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REVIEW

Plant mitogen-activated protein kinases and their roles in mediation of signal transduction in abiotic stresses

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Abstract **Mitogen-activated protein kinase (MAPK) cascade plays a central role in transfer information from diverse receptors/sensors to a wide range of cellular responses in plants. MAP kinases are organized into a complex network for efficient transmission of specific stimuli, including the abiotic stress signaling. In recent years, the mutants of loss-of-function and gain-of-function, and other additional tools are used to investigate the plant MAPK cascades. This review has summarized the recent progress on the MAPK cascade involved in mediation of the transduction of several pronounced abiotic stress signalings, such as salt, drought, low and high temperature, wound, hormone, and deficient nutrients. Currently, although part of the components of the MAPK cascade responding to the abiotic stresses have been identified, the integral molecular mechanisms of the abiotic stresses signaling transduction mediated via MAPK cascade are largely unknown and need to be elucidated further in the future.**

Keywords MAP kinase, abiotic stress, signaling, signal transduction, molecular mechanism

Introduction

Under environmental stress, plants develop complex signaling networks to sense environmental signals and adapt themselves to unfavorable conditions. When plants perceive such stresses, they immediately activate a signaling machinery, including gene expression, to change their physiological status as a defense mechanism.

To date, lots of studies have reported that MAPK cascades are involved in cell response to various growth factors, biotic and abiotic stresses in plants. As one of the most important and conserved signal pathways, the MAPK cascade plays a critical role in gene expression, metabolism, cell death, proliferation, and differentiation in animals, yeasts and plants, by responding to internal and external stimuli (Widmann et al., 1999; Chen et al., 2001). Thus, MAPK cascades play essential roles in the signal transduction pathways involved in numerous eukaryotic cellular processes from cell division to cell death (Davis, 2000). The schematic diagram of MAPK cascade mediating the abiotic stress signaling transduction in plants is shown in Fig. 1.

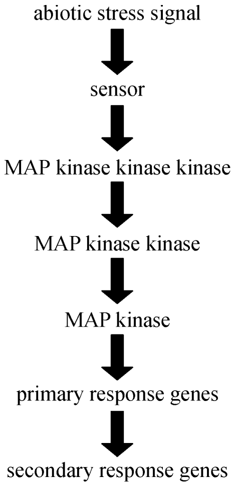

Figure 1 The schematic diagram of MAPK cascade mediating the abiotic stress signaling transduction in plants.

In the past several years, it has been clearly found that the MAPK cascade is evolutionarily conserved from unicellular

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to complex eukaryotic organisms. Usually consisting of protein kinases from three different subfamilies including MAPKKK (MAPK kinase kinase), MAPKK (MAPK keinase), and MAPK, a typical MAPK cascade is composed of three steps: (1) A MAP kinase kinase kinase (MAPKKK) activates a particular MAP kinase kinase (MAPKK) through phosphorylation on two serine/threonine residues, which are generally in a conserved S/T-X3-5-S/T motif. (2) The activated MAPKK can then in turn phosphorylate a MAPK on threonine and tyrosine residues, which are located in the invariant sequence TXY (Cobb and Goldsmith, 1995; Jonak et al., 2002; Nakagami et al., 2005). (3) After that, the MAPK-catalyzed phosphorylation of substrate proteins functions as a switch and turns on or off various ways to adapt organisms to various internal or environmental responses.

To date, more than 100 MAPKs have been isolated and characterized in plants. Among them 23 MAPKs were identified in *Arabidopsis* and 17 MAPKs in rice (Reyna and Yang, 2006), suggesting that the MAPK cascades in plant species would be quite complex (MAPK Group, 2002). Based on the phylogenetic analysis of amino acid sequence and phosphorylation motif, plant MAPKs could be classified at least into four subfamilies (A, B, C, and D). Each subfamily owns various members involved in environmental responses (Jonak et al., 1999). Among the four subfamilies, subfamilies A and B have been intensively studied so far. It is noted that most members in these two subfamilies have a TEY phosphorylation motif in their active sites and are involved in signaling of biotic and abiotic stress such as cold, drought, wound, pathogen attack (Jonak et al., 1996; Xiong and Yang, 2003; Jonak et al., 2004) and hormones (Ouaked et al., 2003).

