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REVIEW

Signal convergence through the lenses of MAP kinases: paradigms of stress and hormone signaling in plants

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Abstract Common mechanisms plants use to translate the external stimuli into cellular responses are the activation of mitogen-activated protein kinase (MAPK) cascade. These MAPK cascades are highly conserved in eukaryotes and consist of three subsequently acting protein kinases, MAP kinase kinase kinase (MAPKKK), MAP kinase kinase (MAPKK) and MAP kinase (MAPK) which are linked in various ways with upstream receptors and downstream targets. Plant MAPK cascades regulate numerous processes, including various environmental stresses, hormones, cell division and developmental processes. The number of MAPKKs in *Arabidopsis* and rice is almost half the number of MAPKs pointing important role of MAPKKs in integrating signals from several MAPKKKs and transducing signals to various MAPKs. The cross talks between different signal transduction pathways are concentrated at the level of MAPKK in the MAPK cascade. Here we discussed the insights into MAPKK mediated response to environmental stresses and in plant growth and development.

Keywords environmental stresses, abiotic stress, biotic stress, mitogen-activated protein kinase kinase, MAPKK, mitogen-activated protein kinase, MAPK, plant signaling

Introduction

In plants, mitogen-activated protein kinases (MAPKs) are known to be involved in the transduction of a variety of extracellular signals and the regulation of different developmental processes. A canonical MAPK signaling cascade consists of MAP kinase kinase kinases (MAPKKK, MKKK, MEKK, MAP3K), MAP kinase kinase (MAPKK, MKK, MEK, MAP2K) and MAP kinase (MAPK, MPK) as shown in Fig. 1. The MAPKKK, activated by upstream plasma membrane receptor kinase, activate MAPKK by phosphorylation on a conserved S/T-X_{3-5}-S/T motif between subdomain VII and VIII and it also has a putative MAPK docking domain K/R-K/R-K/R-X_{1-6}-LX-L/V/I. Upon activation MAPKKs in turn activate MAPKs by phosphorylation on threonine and tyrosine residues at a conserved T-X-Y motif (Chang and Karin, 2001). Activated MAPKs phosphorylate and activate various cytoplasmic or nuclear substrates, which include other kinases, enzymes, or transcription factors with significant effect on their localization, stability, and transcript levels (Khokhlatchev et al., 1998; Whitmarsh, 2007). These MAPK substrates play crucial role in regulation of several essential cellular processes in response to stimuli.

Analysis of *Arabidopsis* genome revealed presence of 20 members of MAPKs, 10 MAPKKs and around 80 MAPKKKs as shown in Fig. 1 (Jonak et al., 2002; MAPK Group, 2002). The 10 MAPKK genes of *Arabidopsis* are classified into four groups. The MAPKKs of group A includes *MKK1*, *MKK2* and *MKK6*. Group B include *MKK3* possess 3′ extension encoding a NTF (nuclear transfer factor) domain. Group C includes *MKK4* and *MKK5* and group D includes *MKK7*, *MKK8*, *MKK9* and *MKK10*. *In silico* analysis of rice genome revealed 15 members of MAPKs and 8 MAPKKs (Hamel et al., 2006; Kumar et al., 2008). The eight members of rice MAPKKs are again classified into four groups. Group A includes *MKK1* and *MKK6*, group B includes *MKK3*, group C includes *MKK4* and *MKK5* and group D includes *MKK10-1*, *MKK10-2* and *MKK10-3*.

In past decade, several members of plants MAPK cascade have been characterized and their role in stress as well as growth and development was established. The MAPKKs are reported to be involved in producing various biotic and

