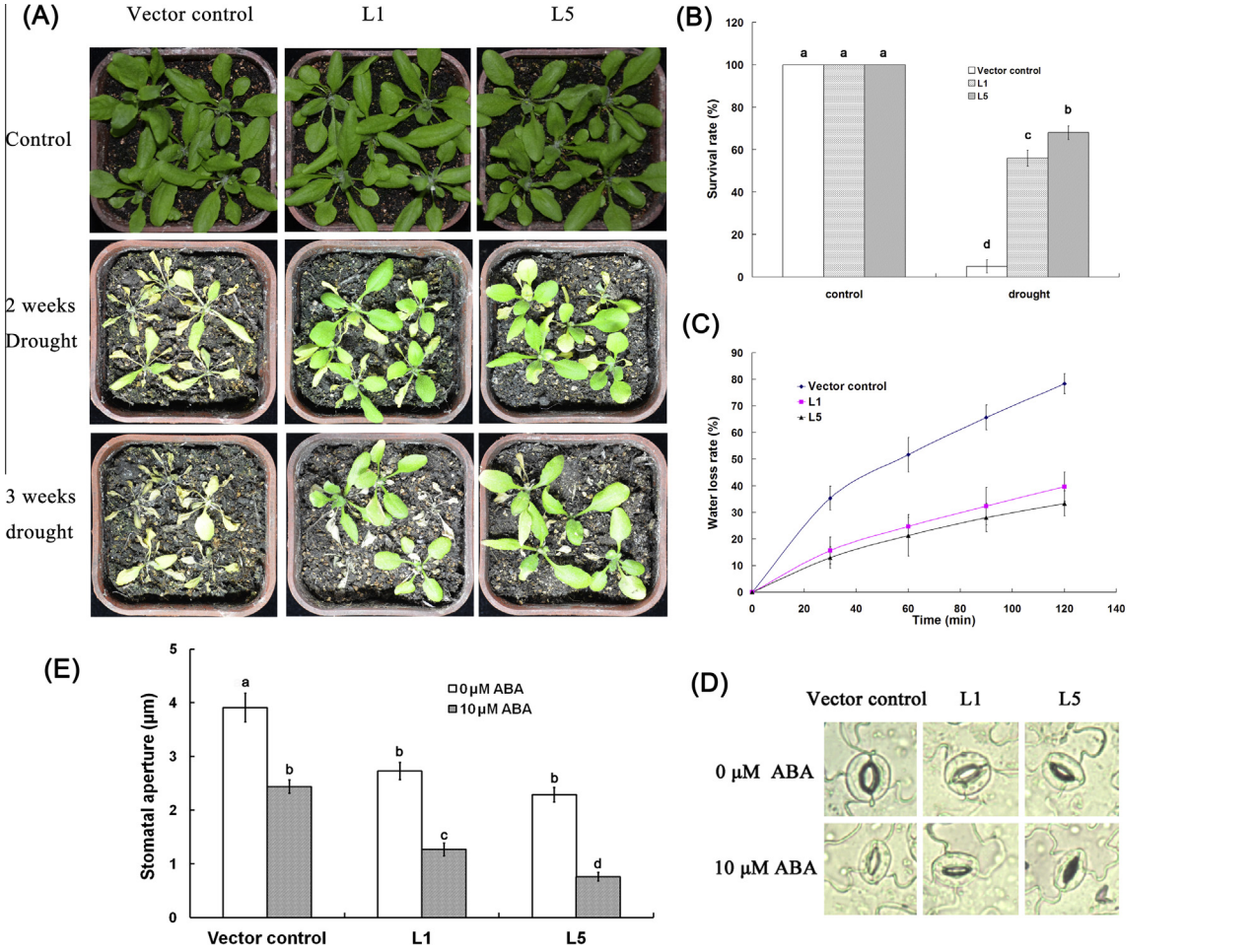

Fig. 3. Phenotype tests of vector control and 35S-*PeCPK10* transgenic lines (L1 and L5) under drought stress. (A) 1-week-old seedlings were grown for a 3-week treatment with or without irrigation. Photographs were taken at the end of the 2- or 3-week treatment. (B) Survival rates of the transgenic plants after a 3-week drought stress. Each data point is the average of three experiments. (C) Time courses of the water loss rate from the detached leaves of vector controls, L1, and L5. (D) ABA-inhibited stomatal opening. Concentrations of 10 μM ABA were added to the incubation medium in the dark, and the tested epidermis was then incubated in the above buffer for 2 h in the light. (E) Stomatal aperture measurements of vector control and transgenic lines in response to 0 and 10 μM ABA. Each bar in (B), (C) and (E) represents the mean ± SE. The different letters on the top of the columns indicate significantly differences at $P \leqslant 0.05$.

ABI5, and *MYB101* are both stress- and ABA-inducible. *LTP3* and *LTP4*, which are involved in ABA responses, showed the highest induction among the 17 upregulated *Arabidopsis* genes. In addition to the genes mentioned above, *LOS1* (*At1g56070*) and *KIN1* (*At1g14370*) exhibited slight induction in transgenic lines (absolute fold change of 1.5–2.0), eight stress or ABA-inducible genes, such as *ABI1-4* (*At4g26080*, *At5g57050*, *At3g24650*, *At2g40220*), *ERD10* (*At1g20450*), *CBF1* (*At4g25490*), *OST1* (*At4g33950*) and *MYB2* (*At2g47190*), showed no induction in transgenic lines, and a salt-responsive gene, *STO* (*At1g06040*), was even down-regulated in transgenic lines. These results indicated that *PeCPK10* copes with various abiotic stresses by regulating a specific set of stress- and ABA-response genes.

4. Discussion