Signal transduction of salt and drought stresses mediated by MAPK cascade

Mediation of the signal transduction by MAPK in salt stress

When exposed to salt stress, plant responses such as hyperosmotic and oxidative stress are induced, although to varying degrees. Consequently, molecular events in response to salt stress are convergent, involving abscisic acid (ABA), reactive oxygen species (ROS), and Ca^{2+} signaling that activate various downstream signaling cascades. So far, several literatures have demonstrated that distinct MAPK cascades were also involved in the salt signal transduction (Wang et al., 2003).

Early studies on MAPK in yeast have shown that the HOG1 MAPK pathway could be activated in response to hyperosmolarity and is responsible for increased production of osmolytes (Pöpping et al., 1996). Two tobacco (*Nicotiana tabacum*) MAPKs and their homologs are rapidly activated after salt stress (Droillard et al., 2000), suggesting that part components of MAPK cascade are involved in the transduction of salt stress signaling.

Later, intensive studies in the model plant *Arabidopsis* have shed light on determining how the MAPK cascade regulates the salt signal transduction. In *Arabidopsis*, the mitogen-activated protein kinase (MAPK) kinase 2 (MKK2) and MKK9, are upstream activators of the MPK3 and MPK6. The expression of active MKK9 protein in transgenic plants induces the synthesis of ethylene and camalexin through the activation of the endogenous MPK3 and MPK6 kinases (Zhang et al., 2006). Meanwhile, expression of active MKK9 protein enhances the sensitivity of transgenic seedlings to salt stress, whereas loss of MKK9 activity reduces salt sensitivity, indicating a role of MKK9 in the salt stress response. It is also ascertained that one MAPK pathway in *Arabidopsis*, AtMEKK1-MEK1/AtMKK2-AtMPK4, is involved in salt stress signal transduction (Ichimura et al., 2000), based on the observation that AtMPK4 is rapidly activated by hyposmolarity.

Similar studies in several other plant species also explored the MAPK cascade to be widely involved in the salt signal transduction. In rice, a time course (30–120 min) experiment under salt stress revealed that the OsSIPK mRNA is strongly induced by sodium chloride (Lee et al., 2008). Similarly, in *Chorispora bungeana*, a MAP kinase gene family member, *CbMAPK3*, accumulated rapidly more transcripts when treated with salinity stress. In *Malus micromalus*, two members of the MAP Kinase gene family, MPK4 and MPK6 isolated by functional complementation of osmosensitive yeast mutants, are related to the salt stress signaling. The yeast two-hybrid, *in vitro* and *in vivo* protein kinase assays revealed that MKK2 directly targets MPK4 and MPK6. The mutant mkk2 null plants and MKK2 overexpression all suggest that the interaction components of MKK2-MPK4 and MKK2-MPK6 are involved in the salt signal transduction in *Malus micromalus* plants (Peng et al., 2003).

Further studies noted that salt stress can activate different MAPKs at different times after the onset of stress, and the activities of these MAPKs also last for different time periods (Xu et al., 2008). Additionally, different levels of salt stress can cause the activation of distinct MAPKs (Munnik et al., 1999). Under salt stress, the transcript of *GhMAPK* begins to accumulate after 1 h and increases to a high level within 4 h, and then declines. But there is another *GhMAPK* transcript peak at 8 h (Wang et al., 2007), showing a similar pattern to that of *BnMPK3* in oilseed (Yu et al., 2004). These results may suggest an existence of a feedback adjustment in salt-stress signaling pathway. Therefore, distinct MAPK cascades exist in various plant species. The diversity of salt tolerance among the cultivars or genotypes in same plant species may be also largely resulted from the variations of MAPK cascade signaling response to the salt stress.

Mediation of the signal transduction by MAPK in drought stress

Less water availability and drought are becoming limiting factors for crop production worldwide. Crop plants have evolved specific mechanisms to respond and withstand drought stress. The adaptive strategies for drought tolerance in plants mainly depend on the expression of specific sets of genes that result in changes in the composition of the major cell components. It has been demonstrated that the conserved MAPK cascade is involved in the transduction of drought stress signaling in plant species.

