

MAPK cascades and major abiotic stresses

Khaled Moustafa · Synan AbuQamar ·
Mohammad Jarrar · Abdul Jabbar Al-Rajab ·
Jocelyne Trémouillaux-Guiller

Received: 27 March 2014 / Revised: 22 April 2014 / Accepted: 26 April 2014 / Published online: 15 May 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract Plants have evolved with complex signaling circuits that operate under multiple conditions and govern numerous cellular functions. Stress signaling in plant cells is a sophisticated network composed of interacting proteins organized into tiered cascades where the function of a molecule is dependent on the interaction and the activation of another. In a linear scheme, the receptors of cell surface sense the stimuli and convey stress signals through specific pathways and downstream phosphorylation events controlled by mitogen-activated protein (MAP) kinases and second messengers, leading to appropriate adaptive responses. The specificity of the pathway is guided by scaffolding proteins and docking domains inside the interacting partners with distinctive structures and functions. The flexibility and the fine-tuned organization of the signaling molecules drive the activated MAP kinases into the appropriate location and connection to control and integrate the information flow. Here, we overview recent findings of the involvement of MAP kinases in major

abiotic stresses (drought, cold and temperature fluctuations) and we shed light on the complexity and the specificity of MAP kinase signaling modules.

Keywords MAP kinases · Mitogen-activated protein · Environmental abiotic stresses · Plant adaptation

Abbreviations

MAP	Mitogen activated protein
MAPK	Mitogen activated protein kinase
MAP2K, MEK, MKK, or MAPKK	MAPK kinase
MAP3K, MEKK, MKKK or MAPKKK	MAPK kinase kinase
MAP4K, MEKKK, MKKKK or MAPKKKK	MAPK kinase kinase kinase
S/T	Serine/threonine
T(E/D)Y	Threonine(glutamate/aspartate)tyrosine

Communicated by N. Stewart.

K. Moustafa (✉)
Institut National de la Santé et de la Recherche Médicale (INSERM), Créteil, France
e-mail: khaled.moustafa@inserm.fr

S. AbuQamar
United Arab Emirates University, Al Ain, United Arab Emirates

M. Jarrar
University of Modern Sciences, Dubai, United Arab Emirates

A. J. Al-Rajab
Jazan University, Jazan, Saudi Arabia

J. Trémouillaux-Guiller
Université François-Rabelais, Tours, France

Introduction

Drought, salinity, and temperature shifts are major abiotic environmental stresses that affect civilizations since the dawn of agriculture with recurring waves of different intensities and amplitude. Mitogen-activated protein (MAP) kinase cascades convey stress signals from receptors to specific effectors to regulate gene expression, cell activities and protein functions in various developmental and adaptive processes. The achievement of sequencing of some plant genomes reveals that MAP kinases are tiered into four gene family classes. Arabidopsis genome, for example, encodes for 10 MAP4K, 80 MAP3K, 10 MKK

and 20 MPK genes for which a new nomenclature and group classification were established (Ichimura et al. 2002). Due to the lack of information of the MAP4Ks, MAP kinase signaling modules are typically described to be composed of three proteins, starting with a MAP kinase kinase kinase (known also as MAP3K, MEKK, MKKK, or MAPKKK), which activates a MAP kinase kinase (MAP2K, MEK, MKK or MAPKK), which, in turn, activates a MAP kinase (MAPK, also called MPK). The activation of these MAP kinases is a sequential process through the phosphorylation of some conserved residues within an activation loop in their kinase domains. Following an internal or external signal, a MAP3K protein phosphorylates a MAP2K in two serine/threonine residues, in the consensus motif S/TxxxxS/T, which in turn activates a MAPK by phosphorylation on threonine and tyrosine residues in T(E/D)Y conserved motif. MAP kinases, with phosphatases, function as On/Off signal switchers to regulate the activity of many downstream targets such as cytoskeletal proteins in the cytosol or transcription factors in the nucleus to control cell signaling in plant adaptation to environmental oscillations.

Although environmental factors are natural components of the ecosystem, hence hardly unavoidable hazards, sub- and supra optimal ranges of these factors disturb plant growth and reduce plant productivity by up to 50 % (Bray et al. 2000). To maintain plant productivity and sustain ecology under environmental harsh conditions, the development of new crop varieties is of great importance to secure food availability and quality under exacerbate climate change. The first step towards such an objective is to understand the molecular and genetic bases of plant interactions with its adverse environmental conditions. The integration of genetic and Omics approaches such as genomics, transcriptomics, proteomics and metabolomics have opened wide avenues towards the dissection of the adaptation mechanisms in several living organisms. Deeper understanding of such mechanisms helps the bioengineering of new adaptable plant cultivars in a relatively short time compared to classical breeding approaches. Here, we summarize recent findings in the involvement of MAP kinases signaling pathways in response to the most devastating abiotic stresses worldwide; drought, salt and extreme temperatures. The complexity of MAP kinase modules are also debated in the light of new findings.

MAP kinase complexity in environmental stresses' signaling

Unlike the interaction with a biotic stress, which is mostly provoked by one living factor, plant interaction with abiotic stresses is multivariable complex trait. In natural

ecosystem, one type of abiotic stresses comes rarely alone. Drought, salt and temperatures are closely related with overlapping physiological effects (Krasensky and Jonak 2012). High temperatures, for example, frequently come with drought and water deficits, aggravating the effects of salinity and osmotic shifts on plant cells (Bitá and Gerats 2013; Xiong et al. 1999). Salinity is also a common feature in arid and semiarid lands where plants are convicted to tolerate osmotic stress caused by both salinity and low water potential (Rengasamy 2006). As a result, a complex signaling network should operate and activate relevant signaling pathways to allow plants survival.

Plant survival under multiple and simultaneous environmental conditions implies complex processes of signal reception, transmission, amplifications and interaction. The transmission of information in biological systems is rarely one-way direction. Signal transmission is rather a complex crosstalk activity involving different molecules at different levels of organization. The large number of MAP kinases and stress-responsive genes outlines this complexity and suggests how stressed plants struggle to adapt to diverse and adverse conditions.

