MAPK cascades and major abiotic stresses

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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 stimulus and convey the stress signal 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 the 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: MAPK kinase kinase

MAP4K, MKKK or MEKKK: MAPK kinase kinase kinase.

S/T: Serine/Threonine, T(E/D)Y: Threonine(Glutamate/Aspartate)Tyrosine

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. 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 reveal that MAP kinases are tiered into four gene family classes. Arabidopsis genome for example encodes for 10 MAP4Ks, 60 MAP3Ks, 10 MKKs, and 20 MPKs genes for which a new nomenclature and group classification was 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 by a MAP kinase kinase kinase (known also as MAP3K, MEKK or MAPKKK), which activates a MAP kinase kinase (MAPKK, MAP2K or MKK), 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 ([Bita 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 ([Alzwi 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 silent MAP kinases, and then characterizing the resulting phenotypes. This should provide evidence 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 co-localization of interacting partners would save cells an important amount of energy and reduces 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.* ([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.* ([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, 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 posttranslational 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.* ([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*, indicating that *MaMPK* could be associated with the natural drought tolerance trait in apple. ([Peng, et al. 2006](#))

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.* ([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 MKKs and MPKs 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 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 to 10 min ([Mikolajczyk, et al. 2000](#)).

In maize, Kong *et al.* 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 ([Kong, et al. 2011](#)).

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* exhibit 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, an 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. 2011](#)) 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.

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 (figure 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 synergetic 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

Figure 1. Hypothetic MAP kinase cascade inferred from gene expression data and co-regulation paterns.

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.

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.

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