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Cloning and Characterization of a Mitogen-Activated Protein Kinase Gene 84*KMPK*14 in Hybrid Poplar (*Populus alba* × *P. glandulosa* cv. "84K")

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Abstract

Mitogen-activated protein kinases (MAPKs) are important components in signal transduction modules which play crucial roles in regulation of many biological processes in plants. Although genome-wide analysis of MAPK and MAPKK family has been carried out in poplar species, few data about the biological function analysis of this gene family are available to date. In this study, a group C MAPK gene 84*KMPK*14 was cloned from hybrid poplar (*Populus alba* × *P. glandulosa* cv. "84K"). It contained a typical protein kinase domain, a conserved TEY-motif and an atypical conserved common docking (CD) domain. Sequence alignment revealed that 84*KMPK*14 was the most homologous to *Populus trichocarpa PtMPK*14. Expression analysis indicated that the transcript of 84*KMPK*14 in roots and young leaves was higher than that in other tissues. The expression of 84*KMPK*14 was down-regulated by low or high temperature and was induced by H_2O_2 significantly. It was suppressed by drought and salinity stresses slightly one hour after treatment and then increased quickly three hours after treatment. These results indicated that 84*KMPK*14 may be involved in environmental stresses, which provides basis for further characterization of the physiological analysis on this gene.

Keywords

MAPK, Expression Analysis, Abiotic Stress, 84K Poplar, *MPK*14

1. Introduction

The Mitogen-activated protein kinase (MAPK) cascade is a conserved signaling

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transduction pathway, which plays important roles in the regulation of the cell cycle, plant growth and development, and responses to hormones and environmental stresses [1] [2]. The core MAPK cascade pathway consists of three proteins kinases: MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK, which phosphorylates and activates the latter one in a specific way [3]. Besides, the fourth level of kinases, MAPKKK kinases (MAPKKKKs), may function as adapters linking upstream signals to the core MAPK cascade pathways [4]. In the process, a variety of external stress signals are cascaded, amplified and are transmitted into the cell, which subsequently activates multiple downstream regulatory factors.

The MAPKs can be categorised into four subfamilies (A - D groups) based on amino acid sequences similarities. Groups A, B and C possess a common phosphorylation motif TEY in their activation loop, whereas group D MAPKs display TDY motif instead of TEY motif [5] [6]. Besides, the four groups possess diverse CD domain in their C-terminal extension, which functions as a docking site for MAPKKs, phosphatases and protein substrates [7]. CD domain in group A and B MAPKs possesses common sequence characteristics [LH][LHY]Dxx[DE]xx[DE]EPxC (where x represents any amino acid) that includes two adjacent acidic residues (D and E) [5], whereas group C MAPKs show modified sequence features in the hydrophobic residues [8], and the CD domain is not found in the Group D MAPK sequences [5].

Group C MAPKs contain 4 members, *MPK*1, *MPK*2, *MPK*7 and *MPK*14. The function studies of group C MAPKs appeared in the recent 10 years, which lags behind group A and B. Studies reveals that group C MAPKs are involved in various signaling processes and have important biological functions in the response of stress resistance and the regulation of plant growth and development. The transcriptional expression of Arabidopsis *MPK*1 and *MPK*2 and pea *PsMPK*2 are induced by wounding, JA, abscisic acid, and H_2O_2 [9] [10]. The *ZmMPK*7 in maize is transcriptionally induced by ABA and H_2O_2 [11]. The expression of *GhMPK*7 in cotton is induced by chemical, biotic and abiotic inducers, such as NaCl, wounding, pathogen, SA, MeJA and H_2O_2, and genetic evidence showed that *GhMPK*7 might play an important role in SA-regulated broad-spectrum resistance to pathogen infection as well as in regulation of plant growth and development [12]. The transcription of the *SlMPK*7 in tomato leaves was triggered by hydrogen peroxide (H_2O_2) anabiotic stresses and exogenous signaling molecules. Moreover, overexpression of *SlMPK*7 enhanced tolerance to chilling stress in tomato [13]. Tomato *MPK*1 and *MPK*2 are involved in plant disease resistance and abiotic stresses, and *MPK*2 played a more critical role than *MPK*1 in 24-epibrassinolide (EBR)-induced apoplastic H_2O_2 accumulation [14]. Studies showed that the Arabidopsis four group C MAPKs are activated by upstream *MKK*3, and *MKK*3-*MPK*7 is involved in pathogen signaling [15]. Besides, the ABA core machinery is required for the activation of the *MAP*3*K*17/18-*MKK*3-*MPK*1/2/7/14 [15].