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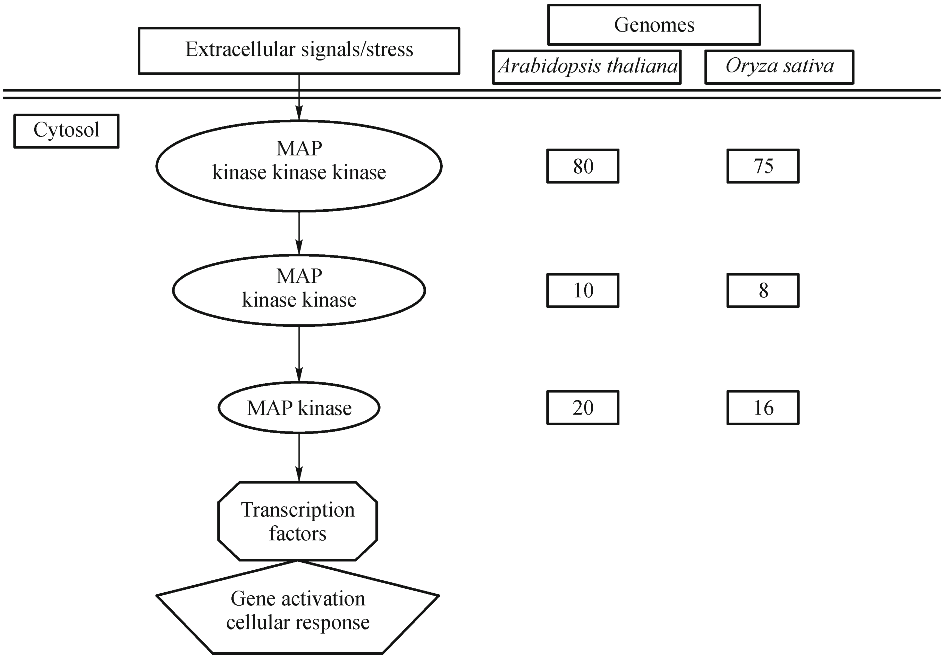

Figure 1 A typical MAPK cascade and the correspoding number of genes in *Arabidopsis thaliana* and *Oryza sativa*. Mitogen-activated protein kinase (MAPK) pathway is composed of 3 kinases (MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK) that are sequentially activated by phosphorylation. The corresponding number of genes encoding these kinases in *Arabidopsis* and *O. sativa* genome in the MAPK cascade are presented in right.

Table 1 List of MKKs involved in different biologic processes in plants

MKKs	Involvement	Plants	References
MKK1	Cold, salt, drought	Rice	Wen et al., 2002; Kumar et al., 2008
MKK1	H_2O_2	*Arabidopsis*	Xing et al., 2008
MKK2	Cold, salt	*Arabidopsis*	Teige et al., 2004
MKK4, *MKK6*	Cold, salt	Rice	Kumar et al., 2008
MKK4	Arsenite	Rice	Rao et al., 2011
MKK9	Salt, dehydration	*Arabidopsis*	Alzwiy and Morris, 2007; Xu et al., 2008
MKK10-2	Cold	Rice	Kumar et al., 2008
SIMKK	Salt	Alfaalfa	Kiegerl et al., 2000
MKK1	Innate immunity	*Arabidopsis*	Mészáros et al., 2006
MKK1	Non-host resistance	Tobacco	Takahashi et al., 2007
MKK1	Blast and insect resistance	Rice	You et al., 2007
MEK1/NQK1	Resistance to tobacco mosaic virus (TMV)	Tobacco	Liu et al., 2003
MKK2	SA and JA	*Arabidopsis*	Brader et al., 2007
MKK2, *MKK3*	Pto-mediated resistance	Tomato	Ekengren et al., 2003
MKK2	Various pathogen elicitors	Tobacco	Yang et al., 2001
MKK3	*P. syringae*	*Arabidopsis*	Dóczi et al., 2007
MKK4	Chitin elicitor	Rice	Kishi-Kaboshi et al., 2010
MKK7	*P. syringae, H. parasitica*	*Arabidopsis*	Zhang et al., 2007
MKK9	Ethylene signaling, Camelexin biosynthesis	*Arabidopsis*	Yoo et al., 2008; Xu et al., 2008
MEK1	Cell division	Tobacco	Calderini et al., 2001
MKK3	JA	*Arabidopsis*	Takahashi et al., 2007
MKK4, *MKK5*	Stomatal differentiation	*Arabidopsis*	Wang et al., 2007
SIPKK	Wounding, ozone	Tobacco	Gomi et al., 2005
MKK7	Auxin transport	*Arabidopsis*	Mou et al., 2002

abiotic stress responses, growth and developments (Table 1). In this review, we will particularly focused on involvement of MAPKKs in response to an array of environmental stresses and in plant growth and development.