In *Arabidopsis*, a mitogen-activated protein kinase family member, ATMPK3, has been observed to be induced under drought stress (Mizoguchi et al., 1996). Similarly, a MAPK family member, MMK4, identified in alfalfa, has also shown more mRNA accumulation when exposed to drought stress (Jonak et al., 1996). In *Malus*, a MAPK (MaMAPK, GenBank accession No. AF435805) was identified to be involved in the drought tolerance. *MaMAPK* expressed in both roots and leaves of seedlings of three *Malus* species when treated with 20% polyethylene glycol, with the expression level peaking at 1.5 h after polyethylene glycol treatment. *In vitro* kinase activity assays indicated that the dynamic changes of MAPK activity were very similar to those of the relative expression of *MaMAPK* mRNA (Peng et al., 2006). These results suggested that *MaMAPK* was regulated not only by water stress at transcription level, but also by the status of phosphorylation and dephosphorylation at protein level. In addition, of the tested three apple genotypes, the highest MAPK activity and *MaMAPK* expression level was found in genotype *M. sieversii*, followed by genotype *M. micromalus* and genotype *M. hupehensis*, in accordance with the capacities of drought tolerance in these three genotypes (Peng et al., 2006). These results suggest that MaMAPK plays an important role on regulating the drought tolerance based on the mediation of the transduction of drought stress signaling in *Malus*.

Two ways of drought stress signal transduction could be classified based on whether the abscisic acid (ABA) is dependent. Further studies on alfalfa MMK4 reveal that this component in MAPK cascade is not activated by exogenous ABA (Jonak et al., 1996), suggesting that MKK4 plays a role in the signaling of drought stress independent of ABA. Though some results ascertain that the MAPK cascade is involved in the transduction of drought stress cue, the distinct cascade and the components in plants need to be explored clearly in the future.

MAPKs involved in high- or low-temperature stress signal transduction

Mediation of the signal transduction by MAPK in cold stress

It is now clearly known that corresponding metabolic and physiologic responses occur when plants are exposed to the stresses of low or high temperature. These modifications in metabolic and physiologic processes are related largely to the capacities of plants to withstand the cold and heat stresses. A comparative examination of plant responses to high and low temperature revealed many similarities and parallels as well as differences.

Recently, more and more studies have suggested that the MAPK cascades play important roles in regulating plant responses to low and high temperature stresses. Under cold stress condition, many genes encoding the components of MAPK cascade are detected to be activated. Wang et al. (2007) observed that cold temperatures (4°C) lead to an elevation of *GhMAPK* mRNA level. The accumulation of *GhMAPK* transcripts in leaves of cotton seedling was substantially induced by 4°C treatment during an 8-h period. MAPK members of *Arabidopsis* ATMPK3 (Mizoguchi et al., 1996) and alfalfa MMK4 (Jonak et al., 1996) both showed more mRNA accumulation under cold stress. When exposed to low temperature, the activated alfalfa MMK4 was also correlated with a decrease in electrophoretic mobility, consistent with the activation capacity derived from the kinase phosphorylation (Jonak et al., 1996). This result suggested that alfalfa MKK4 responds to cold at two step regulations, including the transcriptional and the kinase posttranslational activation process.

As a noted plant species with high level resistance to the freezing environment, plants of *Chorispora bungeana* Fisch have been widely used as the materials for exploration of the physiologic and molecular mechanism for cold tolerance in plants. In *Chorispora bungeana*, a new MAPK cDNA, *CbMAPK3*, was detected to accumulate more transcripts when treated with cold stress (4°C) (Zhang et al., 2006), suggesting that CbMAPK3 may play an important role in response to low temperatures. However, it is necessary for the detailed mechanism of CbMAPK3 in mediating the cold signaling in *Chorispora bungeana* to be studied further.

In the past several years, the interaction mechanism of the components in the MAPK cascade has gradually been revealed. In *Arabidopsis*, it was found that the MAPK kinase 2 (MKK2) mediation in cold stress responses is through activation of the two MAP kinases MPK4 and MPK6 (Teige et al., 2004). The transduction of the cold signaling depends on the interactions of MKK2-MPK4 and MKK2-MPK6.