Populus trichocarpa is an important genus of tree that plays a vital role in economic and ecosystem services all over the world. Environmental biotic and abiotic stresses could have harmful effects on the development and growth of poplars. Therefore, the insights into the molecular mechanisms of environmental adaptability of poplar are highly regarded. It has been reported that the genome of *Populus trichocarpa* encodes 21 MAPKs (*PtMPK*), and these MAPKs are divided into A-D groups [16] [17]. The group C MAPKs consist of *PtMPK*1, *PtMPK*2, *PtMPK*7 and *PtMPK*14 [16] [17]. However, the biological function of these MAPKs is completely unknown.

Poplar 84K (*Populus alba* × *P. glandulosa* cv. "84K") is a new hybrid variety. Poplar 84K is easy to grow roots and grows fast in young seedling stage. Besides, it has advantage of good material, strong resistance and wide adaptability, and is an important fast-growing timber species [18]. In this study, a group C MAPK gene, 84*KMPK*14 was cloned from hybrid poplar 84K. The expression pattern of 84*KMPK*14 gene in plant tissues was analyzed using quantitative realtime RT-PCR (qRT-PCR). The results indicated that 84*KMPK*14 might have different developmental function with *PtMPK*14 in other poplar species *Populus trichocarpa.* Moreover, the expression response analysis of 84*KMPK*14 gene to low temperature, high temperature, high salinity, drought and exogenous H_2O_2 revealed that 84*KMPK*14 is regulated by H_2O_2 and might be a candidate gene underlying these abiotic stresses. This study is meaningful to reveal that 84*KMPK*14 might be an important regulator in development and abiotic stress response in 84K poplar.

2. Materials and Methods

The experiment was conducted at Shandong Agricultural University and the Flower Institute of Forestry College.

2.1. Plant Materials

The 84K poplar plantlets transformation was set up by the Flower Institute of Forestry College. The 84K poplar plantlets were propagated by tissue culture. The 2 cm shoot tips were cut and cultured in sterilizing containers containing basal MS medium supplemented with 0.01 mg/L NAA and 0.1 mg/L IBA, at 25˚C/25˚C under 16 h light/8 h dark photoperiod and 12,000 lux light intensity. After 4 weeks, the seedlings were transferred to polystyrene trays containing Hoagland's solution.

Two week-old hydroponic polar plantles were submerged to various treatments. For stress treatments, 200 mM NaCl, 10% PEG 6000 and 50 mM H_2O_2 was applied in the Hoagland's nutrient solution to examine adaptive responses to NaCl, dought and H_2O_2, and the seedlings were placed in a 4˚C or 42˚C growth chamber to examine adaptive responses to cold or heat stress, at the same time, seedlings in the basic nutrient solution under normal condition were used as mock control. Shoot apexs were collected directly into liquid nitrogen

after 0 h, 1 h, 3 h, 6 h and 12 h of all the treatments and stored in a −80˚C freezer.

2.2. RNA Extraction and cDNA Synthesis

The total RNA was isolated and was treated with Recombinant DNaseI (RNase-free) with TRNzol reagent (TransGen Biotech, China) according to the manufacturer's protocol. Then the first-strand cDNA was synthesized from 2 μg total RNA using M-MLV reverse transcriptase and oligo (dT) 15 as primer with the PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, China).

2.3. PCR Cloning of 84*KMPK*14 Gene

The full-length cDNA of 84*KMPK*14 was amplified from the cDNAs by using the following primer pairs: 84*KMPK*14-F (5'-GAAATGGCAACTTTAGTGGAGC-3') and 84*KMPK*14-R (5'-GCCGGAGTTTCATTGATATTGC-3'). The PCR amplification was performed as follows: the reaction mixture was first pre-denatured at 94˚C for 3 min; then at 94˚C for 15 s, 58˚C for 15 s, and 72˚C for 2 min for a total of 30 cycles; followed by 72˚C for 10 min. The PCR products were detected with 1% agarose gel electrophoresis. The target PCR fragment was recovered with the Nucleo Trap Gel Extraction Kit (TaKaRa) and was ligated to the Blunt3 vector and then was transformed into *Escherichia coli* DH5*α*. The positive clones were selected and identified by sequencing.

2.4. Bioinformatics Analysis

The BLASTP program on the National Center for Biotechnology Information was used to predict the conservative domain of 84*KMPK*14 protein. BLASTX (NCBI) was used to study the homology of the nucleotide sequences. DNAMAN was used to conduct multiple sequence alignment. Phylogenetic tree was constructed by MEGA5.0 software according to the neighbor-joining method, and tested by bootstrap, which was repeated 1000 times. Furthermore, the protParam tool was used to predict the physicochemical properties of 84*KMPK*14 protein and ExPaSy-SOPMA was used to predict protein secondary structure.

