Cloning and Expression Analysis of a Mitogen-Activated Protein Kinase Gene OsMPK14 from Rice

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Abstract: Mitogen activated-protein kinases (MAPKs) are important components in signal transduction pathways responding to various biotic and abiotic stresses. An MAPK gene OsMPK14 (GenBank Accession No. GQ265780) from rice (Oryza sativa L.) was cloned by RT-PCR. The full-length cDNA of OsMPK14 consists of 1660 bp in size, containing an open reading frame of 1629 bp, which encodes a 542-amino-acid polypeptide and has a typical protein kinase domain and a phosphorylation activation motif TDY. Sequence alignment and analysis revealed that OsMPK14 is located on rice chromosome 5, and composes of nine exons and eight introns in the coding region. Semi-quantitative RT-PCR was performed to detect its expression patterns in rice shoots and roots treated under darkness, drought, high salinity, low temperature and abscisic acid treatments. The OsMPK14 mRNA was induced by abscisic acid, low temperature and high salinity, but weakly inhibited by drought. Light could up-regulate its expression in roots, but down-regulate in shoots. The results indicate that OsMPK14 could be implicated in diverse rice stimuli-responsive signaling cascades, and its expression might be regulated by multiple factors.

Key words: rice; mitogen-activated protein kinases gene; gene clone; abiotic stress; expression analysis

Mitogen-activated protein kinase (MAPK) is one of the largest and most important kinases, which has been identified in both unicellular and multicellular eukaryotes. As the terminal component of the MAPK cascade, MAPK is activated by its upstream MAP kinase kinase (MAPKK) and MAP kinase kinase kinase (MAPKKK). The activated MAPK can then phosphorylate a substrate, resulting in signal amplification. Accordingly, these three kinases are functionally interlinked and have been found to play a central role in signal transduction mechanisms of many different eukaryotic organisms.

The MAPK was first discovered in 1986 from animal cells, later this kinase was found to be related to a set of proteins that are phosphorylated at tyrosine residue in response to mitogens. After that, more and more research indicated that MAPK could also mediate the transduction of hormone, environmental stress and cell differentiation (Liu et al, 2000). In plants, MAP kinase genes were first reported from pea in 1993 (Stafstrom et al, 1993). Plant MAPK cascade involved complicated crosstalks with other signaling pathways and played crucial roles in signal transduction of extracellular stimuli in plants. After integrating various extracellular and intracellular signals, plants can control varieties of stress responses, defense responses, as well as hormone regulation accurately through MAPK cascade (Gustin et al, 1998; Petersen et al, 2000).

Because rice is a major crop and important model organism, the research on rice MAPK received increasing attention. By bioinformatic analysis of the rice genome, several genes encoding putative MAPK were identified. So far, some studies showed that rice MAPK was related to the development and growth, and could be induced by a variety of biotic and abiotic stresses as well (Agrawal et al, 2003b; Reyna and Yang, 2006). Previously, we cloned a cDNA fragment of potential OsMPK14 gene from rice panicles by cDNA library screening, named as OsRP19 (GenBank Accession No. FJ919600). In this study, the full-length cDNA coding region of OsMPK14 was cloned by RT-PCR, and its expression patterns were analyzed when treated with light, drought, high salinity, low temperature and abscisic acid (ABA) using the semi-quantitative RT-PCR technique, in order to lay the foundation for further studying the physiological function of OsMPK14 in rice growth and stress.

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response.

MATERIALS AND METHODS

Plant materials

Rice (Oryza sativa L.) cultivar ‘Huangjinqing’ was kept by our laboratory. Shoots and roots at the one-leaf, two-leaf stage and three-leaf stages were collected for RT-PCR analysis.

Enzymes and reagents

Tag DNA polymerase, dNTPs, TRNzol total RNA reagent, M-MLV reverse transcriptase and DNA marker were purchased from Tiangen Biotech Co., Beijing, China; DNA Gel Extraction Kit were bought from Sangon Biotech Co., Shanghai, China; and DNA sequencing was determined by Shanghai Invitrogen Biotechnology Co., Shanghai, China.

Rice materials treatment

After disinfection, soak and pregermination, rice seeds were grown in two plastic vessels containing water. Then one was cultivated in a darkroom at 28°C, the other in a HP300GS-C artificial climate chamber at 28°C with light intensity of 16 000 lux, under a 16 h light/8 h dark period. Collected shoots and roots at the one-leaf, two-leaf and three-leaf stages, respectively and froze them in liquid nitrogen and stored at -80°C.

Rice seedlings at trefoil period were used for treatment with 1%Nacl, 30% PEG6000, 4°C and 100 µmol•L-1 ABA, respectively. Collected shoots and roots of rice seedlings treated after 0h, 1h, 6h and 12h. Froze them in liquid nitrogen and stored at -80°C. You can find the detailed method according to Cao et al (2003).