Abiotic stress

MAPK signaling cascade is known to mediate cold and salt stress tolerance in plants (Sinha et al., 2011). *Arabidopsis* group A MAPKK, MKK2 and the downstream MAPKs MPK4, and/or MPK6 have been isolated by functional complementation of osmosensitive yeast mutants. The MKK2- MPK4/MPK6 cascade was shown to be activated by cold, salt stress and stress-induced MKKK, MEKK1 (Teige et al., 2004). Further MKK2 overexpression lines showed constitutive activity of MPK4 and MPK6, upregulated expression of stress-induced marker genes, and enhanced freezing and salt tolerance. Inversely, *mkk2* null plants showed deficient activity of MPK4 and MPK6 and were hypersensitive to salt and cold stress. Demonstrating importance of MAPKK in regulation of gene expression pattern, it was observed using full genome transcriptome analysis that *MKK2*-overexpressing plants showed altered expression of 152 genes involved in transcriptional regulation, signal transduction, cellular defense, and stress metabolism. In subsequent study it has been demonstrated that MKK1 and MKK2 have overlapping functions in defense signaling mediated by MEKK1, MPK4, and MKS1 (Qiu et al., 2008).

Abscisic acid (ABA) is one of the important cellular signals and stress hormones and mediate crucial role in providing information about environmental stresses and prepare plants to cope with the stresses. ABA has been shown in triggering the production of H_2O_2 which acts as a signal mediating several stress tolerance responses (Neill et al., 2002; Vanderauwera et al., 2005; Gadjev et al., 2006; Pitzschke and Hirt, 2006). AtMKK1 and AtMPK6 cascade was shown to mediate ABA-induced CAT1 expression and H_2O_2 production (Xing et al., 2008). *CAT1* transcript was induced in an ABA-dependent way and the induction was abolished in the *mkk1* mutant, while *AtMKK1* overexpression significantly enhanced the ABA-induced CAT1 expression and H_2O_2 production. Further, *mpk6* mutant blocked, and *AtMPK6* overexpression enhanced the ABA-dependent expression of CAT1 and H_2O_2 production. The ABA induced AtMPK6 activity was increased in an AtMKK1-dependent manner. Further, role of CAT1 in stress responses was shown not only in ROS scavenging, but also in its feedback regulation of the H_2O_2 signaling.

In *Arabidopsis*, MKK9 was found to be a negative regulator of the abiotic stress response (Alzwiy and Morris, 2007). The *mkk9* mutant of *Arabidopsis* is salt and dehydration insensitive and could germinate on higher concentration of salts and mannitol. The mutant plants show faster expression of the stress related genes *RD22* and *RD29* after salt treatment and these enhanced stress related gene expression is hypothesized to be the reason for the enhanced ABA and salt tolerance. MKK9 is also considered as an upstream activator of the MAPKs, MPK3 and MPK6. Activation of MKK9 enhances the sensitivity of transgenic seedling to salt stress (Xu et al., 2008).

In Alfalfa a salt stress and elicitor-induced MAPK have been identified and characterized (Munnik et al., 1999; Cardinale et al., 2002). Further a salt stress-induced MAPKK (SIMKK) was found to mediate the salt-induced activation of SIMK (Kiegerl et al., 2000). Another alfalfa, pathogen-responsive MAPKK (PRKK) has been isolated by interaction screening with SIMK in yeast (Cardinale et al., 2002). Unlike SIMKK, recombinant PRKK is inactive form of MAPKK and requires activation by an upstream MAPKKK. Moreover PRKK activates three specific MAPKs in response to pathogen elicitor but not salt stress. In contrast, SIMKK is involved in activation of distinct MAPKs in response to salt stress or pathogen elicitor. The findings suggest that the activation of stress-responsive MAPKs underlies not only pairwise affinities between MAPKKs and MAPKs but also complex stress signal-dependent intracellular mechanisms. In tobacco NtMEK2 was found to be an upstream activator of salicylic acid-induced protein kinase (SIPK) and wounding induced protein kinase (WIPK) (Yang et al., 2001).

Rice breeding programmes have often faced obstacles due to its sensititvity toward low temperature, especially during microspore development stages. Male sterility is the most severe consequence in many cold regions of the world which leads to lower yield of the crop. In rice, a MAPKK, *OsMKK6* (previous name *OsMEK1*) and MAPK, *OsMPK3* (previous name *OsMAP1*) have been identified that are induced specifically by moderately low temperatures (12°C) and not at 4°C. A moderately low temperature is known to cause abnormal development of pollen in many rice growing areas. *OsMEK1* and *OsMPK3* not only show co-regulation of expression pattern but also interact physically as shown by yeast two-hybrid assay (Wen et al., 2002). The result signifies signal transduction pathway, distinct from the existing Lip19-involved pathway, for low-temperature responses in rice. The antagonistic effect of ABA-induced MAPK in rice was also demonstrated in OsMAPK5, where its protein and kinase activity were inducible by ABA as well as various biotic (pathogen infection) and abiotic (wounding, drought, salt, and cold) factors and it also negatively modulate *PR* gene expression and broad-spectrum disease resistance (Xiong and Yang, 2003).