Signal transmission complexity via MAP kinases is further layered by the fact that MAP kinases are multi-tiered gene families recruited for multiple functions such as development, immune defense system, hormones signaling and responses to biotic and abiotic environmental stresses. MAP kinase signaling is also convoluted by opposite regulation patterns under the same or different environmental stresses. In fact, while many MAP kinases are described to be positively regulated under abiotic stresses, some MAP kinases play negative roles in stress tolerance. For example, overexpression of the MAP kinases, *OsMAPK33* in rice (Lee et al. 2011) and *MKK9* in Arabidopsis (Xu et al. 2008), results in high sensitivity of rice and Arabidopsis, respectively, to salt stress. Moreover, the inactivation of the Arabidopsis *MKK9* by the insertion of transferred DNA (T-DNA) generates salt-insensitive phenotypes that germinate at up to 150 mM NaCl, with rapid induction of *RD22* and *RD29* stress genes (Alzwy and Morris 2007). A *mapkkk* knockout mutant of the Arabidopsis MAPKKK20 (MAP kinase kinase kinase) presents an improved salt tolerance, particularly in the later developmental stages (Gao and Xiang 2008). Additionally, the Arabidopsis double mutant *mpk9/mpk12* loses water by transpiration less than the wild type (Jammes et al. 2009).

The dichotomy of positive/negative regulation of MAP kinases in stress responses adds an extra layer of MAP kinases complexity and specificity that need to be elucidated. Due to a large number of homologous MAP kinases in plants, MAP kinases specificity is a tricky question that may lead to a possible redundancy in diverse biological processes. One powerful method to elucidate this question

is to systematically silence MAP kinases and then characterize the resulting phenotypes to have answers to the redundancy or complementarity issue.

The control of a plethora of cellular processes by MAP kinases implies fine tuning specificity mechanisms that are associated with MAP kinase activation and function. Specificity mechanisms have been extensively studied in yeast and mammals, and several proteins have been identified as scaffolding molecules that assemble different MAP kinases into one specific signaling pathway. Looking for analogous scaffolding proteins in plants revealed the presence of some MAP kinases that play dual roles as scaffold protein and kinase enzyme at the same time. For example, the MAPKKK OMTK1 (oxidative stress-activated MAP triple kinase 1), which belongs to the alfalfa MAPKKK family, plays a role as an adapter and a phosphate transporter at the same time (Nakagami et al. 2004). Other studies report that MAP kinases would act as structural adaptors and enzymatic activators in yeast (Edmunds and Mahadevan 2004). The presence of docking domains that form dovetail joints between compatible components in a given signaling pathway is another mechanism of MAP kinase specificity (Tanoue et al. 2000). The spatial localization of MAP kinases may also increase target specificity with the advantage that colocalization of interacting partners would save cells significant amount of energy and reduce physical and functional effects of interfering molecules at long-distance courses.

Among the other unanswered questions in MAP kinase signaling is the identification of MAP kinase substrates in each physiological or biochemical condition. In a high-throughput phosphorylation approach, 48 potential substrates of MPK3 and 39 of MPK6 were identified (Feilner et al. 2005). However, *in vivo* characterization will be required to confirm the potential interaction between these MAP kinases and their corresponding partners.

MAPKs and drought

Drought is one of the major, if not the most, detrimental abiotic stresses worldwide. Drought has devastating impacts not only on plant metabolism and productivity, but also at socioeconomic levels with extended damages. A number of transcriptional and biochemical studies have reported the involvement of MAP kinases in drought responses in many grassy and woody plants. In *Arabidopsis*, for example, it was shown that the MAP kinase MPK6 is required to enhance plant tolerance to dehydration via the regulation of RNA decapping activity (Xu and Chua 2012). In transient expression assays, Hua et al. (2006) found that constitutively active forms of

Arabidopsis MPK and MKK members are able to activate the promoter of *RD29*, a dehydration-responsive gene, suggesting that MAP kinase cascades are involved in drought signaling. The analysis of the transcriptional regulation of 44 MAP kinases under different abiotic stresses demonstrated that *Arabidopsis* MPK2, MPK3, MPK4, MPK5, MPK12 and MAPKKK4 are induced by water stress (Moustafa et al. 2008).

In rice (*Oryza sativa*), Ning et al. (2010) have identified a Raf-like MAPKKK protein, named DSM1 (drought sensitive mutant1) that functions as a potential scavenger of the reactive oxygen species (ROS), increasing plant tolerance to dehydration under water stress. Drought tolerance in rice is also enhanced by overexpression of the MAP kinase *OsMAPK5*, an ortholog of the *Arabidopsis* MPK3 (Xiong and Yang 2003). A rapid induction of another rice MAP kinase, *OsMSRMK2*, was observed within 15 min in response to drought and increased levels of salt (Agrawal et al. 2002), suggesting functional roles of the *OsMSRMK2* in water stress signaling. Moreover, five of the ten members of the rice MAPKK family have been reported as transcriptionally regulated by drought (Kumar et al. 2008).

At protein level, the kinase activity of the alfalfa MAP kinase MMK4 is inducible by drought, though no correlation between protein activity and transcript levels was observed (Jonak et al. 1996). This suggests that post-translational regulation mechanisms may exist to modulate MAP kinase activity under stress conditions. In maize, a new MAP kinase, *ZmMPK3*, was also identified as a drought-responsive gene (Wang et al. 2010), and the genetic transformation with the tobacco MAPKKK *NPK1* enhances the maize tolerance to drought (Shou et al. 2004).

To improve the quality and adaptability of cotton (*Gossypium* spp.), extensive research is undertaken in many research centers. Recently, Zhang et al. (2011) have identified *GhMPK2* as a cotton MAP kinase that functions in reducing water loss and adjusting osmotic pressure under drought conditions. Another new cotton MAP kinase, called *GhMPK16*, belonging to the MAPK group D, was also identified as a drought-responsive gene (Shi et al. 2011). Subcellular localization analysis revealed that both *GhMPK2* and *GhMPK16* translocate into the nucleus, suggesting that both kinases are potential activators of transcription factors that function under drought conditions.