Mediation of the signal transduction by MAPK in heat stress

Up to now, some components in MAPK cascade responding to heat stress have also been identified. A MAPK member (HAMK) activated by heat shock (Sangwan et al., 2002), showed some opposite changes in membrane fluidity coupled with cytoskeletal remodeling, Ca^{2+} influx and the action of Ca^{2+}-dependent protein kinases (CDPK) (Sangwan et al., 2002), when compared to SAMK, a cold-activated MAPK

(Jonak et al., 1996). It is noted that ethylene plays a central role in the response of plants to a combination of heat stress. In *Arabidopsis*, expression analysis of the transcriptional coactivator MBF1c was demonstrated to enhance the tolerance of transgenic plants to heat stress by partially activating or perturbing the ethylene-response signal transduction pathway (Suzuki et al., 2005). Two tobacco (*Nicotiana tabacum*) MAPKs, SIPK and WIPK, were rapidly activated after temperature stress (Jonak et al., 1996; Sangwan et al., 2002). Transcript level of potato *StMPK1* increased after a heat-shock treatment at 42°C (Blanco et al., 2006). All these results suggest that distinct pathways of MAPK cascade are also involved in the transduction of heat stress signaling in plants.

At the level of perception and signal transduction, it is now clear that high and low temperature signals appear to be transduced by nonoverlapping and independent pathway components. Transcriptome analysis of plants expressing constitutively active MKK2 (MKK2-EE plants) showed altered expression of genes induced by abiotic stresses, but also a significant number of genes involved in defense responses (Brader et al., 2007). Therefore, more studies are needed for elucidation of the genetic or metabolic network controlling the response and tolerance of temperature stresses in plants.

MAPKs involved in wounding stress signal transduction

One of the most severe environmental stresses that plants encounter is wounding, generally caused by mechanical injury, pathogen or herbivore attack. To counter this kind of stress, plants have developed the related defense systems that are mostly based on activation of particular sets of genes encoding a variety of enzymes. It is noted that some of these genes are expressed systemically throughout the plant and protect the plant from attack at distant sites, though some are only induced locally at the site of attack.

More and more literatures have noted that MAPK cascades are involved in the wound signaling transduction in plants. In a separate study, Seo et al. (1995) found that a tobacco MAPK gene, termed *WIPK*, was rapidly transcripted in leaveed after being wounded. Transgenic analysis of WIPK suggested that the wound-induced JA biosynthesis was related to the overexpression of this *MAPK* gene (Seo et al., 1999). Similarly, in wounded tomato (Solanum lycopersicum) and alfalfa, two MAPK members, SIPK and WIPK, were activated both locally and systemically (Bogre et al., 1997; Stratmann et al., 1997). Overexpression of the WIPK in transgenic tobacco led to inactivation of the endogenous copies and as a consequence to suppression of the wound response. Specific antisera analysis demonstrated that the activation of wound-induced MAPKs is a posttranslational process, whereas the inactivation is dependent on *de novo* transcription and protein synthesis (Usami et al., 1995). After cutting, the transcript level of GhMAPK increased transiently and rapidly, the mRNA accumulation increased markedly within 15 min, and then decreased to the base level in 1 h (Wang et al., 2007). This response is much similar to the response of WIPK to wound in tobacco (Zhang and Klessig, 1998).

Another wounding type caused by insect attack also activates some MAPK members in plants. When the host *N. attenuata* is attacked by *M. sexta* larvae, the response is reconfigured at transcriptional, phytohormonal, and defensive levels due to the introduction of oral secretions (OS) into wounds during feeding. Distinct MAPK members are involved in the above process via mediation of the wounding signaling. A *Solanum tuberosum* MAPK, StMPK1, was differentially accumulated in potato tuber in response to *Fusarium solani*. Transcript accumulation after infection was transient and started earlier than what was observed in wounded tubers. The results suggested that StMPK1 may participate in the cellular response against multiple environmental stimuli in potato tubers (Blanco et al., 2006).

Currently, the mechanism of the interaction among the MAPK cascade components and the signaling process has been gradually explored. Wu et al. (2007) demonstrated that both SIPK and WIPK were upstream signaling components regulating wound- and OS-elicited JA, salicylic acid (SA), JA-Ile/JA-Leu conjugate, and ethylene biosynthesis in *N. attenuata*. Transcriptional analysis also revealed that SIPK and WIPK mediated the wounding- and OS-elicited accumulation of many defense-related genes, including three MAPKs and four calcium-dependent protein kinases (CDPKs). Moreover, the transcript accumulations of MAPKs and CDPKs regulate each other, highlighting the complicated transcriptional crosstalk that occurs among protein kinases (Wu et al., 2007). In *Arabidopsis*, the activation of subgroup C1 MAPKs (AtMPK1/AtMPK2) in response to mechanical injury was detected. An increase in their kinase activity in response to wounding was blocked by cycloheximide. Jasmonic acid (JA) activated AtMPK1/AtMPK2 in the absence of wounds. Wounding and JA-induction of AtMPK1/2 kinase activity were not prevented in the JA-insensitive coi1 mutant. Other stress signals, such as abscisic acid (ABA) and hydrogen peroxide, activated AtMPK1/2. Regulation of AtMPK1/2 kinase activity in *Arabidopsis* might be under the control of the signals involved in different kinds of stresses (Ortiz et al., 2007).

