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Research article

Molecular characterization of *RsMPK2*, a C1 subgroup mitogen-activated protein kinase in the desert plant *Reaumuria soongorica*

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ABSTRACT

Reaumuria soongorica (Pall.) Maxim. is a short woody shrub widely found in semi-arid areas of China, and can survive severe environmental stresses. To understand its potential signaling transduction pathway in stress tolerance, we investigated the participation of mitogen-activated protein kinases (MAPKs) as possible mediators of abiotic stresses. A novel MAP kinase cDNA (*RsMPK2*) that encodes a 374 amino acid protein was isolated from *R. soongorica*. *RsMPK2* belongs to the C1 subgroup, which is still functionally uncharacterized compared to groups A and B; and contains all 11 of the conserved MAPK subdomains and the TEY phosphorylation motif. *RsMPK2* is expressed in vegetative (root, stem, leaf and callus) and reproductive (flower) organs. The transcripts of *RsMPK2* were rapidly accumulated at high levels when *R. soongorica* was subjected to dehydration, salinity conditions and treatment with abscisic acid or hydrogen peroxide. Growth analysis of *Escherichia coli* (srl::Tn10) cells transformed with pPROEXHT-*RsMPK2* showed that the expression products of *RsMPK2* do not act as an osmoprotectant. But, the inhibition of *RsMPK2* expression by the inhibitor U0126 induced a decrease of antioxidant enzyme activity under stresses, indicating that *RsMPK2* is involved in the regulation of the antioxidant defense system in the response to stress signaling.

1. Introduction

Plant mitogen-activated protein kinase (MAPK) cascades play an important role in mediating biotic and abiotic stress responses, and is a major pathway through which extracellular stimuli are transduced into intracellular signalling. MAPKs have been identified and classified into four major groups (A–D) [17]; among them, groups A and B have been extensively studied, whereas there is limited functional information on groups C and D to date. The first report of group C function demonstrated that the activation of *Arabidopsis* C1 subgroup MAPKs (*AtMPK1/AtMPK2*) might be under the control of signals involved in different types of stresses [20]. Studies on *Gossypium hirsutum* MAPK (*GhMAPK*), which has a high degree of identity with plant group-C MAPKs, show that it may also play an important role in response to environmental stresses [24]. Ortiz-Masia et al. [21] demonstrated that the first C1 subgroup MAP kinase from pea (*Pisum sativum* L.), *PsMPK2*, plays a role in response to mechanical injury and other stress signals such as abscisic acid, jasmonic acid and hydrogen peroxide. A recent article by Zong et al. [31] provides evidence that *ZmMPK7*, a novel group C MAPK gene from *Zea mays*, which is induced by abscisic acid and hydrogen peroxide, is responsible for the removal of reactive oxygen species. These findings indicate that the C group of MAPKs may be involved in the response to stress.

Reaumuria soongorica (Pall.) is a desiccation-tolerant perennial semi-shrub that is exposed to multiple environmental stress conditions, including low water availability, extreme temperature fluctuations, high irradiance and nutrient deprivation. The desiccation-tolerant traits of *R. soongorica* have recently been investigated at the physiological level [14–16]), and qualified *R. soongorica* as a resurrection plant since it is dormant during desiccation. Because of their long life span, trees may have evolved adaptive strategies differing from those of annual plants, to survive long periods of stress; consequently, there is an interest in studying stress resistance in shrubs. *R. soongorica* is one of the most promising plants for such studies. To investigate the signaling transduction of stress tolerance in *R. soongorica*, we isolated a C1 subgroup MAPKs gene through

Abbreviations: MAPKs, mitogen-activated protein kinases.

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rapid amplification of cDNA ends (RACE) cloning. Our study of the regulation of this gene's transcripts in response to diverse stress signals suggests that the C1 subgroup gene *RsMPK2* may be involved in the plant defense response to environmental stress. Further experiments show that *RsMPK2* does not provide osmo-protection but involved in the activation of the activities of antioxidant enzymes.

2. Materials and methods

2.1. Plant material and treatments

Seeds of *R. soongorica* were obtained from the northern foothills of Lanzhou City, Gansu, China (36°17′N, 103°48′ E, 1700–1900 m a.s.l.) and were planted in individual 9-L plastic pots containing soil. The pots were placed in the experimental field of the Botanical Garden of Lanzhou University. In the second year, the regenerated plants were used for experiments. For drought treatment, plants were dehydrated by withholding water at air temperature and ambient photoperiod in July. For other treatments, plants were excised at the base of the stem and placed in the distilled water for 1 h to eliminate wound stress (according to [28]). After treatment, the cut ends of the stems were placed in beakers wrapped with aluminum foil containing either 100 μM ABA, 10 mM H_2O_2 or 200 mM NaCl solution for 24 h at 25 °C; the stems were exposed to continuous light at an intensity of 200 mmol m^{-2} s^{-1}. To study the effect of MAPKK inhibition on the expression of the gene, the detached plants were pretreated with 10 mM U0126 (Sigma–Aldrich), for 8 h and then exposed to either 100 μM ABA, 10 mM H_2O_2, or 10% PEG (PEG 6000) treatment for 12 h, under the same conditions as described above. The detached plants were treated with distilled water under the same conditions for the whole period and served as controls for the above. After the respective treatments, the leaves of detached plants were harvested at different times and immediately frozen in liquid N_2 until further analysis.