Sequence prediction and analysis

Submitted the cDNA fragment OsRP19 obtained by cDNA library screening into GenBank database to predict and splice out the full-length cDNA sequence. Analyzed the deduced protein by BlastP and searched its conserved domain by the network Conserved Domains. Chromosome location was determined by searching the full-length cDNA in rice genome database, and then gene structure was analyzed by the biological software Genetyx.

Cloning cDNA encoding region of OsMPK14 gene

Total RNA was isolated from rice panicles with TRNzol reagent. Following the manufacturer’s protocol, cDNA was synthesized from 3 µg total RNAs with M-MLV reverse transcriptase using oligo(dT)15 as primer. Two primers, used for PCR amplification of the OsMPK14 cDNA, were designed and synthesized according to the open reading frame of deduced OsMPK14 gene, which were P1 (5' -CGACACCTCTGAGATGGATT-3') and P2 (5' -TGGTGCGCTGCAGAAATTTA-3'). PCR was performed as follows: the reaction mixture was first pre-denatured at 94°C for 3 min; then in each cycle denaturation was at 94°C for 45 s, annealing at 57°C for 30 s and extension at 72°C for 2 min, involving a total of 30 cycles followed by 72°C for 5 min. Extracted the bands of interest from gel using Nucleo Trap Gel Extraction Kit, and cloned the purified products into a pGM-T vector, then transferred to a biotechnology company to sequence.

Semi-quantitative RT-PCR analysis of OsMPK14

Using the method described above, we obtained the cDNA of samples treated with NaCl, PEG6000, 4°C and ABA, respectively. The rice Actin gene OsAct1 was used to normalize samples. The primers of OsAct1 were P3 (5' -CATGCTATCCCTCGTCTCGACCT-3') and P4 (5' -CGCACTTCATGATGGAGTTGTTA-3'). The PCR was carried out as follows: pre-denaturation at 94°C for 3 min; then 25 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min; and the final extension at 72°C for 7 min (Liang et al, 2008). The primers of OsMPK14 were P5 (5’-AGCCTTCTGCTCAACCTATC-3') and P6 (5’-CACCCTTTACCCCTGCT-3'). The thermocycler program had an initial 94°C denaturation step followed by 30 cycles consisting of denaturation at 94°C for 45 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s, and then with a final extension at 72°C for 5 min. PCR products were electrophoresed on 1.5% agarose gel. The expression intensity of OsMPK14 and OsAct1 was quantified with the Bandleader software. Then the expression tendency could be found after calculating
the results of OsMPK14/OsAct1 in the EXCEL software.

RESULTS

Prediction and analysis of OsMPK14

The results showed that a cDNA fragment OsRP19 had high homology with a cDNA sequence numbered AK063944.1. Thus, we spliced out a sequence including 2221 bp nucleotides. Translating the sequence into corresponding amino acid polypeptide, we found they were the same with OsMPK14 forecasted by bioinformation. Apparently, the cDNA fragment OsRP19 is OsMPK14.

Cloning the cDNA encoding region of OsMPK14

Using the RT-PCR method, we cloned an integral cDNA encoding region of OsMPK14 (Fig. 1). The full-length cDNA of OsMPK14 consisted of 1660 bp in size, containing an open reading frame of 1629 bp, which encodes 542 amino acid polypeptide (Fig. 2-A). The results of protein conserved domain search suggested that amino acids from 12 to 304 were corresponded with a typical serine/threonine protein kinase domain (Fig. 2-B). Sequence alignment and analysis revealed that OsMPK14 is located on chromosome 5, composed of 9 exons and 8 introns in the coding region (Fig. 2-C).

Relationship between light and expression of OsMPK14

The results of semi-quantitative RT-PCR showed that OsMPK14 could be expressed both in rice shoots and roots, but the quantity was different (Fig. 3). The expression in shoots of green seedlings declined gradually as seedlings grew, whereas the expression in shoots of yellow seedlings was opposite. Tendency of OsMPK14 expressed in roots of green and yellow seedlings was similar. They all presented fluctuation, but the expression in roots of yellow seedlings was lower than that of green seedlings at the same period. All of these suggest that light maybe controls the expression of OsMPK14 negatively in shoots, but positively in roots of rice.