In rice, the negative regulators of MAPKs responding to the wounding stress were identified using mutant analysis (Katou et al., 2007). Based on the study of osmkp1, a loss-of-function mutant, the biological function of MAP KINASE PHOSPHATASE1 (OsMKP1) responding to wounding was determined. It was observed that the activity of two stress-responsive MAPKs, OsMPK3 and OsMPK6, was higher in osmkp1 than that in the wild type after wounding (Katou et al., 2007). This result suggests that there exists a coordinated regulation of the MAPK and MPK6 by the OsMKP1 (Bartels et al., 2009). Further studies on ptp1, a mutant of PROTEIN TYROSINE PHOSPHATASE1 (PTP1), in combination with the results from mutant osmkp, indicate that the mutations are associated with a deregulation of MPK6 that causes constitutive wounding defense responses.

MAPKs involved in ROS signal transduction

Over the last years, it has become evident that reactive oxygen species (ROS) plays an important role in various physiologic responses, such as pathogen defense and stomata regulation. However, ROS overproduction is detrimental for proper plant growth and development. In plants, ROS can be formed as a metabolic by-product under various abiotic stress conditions as well as pathogen attacks (Apel and Hirt, 2004).

Among various ROS, hydrogen peroxide (H_2O_2) is one that can cross plant cytosolic membranes and therefore directly function in cell-to-cell signaling. Two reports have proposed that distinct MAPK cascades can be activated by H_2O_2 in *Arabidopsis* and soybean (Kovtun et al., 2000), respectively. Wang et al. (2007) examined the expression of the *GhMAPK* gene in response to H_2O_2 treatment, and the results showed that increasing accumulation of *GhMAPK* transcripts was detected 1 h after treatment, reaching a maximum in 6 h. A specific *Arabidopsis* MAPKKK, designated ANP1, was activated by H_2O_2, which then initiated a phosphorylation cascade involving two stress MAPKs, AtMPK3 and AtMPK6 (Kovtun et al., 2000). Though constitutively active ANP1 mimicked the H_2O_2 effect and initiated the MAPK cascade inducing specific stress-responsive genes, the action of auxin was blocked. This result provided a molecular link between oxidative stress and auxin signal transduction. Furthermore, transgenic tobacco plants that constitutively express an active tobacco ANP1 ortholog, NPK1, showed enhanced tolerance to multiple environmental stress conditions without activating drought, cold, and abscisic acid signaling pathways. Therefore, manipulation of key regulators of an oxidative stress signaling pathway, such as ANP1/NPK1, provides a strategy for engineering multiple stress tolerance that may greatly benefit agriculture.

In *Arabidopsis*, ROS generated from ozone treatment leads to activation and nuclear translocation of MPK3 and MPK6 (Ahlfors et al., 2004). RNAi lines silenced for either of these MAPKs are shown to be hypersensitive to ozone. Among them, MPK6-RNAi plants display a stronger and prolonged activation of MPK3 compared to wild type. Reciprocally, MPK3-RNAi lines show a stronger and prolonged MPK6 activation (Miles et al., 2005). Like MPK3 and MPK6 in *Arabidopsis*, the putative tobacco orthologs SIPK and WIPK also become activated by ozone (Kumar and Klessig, 2000; Samuel et al., 2000). Paradoxically, overexpression as well as suppression of SIPK finds that SIPK renders plants hypersensitive to ozone treatment (Samuel and Ellis, 2002). Overlapping but not completely interchangeable actions are known for SIPK and WIPK, and the WIPK activation usually is accompanied by SIPK activation (Kumar and Klessig, 2000; Samuel et al., 2000). Overexpression of a SIPK-interacting MAPKK, *SIPKK*, also enhances the resistance to ozone in plants (Miles et al., 2005), suggesting that this MAPK cascade component plays an important role in improving the ozone stress tolerance.