In woody and higher plants, MAP kinases also respond to drought stress. In apple (*Malus* spp.), the MAP kinase *MaMPK* is upregulated at transcriptional and protein levels in three apple species with strong activity in the drought-tolerant species *Malus sieversii* (Peng et al. 2006), indicating that *MaMPK* could be associated with the natural drought-tolerance trait in apple.

MAPKs and osmotic stress

Plant responses to salt stress involve different adaptive mechanisms such as ion exclusion and sequestration into the vacuole, particularly Na^+ and Cl^- to reduce their cytotoxicity. The synthesis of protective proteins, such as LEA (late embryogenesis abundant) proteins and chaperones (e.g., heat shock proteins) is also accelerated to buffer the negative effects of the toxic ions. As signal transducers, MAP kinases convey osmotic stress signals to appropriate effectors and contribute to the adaptation of plant cells to the high salt concentrations. Research data from several studies support this assumption and show that MAP kinases are regulated by osmotic and salt stresses at both transcriptional and protein activity levels. Recently, Hoang et al. (2012) have reported that the MAP kinase MPK6 activates, and interacts with, MYB41 and results in enhanced salt tolerance in Arabidopsis. In addition, the regulation of MPK6 by the phosphatidic acid (PA) under salt stress suggests that MPK6 and PA may function in the same signaling pathway in response to salt stress (Yu et al. 2010).

A potential MAP kinase signaling pathway mediating salt stress responses, composed of *MEKK1*, *MKK2* and the interchangeable *MPK4/MPK6* genes, was previously identified in Arabidopsis (Teige et al. 2004). Transgenic plants overexpressing *MKK2* exhibit constitutive upregulation of stress marker genes and show enhanced salt tolerance. The expression of different active forms of the MKK and MPK gene family members results in the activation of *RD29A* and *RD29B* gene promoters (Hua et al. 2006), suggesting that MAP kinases could be upstream activators of the stress marker genes under salinity conditions. Additionally, five MPKs (*MPK9*, *MPK10*, *MPK11*, *MPK17* and *MPK18*), two MKKs (*MKK7* and *MKK9*) and four MEKKs (*MEKK3*, *MEKK5*, *MEKK6* and *MEKK7*) were induced by 200 mM sodium chloride (NaCl) (Moustafa et al. 2008). Arabidopsis transgenic plants that overexpress the maize MAP kinase *ZmSIMK1* showed an enhanced tolerance to high-salinity stress (Gu et al. 2010).

Recently, a new MAP kinase kinase kinase, called MKKK20, has been identified as a potential regulator of osmotic stress in Arabidopsis through the activation of MPK6 (Kim et al. 2012). The Arabidopsis *mkkk20* mutant exhibits high rates of water loss and superoxide accumulation, while transgenic plants overexpressing *MKKK20* exhibit an enhanced tolerance to osmotic stress provoked by NaCl, mannitol or sorbitol, suggesting important roles of MAPKKK20 in osmotic adjustment and stress tolerance (Kim et al. 2012). Previously, it has been reported that Arabidopsis MKK4 was induced at transcriptional level in Arabidopsis cells and seedlings and that it mediates the osmotic stress responses via the kinase *MPK3* (Kim et al.

2011). The induction by osmotic stress of the Arabidopsis *MPK17* and its homolog in maize *ZmMPK17* (Pan et al. 2012) suggests that MPK17 and its ortholog could be good candidates for further functional characterizations and involvement in abiotic stress adaptation. Interestingly, under hypoosmotic stress, the Arabidopsis *MPK20* presents an induction pattern similar to that of proline dehydrogenase (ProDH) (Moustafa et al. 2008), suggesting that MPK20 and ProDH may interact with each other and be part of functional hypoosmotic stress-signaling pathway.

In alfalfa, the MKK member, SIMKK, functions as an upstream activator of the SIMK (a MAPK) under salt stress conditions (Kiegerl et al. 2000). Osmotic stress also induces a rapid and transient activation of the salicylic acid-induced kinase (SIPK, an Arabidopsis MPK6 homolog in tobacco) in tobacco cells within 5–10 min (Mikolajczyk et al. 2000).

In maize, Kong et al. (2011a) have recently isolated a novel MAP kinase kinase, named *ZmMKK4*, whose expression is modulated by salinity stress. The overexpression of *ZmMKK4* in tobacco plants results in enhanced osmotic regulation.

In rice, several studies demonstrate that MAP kinases are implicated in salt stress tolerance. For example, the MAP kinases OsMPK44 (Jeong et al. 2006), OsMSRMK3 (Agrawal et al. 2003), OsMSRMK2 (Agrawal et al. 2002), OsEDR1 (Kim et al. 2003), OsMAPK5 (Xiong and Yang 2003) and OsMAPK4 (Fu et al. 2002) have been identified as salt stress-responsive genes. Moreover, rice transgenic lines overexpressing *OsMPK5* and *OsMPK44* demonstrate an improved tolerance of salt (Jeong et al. 2006; Xiong and Yang 2003). Similarly, transgenic cotton overexpressing *GhMPK2* exhibits an enhanced osmotic adjustment and increased salt tolerance (Zhang et al. 2011). Finally, it was shown that the MAP kinase *CsNMAPK* is induced in cucumber roots upon salt treatments and the tobacco transgenic seeds overexpressing *CsNMPAK* exhibit higher germination rates compared to the wild type (Xu et al. 2010), suggesting that *CsNMPAK* plays positive roles in plant adaptation to salt stress at early stages.

MAPKs and temperature stresses

Temperature is an essential environmental factor for plant growth, metabolism and productivity. Due to large amount of water in plant tissues, the behavior of cell water under different temperatures is a key factor for cell survival. Depending on species and developmental stages, plants can grow healthy in wide ranges of temperatures (Went 1953). Beyond these ranges, plants could suffer from severe temperature damages. Low temperatures can inhibit germination, growth and metabolic activities (Roberts 1988).

