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Improvement of stress tolerance in plants by genetic manipulation of mitogen-activated protein kinases

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Abstract

Plant stress tolerance depends on many factors among which signaling by mitogen-activated protein-kinase (MAPK) modules plays a crucial role. Reversible phosphorylation of MAPKs, their upstream activators and downstream targets such as transcription factors can trigger a myriad of transcriptomic, cellular and physiological responses. Genetic manipulation of abundance and/or activity of some of these modular MAPK components can lead to better stress tolerance in Arabidopsis and crop plant species such as tobacco and cereals. The main focus of this review is devoted to the MAPK-related signaling components which show the most promising biotechnological potential. Additionally, recent studies identified MAPK components to be involved both in plant development as well as in stress responses, suggesting that these processes are tightly linked in plants.

Keywords: Arabidopsis, Biotechnology, Crops, Mitogen-activated protein kinase, Transgenic plants, Omics approaches, Signaling, Stress tolerance.

Abbreviations: ABA = abscisic acid, ACC = 1-amino-cyclopropane-1-carboxylic acid, ACS6 = ACC synthase 6, BAK1 = BRI1-associated kinase 1, CTR1 = constitutive triple response 1, EF-Tu = elongation factor thermo-unstable, ETI = effector-triggered immunity, ETR1 = ethylene response 1, FLS2 = flagellin sensitive 2, FRK1 = Flg22-induced receptor kinase 1, HR = hypersensitive response, IBR5 = indole-3-butyric acid response 5, JA = jasmonic acid, MAPK = mitogen-activated protein-kinase, MAPKK/MAP2K = mitogen-activated protein-kinase kinase, MAPKKK/MAP3K = mitogen-activated protein-kinase kinase kinase, MKP2 = MAPK phosphatase 2, NLP = Nep1-like protein, NO = nitric oxide, PAD2/3 = phytoalexin deficient 2/3, PAMPs = pathogen-associated molecular patterns, PCD = programmed cell death, PR = pathogenesis related, PRRs = transmembrane pattern recognition receptors, PTI = PAMP-triggered immunity, ROS = reactive oxygen species, SA = salicylic acid, SIMK = stress-induced MAPK, TMV = tobacco mosaic virus, VIP1 = VirE1-interacting protein 1, Y2H = yeast two-hybrid.

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1. Introduction

Plants are sessile organisms which are constantly exposed to a variety of biotic and abiotic stresses in their external environment. In order to survive, plants developed mechanisms for rapid sensing of signals from a changing environment and for transmitting these in specific adaptive/defensive responses. In all eukaryotes, mitogen activated protein kinase (MAPK) pathways play an essential role in signal transduction involved in the regulation of growth, differentiation, proliferation, death and stress responses.

MAPK signaling pathways are regularly assembled into modules which are composed of MAPK kinase kinase (MAPKKK, MAP3K or MEKK), MAPK kinase (MAPKK, MAP2K or MEK) and MAPK. Individual members of these modules are activated by reversible phosphorylation (Figure 1). They are believed to be held together in protein complexes with the help of scaffold proteins. These scaffold proteins along with specific subcellular localization/compartimentalization of scaffolded complexes (e.g. on endomembranes and vesicular compartments) and their individual constituents (e.g. individual MAPKs released from the complex and relocated to the nucleus) might bring certain specificity to the various signaling pathways and perhaps also avoid cross-talk with other signaling pathways. Quite often activated MAPK relocates to the nucleus and regulates transcription factors and/or other proteins involved in transcription with a main consequence of gene expression modulation and reprogramming of plant developmental program and/or stress response. Except for the nuclear proteins, however, plant MAPKs can also regulate proteins involved in cytoskeletal remodeling as well as a large number of cytoplasmic proteins (Figure 1). Taking into account a broad spectrum of triggers and physiological outcomes plant MAPK modules emerged as important regulators of gene expression, plant cytokinesis and development as well as ethylene and camalexin biosynthesis during the last decade. Some molecular substrates of MAPK modules such as transcription factors as well as individual members of these modules are considered as good targets for biotechnological applications. Various tools for in silico database searches including full genome transcriptomic analyses and gene expression correlation studies are available today to disentangle the complex architecture of organization of the MAPK signaling modules. This review summarizes the roles of MAPK signaling pathways with a

main focus on biotic and abiotic stress, and especially on MAPK components and their molecular targets showing a biotechnological potential.