In rice, expression studies have identified differential regulation of five MAPKK in cold, heat, salinity and drought stresses. *OsMKK4* and *OsMKK6* are strongly regulated by cold and salt stresses while *MKK1* is regulated by salt and drought stresses (Kumar et al., 2008). *OsMKK4-OsMPK3* module was also likely to be involved in arsenite stress signal transduction (Rao et al., 2011). A group D member of

MAPKK, *OsMKK10-2* is regulated only by cold stress (Kumar et al., 2008). The study provides the indication of involvement of specific MAPKK in different abiotic stress signaling and also possible cross talks that exist during the signaling processes. A MAPKK reported as *OsMEK1* was identified as *OsWNK1* (With No Lysine Kinase) in rice and its role in circardian rhythm and abiotic stress has been postulated (Kumar et al., 2011).

Biotic stress

First line of inducible defense against microbial infection in plants is the 'innate immunity'. The molecules that are associated with pathogens/microbes, pathogen/microbe-associated molecular patterns (PAMPs/MAMPs) induces signaling pathways including MAPK cascades. The activated MAPK cascade activates downstream components such as transcription factors that leads to changes in global transcriptions to boost immunity (Asai et al., 2002; Boudsocq et al., 2010). The best characterized plant MAMP receptor is the LRR receptor kinase FLS2 that perceives a conserved 22 amino acid peptide (flg22) from bacterial flagellin and activates MAPK cascade (MEKK1, MKK4/MKK5 and MPK3/MPK6) and WRKY transcription factors in *Arabidopsis* (Gómez-Gómez and Boller, 2000; Asai et al., 2002). MEKK1 is required for induction of MPK4 by flg22 and/or reactive oxygen species (ROS) (Ichimura et al., 2006; Nakagami et al., 2006; Suarez-Rodriguez et al., 2007). Further MKK1 also has its role in flg22 induced activation of MPK4 (Mészáros et al., 2006). In *Nicotiana benthamiana* NbMKK1–NbSIPK cascade was shown to control non-host resistance including HR cell death (Takahashi et al., 2007). Silencing of two tomato MKKs, *LeMKK2* and *LeMKK3* and two MAPKs *LeMPK3* and one similar to *NTF6* compromises Pto-mediated resistance (Ekengren et al., 2003). Both LeMKK2 and LeMKK4 show LeMPK1, LeMPK2 and LeMPK3 phosphorylation *in vitro* (Pedley and Martin, 2004). Silencing of *NTF6*/ *NRK1* (MAPK) or *MEK1*/*NQK1* (MAPKK) attenuates N-mediated resistance to tobacco mosaic virus (Liu et al., 2003).

Arabidopsis MKK1 and MKK2 function upstream of MPK4. The double loss of function mutant *mkk1 mkk2* showed marked phenotypes in development and disease resistance similar to those of the single *mekk1* and *mpk4* mutants. *mkk1* or *mkk2* single mutants appear like wild type and basal level of MPK4 activity were not impaired in mutants. By biochemical and molecular analysis, the kinases were shown to have a role in jasmonate and salicylate dependent defense responses, mediated in part via the MPK4 substrate MKS1. Transcriptome analysis revealed overlapping effects of *MKK1* and *MKK2* on global gene expression patterns demonstrating both redundant and unique functions for *MKK1* and *MKK2* (Qiu et al., 2008).