2.2. Cloning of RsMPK2 cDNA from R. soongorica

The degenerate primers P1 and P2 (Table 1) were designed for amplifying *RsMPK2* based on the conserved domains of the C1 subgroup MAPKs of various organisms (Fig. 1). The two primers were used to amplify a cDNA fragment that was reverse transcribed from total RNA samples extracted from *R. soongorica* leaves by the CTAB method [13]. The RT-PCR product, which was about 550 bp, was gel-purified and cloned into the pGEM-T vector (Promega, USA) for sequencing on an automated DNA sequencer (Prism 377, Applied Biosystems). The full-length cDNA was amplified by RACE technology

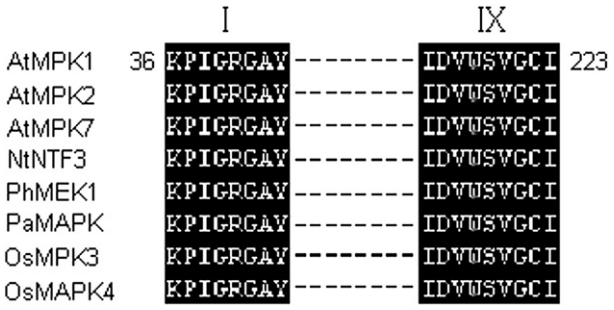

Fig. 1. Consensus amino acid sequences of MAPK used for degenerated primers design.

(RNA ligase-mediated rapid amplification of 5′ and 3′ cDNA end), according to the user manual of the GeneRacer kit (Invitrogen). Total RNA was first treated with calf intestinal phosphatase (CIP) to remove the 5′ phosphates and then with tobacco acid pyrophosphatase (TAP) to remove the 5′ cap structure from the intact, full-length mRNA. The GeneRacer RNA Oligo was ligated to the 5′ end of the mRNA using T4 RNA ligase and reverse transcribed the ligated mRNA using Cloned AMV RT and the GeneRacer Oligo dT Primer to create RACE-ready first-strand cDNA with known priming sites at the 5′ and 3′ ends. To obtain the 5′ end, the first-strand cDNA was amplified using the two nested reverse gene-specific primers 5RP1 and 5RP2, and the GeneRacer 5′ nested end primers 5FP1 and 5FP2. To obtain the 3′ end, the first-strand cDNA was amplified using the two nested forward gene-specific primers 3FP1 and 3FP2, and the GeneRacer 3′ primer 3RP. The RACE PCR products were purified using S.N.A.P.™ columns and subcloned into Pcr4Blunt-TOPO vector for sequencing.

By comparing and aligning the sequences of 3′RACE, 5′ RACE and the middle region products, the full-length cDNA sequence of *RsMPK2* was obtained. This sequence was subsequently amplified via PCR using the P3 and P4 primers, and sequenced. The PCR amplification for generating the full-length *RsMPK2* was repeated three times. The full-length *RsMPK2* was subsequently analyzed for molecular characterization, i.e., the presence of conserved motifs, sequence homology, and the secondary structure were determined.

2.3. Semi-quantitative RT-PCR analysis

To investigate the tissue-specific expression pattern of *RsMPK2*, and the expression patterns of *RsMPK2* under abiotic stresses, total RNA was extracted from the different organs (root, stem, leaf, callus and flower) of 2-year-old plants by the CTAB method and used in RT-PCR analysis. The RNA samples were quantified spectrophotometrically at 260 nm

Table 1
The primers used in this study.

Abbreviation	Sequence (5′-3′)	Description
P1	AT(C/T) GG(C/T) (A/C)(A/G)I GG(A/C/T) GCI TA(C/T) GG	Degenerate primer, Forward
P2	AT(A/G) CA(A/G) CCI GAC (A/G/T)GA CCA IAC (A/G)TC	Degenerate primer, Reverse
5RP1	CTAAGATGCCGAAGCAGTTTCAAT	Reverse primer for 5 RACE, outer
5RP2	AATTCGCGCAACGTCCTCAA	Reverse primer for 5 RACE, Nested
5FP1	CGACTGGAGCACGAGGACAC	Forward primer for 5 RACE, outer
5FP2	TGGAGCACGAGGACACTGACAT	Forward primer for 5 RACE, Nested
3FP1	: ATTGCCAGTATTTCCTGTTCCAGT	Forward primer for 3 RACE, outer
3FP2	GTTGGTATCGAGCCCCAGAGC	Forward primer for 3 RACE, Nested
3RP	GCTGTCAACGATACGCTACGTAACG	GeneRacer 3′ reverse primer
P3	CAAACCCGGGGAAGCATTACT	Gene specific primer, Forward
P4	GCTTCAGGGTGGTAGTGGAGAATC	Gene specific primer, Reverse
P5	CGATGCCAAATATGTGCCCATTA	Gene specific primer, Forward
P6	CAGCTTCAG GGTGGTAGTGGAGAA	Gene specific primer, Reverse
18SF	ATGATAACTCGACGGATCGC	18S gene specific primer, Forward
18SR	CTTGGATGTGGTAGCC GTTT	18S gene specific primer, Reverse
P7	ACGCGTCGACATGGCGACTCCTATC GAACCTC	Gene specific primer, Forward, SalI site underlined
P8	CGGGATCCCAAGGGAGATTTATTGTTGTGAGA	Gene specific primer, Reverse, BamHI site underlined