Cold stress also provokes water deficit and disturbs the membrane integrity by the formation of icy crystals (Yamazaki et al. 2009). High temperatures, on the other hand, provoke heat stress and accelerate the respiration (Abele et al. 2002) and the photosynthetic rates (Pastenes and Horton 1996), leading to overproduction of reactive oxygen species that affect plant growth and productivity.

So far, no specific receptor has been identified in plants in response to temperature stresses. However, the plasma membrane itself could play the role of primary sensor of temperature fluctuations (Sangwan et al. 2002). In fact, the structural modifications in plasma membrane caused by temperature shifts can trigger downstream-specific signaling events. While low extreme temperatures increase the rigidification of the plasma membrane, high temperatures increase its fluidization. Such fluctuations alter the activity of the membrane-associated proteins such as ATPases, ions transporters, H⁺ pumps and protein channels. Both changes (rigidification and fluidization) are perceived as primary stimuli signals that trigger fluxes of second messengers (e.g., Ca⁺²) to activate specific heat response. Heat responses include the production and activation of heat shock proteins (HSPs) (Vierling 1991); heat shock transcription factors (HSFs); cold shock domain containing proteins (CSDPs) (Karlson and Imai 2003); ROS scavengers; antioxidants; compatible solutes; calcium-dependent protein kinases (CDPKs) and MAP kinases.

Although little is known about MAP kinase signaling in responses to heat in plant, specific MAP kinases are upregulated and/or activated by temperature stresses. The heat shock activated MAP kinase (HAMK) was identified as the first heat responsive MAP kinase in alfalfa cells (Sangwan and Dhindsa 2002). The activation of HAMK in tobacco cells was observed conjunctly with the accumulation of heat shock protein, HSP70, suggesting that HAMK may trigger a heat signaling cascade via HSP genes (Suri and Dhindsa 2008). The activation of HAMK also seems to be calcium dependent in tomato leaves and cells exposed to heat stress (Link et al. 2002). Heat and calcium-dependent activation of MAP kinases is also observed for MPK6 in Arabidopsis to regulate the activity of vacuolar processing enzyme (VPE) (Li et al. 2012). In a recent study (Kong et al. 2012), it was shown that the transcript abundance of the tomato MAP kinase SIMAPK increases after heat treatment.

In rice, a part of potentially interacting MAP kinases cascade, composed of OsMKK6-OsMPK3, has been recently identified in response to chilling (Xie et al. 2012). In the same way, OsMEK1 (a putative rice MAP kinase kinase, isolated by subtractive screening of cDNA library) has been shown to interact with OsMAP1 at 12 °C, suggesting that both kinases, OsMEK1 and OsMAP1, may be involved in the temperature stress signaling (Wen et al.

2002). Another MAP kinase, called OsMAPK4, has also been identified in rice leaves and roots with strong upregulation by cold stress (Fu et al. 2002). In a recent transcriptional profiling study carried out on cassava, a tropical drought-tolerant crop, homologous of the Arabidopsis MPK4, was identified as one of the genes upregulated by cold stress (7 °C) (An et al. 2012). Surprisingly, the mRNA transcripts of the rice OsMSRMK2 are regulated at both high (37 °C) and low-moderate (12 °C) temperatures (Agrawal et al. 2002). OsMKK4 and OsMKK6 are also strongly regulated by cold and heat treatments (Kumar et al. 2008). Such contradictory results in opposite conditions could be explained by great functional plasticity of the MAP kinase signalling pathways.

In maize, a MPKK, ZmMKK4 (Kong et al. 2011b) and a MPK ZmMPK17 (Pan et al. 2012) have been described to be inducible by cold stress. Both proteins are mainly located in the nucleus, suggesting that they regulate the activity of transcription factors functioning in plant adaptability to cold stress. This assumption may be supported by a high germination rate of tobacco seeds that overexpress *ZmMPK17* and accumulate high levels of proline. Five other MAP kinases were upregulated in maize roots at low temperatures (Wu et al. 2011). In cotton, the transcript level of the MAP kinase, *GhMAPK*, increased markedly in plant seedlings subjected to cold temperature (4 °C) (Wang et al. 2007), suggesting that MAP kinases may play protective roles against cold effect on root cells.

The previously mentioned MAPK cascade (composed of MEKK1, MKK2 and MPK4/MPK6), which functions in salt stress signaling in Arabidopsis, is also functional in cold stress pathway (Teige et al. 2004). The *mkk2* mutant presents a freezing sensitive phenotype with no observable activity neither for MPK4 nor MPK6, suggesting that MKK2 is an upstream activator of MPK4 and MPK6 in cold signaling. This finding is consistent with previous studies showing that cold stress activates MEKK1, MPK3, MPK4 and MPK6 (Mizoguchi et al. 1996; Ichimura et al. 2000).

Finally, the MAP kinase kinase *SbMAPKK* isolated from salt-tolerant species, *Salicornia brachiata*, is induced by many abiotic stresses with maximum expression levels at cold treatment (Agarwal et al. 2010), suggesting that cold and salt stresses may have common kinase components that allow plants to cope with these harsh environmental conditions. It is also worthy to note that some MAP kinases are involved in the physiological recovery after the exposure to cold stress. For example, the transcript levels of the maize MAP kinase *ZmMPK5* increase after a temperature shift from 5 to 25 °C (Berberich et al. 1999). Table 1 summarizes a list of MAP kinase genes involved in the responses to multiple abiotic stresses in different plant species, based on changes in transcriptional regulation and/or enzymatic activities.