The endogenous *Arabidopsis* MKK1 is activated in cells treated with flg22, phosphorylates MPK4 *in vitro*, and activates MPK4 *in vivo* in protoplasts. In *mkk1* mutant plants, the activation by flg22 of MPK4 and two other flg22-induced MAPKs (MPK3 and MPK6) is impaired. In *mkk1* mutant, a battery of both flg22-induced and flg22-repressed genes show altered expression, indicating that MKK1 negatively regulates the activity of flagellin-responsive genes. Intriguingly, in contrast to the *mpk4* mutant, *mkk1* shows no morphological anomalies and is compromised in resistance to both virulent and avirulent *Pseudomonas syringae* strains. Thus, the MKK1 signaling pathway modulates the expression of genes responding to elicitors and plays an important role in pathogen defense (Mészáros et al., 2006). In rice, a gene coding for a blast (*Magnaporthe grisea*) and insect (*Nilaparvata lugens*) responsive putative MAPKK, *OmMKK1* (*Oryza minuta* MKK1), have been identified (You et al., 2007) which is represented by two copies in *O. minuta* genome. Expression pattern of *OmMKK1* is related to that of defense response signaling pathways. An *OmMKK1* ortholog in *O. sativa, OsMKK1* also showed similar induction by stress-related hormones and biotic stresses to that of *OmMKK1* albeit with different kinetics (You et al., 2007).

Role of MKK2 in both abiotic stress tolerance and plant disease resistance have been demonstrated in *Arabidopsis*. MKK2 is an upstream activator of MPK4 and MPK6 and plays a critical role in the cold and salt stress response, but does not mediate activation of these MAPKs by the elicitors flagellin and laminarin. MPK4 and MPK6 are also activated upon infection by *P. syringae*. However plants expressing constitutively active *MKK2* have only enhanced MPK4 activity. These plants are more resistant to a virulent strain of *P. syringae* and *Erwinia carotovora*, but hypersensitive to *Alternaria brassicicola*. In contrast, no differences in sensitivity were observed in *mkk2* plants against *E. carotovora* or *A. brassicicola*. Hormone analysis revealed that MKK2-EE plants were compromised in the production of jasmonic acid (JA) and salicylic acid (SA) upon infection by *P. syringae* pv. tomato DC3000 (Brader et al., 2007). Tobacco MAPKK, NtMEK2, is upstream of SIPK and WIPK, two tobacco MAPKs that are activated by various pathogens or pathogen-derived elicitors (Yang et al., 2001).

In one of the initial studies on group B MKKs, Dóczi et al (2007) postulated novel pathogen-signaling pathway showed MKK3 as an activator of group C MAPKs and a positive regulator of *PR* gene expression. MKK3 interacts with the group C MAPKs 1, 2, 7, and 14 as seen from study of mutant plants and protoplast expression analyses. Among the MAPKs which showed interaction, MPK7 was a preferential substrate of MKK3 in presence of H_2O_2, and the MKK3-MPK7 module positively regulate *PR1* expression independent of flg22 elicitation. *MKK3* overexpression plants were tolerant to *P. syringae* whereas *mkk3* mutant showed susceptible reaction for the pathogen. However, role of MKK3-MPK7 module in defense signaling is not through MAMP induction and thought to be part of a secondary response triggered by the oxidative burst. In agreement with the hypothesis, role of MKK3 has also been shown in JA-mediated developmental processes (Takahashi et al., 2007).

The *Arabidopsis MKK7* positively regulates plant basal and systemic acquired resistance (SAR). The activation tagged *bud1* mutant which show high *MKK7* expression accumulates elevated levels of SA, constitutive *PR* gene expression, and displays enhanced resistance to both *P. syringae* and *Hyaloperonospora parasitica*. Reducing mRNA levels of *MKK7* by antisense RNA expression not only compromises basal resistance, but also blocks the induction of SAR. Moreover mere ectopic expression of *MKK7* in local tissues induces *PR* gene expression and resistance to Psm ES4326 in systemic tissues, indicating the activation of MKK7 is sufficient for generating the mobile signal of SAR (Zhang et al., 2007).

Constitutively active forms of MKK4 and MKK5 activate MPK3 and MPK6, which leads to the transcriptional activation of flg22-induced receptor like kinase (*FRK1*) and transcription factors, WRKY22 and WRKY29 (Asai et al., 2002). In rice cell culture, MKK4 ortholog, OsMKK4 is also shown to be involved in PAMP triggered pathway signaling (Kishi-Kaboshi et al., 2010). A chitin elicitor (a fungal MAMP) was shown to activate OsMKK4 which in turn activates two rice MAPKs (OsMPK3 and OsMPK6). OsMPK6 was found to be essential for chitin elicitors induced biosynthesis of diterpenoid phytoalexins. Conditional expression of active OsMKK4 (OsMKK4^{DD}) led to enhanced secondary metabolite biosynthesis and induction of defense responses such as cell death, expression of diterpenoid phytoalexin biosynthetic pathway genes as well as accumulation of these phytoalexins and lignins without generation of extracellular ROS (Kishi-Kaboshi et al., 2010).

