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# Cloning of a Calcium-Dependent Protein Kinase Gene *NtCDPK12*, and Its Induced Expression by High-Salt and Drought in *Nicotiana tabacum*

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## Abstract

Calcium-dependent protein kinases (CDPKs, EC 2.7.1.37) comprise a large family of Ser/Thr kinases in plants and play an important role in plant $Ca^{2+}$ signal transduction. A full-length CDPK gene, *NtCDPK12* (GenBank accession number GQ337420), was isolated from common tobacco (*Nicotiana tabacum*) leaves by rapid amplification of cDNA ends (RACE). The *NtCDPK12* cDNA is 1 816 bp length and contains an open reading frame (ORF) of 1 461 bp encoding 486 amino acids. Sequence alignments indicated that NtCDPK12 contains all conserved regions found in CDPKs and shows a high level of sequence similarity to many other plant CDPKs. The results of real-time quantitative reverse transcription-PCR (qRT-PCR) showed that *NtCDPK12* was highly expressed in stems and increased in roots treated with high-salt or subjected to drought stress, which indicates that *NtCDPK12* was induced by high-salt and drought stresses.

Key words: abiotic stress, CDPK, *Nicotiana tabacum*, RACE, real-time qRT-PCR

## INTRODUCTION

Common tobacco (*Nicotiana tabacum*) is an agricultural leaf product and valuable cash crop, and has great contribution to national economy. However, tobacco production is facing severe challenges ascribed to the deteriorative living environments including abiotic or biotic stresses. Therefore, exploring the stress resistance is important for tobacco production.

Calcium ($Ca^{2+}$) is an important second messenger in plant cells and plays a key role in regulating the processes of plant growth and developments (Rudd and Franklin-Tong 2001). Among the earliest cellular responses to environmental stresses, cytoplasmic calcium concentration changes instantaneously, and a specific calcium signature is often established (Knight and Knight 2001). A variety of intracellular $Ca^{2+}$-binding proteins can identify and transfer calcium signals, and trigger cell-specific adaptive responses to the stresses (Cheng *et al*. 2002; Luan *et al*. 2002).

Calcium-dependent protein kinases (CDPKs, EC 2.7.1.37) are a large family of Ser/Thr protein kinases in plants. CDPKs consist of four characteristic domains: an N-terminal variable domain, a protein kinase domain, an autoinhibitory domain (junction region), and a calmodulin (CaM)-like regulatory (calcium-binding) domain (Hrabak *et al*. 2003; Liu *et al*. 2006). Such kinases were first discovered in soybean (Harmon *et al*. 1987) and existed in a wide variety of plants. They are encoded by a multi-gene family. There are 34 and 31 CDPKs in *Arabidopsis thaliana* and rice (*Oryza sativa*)

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genomes, respectively (Cheng *et al*. 2002; Hrabak *et al*. 2003; Asano *et al*. 2005; Ray *et al*. 2007; Ye *et al*. 2009). The number of CDPKs in diploid wheat (*Triticum aestivum*) genome is also estimated to be at least 26 (Li *et al*. 2008). At present, more than 160 CDPK genes are isolated from at least 24 kinds of higher plants, 7 kinds of lower plants, and 5 kinds of protozoans at www.ncbi.nlm.nih.gov.

Increasing evidence has demonstrated that CDPKs are involved in environmental stress signal transduction. An elevation in transcription levels as well as an increase in the activity of CDPKs has been observed as a response to different stresses (Ye *et al*. 2009). The expression of *OsCPK7* (*OsCDPK13*) was observed to be increased in the leaf sheath response to cold stress, suggesting that *OsCPK7* may be an important signalling component in rice seedlings under cold stress (Abbasi *et al*. 2004). Expressions of *VfCPK1* at both the mRNA and protein levels are increased in leaves treated with abscisic acid or subjected to drought stress (Liu *et al*. 2006). The levels of *ZmCPK11* transcription accumulation are stimulated by wounding both in leaves and noninjured neighboring leaves, indicating that the maize (*Zea mays*) protein kinase is involved in a systemic response to wounding (Szczegielniak *et al*. 2005). The *PaCDPK1* gene is transcriptionally activated in response to low temperature, wounding, and pathogen infection (Tsai *et al*. 2007). Similarly, in common tobacco, *NtCDPK1*, *NtCDPK2*, and *NtCDPK3* are involved in signalling transduction induced by both biotic and abiotic stresses (Yoon *et al*. 1999; Romeis *et al*. 2001), and *NtCPK4* expression is increased in response to the treatment of gibberellin or NaCl (Zhang *et al*. 2005).

The objective of the present study was to clone a *CDPK* gene in common tobacco leaves and to investigate its possible responses to abiotic stresses.

## MATERIALS AND METHODS

### Plant materials and stress-treatments

Common tobacco cultivar K326 was grown in the greenhouse with a natural light period, the daytime temperature about 25-26°C, and night temperature about 18°C. Vegetative organs including roots, stems, and leaves were sampled from about 1-mon-old plants with four or five fully expanded leaves, while flowers were collected from 4-mon-old plants at budding stage. Plants with four or five fully expanded leaves were treated with different test solutions (Liu *et al*. 2006; Li *et al*. 2008). The test solutions consisted of PEG 6000 (20%) and NaCl (200 mmol $L^{-1}$). Leaves and roots for stress-treatments were harvested at 0, 1, 3, 6, 12, and 24 h post-treatments of PEG 6000 and NaCl. For temperature treatments, about 1-mon-old plants were placed at 4°C, and leaves and roots were harvested at 12 h post-treatments. All the samples collected were immediately frozen in liquid nitrogen and maintained at -70°C for RNA extractions.

### RNA extraction

All common tobacco samples including roots, stems, leaves, flowers, and roots or leaves with different stress-treatments were powdered in liquid nitrogen, and the total RNAs were extracted using Total RNA Reagent Kit (Galen Biopharm Co., Ltd., Beijing, China) according to the manufacturer's instruction.

### 5′- and 3′-RACE of *NtCDPK12*

5′-RACE (rapid amplification of cDNA ends)-ready cDNA was prepared by mixing 3 μL RNA, 1 μL 5′-RACE CDS Primer A [5′-$(T)_{25}N_{-1}$N-3′, N=A, C, G, or T, $N_{-1}$=A, G or C] and 1 μL SMART II A Oligo (5′-AAG CAG TGG TAT CAA CGC AGA GTA CGC GGG-3′). 3 μL RNA, 1 μL 3′-RACE CDS Primer A [5′-AAG CAG TGG TAT CAA CGC AGA GTA $C(T)_{30}N_{-1}$N-3′, N=A, C, G, or T, $N_{-1}$=A, G or C] and 1 μL sterile water were mixed for 3′-RACE-ready cDNA preparation.

According to the *NtCDPK12*, 618-bp middle fragment (GenBank accession no. FJ594482) (Tai *et al*. 2009), two gene-specific primers (GSP) GSP1 (5′-TGC GGT AAG TCG CTC TCT GGG GTC C-3′) and GSP2 (5′-TCC CTG GGT GAA GAT TGA TGG TGT GGC T-3′) were designed for 5′- and 3′-RACE, respectively. 5′- and 3′-RACE were performed with the SMART™ RACE cDNA Amplification Kit (Clontech, Mountain View, CA, USA) according to the manufacturer's instruction. 5′-RACE was performed by using GSP1 and universal primer A mix (UPM; long: 5′-CTA ATA CGA CTC ACT ATA GGG CAA GCA GTG GTA TCA ACG CAG AGT-