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Research article

Molecular characterization of a potato MAP kinase transcriptionally regulated by multiple environmental stresses

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Abstract

The MAPK cascade is an evolutionary conserved signaling pathway that links external stimuli with cellular responses. Using polymerase chain reaction (PCR), a DNA fragment corresponding to a *Solanum tuberosum* MAPK, StMPK1, was isolated. StMPK1 amino acid sequence displayed over 90% identity with tomato MPK1 (LeMPK1) and tobacco SIPK. Southern blot analysis indicated that the gene encoding StMPK1 is present in a single copy in the potato genome. StMPK1 mRNA levels differentially accumulated in potato tuber in response to wounding and to wounding plus *Fusarium solani* f. sp. *eumartii*. Transcript accumulation after infection was transient and started earlier than what was observed in wounded tubers. StMPK1 mRNA levels also increased in potato tuber after 24 h of treatment with jasmonic acid (JA) and abscicic acid (ABA), but not in response to ethylene or salicylic acid. In addition, StMPK1 transcript levels increased after a heat-shock treatment at 42 °C. The results suggest that StMPK1 may participate in the cellular responses against multiple environmental stimuli in potato tubers.

Keywords: Abscicic acid; Fungal infection; *Fusarium eumartii*; Heat shock; Jasmonic acid; MAP kinase, *Solanum tubersoum*; Wounding

1. Introduction

Plant defense responses to environmental stresses involve activation of complex and intricate networks of signaling pathways. One of these pathways is the MAPK (mitogen activated protein kinase) cascade. This signaling module links external stimuli with several cellular responses and is evolutionary conserved among eukaryotic organisms [13,24]. The last component of this cascade, a MAPK, is a serine/threonine kinase activated by a dual phosphorylation of threonine and tyrosine residues of a TXY motif. These phosphorylations are performed by a MAPK kinase (MAPKK), which is in turn activated by an upstream MAKK kinase (MAPKKK).

In the last decade, an increasing number of plant MAPKs have been isolated and characterized in dicotyledonous, as well as in monocotyledonous species [1,11,23,24]. Twenty-three MAPKs have been identified in the *Arabidopsis thaliana* genome and classified according to their sequence homology into four major groups, from A to D [12,13]. Groups A–C contain the phosphorylation motif TEY whereas family D contains the motif TDY.

Plant MAPKs can be regulated at mRNA, protein or enzymatic activity levels in response to several abiotic and biotic stresses, plant hormones, and cell division [4,13,29]. The group A of MAPKs has been implicated in response to oxidative stress, cold, drought, wounding, as well as fungal and bacterial elicitors. AtMPK3 and AtMPK6 are representative members of group A in Arabidopsis [2,15,19]. Two MAPKs closely related to AtMPK3 and AtMPK6, the tobacco WIPK (wounding-induced protein kinase) and SIPK (salicylic-induced protein kinase), had been previously reported as activated in response to wounding and salicylic acid, respectively [23,32]. Both MAPKs are also activated in tobacco plants in response to pathogen stimuli, including *Phytophthora infestans* elicitin and tobacco mosaic virus [27,31]. Later on, Holley et al. [11] isolated three MAPKs from tomato plants and charac-

Abbreviations: ABA, absicic acid; JA, jasmonic acid; MAPK, mitogenic activated protein kinase; StMPK1, *Solanum tuberosum* mitogen activated protein kinase.

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terized their inducibility by different elicitors and stress conditions. Two of them, LeMPK1 and LeMPK2, are activated by UV-B radiation, systemin and oligosaccharides elicitors, whereas LeMPK3 is only activated by UV-B radiation. More recently, Mayrose et al. [17] reported that LeMPK3 is specifically induced at mRNA level in resistant plants infected with avirulent strains of *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato*. LeMPK3 transcripts are also rapid and transiently accumulated in response to mechanical stress and wounding.

In the past years, our laboratory has been investigating the interaction between a commercial potato cultivar, *Solanum tuberosum* L. sp. *tuberosum* cv Spunta (*S. tuberosum*) and the pathogenic fungus *Fusarium solani* f. sp. *eumartii* (*F. eumartii*), which causes significant looses during production and commercialization of potato tubers in Argentina, south of Brazil, North America and Europe [21]. Previous results obtained by our group showed an increment in the phosphorylation of myelin basic protein by potato protein extracts in response to elicitors derived from *P. infestans* and *F. solani* f. sp. *eumartii* (*F. eumartii*). The activity was calcium independent, suggesting that MAPKs might be involved in the responses to these pathogens (F.A. Blanco, 2001. PhD Thesis, University of Mar del Plata, Argentina). Also in potato, Katou et al. [14] showed that a MAPKK, StMEK1, is activated by elicitors derived from *P. infestans*. Expression of a constitutively-active form of StMEK1 in *Nicotiana benthamiana* leaves activated WIPK and SIPK kinases, and induced hypersensitive response and accumulation of defense response transcripts [14]. Nevertheless, the evidence of involvement of MAPKs in defense-related signaling pathways in potato is still very limited. In this study, we present the cloning of a cDNA fragment and expression characterization of a potato MAPK, whose transcripts showed differential accumulation in potato tubers in response to several stress conditions, including fungal infection, wounding and heat shock, as well as phytohormone treatments.