Cell division and differentiation

The coordinated expression and activation of MAPK cascade clearly suggests its role in cell division process. In synchronized tobacco cell cultures, the p43NTF6 MAPK is activated at a late stage in mitosis, around the anaphase/early telophase transition, and localizes in the middle of two microtubule arrays, characteristic of the phragmoplast, a plant-specific structure involved in laying down the new cell wall. The timing of both its activation and intracellular localization suggest a role of p43NTF6 in cell plate formation during cell division. With the aim of identifying possible partners for p43NTF6 two-hybrid screening of a tobacco BY-2 cell suspension cDNA library using the p43NTF6 MAPK as bait resulted in the isolation of a cDNA encoding a protein with features characteristic of a MAPKK, which has been named NtMEK1. Protein–protein interaction analysis experiments showed a physical interaction between NtMEK1 and the tobacco MAPK, p43NTF6 and p45NTF4, but not p43NTF3. In kinase assays, NtMEK1 preferentially phosphorylated p43NTF6. Functional studies in yeast showed that p43NTF6 could complement the yeast MAPK mutant *mpk1* when co-expressed with NtMEK1, and that this complementation depended on the kinase activity of p43NTF6. Expression analysis showed that the NtMEK1 and NTF6 genes are co-expressed both in plant tissues and following the induction of cell division in leaf pieces. This work showed that the role of MAPK cascade in facilitating its disassembly from the phragmoplast and thereby allows the formation of the cell plate (Calderini et al., 2001).

In *Arabidopsis*, role of YODA (YDA) in embryo development and stomatal differentiation is known (Bergmann et al., 2004; Bayer et al., 2009; Suarez-Rodriguez et al., 2010). Subsequent studies have shown that the MAPKKs, MKK4 and 5 and the MAPKs, MPK3 and 6 function downstream of YDA (Wang et al., 2007)). The rescued double mutants of either *mkk4 mkk5* or *mpk3 mpk6* showed clustered stomatal patterning indicating that the coordinated cell fate specification of stomata versus pavement cells was disrupted in the mutant plants. In contrast, transgenic plants expressing constitutively active MKK4 or MKK5 showed no stomatal differentiation (Wang et al., 2007). Use of MAPKK inhibitor (PD98059) partially inhibits stomatal closure in *Commelina communis*. Further, PD98059 also abolishes the ABA induced H_2O_2 generation and ABA-mediated stomatal closure in epidermal peels of *Vicia faba* (MacRobbie and Kurup, 2007). Role of MAPK cascade in stomatal development and movement has recently been reviewed (Liu et al., 2010).

Hormonal signaling

Plants being sessile have developed a network of signaling events leading to defensive responses by producing defensive compounds. JA or its methyl ester (MeJA) are plant signaling compounds involved in the regulation of many stress responses and development (Turner et al., 2002). Several studies have revealed that some MAPK cascades are involved in the wound/JA signaling pathway (Zhang and Klessig, 1998; Seo et al., 1999; Ichimura et al., 2000; Petersen et al., 2000; Matsuoka et al., 2002; Thoma et al., 2003; Andreasson and Ellis, 2010; Sinha et al., 2011).

In tobacco NtMPK4 was activated by wounding along with two other wound responsive tobacco MAPKs, WIPK and SIPK. NtMPK4 was activated by SIPKK, which has been isolated as an SIPK interacting MAPK kinase. With suppressed NtMPK4 activity in tobacco wound induced expression of JA responsive genes were also inhibited. Further, *NtMPK4* silenced plants showed enhanced sensitivity to ozone caused by an abnormal regulation of stomatal closure in an ABA independent manner. Additionally, overexpression of constitutively active type *SIPKK*^{EE} in tobacco plants displayed greater responsiveness to wounding and enhanced resistance to ozone. These results suggest that

NtMPK4 is involved in JA signaling and in stomatal movement (Gomi et al., 2005).