2.5. Real-Time Quantitative PCR Analysis

The qRT-PCR reactions were performed on 2 μL of each cDNA dilution using the SYBR® Premix ExTaq (TaKaRa) and CFX96™ Real-time PCR Detection System (Bio-Rad, America) according to the manufacturer's instructions. The reaction procedure was as follows: started with an initial step of 95˚C for 30 s; 39 cycles at 95˚C for 30 s, 60˚C for 30 s to calculate cycle threshold values; finally 60˚C for 30 s and 95˚C for 30 s to obtain the melting curve. The samples from each biological replicate were assayed in triplicate and included three negative controls using water instead of cDNA. The *Actin* gene (POPTR_0001s31700g) was used as the reference gene. The specific primers used for quantitative analysis was designed using the Primer 5.0 software. The primers in qPCR were used

as follows: 84*KMPK*14q-F (5'-CCTTATGCAAGGAGAATCCATTT-3'), 84*KMPK*14*q*-R (5'-GCTTCTGTCACACTAATTCTCTTAG-3'), *Actin*-F (5'-TTCTACAAGTGCTTTGATGGTGAGTTC-3'), *Actin*-R (5'-CTATTCGATACATAGAAGATCAGAATGTTC-3'). Relative transcript levels were calculated using the comparative Ct method and results were normalized to the *Actin* gene.

3. Results

3.1. Cloning and Sequence Analysis of 84*KMPK*14 Gene

To study the roles of group C MAPKs in poplar, we cloned the candidate gene homologous to *MPK*14 gene from young leaves of 84K poplar by RT-PCR (**Figure 1**), and it was designated as 84*KMPK*14.

The full-length cDNA of 84*KMPK*14 consisted of 1107 nucleotides, encoding a polypeptide of 369 amino acids and having the isoelectric point of 7.66 and the relative molecular weight of 42,693.09 Da. The instability index is computed to be 35.04 and the grand average of hydropathicity was −0.222. The phosphorylation site prediction results demonstrated that the 84*KMPK*14 protein sequence possesses 16 serine (Ser) phosphorylation sites, 15 threonine (Thr) phosphorylation sites, and 16 tyrosine phosphorylation sites. Protein sequence and structural analysis showed that 84*KMPK*14 contains a conserved-TEY-signature was found in its activation loop (**Figure 2**). Moreover, a variant conserved common docking (CD) domain was found in the C-terminal part of the protein (**Figure 2**, **Figure 3**).

Phylogenetic tree of plant group C MAPKs was constructed by using the full-length protein sequences (**Figure 4**). The dendrogram showed that the 84*KMPK*14 protein was most closely related to *Populus trichocarpa PtMPK*7 and *PtMPK*14, *Arabidopsis thaliana AtMPK*7 and *AtMPK*14 (**Figure 4**). Amino acid sequence identity analysis indicated that 84*KMPK*14 protein was highly homologous to *MPK*14 in *Populus trichocarpa* (*PtMPK*14; XP002310268.1), with 96.75% identity. Thus, it is most likely that the 84*KMPK*14 encodes a member of a putative group C *MPK*14 in 84K poplar, and it was renamed as 84*KMPK*14.

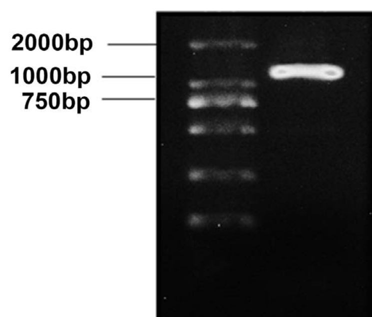

Figure 1. PCR products of 84*KMPK*14 full-length cDNA. M, DNA Marker DL2000.

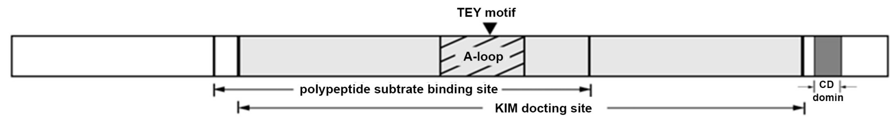

Figure 2. The protein structural organization of 84*KMPK*14. Polypeptide subtrate binding site TEY motif CD domain A-loop (activation loop) and the conserved functional domains family-specific protein signatures for 84*KMPK*14.