P. euphratica Oliv. is considered to be abiotic stress tolerant compared to other *Populus* species [22]. Thus, much interest has been focused on elucidating the molecular and cellular processes underlying its acclimation to abiotic stresses [23]. Here, a gene encoding a member of the CDPK protein family that is induced in leaves of *P. euphratica* during the early stages of severe salt stress was isolated and characterized. Expression analysis showed that *PeCPK10* mRNAs were induced rapidly under various environmental stresses, especially when suffered with cold (Fig. 2). Analyses of the PeCPK10-GFP fusion protein indicated that highly-homologous family members did not necessarily share the same cellular localization. Although shared 81.26% amino-acid identity with AtCPK10, PeCPK10 proteins were expressed in the cytoplasm and nucleus of *Arabidopsis* mesophyll protoplasts, making a difference from AtCPK10 that was reported to be localized in the plasma membrane [24]. These localization patterns may facilitate their functions in both early and later responses to abiotic stresses. For example, cytoplasm-localized PeCPK10 may more easily mediate a quick response by sensing Ca^{2+} and phosphorylating downstream messengers, such as guard cell regulation, whereas the nuclear-localized PeCPK10 could more easily phosphorylate nuclear-localized regulators, such as transcription factors.

To further understand *PeCPK10* function *in vivo*, we generated *PeCPK10*-overexpressing *Arabidopsis* under control of the constitutive CaMV 35S promoter. The growth of transgenic plants was analyzed under different abiotic stress conditions. As shown in Figs. 2 and 3, *PeCPK10*-overexpressing plants displayed tolerance against drought and freezing stresses, respectively. We also examined the rapid responses of *PeCPK10*-overexpressing lines after water loss treatment as well as physiological responses to long-term water withholding. The results of both experiments suggested that