The MKK3–MPK6 cascade was shown to play a role in jasmonate dependent negative regulation of ATMYC2/JASMONATE-INSENSITIVE1 (JIN1) (Takahashi et al., 2007). ATMYC2/JIN1 is a major positive regulator of JA inducible gene expression and essential for JA dependent developmental processes in *Arabidopsis thaliana*. JA negatively controls ATMYC2/JIN1 expression. Moreover, JA regulated root growth inhibition is affected by mutations in the MKK3–MPK6 cascade, which indicates their important roles in JA signaling.

MKK also play a crucial role in auxin transport. *bud1*, a semi-dominant *Arabidopsis* activation tagged mutant which show increased *MKK7* expression (Mou et al., 2002) show deficiency in auxin transport. Whereas, *MKK7* antisense plants showed enhancement in auxin transport which indicate that MKK7 negatively regulates polar auxin transport (PAT) (Dai et al., 2006). The *bud1* mutant has an elevated level of SA, and exhibits constitutive *PR* gene expression and enhanced resistance to both Psm ES4326 and *H. parasitica* Noco2. *MKK7* expression induced by pathogen infection in wild type plants and silencing of *MKK7* by antisense RNA expression not only compromises basal resistance but also blocks the induction of SAR, demonstrating that MKK7 is a positive regulator required for both basal resistance and SAR. Ectopic expression of *MKK7* in local tissues induces *PR* gene expression and resistance to the bacterial pathogen Psm E4326 in systemic tissues, indicating that MKK7 activation may be involved in generating the mobile signal for SAR (Zhang et al., 2007). Activation of MAPK in response to auxin (IAA, NAA, and 2,4-D) have been investigated in *Arabidopsis* roots (D-99). Application of MAPKK inhibitors (U0126 and PD098059) reduced the expression of auxin inducible reporter constructs, indicating the activation of an auxin-responsive MAPK cascade in roots (Mockaitis and Howell, 2000).

Ethylene (C_2H_4) was the first example of a gaseous signaling molecule in biological systems, discovered more than a century ago. Extensive studies of ethylene signal transduction in *Arabidopsis* has established a linear pathway connecting five receptors 2–7 to a single negative regulator, constitutive triple response 1 (CTR1) (Kieber et al., 1993), and important downstream positive components, EIN2 (Alonso et al., 2003) and EIN3 transcription factors (Chao et al., 1997). CTR1, a putative Raf-like MAPKKK interacts with the ethylene response 1 (ETR1) receptor (Clark et al., 1998; Huang et al., 2003). The application of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) activates of MPK3 and 6, further MPK6 seems to interact with EIN3. Involvement of MPK6 was also shown in phosphorylation mediated stabilization of ethylene biosynthetic enzymes, ACC synthase (ACS2/6), to promote ethylene synthesis (Liu and Zhang, 2004). Evidence of involvement of a MAPKK, MKK9 in this pathway was given by Yoo et al. (2008). CTR1 in an unconventional way block MKK9–MPK3/MPK6 activation and simultaneously enhancing EIN3 degradation by distinct MAPK phosphorylation (Yoo et al., 2008).

Xu et al. (2008) identified MKK9 as the upstream kinase of both MPK3 and MPK6 in planta. Activation of MKK9 causes activation of the endogenous MPK3 and MPK6, and the subsequent upregulation of multiple genes in ethylene and camalexin biosynthetic pathways and induces high levels of ethylene and camalexin. Mutation of either *MPK3* or *MPK6* compromises MKK9 induced ethylene and camalexin accumulation. As mentioned in earlier section, activation of MKK9 enhances the sensitivity of transgenic seedling to salt stress. The results suggested that MKK9 plays an important role in ethylene and camalexin biosynthesis, and salt stress response in *Arabidopsis*.

Nitric oxide (NO) is an important signal molecule that mediates many developmental and physiological processes in plants. NO functions downstream of the branch point of MeJA and ABA signaling in *Arabidopsis* guard cells (Saito et al., 2009). There has been debate whether NO is upstream or downstream parts of MAPK modules. Previously researchers have indicated that NO can activate MAPK cascade. NO activates SIPK in tobacco and MAPK in maize and *Arabidopsis* (Clarke et al., 2000; Kumar and Klessig, 2000; Zhang et al., 2007a). Others however, studied the MAPK module in regulating the production of NO (Asai et al., 2008). In tobacco, the elicitor INF1 produced by *P. infestans*, can induce the activation of MAPK or MAPKK, MEK1 and MEK2 thus enhances the production of NO (Yamamoto et al., 2004). Recent findings (Wang et al., 2010) showed that MAPK, MPK6 is involved in modulating NO production and signal transduction in response to H_2O_2 during *Arabidopsis* root development. This hypothesis favored that MAPK modules are involved in the regulation of NO production in plants.

