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Short communication

Molecular cloning and characterization of a novel MAP kinase gene in *Chorispora bungeana*

Tengguo Zhang [a], Yubing Liu [a], Lingui Xue [a], Shijian Xu [a], Tuo Chen [b], Tongwen Yang [a], Lijing Zhang [a], Lizhe An [a,b,*]

[a] *Key Laboratory of Arid and Grassland Agroecology of Ministry of Education, School of Life Sciences, Lanzhou University, Lanzhou 730000, China*
[b] *Cold and Arid Regions Environmental and Engineering Research Institute, Chinese Academy of Sciences, Lanzhou 730000, China*

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Abstract

Chorispora bungeana Fisch. and C.A. Mey (*Chorispora bungeana*) is a rare alpine subnival plant species that is highly capable of resisting freezing environment. Since it is a stress-tolerant plant, we investigated the participation of mitogen-activated protein kinases (MAPKs) as possible mediators of abiotic stresses. We have isolated from *Chorispora bungeana* a new MAPK cDNA *CbMAPK3* which encodes a 369 amino-acid protein with moderate to high nucleotide sequence similarity to previously reported plant MAPK genes. *CbMAPK3* contains all 11 of the MAPK conserved subdomains and the phosphorylation motif TEY. The transcripts of *CbMAPK3* were detected and no tissue-specific expression were observed in both roots and leaves, The transcripts of *CbMAPK3* accumulated highly and rapidly when *Chorispora bungeana* treated with cold (4 and –4 °C), ABA and salinity stress. These results indicate that the *CbMAPK3* may play an important role in response to environmental stresses.

Keywords: RACE; Mitogen-activated protein kinases; *Chorispora bungeana*; Environmental stresses

1. Introduction

To survive biotic and abiotic stresses, plants have developed elaborate mechanisms to perceive external signals and adjust metabolic pathways by modulating the expression of genes. Activation and/or inactivation of appropriate genes in response to particular stimuli are mediated through well-tuned signal transduction systems [1]. The phosphorylation cascades including mitogen-activated protein kinases (MAPKs), MAPK kinases (MAPKKs), and MAPKK kinases (MAPKKKs) have been reported to function in various signal transduction pathways from yeasts to vertebrates [2]. MAPKs are activated when both tyrosine and threonine residues in the TXY motif are phosphorylated by dual-specificity kinases MAPKKs. MAPKKs are activated when serine and serine/threonine residues in the S/T–$X_{3–5}$–S/T motif are phosphorylated by serine/threonine kinases MAPKKKs [3].

In plants, a variety of genes encoding MAPKs have been identified [4–10]. The analysis of *Arabidopsis* genome sequence has revealed the presence of 24 MAPK genes in the genome, which suggests that the MAPK cascades in plants may be quite complex [9]. Compared with mammalian MAPKs, all plant MAPKs have highest homology to the extracellular signal-regulated kinase (ERK) subfamily [11]. Most of isolated plants MAPKs have the TEY motif as the dual-phosphorylation site. The predicted amino acid sequences of these plant MAPKs show high conservation over the entire length with highest similarity in the eleven domains that are necessary for the catalytic function of serine/threonine protein kinase.

Comparisons of deduced amino acid sequences indicate that plant MAPKs can be grouped into at least five distinct families (Fig. 3). MAPKs within one branch serve similar function in different species [12], the MAPKs in family A and B are

Abbreviations: ABA, Abscisic acid; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; MAPKKK, MAPKK kinase; ORF, Open reading frame; RACE, Rapid amplification of cDNA ends; RT-PCR, Reverse transcription polymerase chain reaction; UTR, untranslated region.

* Corresponding author. Tel.: +86 931 496 7181; fax: +86 931 496 7181.
E-mail address: lizhean@lzu.edu.cn (L. An).

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mostly involved in environmental and hormonal responses, some of the MAPKs of family C are involved in cell cycle regulation and some involved in environmental stress responses, the function of MAPKs in family D are little known at present, family E MAPKs are all have the TDY motif instead of TEY in their T-loop and also have the C-terminal extension domain.

Chorispora Bungeana Fisch. and C.A. Mey. (*Chorispora bungeana*) is a representative alpine subnival plant which can survive under frequent temperature fluctuations, and freezing temperatures. *Chorispora Bungeana* is a perennial herb that belongs to Brassicaceae. It grows in the freeze-thaw tundra in border of glacier where almost all the other flowering plants have great difficulty for growing, and it mainly distributes in an ice free cirque (with a 3800–3900 m height above sea level) beside the Glacier No. 1 in the source area of Urumqi River in Tianshan mountains, Xinjiang province, China, where the average temperature is below subzero during the growth period from June to September [13]. Some works had been done for the exploration of its special cold-hardiness mechanism. Previous research showed that the alpine subnival plant did not possess special morphological characteristics that helped it survive under freezing environment [14]. Therefore, molecular and physiological mechanisms were assumed to account for its adaptation to the freezing environment.