2. Short overview of abiotic stress factors triggering MAPK activity

MAPK pathways are known to be activated by diverse abiotic stresses such as cold, salt, heat, drought, wounding, UV irradiation, osmotic shock, ozone or heavy metal intoxication. The main Arabidopsis MAPKs activated by salt, cold, drought, touch and wounding are MPK4 and MPK6 (Ichimura et al., 2000, Teige et al., 2004). For cold and salt stresses, one complete MAPK signal transduction module was identified in Arabidopsis. This module consists of the MEKK1 as upstream activator of MKK2 and the downstream MAPKs MPK4 and MPK6 (Teige et al., 2004). Additionally, also MKK1 is activated by salt, drought and wounding stress and can phosphorylate MPK4, thus it might be also involved in abiotic stress signaling (Teige et al., 2004, Xing et al., 2007). Hypoosmolarity was shown to activate MPK3, MPK4 and MPK6 in cell suspensions and plantlets of Arabidopsis (Droillard et al., 2004).

Ozone, as a major pollutant and potent reactive oxygen species (ROS) generator, activated MAPK signaling pathways through triggering ROS production and accumulation of ethylene, jasmonic acid (JA) and salicylic acid (SA) resulting in local programmed cell death (PCD). Ozone activated MPK3 and MPK6 and it caused the nuclear translocation of these MAPKs in Arabidopsis (Ahlfors et al., 2004). Such activation is independent of ethylene and JA, but activity of MPK3 is dependent on salicylic acid. Later, it was shown that MKP2 (MAPK phosphatase 2) is an important positive regulator of the cellular response to ozone since it can affect the activation state of MPK3 and MPK6 (Lee and Ellis, 2007). Suppression of MKP2 creates hypersensitivity to ozone with prolonged activation of MPK3 and MPK6. Also in tobacco, NtMPK4 plays an important role in ozone sensitivity and JA signaling. Using transgenic plants it was shown that NtMPK4 played a main role in the response to wounding, and was also involved in ozone tolerance by regulating stomatal closure (Gomi et al., 2005). Oxidative stress induced by exogenous H₂O₂ can also activate MPK1 and MPK2 (Ortiz-Masia et al., 2007), MPK3 and MPK6 (Kovtun et al., 2000), MPK4 (Nakagami et al. 2006) and MPK7 (Doczi et al. 2007) in Arabidopsis suggesting that ROS act upstream of several MAPK cascades.

In higher plants, MAPKs can be activated also by toxic levels of heavy metals. Cadmium and copper treatment induced *OsMAPK3* and *OsMPK6* in rice (Yeh et al., 2007). This result implies that a MAPK cascade may function in cadmium and copper signaling pathway in rice. Additionally, an activation of four distinct MAPKs such as SIMK, MMK2, MMK3 and SAMK was observed after exposure of *Medicago sativa* seedlings to the excess of copper or cadmium ions (Jonak et al., 2004). Nevertheless, distinct MAPK pathways seemed to be involved in the response to copper and cadmium stress.

Thus MAPK signaling pathways appear as universal transducers of diverse abiotic stresses in plants (Table 1).

3. Short overview of biotic stress factors inducing MAPK activity

During evolution, higher plants developed an innate immune system (Jones and Dangl, 2006) to detect pathogen attacks and to activate rapid multistep defense responses, such as the production of ROS, the induction of the hypersensitive response (HR), a localized cell death at the sites of infection or the synthesis of pathogenesis related (PR) proteins and anti-microbial phytoalexins.

The plant innate immune system involves two levels of defense responses against pathogens. The first level is based on specific detection of conserved pathogen-associated molecular patterns (PAMPs) with transmembrane pattern recognition receptors (PRRs) and triggers defense responses of plant cells. Such defense responses include changes of enzymatic activity, gene expression reprogramming and production of antimicrobial compounds. This leads to the activation of PAMP-triggered immunity (PTI) that can stop further colonization of plant by pathogen. To suppress PTI, pathogens produce effector proteins that can overcome the activities of PTI signaling components and effectively stop PTI. At a second level of defense, plants can recognize pathogen effectors through resistance (R) proteins. These proteins function as immune receptors and trigger HR with localized cell death restricting the spread of the pathogen. This second level of plant immunity is called effector-triggered immunity (ETI) and is an accelerated and amplified PTI response leading to disease resistance (Jones and Dangl, 2006). In recent years, it has been established that MAPKs play important roles in innate immune response and resistance to pathogens in *Arabidopsis*, rice, tobacco, parsley, tomato and maize. In this

respect, the best characterized Arabidopsis MAPKs are again MPK3, MPK4 and MPK6 which were found to be activated by bacterial and fungal PAMPs during plant-pathogen interactions (Nuhse et al., 2000, Desikan et al., 2001). Hence, this orchestrated network of MAPK cascades represents the basal level of plant innate immunity involved in responses to microbial pathogens and their effectors as well as in plethora of abiotic stress responses.