MAPKK— a signal convergence point

Many of the MAPK signaling modules have been reported to be overlapping (Andreasson and Ellis, 2010). Literature available regarding plant MAPK cascade gene models clearly indicate that plants use a relatively less number of MAPKKs compared to other MAPK components. The number of MAPKKs in *Arabidopsis* and rice is almost half of the number of MAPKs. *Arabidopsis* genome shows presence of 80 MAPKKKs and 20 MAPKs whereas only 10 MAPKKs (Jonak et al., 2002; MAPK Group, 2002). Similarly in dicot model plant rice 75 MAPKKKs, 8 MAPKK and 15 MAPKs gene models (Hamel et al., 2006; Rao et al., 2010) have been predicted. Further a survey of algal genomes has revealed a single MKK each for the *Chlamydomonas* and *Volvox* (Suarez-Rodriguez et al., 2010). However these algae show

8 to 10 annotated MAPKKKs and 8 MAPKs each for the *Chlamydomonas reinhardtii* and *Volvox carteriin*. These findings suggest that the same MAPKK may function in several MAPK modules. The points of signal convergence may be MAPKKs in at least some cases because of their abilities to activate more than one MAPK (Cardinale et al., 2002; Suarez-Rodriguez et al., 2010). These facts point at the important role of MAPKKs in integrating signals from several MAPKKKs and transducing to various MAPKs. Being central component of the MAPK cascade MAPKKs link a few upstream MAPKKK to the several downstream MAPKs.

Genetic analysis and comprehensive protein phosphorylation and protein-protein interaction studies involving *A. thaliana* MAPKKs and MAPKs indicate that closely related members of plant MAPKKs have similar functions and target MAPKs. For example, *Arabidopsis* group A MAPKK, MKK1 and MKK2 are proposed to activate the MPK4 (Qiu et al., 2008), whereas MKK4 and MKK5 act upstream of MPK3 and MPK6, apparently in a redundant manner (Asai et al., 2002). This hypothesis is exemplified by two studies showing protein-protein interaction of *Arabidopsis* MAPKK and MAPK using yeast two-hybrid analyses and functional protein microarrays (Lee et al., 2008; Popescu et al., 2009). In protein microarray each of the nine of ten *Arabidopsis* MAPKKs (excluding MKK8) was found to phophorylate minimum of four MAPKs. MKK7 was shown to phosphorylate maximum 8 MAPKs. The information about MKK8 targets could not be available since it was not represented in protein microarray experiment (Popescu et al., 2009). MKK8 in spite of having all the canonical MAPKK motifs lacks active expression (Hamel et al., 2006). Nevertheless, yeast two hybrid analyses by Lee's group found no interacting partner of MKK8 (Lee et al., 2008).

Conclusions

MAPK cascades are known to be involved in the transduction of a variety of extracellular signals and the regulation of different developmental processes in plants. In past decade, several members of plants MAPK cascade have been characterized and their role in environmental stresses as well as growth and development was shown. In this review article we described several aspects of role of plant MAPKK in abiotic stress, biotic stress, cell division and differentiation and hormonal signaling. In plants so far only few components of MAPK cascades have been thoroughly studied and the upstream modules in the cascade, i.e. MAPKKK and MAPKK are not thoroughly investigated. It is however not clear that which factor decides a certain MAPKK to phosphorylate and activate which downstream target/s under most of the stresses. It is plausible that spatial, temporal and stress specific expression of the target proteins and co-localization of respective proteins in subcellular components, dynamics of protein-protein interactions may render a level of specificity. Role of upstream phosphorylating MAPKKK in defining MAPKK-MAPK interaction also needs to be investigated to decipher complete MAPK cascade involvement in various response stimuli. The identification of the role of MAPKKs in integrating signals from several MAPKs and the cross talks between different signal transduction pathways will be a future challenge to disentangle the sophisticated network of plant MAPK signaling pathways.

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Abbreviations

MAPK/MPK: mitogen-activated protein kinase; MAPKK/MKK/MEK/MAP2K: mitogen-activated protein kinase kinase; MAPKKK/MKKK/MEKK/MAP3K: mitogen-activated protein kinase kinase kinase

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