Table 1 A non-exhaustive list of MAP kinases involved in abiotic stress responses, based on transcriptional regulation and/or protein activity in different plant species

Plant	MAP kinase	Locus or accession number	Type of abiotic stress	References
Arabidopsis	<i>AtMEKK1</i>	NM_100771	Cold, salinity, dehydration	Mizoguchi et al. (1996), Moustafa et al. (2008), Teige et al. (2004)
	<i>AtMEKK6</i>	NM_111677		
	<i>AtMPK4</i>	AAK64089	Hypoosmolarity	Droillard et al. (2004), Moustafa et al. (2008)
	<i>AtMPK20</i>	AAD21721		
	<i>AtMPK1</i>	AAD32871	Cold, dehydration,	Ichimura et al. (2000), Droillard et al. (2002), Yu et al. (2010)
	<i>AtMPK3</i>	AAK62406	hyperosmolarity, salinity	
	<i>AtMPK6</i>	AAB64027		
	<i>AtMKK2</i>	AAK60281	Cold, salinity	Teige et al. (2004)
	<i>AtMKK7</i>	AAF25995	Dehydration,	Moustafa et al. (2008)
	<i>AtMKK9</i>	AAG30984	hyperosmolarity, salinity	
	<i>AtMPK9</i>	BAA92223		
	<i>AtMPK17</i>	AAC67338		
	<i>AtMPK18</i>	AAG51978		
	Rice	<i>OsMPK3</i>	DQ826422	Drought, temperatures, salinity
<i>OsMPK7</i>		DQ826424		
<i>OsMKK4</i>		DQ848674	Cold, heat	Kumar et al. (2008), Wen et al. (2002)
<i>OsMKK6</i>		DQ779790		
<i>OsMKK6</i>		DQ779790	Cold, drought, salinity	
<i>OsMPK3</i>		DQ826422		
<i>OsMKK10-2</i>		EF666056	Cold	
<i>OsMPK4</i>		FJ621301	Cold, drought, salinity	Xiong and Yang (2003), Fu et al. (2002)
<i>OsMPK5</i>		NM_001056305		
<i>OsMKK6</i>		DQ779790	Cold	Xie et al. (2012)
<i>OsMPK3</i>		DQ826422		
<i>DSM1</i>		Os02g50970	Drought	Ning et al. (2010)
<i>OsEDR1</i>		Os10g0430900	Salinity	Kim et al. (2003)
<i>OsMAPK44</i>		Os05g49140	Salinity	Jeong et al. (2006)
Alfalfa	<i>MMK4</i>	X82270	Cold, drought	Jonak et al. (1996)
	<i>HAMK</i>	–	Heat	Sangwan et al. (2002)
Maize	<i>ZmMPK3</i>	EU130900	Cold, drought, salinity	Wang et al. (2010)
	<i>ZmMPK5</i>	AB016802	Cold, salinity	Berberich et al. (1999)
	<i>ZmSIMK1</i>	AY433815	Salinity	Gu et al. (2010)
	<i>ZmMKK4</i>	NM_001154404	Cold, salinity	Kong et al. (2011)
	<i>ZmMPK17</i>	NM_001154688	Cold	Pan et al. (2012)
Cotton	<i>GhMPK2</i>	FJ966890	Cold, drought, salinity	Wang et al. (2007), Zhang et al. (2011)
	<i>GhMPK16</i>	FJ966889	Drought	Shi et al. (2011)
Tomato	<i>SIMAPKs</i> (16 genes)	Ex. SIMPK1 (AAP20419) SIMPK16 (NP_001233915)	Heat	Kong et al. (2012)
Cucumber	<i>CsNMAPK</i>	DQ812086	Salinity	Xu et al. (2010)
Potato	<i>StMPK1</i>	AB062138	Heat	Blanco et al. (2006)
Tobacco	<i>SIPK</i>	AB098730	Osmotic, salinity	Mikolajczyk et al. (2000)
Apple	<i>MaMPK</i>	AF435805	Drought	Peng et al. (2006)
<i>Chorispora bungeana</i>	<i>CbMAPK3</i>	AY805424	Cold, salinity	Zhang et al. (2006)
<i>Salicornia brachiata</i>	<i>SbMAPKK</i>	EB484994	Dehydration, cold, salinity	Agarwal et al. (2010)

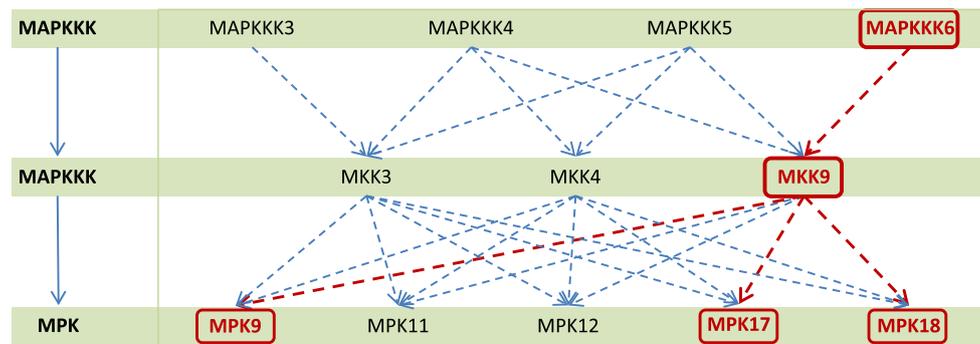


Fig. 1 Hypothetic MAP kinase cascade inferred from gene expression data and co-regulation patterns. Based on gene expression profiling, gene co-regulation and induction patterns by major abiotic stresses (drought, salt and temperatures) (Table 1), a hypothetic MAP

kinase cascade (in red) could be investigated for plant tolerance to abiotic stresses: MAPKKK6→MKK9→MPK9/MPK17/MPK18. Dotted arrows suggest potential interactions between MAP kinases in abiotic stress responses

Perspective and concluding remarks

Several MAP kinases have been identified in response to multiple environmental stresses. MAP kinases serve as signal carriers and scaffolding proteins, linking complex arrays of interacting molecules in developmental and adaptive responses. In the forthcoming years, there will be an urgent need to identify MAP kinase substrate and develop new biotechnological approaches that accelerate the breeding of new plant species that should be well adapted to environmental fluctuations to secure food supply for growing population. Mainly, it would be essential to identify and quantify interacting proteins that act coordinately under multiple environmental stresses. With their mode of action in a sequential way (MAPKKK-MAPKK-MAPK), MAP kinases offer an interesting potential to be assembled into one functional “MAPK transgenic circuits” to be delivered into target cells as a ‘gene kit’ or a ‘gene circuit’. To accomplish such an approach, research is needed to determine the specific interacting MAP kinases and their targets and to define where and when these interacting partners may provide new characteristics of interest. Based on gene expression data and information presented in Table 1, an inferred MAP kinase cascade can be designed (Fig. 1) and tested in future research for the assessment of plant tolerance to abiotic stresses. The identification of MAPK targets would also help in the construction of complete functional proteins circuit from receptor to final target genes. Transgenic circuit directing protein–protein interactions hold the promise of consequential effects and may provide a better transformation efficacy than the conventional genetic transformation based on only one single gene. Rational designing of transgenic circuit and taking into account the specific molecules that are most likely to interact with each other, is an important criterion for the success of such an approach. Wiring interacting molecules in a transgenic circuit and delivering

it into plant cell would maintain the functional equilibrium of the interacting partners and result in efficient and synergistic adaptive responses.