In this report, we describe the cloning and characterization of a plant MAPK from *Chorispora bungeana*, *CbMAPK3*. The deduced protein sequences of *CbMAPK3* were most closely related to *Arabidopsis AtMPK3*, and all belonged to subgroup A. To get an insight into its behavior and regulation by environmental stimuli, we have characterized the mRNA expression profile of *CbMAPK3* against cold (4 and –4 °C), ABA and salinity stress. The results obtained in this study demonstrate that *CbMAPK3* is transcriptionally regulated by abiotic stress.

2. Results and discussion

2.1. Cloning and sequence analysis of the full-length cDNA of CbMAPK3

Based on cDNA sequences of the conserved regions of plant MAPK genes, primer P1 and P2 were designed and synthesized for the amplification of the middle region of MAPK-like cDNA from *Chorispora bungeana*. A single fragment of about 1030 bp showing high homology to *AtMPK3* was obtained. Based on the middle region sequences, a reverse specific primer CbRACE5 and two pairs of PCR primers were designed for the 5′RACE, a single and specific fragment of about 300 bp was obtained in which a 5′ untranslated region (UTR) of 91 bp was found upstream of the first ATG codon. A reverse specific primer CbRACE3 and a pair of PCR primers were designed and used in the 3′RACE, a single and specific fragment of about 400 bp was obtained, and a 3′ UTR of 218 bp was found downstream from the stop codon. Based on the sequences of the middle region sequences and the 3′,5′RACE products, the full-length cDNA was deduced and amplified using a pair of primer P3 and P4, which was confirmed by sequencing for three times. The cloned full-length cDNA of mitogen-activated protein kinase gene from *Chorispora bungeana* was 1419 bp with a polyA tail of 15 bp (Fig. 1). The cDNA contained a 1110-bp ORF encoding a protein of 369 amino acids with a calculated molecular weight of about 42.5 kDa and with a p*I* of 5.79. The protein exhibit closest homology to a subgroup of plant MAPKs containing *Arabidopsis AtMPK3* and we thus refer to them as *CbMAPK3* (GenBank accession number: *AY805424*).

An alignment of the predicted amino acid sequences of *CbMAPK3* with the cloned MAPKs from various plants were performed using DNAStar software shown in Fig. 2. The *CbMAPK3* protein contained all 11 conserved amino acid and peptide motifs characteristic of 11 subdomains of protein kinases with serin/threonine specificity. The TEY motif, which includes the threonine and tyrosine residues whose phosphorylation is necessary for MAP kinase activation and is a characteristic feature of MAP kinase, is also conserved in the *CbMAPK3* protein sequence (Fig. 2).

A phylogenetic tree based on the genetic distance of the protein sequences was constructed by the Clustal method using DANStar software, it has been proposed from the analysis of sequences homology of the predicted amino acid sequences that plant MAPKs can be grouped at least five distinct groups. Based on the relationship tree of cloned plant MAPKs, *CbMAPK3* can be grouped into subgroup A (Fig. 3). Comparison of the predicted protein sequences of the *CbMAPK3* with MAP kinases of other plants shows that CbMAPK3 is most homologous to the *Arabidopsis AtMPK3* (95%), *Nicotiana tabacum NtWIPK* (82%), *Capsicum annuum CaMPK1* (81%), *PsMAPK3* (80%), *Medicago sativa MsMMK4* (80%), *Petrosecinum crispum PARSLEY MAPK* (80%) (Fig. 2).

The secondary structure of the putative *CbMAPK3* protein was analyzed by SOPMA [http://npsa-pbil.ibcp.fr/cgi-bin] and the result showed that the putative *CbMAPK3* peptide contained 46% alpha helix, 13% extended strand, 6% beta turn, and 35% random coil (Fig. 4). The alpha helix and random coil constituted interlaced domination of the main part of the secondary structure. From the above sequence analyses, *CbMAPK3* was found to have many characteristics common to the MAPKs in plant and family A members tend to share highly similar sequences.

2.2. Expression patterns of CbMAPK3 in different tissue and under abiotic stresses

To investigate *CbMAPK3* expression pattern in various tissues of *Chorispora bungeana*, total RNA was isolated from 1-month-old leaves and roots, and subjected to RT-PCR analysis, the result shown that its expression has almost no tissue specificity (Fig. 5).

Chorispora bungeana was a rare and typical alpine subnival plant growing near the No. 1 glacier, the origin of Urumqi River on Tianshan Mountains in china. The annual average temperature therein was lower than 5 °C in daytime and