Arabidopsis FLS2 (flagellin sensitive 2) is a well characterized plant PRR from the leucine-rich repeat receptor kinase family which is required for flagellin signaling (Gomez-Gomez and Boller, 2000, Chinchilla et al., 2007). Application of the bacterial elicitor flagellin (flg22 represents a 22-amino-acid-long peptide derived from flagellin), the first general elicitor produced by eubacteria (Felix et al., 1999), causes internalization of plasma membrane receptor FLS2 via receptor mediated endocytosis (Robatzek et al., 2006). Flg22 also triggers rapid and strong activation of Arabidopsis MPK3, MPK4 and MPK6 (Asai et al., 2002, Droillard et al., 2004, Suarez-Rodriguez et al., 2007). The signal perception of flg22 occurs at the plasma membrane through receptor-like kinase complex of FLS2 and BAK1 (BRI1-associated kinase1) which triggers at least two parallel MAPK signaling cascades (Chinchilla et al., 2007, Zipfel et al., 2004, see below). The first MAPK module acting downstream of the FLS2-BAK1 receptor complex was identified using transient expression in protoplasts together with biochemical and genetic approaches (Asai et al., 2002). It is composed of the MAP3K MEKK1, the two MAP2Ks MKK4/5 and the two MAPKs MPK3/6. The activation of MPK3 and MPK6 via MKK4 and MKK5 leads to the phosphorylation-dependent activation of the transcription factors WRKY22 and WRKY29 (WRKY DNA-BINDING PROTEIN 29) and FRK1 (Flg22-induced receptor kinase 1) and early flg22-induced expression of genes such as *WRKY29*, *FRK1* and *GST1* (Asai et al., 2002). A MAPK cascade containing MPK3 and MPK6 is also involved in camalexin biosynthesis functioning upstream of PAD2 (phytoalexin deficient 2) and PAD3 (Ren et al., 2008). The interplay of these MAPK signaling modules confers resistance to several bacterial and fungal pathogens.

It was shown that flg22 activated MPK6 phosphorylates and stabilizes ACC (1-amino-cyclopropane-1-carboxylic acid) synthase 6 (ACS6) an enzyme that is involved in the biosynthesis of the phytohormone ethylene (Liu et al., 2004; Joo et al., 2008). However, MPK6 not only seems to be involved in PAMP triggered ethylene biosynthesis but also

gets activated by ethylene via MKK9 (Yoo et al., 2008). Flg22-induced activation of MPK3 leads to the activation of VirE1-interacting protein 1 (VIP1), a transcription factor, that after phosphorylation is relocated from the cytoplasm to the nucleus to induce the expression of pathogenesis-related genes such as *PRI* (Djamei et al., 2007).

The second MAPK cascade, comprised of MEKK1, MKK1/MKK2 and MPK4, negatively regulates defense responses. In this defense response, MEKK1 is required for specific activation of MPK4, but not for MPK3/MPK6 signaling pathway (Ichimura et al., 2006). Elevated levels of SA in *mpk4* mutant led to the expression of pathogenesis-related genes and increased resistance to pathogen (Petersen et al., 2000). In addition, MPK4 is also required for JA-driven expression of defensive proteins because activation of specific genes was blocked in the *mpk4* mutant (Petersen et al., 2000). However, induction of systemic acquired resistance in *mpk4*, *mekk1* or *mkk1mkk2* double mutants, produces a characteristic dwarf phenotype which is probably caused by a smaller cell size (Ichimura et al., 2006, Petersen et al., 2000, Qiu et al., 2008b, Suarez-Rodriguez et al., 2007). This SA-dependent mechanism of action is based on the activation of the WRKY transcription factors WRKY25 and WRKY33 by MKS1, which was confirmed to interact with MPK4 (Andreasson et al., 2005, Petersen et al., 2010).