2. Results

2.1. Isolation and sequence analysis of StMPK1 clone

A partial cDNA clone encoding a potato MAPK was obtained using a polymerase chain reaction (PCR) approach. Two degenerated primers that expand from 500 to 1000 bp of the coding region of selected MAPKs (see Section 4) were designed. To increase the specificity of the primers, the region corresponding to the conserved catalytic regions of Ser/Thr kinases was excluded. These primers were used in a PCR amplification reaction with a cDNA library of potato tubers (*S. tuberosum* cv Spunta) infected with *F. eumartii* for 24 h as template [8]. The amplified fragment was cloned, sequenced and compared with potato TIGR's *Solanum tuberosum* Gene Index (TIGR's StGI) database using the BLASTN program. The 500 bp nucleotide sequence was 98% identical to an EST sequence (EST ID: NP461062 and GenBank accession no. AB062138) annotated as StMPK1, which was publicly released during the course of this work. Another three potato MAPKs were annotated as StMPK2, StMPK3 and StMPK4 (GenBank accession nos. AB062139, AB062140 and AB062141, respectively). The 500 bp nucleotide sequence isolated here displayed 89% of identity with the nucleotide sequence of StMPK2, but only 64% and 65% of identity with the nucleotide sequences of StMPK3 and StMPK4, respectively.

The StMPK1 full length cDNA and deduced amino acid sequences are presented in Fig. 1. StMPK1 mRNA is 1485 nt in length and contains a 5′- untranslated region (UTR) of 44 nt, an open reading frame (ORF) of 1191 nt and a 3′-UTR of 251 nt. The ORF encodes a predicted polypeptide of 396 amino acids and a molecular mass of 47.2 kDa. The predicted amino acid sequence contains the catalytic domain conserved among all Ser/Thr kinases (Smart PSSMId: 17776) and a TEY domain characteristic of MAPKs of group A–C. A phylogenetic analysis of the amino acid sequences of cloned plant MAPKs showed that StMPK1 is closely related to MAPKs in group A (Fig. 2A). It displayed 97% of identity to *Lycopersicon esculentum* MPK1 (LeMPK1), 93% to tobacco salicylic acid-induced protein kinase (NtSIPK), 89% to *Oryza sativa* MAPK6 and 87% to *Arabidopsis thaliana* MAPK6 [1,11,19,32]. A local alignment of the deduced amino acid sequence of the 500 bp cloned-fragment of StMPK1 with the closest sequences from other plant species revealed a high conservation in this region, which comprises the catalytic domain of MAPKs, reaching 100% of identity with LeMPK1 and 98% with NtSIPK (Fig. 2B).

2.2. Southern blot analysis of StMPK1

The copy number of StMPK1 gene was analyzed by Southern blot analysis. Genomic DNA was extracted from potato leaf tissue, digested with *Sac*I, *Eco*RI, *Hin*dIII, *Bam*HI and *Bgl*I restriction enzymes, and hybridized to the PCR-amplified StMPK1 fragment described above. Under high stringent conditions, a single prominent band was detected in the DNA samples digested with each of the five restriction enzymes (Fig. 3). A faint second band was observed for two of the restriction enzymes used, *Eco*RI and *Hin*dIII. In the DNA sample digested with *Eco*RI the second band is most likely consequence of the presence of an internal restriction site in the 500 bp probe; and the second band observed in the *Hin*dIII digested DNA could indicate restriction site in the intronic sequences of StMPK1 gene. The simplicity of the hybridization pattern suggested that the gene encoding StMPK1 might be present in the potato genome as single copy, and that the probe used in hybridizations only recognized one member of the potato MAPK family.

2.3. Expression pattern of StMPK1 under biotic and abiotic stress conditions

The spatial expression pattern of StMPK1 was investigated in different potato organs by Northern blot analysis. A transcript of approximately 1.6 kb was detected in tubers buds,