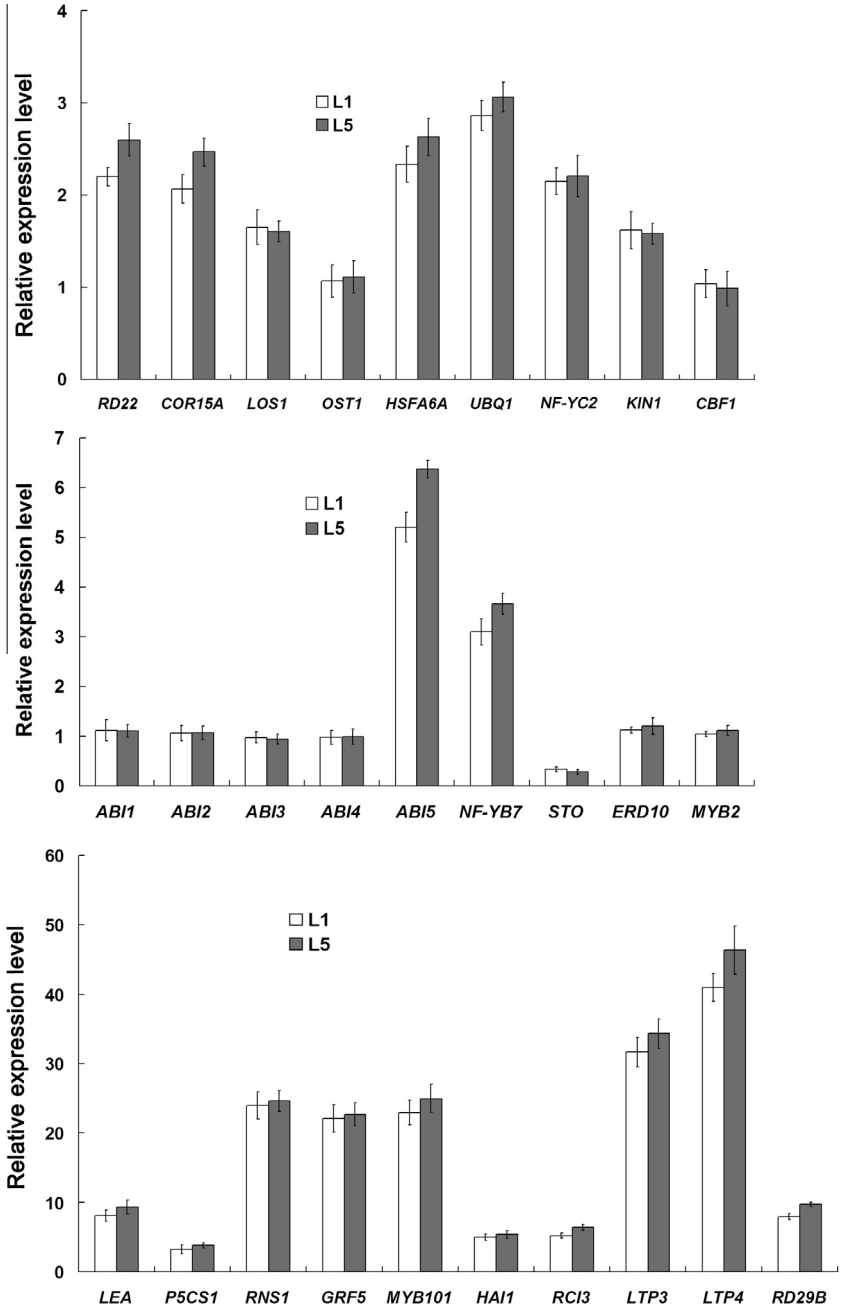

Fig. 4. Quantitative PCR analysis of 28 marker genes in the vector control plants and *PeCPK10*-transgenic *Arabidopsis*. *AtActin* (At5g09810) was used as an internal control. The gene expression level in the vector control plants was assigned a value of 1. Each column represents an average of five replicates. Each bar represents the mean ± SE.

PeCPK10 transgenic plants had increased tolerance to drought stress. This phenomenon may be attributed to expressions of some drought response genes (*RD22*, *RD29B*, *UBQ1*, *HSFA6A*, *NF-YC2*, and *NF-YB7*) that up-regulated in *PeCPK10*-overexpressing lines.

In contrast to drought stress [14,24,25], less is known about the CDPK-mediated freezing signaling pathway. Here, functional analysis showed that overexpression of *PeCPK10* conferred freezing tolerance to transgenic *Arabidopsis* and resulted in the accumulation of more proline (Fig. 2C), which may correlate to the high level of *P5CS1* expression [26]. Downstream gene expression analyses also revealed that cold/freezing-responsive genes, such as *COR15A* and *RD29B*, were significantly upregulated in the transgenic plants compared to vector control plants. These specifically induced genes may contribute to enhanced freezing tolerance in the *PeCPK10*-overexpressing plants. However, overexpression of *PeCPK10* did not affect the expression of *CBF1*, which has established a key role in cold response. We presumed *PeCPK10* could affect the expression of other transcriptional activators to regulate freezing-responsive genes, since only 12% of cold/freezing responsive genes are members of CBF regulon [27,28].