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84KMPK14   MATLVEPPNGIRPRGKQYYSMWETVFEVDSKYVPIKPIGRGAYGVVCSSINRETNEKVAIKKINNVLENKIDALRTLREL   80
AtMPK7     MAMLVEPPNGIKQQGKHYYSMWQTLFEIDTKYVPIKPIGRGAYGVVCSSINRETNERVAIKKIHNVFENRVDALRTLREL   80
AtMPK14    MAMLVDPPNGIRQEGKHYYTMWQTLFEIDTKYVPIKPIGRGAYGVVCSSINSETNERVAIKKIHNVFENRIDALRTLREL   80
PtMPK7     MATLVEPPNGIRPSGKQYYSMWHTVFELDSKYVPIKPIGKGAYGVVCSSINRETNEKVAIKKINNVFENKIDALRTLREL   80
PtMPK14    MATLVEPPNGIRPRGKQYYSMWETVFEVDSKYVPIKPIGRGAYGVVCSSINRETNEKVAIKKINNVLENKIDALRTLREL   80
ZmMPK7     MAMMVDPPNGIGNQGKHYYSMWQTLFEIDTKYVPIKPIGRGAYGIVCSSINRETNEKVAIKKIHNVFDNRVDALRTLREL   80
ZmMPK14    MAMMVDPPNGIGNQGKHYYSMWQTLFEIDTKYVPIKPIGRGAYGIVCSSINRETNEKVAIKKIHNVFDNRVDALRTLREL   80

84KMPK14   KLLRHIRHENVIALKDVMMPIHRTSFKDVYLVYELMDTDLHQIIKSTQPLSNDHCKYFIFQLLRGLNYLHSANILHRDLK   160
AtMPK7     KLLRHVRHENVIALKDVMLPANRSSFKDVYLVYELMDTDLHQIIKSSQSLSDDHCKYFLFQLLRGLKYLHSANILHRDLK   160
AtMPK14    KLLRHVRHENVISLKDVMLPTHRYSFRDVYLVYELMDSDLNQIIKSSQSLSDDHCKYFLFQLLRGLKYLHSANILHRDLK   160
PtMPK7     KLLRHIRHENVIALKDVLMPIHRTSFKDVYLVYELMDTDLHQIIKSSQPLSSDHCKYFIFQLLRGLNYLHSANILHRDLK   160
PtMPK14    KLLRHIRHENVIALKDVMMPIHRRSFKDVYLVYELMDTDLHQIIKSSQPLSNDHCKYFIFQLLRGLNYLHSANILHRDLK   160
ZmMPK7     KLLRHLRHENVIALKDIMMPIHRRSFKDVYLVYELMDTDLHQIIKSPQGLSNDHCQYFLFQLLRGLEYLHSAEILHRDLK   160
ZmMPK14    KLLRHLRHENVIALKDIMMPIHRRSFKDVYLVYELMDTDLHQIIKSPQGLSNDHCQYFLFQLLRGLKYLHSAEILHRDLK   160

                                          TEY
84KMPK14   PGNLLVNANCDLKICDFGLARTSRGNEQFMTEYVVTRWYRAPELLLCCDNYGTSIDVWSVGCIFAEILGRRPIFPGTECL   240
AtMPK7     PGNLLVNANCDLKICDFGLARTSQGNEQFMTEYVVTRWYRAPELLLCCDNYGTSIDVWSVGCIFAEILGRKPIFPGTECL   240
AtMPK14    PGNLLVNANCDLKICDFGLART...YEQFMTEYVVTRWYRAPELLLCCDNYGTSIDVWSVGCIFAEILGRKPIFPGTECL   237
PtMPK7     PGNLLVNANCDLKICDFGLARTSRGNEQFMTEYVVTRWYRAPELLLCCDNYGTSIDVWSVGCIFAEILGRKPIFPGTECL   240
PtMPK14    PGNLLVNANCDLKICDFGLARTSRGDEQFMTEYVVTRWYRAPELLLCCDNYGTSIDVWSVGCIFAEILGRRPIFPGTECL   240
ZmMPK7     PGNLLVNANCDLKICDFGLARTNSSKGQFMTEYVVTRWYRAPELLLCCDNYGTSIDVWSVGCIFAELLGRKPIFPGTECL   240
ZmMPK14    PGNLLVNANCDLKICDFGLARTNSSKGQFMTEYVVTRWYRAPELLLCCDNYGTSIDVWSVGCIFAELLGRKPIFPGTECL   240