It is noted that there is a tight relation between the ROS and salicylic acid regulating pathway. In tobacco, it is found that the oxidative stress leads to the activation of the salicylate-induced protein kinase (Samuel et al., 2000; Samuel and Ellis, 2002). Recently, Jiang et al. (2007) showed that SB202190, a special inhibitor of p38 MAPK, can modulate salicylic acid-induced H_2O_2 generation in *Vicia* guard cells. This verifies that SB202190 regulates SA- induced ROS signaling in plant cells.

Stomata opening and closing are mediated partly by ROS. Currently, there is evidence for MAPKs to be involved in stomata regulation. It is demonstrated that *NtMPK4* is preferentially expressed in the epidermis, and *NtMPK4*-silenced plants are hypersensitive to ozone due to an ABA-independent misregulation of stomatal closure (Gomi et al., 2005). Thus, NtMPK4 might function at early stages of ROS signaling by controlling the entrance gate for ozone uptake from the environment.

The pathway of the MAPK cascade involved in the ROS signaling transduction has also been identified. Nakagami and colleagues (2005) defined MPK4 as downstream target of MEKK1 and showed that MEKK1 functioned in integrating ROS homeostasis with plant development and hormone signaling. MEKK1, predominantly via the MEKK1-MKK1/2-MPK4 pathway, is responsive to multiple ROS-inducing conditions by regulation of more correlated transcription factors (Pitzschke et al., 2009).

ROS can be detected by at least three mechanisms (ROS receptors, redox sensitive transcription factors and phosphatases). Detection of ROS by receptors results in the generation of Ca^{2+} signals and the activation of a phospholipase C/D (PLC/PLD) activity that generates phosphatidic acid (PA) (Xu et al., 2007). Recently, Zong and colleagues (2009) isolated a novel group C MAPK gene, *ZmMPK7*, from *Zea mays*. It is observed that *ZmMPK7* is induced by exogenous ABA and H_2O_2 via calcium-dependent transcription pathway. The response to ABA of this gene is blocked by several reactive oxygen species (ROS) manipulators such as imidazole, tiron, and dimethylthiourea (DMTU), indicating that endogenous H_2O_2 may be required for ZmMPK7-mediated ABA signaling and calcium signaling are also involved in this process. The regulation mechanism in response of the ROS signaling related to MAPK cascade and Ca^{2+} signaling need to be elucidated further.

MAPKs involved in hormones signal transduction

Ever since the discovery of plant hormones, their roles in plant development and physiologic responses have been intensely studied. Despite lots of efforts to understand how plant hormone signals are perceived and transmitted, the corresponding molecular mechanisms have still remained largely unclear. Increasing evidence now suggests that MAPK pathways are involved in mediating abscisic acid, JA and ethylene responses.

Many studies on the dissection of the MAPK cascade functions in hormone response have been focused on ABA, JA, and ethylene. It is observed that ABA treatment activates AtMPK3 and p46MAPK in *Arabidopsis* (Lu et al., 2002), OsMAPK5 in rice (Xiong and Yang, 2003), p45MAPK in pea (Burnett et al., 2000), and several MAPK isoforms between 40 and 43 kDa in barley (Knetsch et al., 1996). A MAPK activated by ABA was also identified in pea (*Pisum sativum*) guard cells (Burnett et al., 2000). In stomata regulation, the aperture and the density of stomata related to hormone regulations are also involved in the MAPK modules (Bergmann et al., 2004). In maize, the role of MAPK in ABA-induced antioxidant defense was investigated in the leaves. The results clearly suggest that MAPK is involved in the ABA-induced antioxidant defense, and a cross talk between H_2O_2 production and MAPK activation plays a pivotal role in ABA signaling. In the signaling cascade, the H_2O_2 induced by ABA activates MAPK, which in turn induces the expression and the activities of antioxidant enzymes (Zhang et al., 2006). In *Arabidopsis*, an ABA-dependent signaling pathway in abiotic stresses response is detected by connecting *CAT1* expression and the MAPK cascade components through a phosphorelay including AtMKK1 and AtMPK6 (Xing et al., 2008). On the other hand, mkk1 mutant reduced both the sensitivity to ABA during germination and the drought tolerance of seedlings, whereas the *AtMKK1* overexpression line showed the opposite responses when compared with the wild type. The data suggest AtMKK1–AtMPK6 to be a key module in an ABA-dependent signaling cascade causing H_2O_2 production and stress responses.