Although *PeCPK10* was only slightly induced by ABA, overexpression of *PeCPK10* definitely enhanced the expressions of some ABA-responsive genes, for example *ABI5* (Fig. 4). Detailed work will be performed in the future to confirm the regulatory network of *PeCPK10* in ABA and abiotic stress signaling, which have been regarded as complex networks.

In conclusion, we isolated, for the first time, a specific poplar CDPK member from *P. euphratica*. We showed the molecular evidence that *PeCPK10* is involved in drought and cold stress response. These results suggest that *PeCPK10* may be target for cold- or

drought-tolerance breeding by generating poplar plants with enhanced activity of *PeCPK10*.

Acknowledgments

This work was supported by Fundamental Research funds for the Central Universities (Nos. YX2010-18, YX2011-22 and DT2012-01), National Natural Science Foundation of China (Nos. 31100492, 31070597 and 31370597), National R&D Key Project of China (2011BAD38B01) and Research Fund for the Doctoral Program of Higher Education of China (20110014120003). We thank Houling Wang and Jiayi Zheng for assist in transient expression analysis and transgenic lines screening, respectively.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.10.103.

References

[1] J.J. Balatinecz, D.E. Kretschmann, A. Leclercq, Achievements in the utilization of poplar wood-guideposts for the future, Forest. Chronicle 77 (2001) 265–269.
[2] J. Zhuang, B. Cai, R.H. Peng, B. Zhu, X.F. Jin, Y. Xue, F. Gao, X.Y. Fu, Y.S. Tian, W. Zhao, Y.S. Qiao, Z. Zhang, A.S. Xiong, Q.H. Yao, Genome-wide analysis of the AP2/ERF gene family in *Populus trichocarpa*, Biochem. Biophys. Res. Commun. 371 (2008) 468–474.
[3] E. Dreyer, M. Bogeat-Triboulot, D. Le Thiec, J. Guehl, F. Brignolas, M. Villar, C. Bastien, F. Martin, A. Kohler, Drought tolerance of poplars: can we expect to improve it?, Biofutur (2004) 54–58
[4] C. Duncan, A.L. Chauvenet, L.M. McRae, N. Pettorelli, Predicting the future impact of droughts on ungulate populations in arid and semi-arid environments, PLoS One 7 (2012) e51490.
[5] J. Kudla, O. Batistic, K. Hashimoto, Calcium signals: the lead currency of plant information processing, Plant Cell 22 (2010) 541–563.
[6] J. Sheen, Ca^{2+}-dependent protein kinases and stress signal transduction in plants, Science 274 (1996) 1900–1902.
[7] J.F. Harper, G. Breton, A. Harmon, Decoding Ca^{2+} signals through plant protein kinases, Annu. Rev. Plant Biol. 55 (2004) 263–288.
[8] S. Luan, The CBL–CIPK network in plant calcium signaling, Trends Plant Sci. 14 (2009) 37–42.
[9] J.S. Satterlee, M.R. Sussman, Unusual membrane-associated protein kinases in higher plants, J. Membr. Biol. 164 (1998) 205–213.
[10] T. Asano, N. Tanaka, G. Yang, N. Hayashi, S. Komatsu, Genome-wide identification of the rice calcium-dependent protein kinase and its closely related kinase gene families: comprehensive analysis of the CDPKs gene family in rice, Plant Cell Physiol. 46 (2005) 356–366.
[11] B. Wan, Y. Lin, T. Mou, Expression of rice Ca^{2+}-dependent protein kinases (CDPKs) genes under different environmental stresses, FEBS Lett. 581 (2007) 1179–1189.