Expression of OsMPK14 in response to abiotic stress

Semi-quantitative RT-PCR was used to analyze the expression patterns of OsMPK14 in response to high salt, PEG6000 and low temperature treatments. OsMPK14 was induced highly in rice shoots and roots at 6 h and 12 h after treatment with salt (Fig. 4-A). The expressions of OsMPK14 in rice shoots and roots treated with PEG6000 were fluctuant and indistinctive, but after 12 h treatment, the expressions were lower than 0 h treatment (Fig. 4-B). Interestingly, the expression of OsMPK14 in shoots declined 1 h after treatment with low temperature, and then, ascended 6 h and 12 h after treatment. However, the expression of OsMPK14 in roots treated with low temperature had the feature of steady increase (Fig. 4-C). Thus, we can conclude that OsMPK14 can be induced by salt in rice shoots and roots, but low temperature can only activate its expression after 6 h treatment, and drought inhibits its expression.
Fig. 2. The basic sequence information of rice OsMPK14 gene
A, The nucleotides and deduced amino acid sequences of rice OsMPK14 cDNA. The positions of the nucleotides and amino acids are shown on the left and the right, respectively. The start codon and termination codon are in bold, and the possible TDY phosphorylation site is marked with shadow. The primers P1/P2 and P5/P6 are underlined and defined by double, single line, respectively. B, The protein conserved domain search results of OsMPK14. The positions of amino acids are shown on the top. C, The gene structure of OsMPK14. The exons and introns are indicated by black boxes and lines, respectively. The corresponding figures show the size of them.
Expression of OsMPK14 in response to abscisic acid

The result of semi-quantitative RT-PCR showed that the expression of OsMPK14 in rice shoots and roots increased after treatment with ABA. So, OsMPK14 can also be induced by ABA (Fig. 4-D).

DISCUSSION

As important components in signal transduction pathways, MAPKs play crucial role in responding to various signals. Recently, a total of 17 rice genes encoding MAP kinases have been identified from the rice genome and they were divided into six groups (A, B, C, D, E and F) (Reyna and Yang, 2006). To date, 9 of the 17 rice genes have been cloned (He et al, 1999; Agrawal et al, 2002; Fu et al, 2002; Huang et al, 2002; Song and Goodman, 2002; Wen et al, 2002; Agrawal et al, 2003a; Ning et al, 2006; Song et al, 2006; Rohila and Yang, 2007; Yuan et al, 2007; Lee et al, 2008). Studies have shown that 11 members of rice MAPK family were associated with biotic or abiotic (or both) stress responses (Zhang and Klessig, 2001; Agrawal et al, 2003c; Xiong et al, 2003; Jeong et al, 2006). However, relatively little work has been done to clone OsMPK14 gene and to identify its function in rice growth and abiotic stress responses. Therefore, we cloned and analyzed the entire encoding region of OsMPK14 gene. Results indicated that the OsMPK14 gene encoded 542 amino acid polypeptides and had the typical protein kinase domain and phosphorylation activation motif TDY. OsMPK14 is located on rice chromosome 5, composed of 9 exons and 8 introns in the coding region. Previous studies displayed that members of rice MAPK family were mainly located on rice chromosomes 1, 5 and 6, but different in the number of exons, ranging from 2 to 12 (Liu and Xue, 2007). According to the phylogenetic relationship of the 17 rice MAPKs, OsMPK14 and OsMPK13 have the highest homology, both belonging to the E group (Reyna and Yang, 2006).

Drought, high salinity and low temperature are the stresses that plants often suffer from. OsMPK1, OsMPK4, OsMPK5, OsMPK7, OsMPK8 and OsMPK12 are all involved in the response of these three signals and the transference of hormone ABA which most directly related to stress response (Agrawal et al, 2002, 2003a, 2003c; Huang et al, 2002; Jeong et al, 2006; Reyna and Yang, 2006; Lee et al, 2008). In this study, we surveyed the expression patterns of OsMPK14 in response to drought, high salt, low temperature and ABA in rice shoots and roots. High salt and ABA were found to induce the expression of OsMPK14, but drought could inhibit its expression slightly. Low temperature could also activate OsMPK14 at 6 h after treatment. Thus, OsMPK14 responds to high salt, low temperature and ABA in rice. Some studies have shown that the expression of OsMPK4 was induced by ABA and salt, but drought and low temperature down-regulated its
basal mRNA level (Agrawal et al, 2003a). The expression patterns of OsMPK14 are similar to OsMPK4, except that low temperature can induce the expression of OsMPK14. OsMPK4 is involved in the growth of rice for it exhibits increased expression at late developmental stages in leaves, roots and panicles of rice plants (Fu et al, 2002; Agrawal et al, 2003a). OsMPK14 was just cloned from rice panicles. Whether there are relationships between OsMPK14 and the development of rice panicles needs further research.

In the study, we found that though after treatment with NaCl, PEG6000 and low temperature, the expression of OsMPK14 was obvious, the quantity was fluctuant at different treatment time points. This phenomenon was visible in the expression of other members of rice MAPK family (Agrawal et al, 2002, 2003c, 2008; Ning et al, 2007). Whether it suggests MAPKs have the similar regulatory mechanism in response to various stresses is still unknown. In view of the complexity and importance of MAPK signal transduction cascade, the regulation of the expression of MAPK gene needs to be further explored.

OsMPK14 was inducible in rice shoots and roots before the three-leaf period, but the quantity of expression changed as rice grew, which indicates that the expression of OsMPK14 is strictly regulated by developmental stage. The contrast between light and dark treatment showed that light controlled the expression of OsMPK14 negatively in rice shoots, but positively in roots. To date, the relationship between MAPK and rice light signaling pathway has not been reported. It is worth in-depth study.

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