Much also can be learned about MAP kinase functions, interaction and complexity network from experimental and theoretical studies as well, such as systems biology and in silico predictions. However, it is indispensable to investigate MAP kinase functions and interacting partners in vivo at whole plant levels. In vivo molecular imaging under stress conditions may help identifying interacting molecules at both qualitative and quantitative levels. Such analysis, accomplished by the generation of MAP kinase mutants, will aid the determination of the precise signaling inputs and outputs involved in the organization and the regulation of MAP kinase cascades under multiple environmental abiotic stress conditions.

Conflict of interest None.

References

- Abele D, Heise K, Portner HO, Puntarulo S (2002) Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*. J exp biol 205:1831–1841
- Agarwal PK, Gupta K, Jha B (2010) Molecular characterization of the *Salicornia brachiata* SbMAPKK gene and its expression by abiotic stress. Mol Biol Rep 37:981–986
- Agrawal GK, Rakwal R, Iwahashi H (2002) Isolation of novel rice (*Oryza sativa* L.) multiple stress responsive MAP kinase gene, OsMSRMK2, whose mRNA accumulates rapidly in response to environmental cues. Biochem Biophys Res Commun 294:1009–1016
- Agrawal GK, Agrawal SK, Shibato J, Iwahashi H, Rakwal R (2003) Novel rice MAP kinases OsMSRMK3 and OsWJUMK1 involved in encountering diverse environmental stresses and developmental regulation. Biochem Biophys Res Commun 300:775–783
- Alzwy IA, Morris PC (2007) A mutation in the Arabidopsis MAP kinase kinase 9 gene results in enhanced seedling stress tolerance. Plant Sci 173:302–308