[12] E.M. Hrabak, C.W. Chan, M. Gribskov, J.F. Harper, J.H. Choi, N. Halford, J. Kudla, S. Luan, H.G. Nimmo, M.R. Sussman, M. Thomas, K. Walker-Simmons, J.K. Zhu, A.C. Harmon, The Arabidopsis CDPK–SnRK superfamily of protein kinases, Plant Physiol. 132 (2003) 666–680.
[13] S.Y. Ma, W.H. Wu, AtCPK23 functions in *Arabidopsis* responses to drought and salt stresses, Plant Mol. Biol. 65 (2007) 511–518.
[14] J. Xu, Y.S. Tian, R.H. Peng, A.S. Xiong, B. Zhu, X.F. Jin, F. Gao, X.Y. Fu, X.L. Hou, Q.H. Yao, AtCPK6, a functionally redundant and positive regulator involved in salt/drought stress tolerance in *Arabidopsis*, Planta 231 (2010) 1251–1260.
[15] R. Gu, S. Fonseca, L.G. Puskas, L. Hackler Jr., A. Zvara, D. Dudits, M.S. Pais, Transcript identification and profiling during salt stress and recovery of *Populus euphratica*, Tree Physiol. 24 (2004) 265–276.
[16] S. Chang, J. Puryear, J. Cairney, A simple and efficient method for isolating RNA from pine trees, Plant Mol. Biol. Rep. 11 (1993) 113–116.
[17] J. Chen, X. Xia, W. Yin, Expression profiling and functional characterization of a DREB2-type gene from *Populus euphratica*, Biochem. Biophys. Res. Commun. 378 (2009) 483–487.
[18] S.J. Clough, A.F. Bent, Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*, Plant J. 16 (1998) 735–743.
[19] L. Bates, R. Waldren, I. Teare, Rapid determination of free proline for water-stress studies, Plant Soil 39 (1973) 205–207.
[20] K.E. Hubbard, R.S. Siegel, G. Valerio, B. Brandt, J.I. Schroeder, Abscisic acid and CO_2 signalling via calcium sensitivity priming in guard cells, new CDPK mutant phenotypes and a method for improved resolution of stomatal stimulus-response analyses, Ann. Bot. 109 (2012) 5–17.
[21] C. Dammann, A. Ichida, B. Hong, S.M. Romanowsky, E.M. Hrabak, A.C. Harmon, B.G. Pickard, J.F. Harper, Subcellular targeting of nine calcium-dependent protein kinase isoforms from *Arabidopsis*, Plant Physiol. 132 (2003) 1840–1848.
[22] Q. Qiu, T. Ma, Q. Hu, B. Liu, Y. Wu, H. Zhou, Q. Wang, J. Wang, J. Liu, Genome-scale transcriptome analysis of the desert poplar, *Populus euphratica*, Tree Physiol. 31 (2011) 452–461.
[23] C.Y. Ye, H.C. Zhang, J.H. Chen, X.L. Xia, W.L. Yin, Molecular characterization of putative vacuolar NHX-type Na^+/H^+ exchanger genes from the salt-resistant tree *Populus euphratica*, Physiol. Plant. 137 (2009) 166–174.
[24] J.J. Zou, F.J. Wei, C. Wang, J.J. Wu, D. Ratnasekera, W.X. Liu, W.H. Wu, *Arabidopsis* calcium-dependent protein kinase CPK10 functions in abscisic acid- and Ca^{2+}-mediated stomatal regulation in response to drought stress, Plant Physiol. 154 (2010) 1232–1243.
[25] P. Schulz, M. Herde, T. Romeis, Calcium-dependent protein kinases: hubs in plant stress signaling and development, Plant Physiol. (2013).
[26] H. Liu, Y.H. Zhang, H. Yin, W.X. Wang, X.M. Zhao, Y.G. Du, Alginate oligosaccharides enhanced *Triticum aestivum* L. tolerance to drought stress, Plant Physiol. Biochem. 62 (2013) 33–40.
[27] S. Fowler, M.F. Thomashow, Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway, Plant Cell 14 (2002) 1675–1690.
[28] V. Chinnusamy, J. Zhu, J.K. Zhu, Gene regulation during cold acclimation in plants, Physiol. Plant. 126 (2006) 52–61.