84KMPK14   NQLKLIISVLGSQNDTDLEFIDNPKARRYIKTLPYARGIHFSHLYPHADPLAIDLLLRMLVFDPTKRISVTEALLHPYMS   320
AtMPK7     NQLKLIINVVGSQQESDIRFIDNPKARRFIKSLPYSRGTHLSNLYPQANPLAIDLLQRMLVFDPTKRISVTDALLHPYMA   320
AtMPK14    NQLKLIINVVGSQQDWDLQFIDNQKARRFIKSLPFSKGTHFSHIYPHANPLAIDLLQRMLVFDPTKRISVSDALLHPYME   317
PtMPK7     NQLKLIISVLGSQNDTDLEFIDNPKARRYIKTLPYTRGTHLSHLYPHADPLALDLLQRMLVFDPSKRITVTEALLHPYIS   320
PtMPK14    NQLKLIISVLGSQNDTELEFIDNPKARRYIKTLPYARRIHFSHLYPQADPLAIDLLLRMLVFDPTKRISVTEALLHPYMS   320
ZmMPK7     NQLKLIVNVLGTMSEADLEFIDNPKARRYIKSLPYTPGVPLVSMYPHAHPLAIDLLQKMLIFDPTKRISVTEALEHPYMS   320
ZmMPK14    NQLKLIVNVLGTMSEADLEFIDNPKARRYIKSLPYTPGVPLVSMYPHAHPLAIDLLQKMLIFDPTKRISVTEALEHPYMS   320

                       CD domain
84KMPK14   GLYDPRHDPPARVPVN.LNIDENPGEHVIREMIWDEMLHYHPEVVFANRZ   369
AtMPK7     GLFDPGSNPPAHVPIS.LDIDENMEEPVIREMMWNEMLYYHPEAEISNA.   368
AtMPK14    GLLEPECNPSEHVPVSSLEIDENMEGDMIREMMWEEMLHYLPRA......   361
PtMPK7     GLYDPRRDPPAQVPIN.LDIDENLGEHMIREMIWDEMLHYHPEAVLARR.   368
PtMPK14    GLHDPRHDPPARVPIN.LDIDENLGEHMIREMIWDEMLHYHPEVVFANR.   368
ZmMPK7     PLYDPSANPPAQVPID.LDIDENINSEMIREMMWQEMLHYHPEVATAISM   369
ZmMPK14    PLYDPSANPPAQVPID.LDIDENISSEMIREMMWQEMLHYHPEVATAISM   369
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Figure 3. Alignment of amino acid sequences of 84*KMPK*14 with group C MAPKs in *Populus trichocarpa* and *Arabidopsis thaliana*. Identical amino acid residues are shaded in black, and similar amino acid residues are shaded in gray. TEY, TEY phosphorylation moif; CD domain, atypical conserved common docking domain.

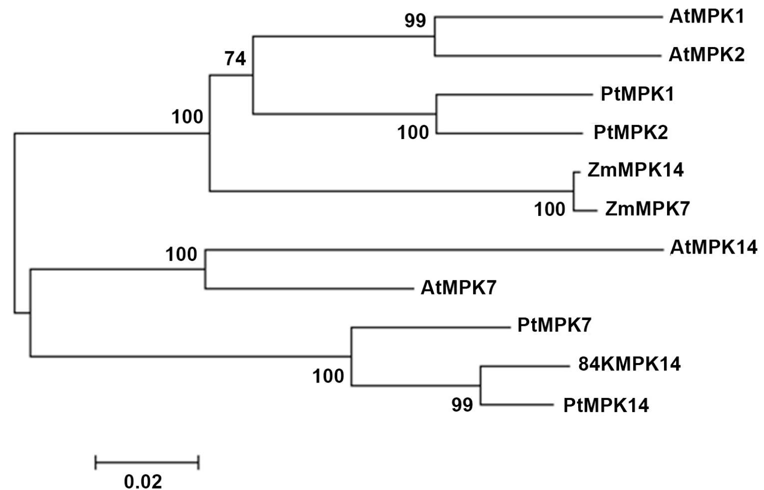

Figure 4. The phylogenetic relationship of 84*KMPK*14 with other plant MAPKs.

3.2. Expression Profile of 84*KMPK*14 Gene

To understand the expression pattern of 84*KMPK*14 gene, qRT-PCR was performed to detect the transcripts in the tissues of 84K poplar. The tissues included young leaves, mature leaves, shoot apex, upper stems, lower stems, and roots. As shown in Figure 5, the 84*KMPK*14 gene transcripts were detected in all of the examined tissues, and were found to be at relatively higher levels in young leaves and roots compared with other tissues. The expression level of 84*KMPK*14 in young leaves is about 3.39 times of that in mature leaves, in which the gene exhibit the lowest expression level. However, the transcripts accumulated in shoot apex, upper stems and lower stems were relatively uniform. These results implied that the 84*KMPK*14 gene is not mainly expressed in young tissues.