The plant hormone JA plays a key role in the environmental stress responses and developmental processes. The roles of JA are performed to some extent via MAPK cascade. In tobacco, overexpression of *WIPK*, a tobacco MPK3 homolog, shows an increased JA level and constitutive expression of JA-inducible proteinase inhibitor II (Seo et al., 1999). However, it is observed that JA activated neither SIPK, a tobacco MPK6 homolog, nor WIPK (Kumar and Klessig, 2000). In rice, a subgroup V member of the MAPK family, *OsSJMK1*, was transiently induced by JA and SA at early stages of treatment. However, this gene was not induced by other hormones, such as ABA, or abiotic stresses, such as drought and salinity. The results suggest that OsSJMK1 might be activated specifically by JA or SA, and involved in defense signaling pathways (Ning et al., 2006). Also in tobacco (*Nicotiana tabacum*), rapid activation of two MAPKs, WIPK and SIPK after wounding causes the subsequent accumulation of JA. Transgenic analysis of overexpressing *NtMKP1*, a gene encoding tobacco MAPK phosphatase, which inactivates WIPK and SIPK exhibited, reduced JA production in response to wounding. Together, these results suggest that WIPK and SIPK perform an important function in JA production in response to wounding, and they cooperatively control SA biosynthesis (Seo et al., 2007).

Although ATMYC2/JASMONATE-INSENSITIVE1 (JIN1) is a major positive regulator of JA-inducible gene expression and essential for JA-dependent developmental processes in *Arabidopsis*, molecular mechanisms underlying the control of ATMYC2/JIN1 expression remain largely unknown. Takahashi et al. (2007) identified a MAPK cascade, MAPK KINASE 3 (MKK3)–MAPK 6 (MPK6), which was activated by JA in *Arabidopsis*. The ATMYC2/JIN1 expression was negatively controlled by JA. Moreover, JA-regulated root growth inhibition was affected by mutations in the MKK3–MPK6 cascade. These results provide a model explaining how MPK6 can convert JA distinct signals into fine different sets of responses in *Arabidopsis*.

As the ethylene precursor, the aminocyclopropane-1-carboxylic acid (ACC) could activate distinct MAPKs in *Medicago trancutula* and *Arabidopsis* (Ouaked et al., 2003). In *Medicago trancutula*, the ACC-activated MAPKs include SIMK and MMK3, while in *Arabidopsis* they contain MPK6 and another MAPK member. Furthermore, it is found that the ACC-induced activation of SIMK and MMK3 is specifically mediated by *Medicago trancutula* SIMKK. Transgenic *Arabidopsis* plants overexpressing *SIMKK* have constitutive MPK6 activation and ethylene-induced target gene expression. These data indicate that a MAPK cascade is composed of part of the ethylene signal transduction pathway in plants (Ouaked et al., 2003). Recently, novel evidence for the involvement of MAPK cascades in ethylene signaling has been put forward. Yoo et al. (2008) found that a MKK9-MPK3/MPK6 module is an integral component of the ethylene signal transduction pathway.

MAPKs involved in nutrients deprivation stress signal transduction

Similar to other abiotic stress, more and more studies have demonstrated that there were complicated and intricate regulatory systems responding to the nutrient stresses in plants. As multicellular organisms, plants have evolved two types of signaling and signal transduction system in whole plant level or cellular level.

In wheat, we have identified a component of MAPK cascade, the designated *TaMPK1a-1*, which shows an upregulated expression pattern under deficient-Pi condition.

Compared to Ji7369, a low-P efficiency cultivar, the high-P efficiency cultivar Shixin828 has much stronger responses to low-Pi stress signal, suggesting that TaMPK1a-1 possibly plays an important role in regulation of wheat phosphorus use efficiency under deficient-Pi condition (Lu et al., 2009).

