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Research article

Molecular characterization of biotic and abiotic stress-responsive MAP kinase genes, *IbMPK3* and *IbMPK6*, in sweetpotato

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ABSTRACT

Plants are continually exposed to numerous environmental stresses. To decrease damage caused by these potentially detrimental factors, various stress-related signaling cascades are activated in plants. One such stress-responsive signaling pathway, the mitogen-activated protein kinase (MAPK) module, plays a critical role in diverse plant stress responses. Here, we functionally characterized biotic and abiotic stress-responsive MAPK genes, *IbMPK3* and *IbMPK6*, from sweetpotato. IbMPK3/6 contain totally 11 MAPK conserved subdomains and the phosphorylating motif TEY. Bacterially expressed IbMPK3/6 could be autophosphorylated *in vitro*, and these proteins phosphorylated universal kinase substrate, such as myelin basic protein. *IbMPK3/6* transcripts were expressed in leaf, stem, and root of sweetpotato cultivars with storage roots of various colors. *IbMPK3* and *IbMPK6* were induced by various biotic/abiotic stress treatments. Furthermore, the kinase activity of IbMPK3/6 was induced during early NaCl, SA, H_2O_2, and ABA treatment. IbMPK3/6 were predominantly localized to the nucleus. To determine the biological functions of IbMPK3/6, we transiently expressed the *IbMPK* genes in tobacco (*Nicotiana benthamiana*) leaves, which resulted in enhanced tolerance to bacterial pathogen and increased expression of pathogenesis-related (*PR*) genes. These data demonstrate that *IbMPK3* and *IbMPK6* play significant roles in plant responses to environmental stress.

1. Introduction

Crop plants are threatened by a variety of stresses throughout their life cycle, including salt, drought, unfavorable temperatures, and pathogen infection, which limit plant development and yield. To overcome these challenges, plants have evolved complicated signaling mechanisms involving various morphological and physiological changes to help them accommodate to the changing environment (Durrant and Dong, 2004; Tuteja, 2007). The recognition of external signals and consequent activation of defense responses demand a complex networking of signaling cascades. Protein phosphorylation event plays an important role in this process at both the transcriptional and translational levels (Teige et al., 2004; Mehlmer et al., 2010; Rodriguez et al., 2010; Dóczi et al., 2012; Meng and Zhang, 2013).

Mitogen-activated protein kinase (MAPK) signaling pathways perform an integral role in signal transduction from extracellular to intracellular compartments through phosphorylation of downstream signaling components. These signaling cascades are highly conserved in both animal and higher plants (Ren et al., 2008; Cargnello and Roux, 2011; Li et al., 2012; Opdenakker et al., 2012; Meng and Zhang, 2013; Samajova et al., 2013; Xu and Zhang, 2015). The phosphorylation cascade consists of three functionally intertwined protein kinases: mitogen-activated protein (MAP) kinase kinase kinase (MAPKKK), mitogen-activated protein kinase kinase (MAPKK), and MAPK (MAPK Group, 2002; Tena et al., 2011;

Abbreviations: ABA, abscisic acid; GST, glutathione S-transferase; H_2O_2, hydrogen peroxide; MAPK, mitogen-activated protein kinase; MBP, myelin basic protein; MeJA, methyl jasmonate; *PR*, pathogenesis-related; *Pta*, *Pseudomonas syringae* pv. *tabaci*; RT-qPCR, quantitative reverse transcription-polymerase chain reaction; SA, salicylic acid; SHM, Sinhwangmi; SZM, Sinzami; YM, Yulmi.

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Samajova et al., 2013). In this phosphorylation process, a MAPKKK phosphorylates and activates a certain MAPKK, which sequentially phosphorylates and activates a particular MAPK. MAPKs, which are composed of 11 subdomains (I–XI) discovered in all serine/threonine protein kinases (Edelman et al., 1987), are activated by the double phosphorylation of Thr and Tyr residues in a TXY (X represents Asp or Glu) motif located in the activation loop (T-loop) between subdomains VII and VIII. Activated MAPKs is often transported into the nucleus, where they induce phosphorylation and activation of distinct downstream effect proteins such as transcription factors and enzymes, initiating downstream stress-related responses (Cargnello and Roux, 2011). The *Arabidopsis*, rice, maize and poplar genomes contain 20, 17, 19 and 21 MAPKs, respectively; by contrast, the yeast and human genomes contain only six and ten MAPKs, respectively (Hamel et al., 2006; Liu et al., 2013). Since plants are sessile and cannot move to avoid unfriendly conditions, the extended number of MAPKs in plants implies that these proteins help plants adapt to various environmental stressors.

MAPKs participate in diverse signaling pathways induced by abiotic and biotic stresses such as low temperature, drought, and salt and pathogens (Zhang and Klessig, 2001; Asai et al., 2002; Droillard et al., 2004; Ding et al., 2009; Pitzschke et al., 2009). For example, MPK is implicated in *R* gene-mediated defense response and plant innate immunity (Zhang and Klessig, 2001; Rodriguez et al., 2010; Dóczi et al., 2012). The roles of AtMPK3, AtMPK4, and AtMPK6 are well known in *Arabidopsis*; these MPKs are implicated in defense against pathogen infection, which is combined with pathogen-associated molecular pattern (PAMP)-triggered immunity. The kinase activities of AtMPK3, AtMPK4, and AtMPK6 are activated by flg22. These kinases are then translocated into the nucleus, where they activate transcription factors involved in inducing the expression of resistance-related genes (such as *pathogenesis-related* [*PR*] *genes*), thereby inducing resistance responses in the plant (Petersen et al., 2000; Asai et al., 2002; MAPK Group, 2002; Droillard et al., 2004; Liu and Zhang, 2004). In addition to activation by flg22, AtMPK1, 2, 7, and 14 are also activated during pathogen signaling (Dóczi et al., 2007; Rodriguez et al., 2010; Dóczi et al., 2012), tobacco MPKs (WIPK and SIPK) regulate the levels of SA and JA (Seo et al., 2007), cotton MPK (GhMAPK) is activated by pathogens, SA, H_2O_2, and abiotic stress (Wang et al., 2007), and rice MPK (OsBWMK1) mediates the defense response by regulating *PR* gene expression (Cheong et al., 2003).