3.3. Expression of 84*KMPK*14 in Response to Abiotic Stress

Abiotic stresses such as low temperature, high temperature, drought and high salinity are common unfavorable environmental conditions, which the plants are frequently subjected to. To investigate whether the 84*KMPK*14 gene responses to abiotic stresses, the expression changes in response to a time course 4˚C, 42˚C, PEG and NaCl stress treatments were analyzed using qRT-PCR technique. To exclude other effect such as temperature and light on the gene expression, a time course H_2O_2 treatment in normal condition was used as control (mock treatment). The results showed that the relative expression of 84*KMPK*14 in normal physiological state significantly unstable with time during the mock treatment (Figure 6).

As shown in Figure 6, the 84*KMPK*14 gene showed remarkably expression response to different treatment compared with the corresponding mock control. After 4˚C treatment, the transcript level of the 84*KMPK*14 gene significantly decreased after 1 h treatment, and the minimum expression level appeared at 3 h treatment (Figure 6(a)). The expression level of 84*KMPK*14 under 42˚C stress

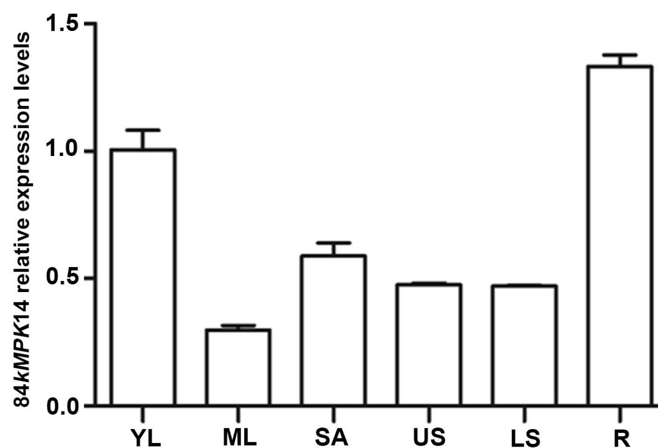

Figure 5. Expression pattern analysis of the 84*KMPK*14 gene in various tissues. YL, young leaves; ML, mature leaves; SA, shoot apex; US, upper stems; LS, lower stems; R, roots.