Copper, as well as cadmium, is a heavy metal with different physiochemical properties and functions. As the cofactor for many physiologic processes including photosynthesis, respiration, superoxide scavenging, ethylene sensing, and lignification, copper is a vital micronutrient essential for plant normal growth and development. However, similar to cadmium, excess copper is harmful due to the production of ROS by autoxidation and Fenton reactions. Under elevated levels of copper and cadmium, nutrient stress signaling is initiated in which distinct signaling transduction systems are involved, including the components of the MAPK cascade. Jonak et al. (2004) demonstrated that four distinct MAPK pathways were activated with the elevation levels of copper and cadmium ions. Excess of copper ions rapidly activated SIMK, MMK2, MMK3, and SAMK, while activation of the four MAPKs to cadmium ions showed similarity but delayed profiles. Transient expression assays showed that the copper-induced activation of SIMK and SAMK, but not of MMK2 or MMK3, was specifically mediated by SIMKK. The MAPK activity level in roots showed that SIMK was highly induced by $CdCl_2$ and $CuCl_2$, and slightly induced by $FeCl_2$ and $Pb(NO_3)_2$. Whereas little or no increase in SIMK activity was observed after incubation with $Al_2(SO_4)_3$, $CoCl_2$ or $ZnCl_2$. MMK2, MMK3, and SAMK were also most strongly activated by $CuCl_2$ and $CdCl_2$. MMK2 was also significantly activated by $FeCl_2$, MMK3 and SAMK poorly responded to this metal.

In rice, the heavy metal-induced signaling pathways derived from copper and cadmium have also been characterized. According to the analysis of the effects of cadmium ($CdCl_2$) and copper ($CuCl_2$) on MBP (myelin basic protein) kinase activities in rice, it is found that Cd^{2+}-induced 42 kDa MBP kinase has the characteristics of MAP kinase, which are partly from the activities of OsMPK3 and OsMPK6. However, the Cd-induced 42 kDa MAP kinase activation has also conferred Cd tolerance in rice (Yeh et al., 2007).

It is well known that zinc (Zn) is another micronutrient essential for normal growth and development of plants. However, the molecular mechanisms responsible for the regulation of plant growth by Zn are still not completely understood. Lin et al. (2005) suggested that Zn elicited a remarkable increase in myelin basic protein (MBP) kinase activities, and that Zn-activated 40- and 42-kDa MBP kinases are MAPK. Pre-treatment of rice roots with reactive oxygen species (ROS) scavenger, sodium benzoate, was able to effectively prevent Zn-induced MAPK activation. However, phosphoinositide 3-kinase (PI-3K) inhibitor, LY294002, was unable to inhibit Zn-induced MAPK activation. These results suggested that the ROS may function in the Zn-triggered MAPK signaling pathway in rice roots.

In conclusion, a common observation both in plants and other organisms is that the MAPK modules are involved in the transmission of multiple signals. More and more studies provide evidence that the MAPK cascade plays important roles in regulation of the responses of abiotic stress signaling, and part of the components of the MAPK cascade have been identified. Moreover, it has been demonstrated that some components of the MAPK modules could be also used to improve the tolerance of crops to freezing, salt, and heat stresses (Kovtun et al., 2000). However, the integral molecular mechanisms of the abiotic stress signaling transduction mediated via MAPK cascade are largely unknown and need to be further elucidated in the future.

Conclusions and perspectives

As one of the major pathways mediating the extracellular stimuli, such as abiotic stresses in plants, the mitogen-activated protein kinase (MAPK) cascades are immediate upstream activators, phosphorylating a variety of substrates including transcription factors, other protein kinases, and cytoskeleton-associated proteins after activation by distinct environmental cues. Components of mitogen-activated protein kinase (MAPK) cascades act as converging points of multiple abiotic stress signaling pathways. Forward and reverse genetic analysis in combination with expression profiling will continue to uncover many signaling components in these cascades. Still, further biochemical characterization of the signaling complexes related to MAPK cascade will be required for determination of the specificity and cross-talk in abiotic stress signaling pathways.

Although conventional genetic screens have yielded valuable insight into abiotic stress signal transduction, modern molecular techniques and genetics approaches will play major roles in the dissection of the signaling transduction pathways mediated by MAPK cascade in plants. Recently, reporter gene-based molecular screens offer a way to systematically identify MAPK signaling components that control subsets of responses, which may not manifest as visible tolerance phenotypes when plants are subjected to the abiotic stresses, such as drought, high salinity, and cold, etc. It is also worthwhile and necessary to further clarify the cross-talk of the signaling pathways between the MAPK cascade and other signal transduction systems in plants in the future.

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