- An D, Yang J, Zhang P (2012) Transcriptome profiling of low temperature-treated cassava apical shoots showed dynamic responses of tropical plant to cold stress. *BMC Genom* 13:64
- Berberich T, Sano H, Kusano T (1999) Involvement of a MAP kinase, ZmMPK5, in senescence and recovery from low-temperature stress in maize. *Mol Gen Genet MGG* 262:534–542
- Bitra CE, Gerats T (2013) Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Front Plant Sci* 4:273
- Blanco FA, Zanetti ME, Casalongue CA, Daleo GR (2006) Molecular characterization of a potato MAP kinase transcriptionally regulated by multiple environmental stresses. *Plant Physiol Biochem PPB/Societe francaise de physiologie vegetale* 44:315–322
- Bray EA, Bailey-Serres J, Weretilnyk E (2000) Responses to abiotic stress. In: Buchanan B, Grussem W, Jones R (eds) *Biochemistry and molecular biology of plants*, p 1158–1203
- Droillard M, Boudsocq M, Barbier-Brygoo H, Lauriere C (2002) Different protein kinase families are activated by osmotic stresses in *Arabidopsis thaliana* cell suspensions. Involvement of the MAP kinases AtMPK3 and AtMPK6. *FEBS Lett* 527:43–50
- Droillard MJ, Boudsocq M, Barbier-Brygoo H, Lauriere C (2004) Involvement of MPK4 in osmotic stress response pathways in cell suspensions and plantlets of *Arabidopsis thaliana*: activation by hypoosmolarity and negative role in hyperosmolarity tolerance. *FEBS Lett* 574:42–48
- Edmunds JW, Mahadevan LC (2004) MAP kinases as structural adaptors and enzymatic activators in transcription complexes. *J Cell Sci* 117:3715–3723
- Feilner T, Hultschig C, Lee J, Meyer S, Immink RG, Koenig A, Possling A, Seitz H, Beveridge A, Scheel D, Cahill DJ, Lehrach H, Kreutzberger J, Kersten B (2005) High throughput identification of potential Arabidopsis mitogen-activated protein kinase substrates. *Mol Cell Proteomics MCP* 4:1558–1568
- Fu SF, Chou WC, Huang DD, Huang HJ (2002) Transcriptional regulation of a rice mitogen-activated protein kinase gene, OsMAPK4, in response to environmental stresses. *Plant Cell Physiol* 43:958–963
- Gao L, Xiang CB (2008) The genetic locus At1g73660 encodes a putative MAPKKK and negatively regulates salt tolerance in Arabidopsis. *Plant Mol Biol* 67:125–134
- Gu L, Liu Y, Zong X, Liu L, Li DP, Li DQ (2010) Overexpression of maize mitogen-activated protein kinase gene, ZmSIMK1 in Arabidopsis increases tolerance to salt stress. *Mol Biol Rep* 37:4067–4073
- Hoang MHT, Nguyen XC, Lee K, Kwon YS, Pham HTT, Park HC, Yun D-J, Lim CO, Chung WS (2012) Phosphorylation by AtMPK6 is required for the biological function of AtMYB41 in Arabidopsis. *Biochem Biophys Res Commun* 422:181–186
- Hua ZM, Yang X, Fromm ME (2006) Activation of the NaCl- and drought-induced RD29A and RD29B promoters by constitutively active Arabidopsis MAPKK or MAPK proteins. *Plant Cell Environ* 29:1761–1770
- Ichimura K, Mizoguchi T, Yoshida R, Yuasa T, Shinozaki K (2000) Various abiotic stresses rapidly activate Arabidopsis MAP kinases AtMPK4 and AtMPK6. *Plant J Cell Mol Biol* 24:655–665
- Ichimura K, Shinozaki K, Tena G, Sheen J, Henry Y, Champion A, Kreis M, Zhang S, Hirt H, Wilson C, Heberle-Bors E, Ellis BE, Morris PC, Innes RW, Ecker JR, Scheel D, Klessig DF, Machida Y, Mundy J, Ohashi Y, Walker JC (2002) Mitogen-activated protein kinase cascades in plants: a new nomenclature. *Trends Plant Sci* 7:301–308
- Jammes F, Song C, Shin D, Munemasa S, Takeda K, Gu D, Cho D, Lee S, Giordo R, Sritubtim S, Leonhardt N, Ellis BE, Murata Y, Kwak JM (2009) MAP kinases MPK9 and MPK12 are preferentially expressed in guard cells and positively regulate ROS-mediated ABA signaling. *Proc Natl Acad Sci USA* 106:20520–20525
- Jeong M-J, Lee S-K, Kim B-G, Kwon T-R, Cho W-S, Park Y-T, Lee J-O, Kwon H-B, Byun M-O, Park S-C (2006) A rice (*Oryza sativa* L.) MAP kinase gene, OsMAPK44, is involved in response to abiotic stresses. *Plant Cell Tissue Organ Cult* 85:151–160
- Jonak C, Kiegl S, Ligterink W, Barker PJ, Huskisson NS, Hirt H (1996) Stress signaling in plants: a mitogen-activated protein kinase pathway is activated by cold and drought. *Proc Natl Acad Sci USA* 93:11274–11279
- Karlson D, Imai R (2003) Conservation of the cold shock domain protein family in plants. *Plant Physiol* 131:12–15
- Kiegl S, Cardinale F, Siligan C, Gross A, Baudouin E, Liwosz A, Eklof S, Till S, Bogre L, Hirt H, Meskiene I (2000) SIMKK, a mitogen-activated protein kinase (MAPK) kinase, is a specific activator of the salt stress-induced MAPK, SIMK. *Plant Cell* 12:2247–2258
- Kim JA, Agrawal GK, Rakwal R, Han KS, Kim KN, Yun CH, Heu S, Park SY, Lee YH, Jwa NS (2003) Molecular cloning and mRNA expression analysis of a novel rice (*Oryza sativa* L.) MAPK kinase, OsEDR1, an ortholog of Arabidopsis AtEDR1, reveal its role in defense/stress signalling pathways and development. *Biochem Biophys Res Commun* 300:868–876
- Kim SH, Woo DH, Kim JM, Lee SY, Chung WS, Moon YH (2011) Arabidopsis MKK4 mediates osmotic-stress response via its regulation of MPK3 activity. *Biochem Biophys Res Commun* 412:150–154
- Kim J-M, Woo D-H, Kim S-H, Lee S-Y, Park H-Y, Seok H-Y, Chung W, Moon Y-H (2012) Arabidopsis MKKK20 is involved in osmotic stress response via regulation of MPK6 activity. *Plant Cell Rep* 31:217–224
- Kong X, Pan J, Zhang M, Xing X, Zhou Y, Liu Y, Li D (2011a) ZmMKK4, a novel group C mitogen-activated protein kinase in maize (*Zea mays*), confers salt and cold tolerance in transgenic Arabidopsis. *Plant Cell Environ* 34:1291–1303
- Kong X, Sun L, Zhou Y, Zhang M, Liu Y, Pan J, Li D (2011b) ZmMKK4 regulates osmotic stress through reactive oxygen species scavenging in transgenic tobacco. *Plant Cell Rep* 30:2097–2104
- Kong F, Wang J, Cheng L, Liu S, Wu J, Peng Z, Lu G (2012) Genome-wide analysis of the mitogen-activated protein kinase gene family in *Solanum lycopersicum*. *Gene* 499:108–120
- Krasensky J, Jonak C (2012) Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *J Exp Bot* 63:1593–1608
- Kumar K, Rao KP, Sharma P, Sinha AK (2008) Differential regulation of rice mitogen activated protein kinase (MKK) by abiotic stress. *Plant Physiol Biochem PPB/Societe francaise de physiologie vegetale* 46:891–897
- Lee SK, Kim BG, Kwon TR, Jeong MJ, Park SR, Lee JW, Byun MO, Kwon HB, Matthews BF, Hong CB, Park SC (2011) Overexpression of the mitogen-activated protein kinase gene OsMAPK33 enhances sensitivity to salt stress in rice (*Oryza sativa* L.). *J Biosci* 36:139–151
- Li Z, Yue H, Xing D (2012) MAP Kinase 6-mediated activation of vacuolar processing enzyme modulates heat shock-induced programmed cell death in Arabidopsis. *New Phytol* 195:85–96
- Link V, Sinha AK, Vashista P, Hofmann MG, Proels RK, Ehness R, Roitsch T (2002) A heat-activated MAP kinase in tomato: a possible regulator of the heat stress response. *FEBS Lett* 531:179–183
- Mikolajczyk M, Awotunde OS, Muszynska G, Klessig DF, Dobrowolska G (2000) Osmotic stress induces rapid activation of a