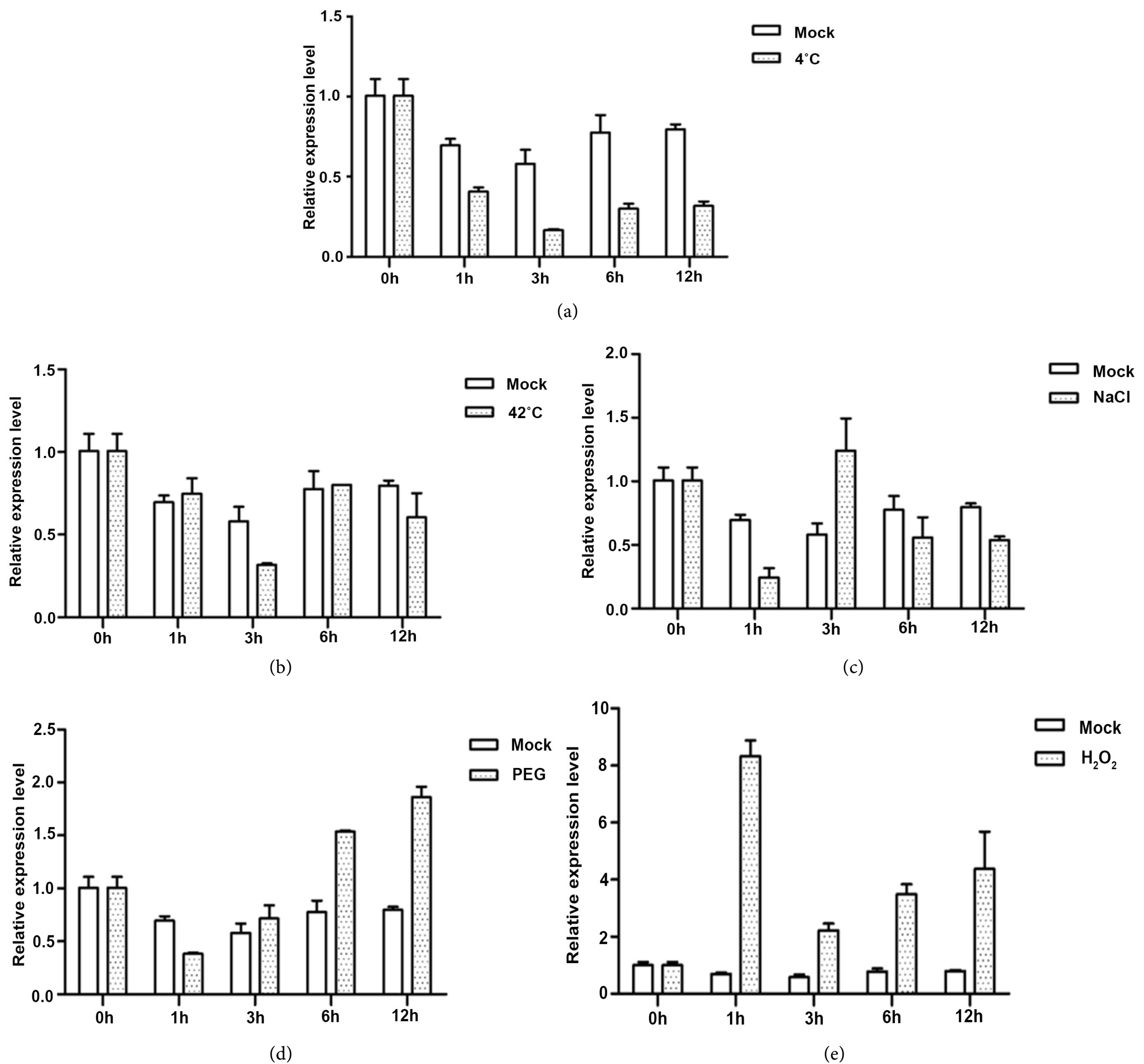

Figure 6. The expression responses of 84*KMPK*14 gene to abiotic stress and H_2O_2 treatment in 84K poplar. All treatments were repeated three times with similar results.

exhibited down-regulated expression only at 3 h treatment, and the expression decreased to 54% of the mock control (Figure 6(b)). When treated with NaCl, the transcripts of 84*KMPK*14 remarkablely decreased at 1 h (35% of the mock control), and then significantly increased and reached to the maximum level at 3 h (2.13-fold of the mock control), and subsequently returned to the mock control level at 6 h (Figure 6(c)). After PEG stress, the expression of 84*KMPK*14 significantly decreased at 1 h treatment, then reverted to the mock level at 3 h treatment, and then, the expression was up-regulate, and reached to the maximum at 12 h (2.33-fold of the mock control) (Figure 6(d)). This findings indicate that the poplar 84*KMPK*14 gene response to 4˚C, 42˚C, NaCl and PEG stress in transcriptional level, and might be a candidate gene underlying these tolerance.

3.4. Expression of 84*KMPK*14 in Response to H_2O_2

Aerobic metabolisms in plant cells continuously produce reactive oxygen species (ROS), and it is in equilibrium in normal conditions. Whereas, biotic stress and abiotic stress could disturbing this balance and exacerbate ROS generation, and as a result, it finally causes oxidative stress in plants [19]. To investigate whether 84*KMPK*14 gene response to oxidative stress, 84K poplars were treated with 50 mM exogenous H_2O_2, then choose shoot apex and used the qRT-PCR to analyze the expression changes in response to a time-course H_2O_2 treatment. The results are shown in **Figure 6(e)**. The expression of 84*KMPK*14 was strong induced at 1 h H_2O_2 treatment, which displayed 12.00-fold of that in mock control. After 3 h of exposure to H_2O_2, the induced expression of 84*KMPK*14 gene decreased to 3.12-fold compared with that in the mock control, and then tended to be increased later. These results prove that the expression of 84*KMPK*14 is regulated by H_2O_2.

4. Discussion

Poplar is one of the main forestation species and commercial tree in the world. The environmental adaptability of poplar is closely related to the timber production. MAPK cascade is a common mode of signal transduction, which can deliver external stimuli to the cells and induce corresponding physiological responses. MAPK gene families in plants are divided into A-D types, and *MPK*14 belongs to C group [16] [17]. In this study, we cloned a new C group MAPK gene, *MPK*14 from 84K poplar. The amino acid sequence alignment showed that *MPK*14 contains typical serine/threonine protein kinase domain and a conserved-TEY-signature in its activation loop (**Figure 2**, **Figure 4**), which is highly conserved in C group MAPKs in plants. The CD domain in MAPKs are known as a docking site for MAPKKs, phosphatases, and protein substrates [7]. Meanwhile, the corresponding modified CD domains in group C *MPK*s is considered as the probable specific interaction site with MKK3 in Arabidopsis [8]. In this study, we found that there are 4 modified residues in the CD domains of 84*KMPK*14 compared with that in *PtMPK*14, indicating that the specific interaction of *MPK*14 with MAPKK in the two poplar tree might be discrepant, and it is to be demonstrated by further experiments.