Sweetpotato [*Ipomoea batatas* (L.) Lam], a perennial dicotyledonous plant, is one of the most important food crops, ranking seventh in annual production worldwide (FAO, 2013). Sweetpotato consists of many nutrients, including carotenoids, carbohydrates, dietary fiber, minerals (calcium, potassium, and iron), and vitamins. In addition, sweetpotato is widely used as a useful source of starch, in animal feed, a staple food, and as a carbohydrate source for bioethanol production. Due to its resistance to a broad range of severe environmental conditions, high yield capability, efficient vegetative propagation techniques, easiness of cultivation, and high nutritional value, sweetpotato is appropriate for growth on marginal lands (Bovell-Benjamin, 2007). However, viral diseases, pests, and various environmental stresses such as unfavorable temperature, drought, and variable climates generally limit the production of sweetpotato in many areas worldwide (Lebot, 2010). Genetic engineering was recently shown to have great potential for improving sweetpotato and generating proper breeding materials with advanced traits, such as virus and nematode resistance, improved nutritional value, and increased salt tolerance, via the expression of native or foreign genes. Transformation with *OCI* from rice improved stem nematode tolerance in transgenic sweetpotato plant (Gao et al., 2011), and transformation of sweetpotato with *BADH* from spinach enhanced tolerance to salt, MV-mediated oxidative stress, and low temperature stress (Fan et al., 2012). Overexpression of sweetpotato genes such as *IbOr*, *IbNFU1*, *IbLCY-ε*, *IbSIMT1*, *IbNHX2*, and *IbMas* increased salt tolerance in this crop (Kim et al., 2013a,2013b; Wang et al., 2013, 2016; Liu et al., 2014a,2014b; 2015). However, the molecular mechanisms involved in resistance to various environmental stresses in sweetpotato remain unclear.

To date, most reported plant MPKs have been isolated and characterized from model plants such as *Arabidopsis*, tobacco, maize and rice. Little is known about MPKs from sweetpotato, a member of the Convolvulaceae family considered one of the most economically important dicotyledonous plants (Dehury et al., 2013). Here, we performed molecular characterization and functional analysis of stress-responsive sweetpotato MPK genes (designated *IbMPK3* and *IbMPK6*). To better understand the functions of these two MPK genes, we analyzed their expression under various stress conditions. The results suggest that *IbMPK3/6* play a role in responses to abiotic stress and hormone signaling in sweetpotato. These genes also act as positive regulators of the disease resistance response, as demonstrated by *Agrobacterium*-mediated transient expression analysis in tobacco leaves.

2. Materials and methods

2.1. Plant materials, growth conditions, and treatments

Sweetpotato [*Ipomoea batatas* (L.) Lam] cultivars Sinzami (SZM), Yulmi (YM), and Sinhwangmi (SHM) were used in this work. Sweetpotato and *Nicotiana benthamiana* plants were cultivated in a growth chamber at 25 °C under a photoperiod of 16 h light/8 h dark. For gene expression analysis in response to abiotic/biotic stresses, 4-week-old sweetpotato plants were treated with 250 mM sodium chloride (NaCl), 10 μM abscisic acid (ABA), 1 mM salicylic acid (SA), 50 mM hydrogen peroxide (H_2O_2), 1 mM methyl jasmonate (MeJA), or water as a control at 25 °C under a photoperiod of 16 h light/8 h dark for 0 (treated water), 5, 10, 20, 30, 60, 180, and 360 min. Cold stress treatment was accomplished at 4 °C under the same light conditions for 0 (untreated control), 5, 10, 20, 30, 60, 180, and 360 min.

2.2. Bacterial strains and culture

Agrobacterium strains GV3101 and LBA4404 were used in the experiments. *Agrobacterium* GV3101 and LBA4404 were streaked onto YEB plates supplied with 25 μg ml^{-1} rifampicin and incubated overnight at 28 °C. The colonies were inoculated in liquid LB medium and grown to $OD_{600} = 1$. For infection purposes, *Pseudomonas syringae* pv. *tabaci* (*Pta*) was streaked onto LB plates supplemented with 20 μg ml^{-1} tetracycline and incubated overnight at 28 °C.

2.3. Isolation of full-length IbMPK3 and IbMPK6 cDNA

The full-length cDNAs of *IbMPK3* and *IbMPK6* were obtained by RT-PCR from total RNA isolated from the anthocyanin-rich sweetpotato cultivar SZM. The coding regions of these *IbMPK* genes were amplified by PCR using the specific primers: for *IbMPK3*, 5′-ATG GTG GGC GGC GGC GAC TTC-3′ and 5′-TTA TGC ATA TCC TGG ATT CAT-3′; for *IbMPK6*, 5′-ATG GAC GCT GGT TCG GCT CAG-3′ and 5′-TCA CAT TTG CAG CTC AAA CTC-3′. The PCR products were purified and initially cloned into the T-blunt vector and sequenced. Plant MPK protein sequences were retrieved from NCBI GenBank. Sequence alignment and phylogenetic tree construction were performed using BioEdit and MEGA software, respectively. To analysis for secondary structure of IbMPK3 and IbMPK6, SOPMA software