- salicylic acid-induced protein kinase and a homolog of protein kinase ASK1 in tobacco cells. *Plant Cell* 12:165–178
- Mizoguchi T, Irie K, Hirayama T, Hayashida N, Yamaguchi-Shinozaki K, Matsumoto K, Shinozaki K (1996) A gene encoding a mitogen-activated protein kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, and water stress in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* 93:765–769
- Moustafa K, Lefebvre-De Vos D, Leprince A-S, Savourée A, Laurière C (2008) Analysis of the *Arabidopsis* mitogen-activated protein kinase families: organ specificity and transcriptional regulation upon water stresses. *Sch Res Exch*. p 12. doi:10.3814/2008/143656
- Nakagami H, Kiegerl S, Hirt H (2004) OMTK1, a novel MAPKKK, channels oxidative stress signaling through direct MAPK interaction. *J Biol Chem* 279:26959–26966
- Ning J, Li X, Hicks LM, Xiong L (2010) A raf-like MAPKKK gene DSM1 mediates drought resistance through reactive oxygen species scavenging in rice. *Plant Physiol* 152:876–890
- Pan J, Zhang M, Kong X, Xing X, Liu Y, Zhou Y, Liu Y, Sun L, Li D (2012) ZmMPK17, a novel maize group D MAP kinase gene, is involved in multiple stress responses. *Planta* 235:661–676
- Pastenes C, Horton P (1996) Effect of high temperature on photosynthesis in beans (I. oxygen evolution and chlorophyll fluorescence). *Plant Physiol* 112:1245–1251
- Peng LX, Gu LK, Zheng CC, Li DQ, Shu HR (2006) Expression of MaMAPK gene in seedlings of *Malus L.* under water stress. *Acta Biochim Biophys Sin (Shanghai)* 38:281–286
- Rengasamy P (2006) World salinization with emphasis on Australia. *J Exp Bot* 57:1017–1023
- Roberts EH (1988) Temperature and seed germination. *Symp Soc Exp Biol* 42:109–132
- Sangwan V, Dhindsa RS (2002) In vivo and in vitro activation of temperature-responsive plant map kinases. *FEBS Lett* 531:561–564
- Sangwan V, Orvar BL, Beyerly J, Hirt H, Dhindsa RS (2002) Opposite changes in membrane fluidity mimic cold and heat stress activation of distinct plant MAP kinase pathways. *Plant J Cell Mol Biol* 31:629–638
- Shi J, Zhang L, An H, Wu C, Guo X (2011) GhMPK16, a novel stress-responsive group D MAPK gene from cotton, is involved in disease resistance and drought sensitivity. *BMC Mol Biol* 12:22
- Shou H, Bordallo P, Wang K (2004) Expression of the Nicotiana protein kinase (NPK1) enhanced drought tolerance in transgenic maize. *J Exp Bot* 55:1013–1019
- Suri SS, Dhindsa RS (2008) A heat-activated MAP kinase (HAMK) as a mediator of heat shock response in tobacco cells. *Plant Cell Environ* 31:218–226
- Tanoue T, Adachi M, Moriguchi T, Nishida E (2000) A conserved docking motif in MAP kinases common to substrates, activators and regulators. *Nat Cell Biol* 2:110–116
- Teige M, Scheikl E, Eulgem T, Doczi R, Ichimura K, Shinozaki K, Dangl JL, Hirt H (2004) The MKK2 pathway mediates cold and salt stress signaling in *Arabidopsis*. *Mol Cell* 15:141–152
- Vierling E (1991) The role of heat-shock proteins in plants. *Annu Rev Plant Physiol Plant Mol Biol* 42:579–620
- Wang M, Zhang Y, Wang J, Wu X, Guo X (2007) A novel MAP kinase gene in cotton (*Gossypium hirsutum L.*), GhMAPK, is involved in response to diverse environmental stresses. *J Biochem Mol Biol* 40:325–332
- Wang J, Ding H, Zhang A, Ma F, Cao J, Jiang M (2010) A novel mitogen-activated protein kinase gene in maize (*Zea mays*), ZmMPK3, is involved in response to diverse environmental cues. *J Integr Plant Biol* 52:442–452
- Wen JQ, Oono K, Imai R (2002) Two novel mitogen-activated protein signaling components, OsMEK1 and OsMAP1, are involved in a moderate low-temperature signaling pathway in rice. *Plant Physiol* 129:1880–1891
- Went FW (1953) The effect of temperature on plant growth. *Annu Rev Plant Physiol* 4:347–362
- Wu T, Kong X-P, Zong X-J, Li D-P, Li D-Q (2011) Expression analysis of five maize MAP kinase genes in response to various abiotic stresses and signal molecules. *Mol Biol Rep* 38:3967–3975
- Xie G, Kato H, Imai R (2012) Biochemical identification of the OsMCK6-OsMPK3 signalling pathway for chilling stress tolerance in rice. *Biochem J* 443:95–102
- Xiong L, Yang Y (2003) Disease resistance and abiotic stress tolerance in rice are inversely modulated by an abscisic acid-inducible mitogen-activated protein kinase. *Plant Cell* 15:745–759
- Xiong L, Ishitani M, Zhu JK (1999) Interaction of osmotic stress, temperature, and abscisic acid in the regulation of gene expression in *Arabidopsis*. *Plant Physiol* 119:205–212
- Xu J, Chua NH (2012) Dehydration stress activates *Arabidopsis* MPK6 to signal DCP1 phosphorylation. *EMBO J* 31:1975–1984
- Xu J, Li Y, Wang Y, Liu H, Lei L, Yang H, Liu G, Ren D (2008) Activation of MAPK kinase 9 induces ethylene and camalexin biosynthesis and enhances sensitivity to salt stress in *Arabidopsis*. *J Biol Chem* 283:26996–27006
- Xu H, Li K, Yang F, Shi Q, Wang X (2010) Overexpression of CsNMAPK in tobacco enhanced seed germination under salt and osmotic stresses. *Mol Biol Rep* 37:3157–3163
- Yamazaki T, Kawamura Y, Uemura M (2009) Extracellular freezing-induced mechanical stress and surface area regulation on the plasma membrane in cold-acclimated plant cells. *Plant Signal Behav* 4:231–233
- Yu L, Nie J, Cao C, Jin Y, Yan M, Wang F, Liu J, Xiao Y, Liang Y, Zhang W (2010) Phosphatidic acid mediates salt stress response by regulation of MPK6 in *Arabidopsis thaliana*. *New phytol* 188:762–773
- Zhang T, Liu Y, Xue L, Xu S, Chen T, Yang T, Zhang L, An L (2006) Molecular cloning and characterization of a novel MAP kinase gene in *Chorispora bungeana*. *Plant Physiol Biochem PPB/ Societe francaise de physiologie vegetale* 44:78–84
- Zhang L, Xi D, Li S, Gao Z, Zhao S, Shi J, Wu C, Guo X (2011) A cotton group C MAP kinase gene, GhMPK2, positively regulates salt and drought tolerance in tobacco. *Plant Mol Biol* 77:17–31