The previous study [12] proposed that the transgenic *Nicotina benthamiana* overexpressing *GhMPK*7 showed obvious phenotypic alterations that germinated earlier and grew faster than the wild-type plants, and these proved that C group MAPKs play an important role in regulating plant growth and development. In this study, the organ-specific expression profile analysis showed that the expression of 84*KMPK*14 was the highest in roots compared with other organs detected in this study (**Figure 5**). This is consist with *PtMPK*14 reported previously [17], and we consider that *MPK*14 in poplar might have important biological roles in roots. The expression of 84*KMPK*14 was almost preferentially expressed in young tissues (**Figure 5**), indicating that 84*KMPK*14 might be in-

volved in the growth and development of poplar. Interestingly, except for roots, we found that the transcription level of 84*KMPK*14 was in the order of young leaves > shoot apex > upper stems, while the expression of *PtMPK*14 in these organs of *Populus trichocarpa* was the exact opposite [17], so, we speculate that an discrepancy might be exist in the developmental function of *MPK*14 between the two poplar species. However, the function of 84*KMPK*14 in poplar growth and development remains to be further investigated.

When carrying out the stress response experiments, the expression of 84*KMPK*14 in 84K seedlings incubated under normal conditions was taken into consideration. The result showed that the transcription of 84*KMPK*14 is unstable during the processing time (1 - 12 h) (**Figure 5**), suggesting a rhythmic expression of 84*KMPK*14 might be existed. Rhythmic expression of Arabidopsis *MPK*7 has been reported On the contrary, no rhythmic expression of *ZmMPK*7 in maize was also been attested [11]. These results give a hint that rhythmic response of group C MAPK genes is differentiated across species and homologue genes.

Several studies have reported that the group C MAPKs response to abiotic stress in several plants [9]-[14] [16]. In this study, the transcription kinetics analysis of 84*KMPK*14 under environmental stress showed that 84*KMPK*14 could be inhibited by low/high temperature, while the transcription level of 84*KMPK*14 dropped first and rose later after drought and high salinity treatment (**Figure 6**). To our interest, different responsive modes to several abiotic stress of group C MAPKs were reported. For example, *OsMPK*14 could be induced by salt, and be inhibited by drought in rice, but low temperature could only activate its expression after 6 h treatment [20]; *ZmMPK*7 was detected no obviously response to PEG or 4˚C in maize seedlings [20]; moreover, *GhMPK*7 accumulated after NaCl treatment, but it showed a negligible response to low/high temperature and PEG [12]. Thus, we deduce that the specific abiotic stress response of group C MAPK genes is not ubiquitous across homologues though they share high sequence similarity, and the abiotic stress responses between orthologs of *MPK*14 are differentiated at least between monocotyledonous and dicotyledonous plants.

Abiotic stress processes, such as drought, heat, salinity, are often associated with the production of ROS, which primarily act as signal transduction molecules in the abiotic stress-response signal transduction network, and then are also toxic byproducts of stress metabolism, which causes oxidative stress leading to cellular damage and ultimately cell death [21] [22]. To date, studies have shown that several group A and B MAPKs are involved in the cross-talk between stresses and hormones [21]. In addition to this, group C MAPKs are also reported to be associated with oxidative stress. The induced accumulation expression of *ZmMPK*7 and *GhMPK*7 was also detected in response to H_2O_2 [11] [12], which is consistent with our results (**Figure 6**). Genetic studies had proved the evidence that tomato *MPK*1/2 may be involved in EBR-induced apoplastic H_2O_2 [14]. NADPH oxidase-produced H_2O_2 regulated *MPK*1/2 activation during ac-

climation apoplastic H_2O_2 and *RBOH*1-mediated *MPK*1/2 activation plays a critical role in induction of cross-acclimation to abiotic stresses in tomato plants [22]. In addition, *ZmMPK*7 in maize is responsible for the removal of reactive oxygen species [11]. Therefore, it is reasonable to speculate that 84*KMPK*14 might also be involved in the oxidative tolerance, and this might be a way by which 84*KMPK*14 participated in several abiotic stress resistance in 84K poplar.But, the mechanism of 84*KMPK*14 in response to abiotic and oxidative tolerance in poplar needs to be further studied through transgenic technology.

84K poplar is a variety of white poplar and it has the advantages of easy to grow roots, fast growing in seedling and young tree stage, good material quality and wide adaptability. Thus, it is valuable to study the adaptable biological mechanism in 84K poplar. Based on the discussion above, the biological function of 84*KMPK*14 in 84K poplar is worthy to be explored in depth through genetics means.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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