Recently, the presence of a nuclear MPK4-WRKY33 complex in Arabidopsis was proven in the absence of pathogen (Qiu et al., 2008a). Employment of *mks1* mutant plants showed dependency of this nuclear complex on MKS1. Treatment with flagellin or *Pseudomonas syringae* caused activation of MPK4 and phosphorylation of MSK1, resulting in release of MKS1 and WRKY33 from MPK4, and WRKY33 targeted the promoter of PAD3. PAD3 encodes for enzyme required for the synthesis of antimicrobial camalexin). More recently, MPK3 and MPK6 were shown to be essential for the induction of camalexin biosynthesis in Arabidopsis infected with fungal pathogen *Botrytis cinerea* (Mao et al., 2011). This occurs through phosphorylation of a pathogen-inducible transcription factor WRKY33 by MPK3/MPK6 which enhances activity of WRKY33 in promoting the expression of downstream camalexin biosynthetic genes. Thus, MPK4 is required for induction of camalexin biosynthesis by bacterial pathogen (Qiu et al., 2008a), but it is not included in camalexin induction by fungal pathogen (Mao et al., 2011).

It seems that both MAPK cascades (MKK4/5-MPK3/6 and MKK1/2-MPK4) activated by flg22 may act antagonistically. However, it was shown that they are interconnected because in the *mkk1* mutant, flg22-dependent activation of MPK4 and also MPK3 and MPK6 is impaired (Mészáros et al., 2006). Thus, the flg22-induced positive regulation of defense responses by MKK4/MKK5-MPK3/MPK6 pathway and the negative one by the MKK1/MKK2-MPK4 pathway are both important players in innate immune response and resistance to pathogens.

In addition to flg22, there is a variety of fungal and bacterial PAMPs, such as chitin, harpin, elongation factor thermo-unstable (EF-Tu), Nep1-like protein (NLP), that can induce activation of MPK3/MPK6 or MPK4, and also trigger regulation of pathogen-related genes (Desikan et al., 1999 and 2001, Kunze et al., 2004, Miya et al., 2007, Qutob et al., 2006).

Involvement of MAPK cascades in pathogen signal transduction is also well studied in tomato, tobacco, rice, parsley and cotton. In tobacco, there are at least two MAPK pathways activated by inoculation with tobacco mosaic virus (TMV). The first one is composed of MEK2-SIPK (salicylic acid-induced protein kinase) and WIPK (wound-induced protein kinase) (Zhang and Klessig, 1998b) and leads to HR. The second one comprises NPK1-MEK1-NTF6 and attenuates resistance to TMV (Liu et al., 2003). Further orthologs of SIPK and WIPK, such as MPK1/2 and MPK3 in tomato, SIMK and SAMK in alfalfa, MAPK5 in rice and MPK6 in parsley also play an important role in defense-related signal transduction (Ren et al., 2006).

A short summary of plant MAP3Ks, MAP2Ks and MAPKs involved in biotic stress responses is provided in Table 1.

4. Plant hormones affecting MAPK activity

MAPK cascades play crucial roles not only in biotic- and abiotic-stress responses and development but also in hormone signaling in plants (Jonak et al., 2002; Nakagami et al., 2005). Recent evidence also suggests that plant hormones are involved in the crosstalk between abiotic and biotic stress signaling (Fujita et al., 2006). Among plant hormones, stress hormones such as ethylene and JA are essential for determining the proper plant

defense mechanism against diverse stress conditions and pathogens. Both of these stress hormones require activation of MAPKs cascade for induction of their biosynthesis.

The ethylene receptor ETR1 (ethylene response 1), showing endoplasmic-reticulum localization, is associated with CTR1 (constitutive triple response 1), a Raf-like MAP3K (Kieber et al., 1993; Clark et al., 1998; Huang et al., 2003; Gao et al., 2003). In the absence of ethylene, ETR1 suppressed signal transduction pathway by activating the negative regulator CTR1 (Hua and Meyerowitz, 1998). Thereafter, degradation of the transcription factor EIN3 occurred by the 26S proteasome leading to the blockage of the downstream transcription cascade. However, the presence of ethylene inactivates the negative regulator CTR1, dissociates CTR1 from the receptor complex and initiates the downstream signaling cascade by stabilizing transcription factor EIN3 in the nucleus, thus activating primary transcription (Chao et al., 1997; Yanagisawa et al., 2003; Binder et al., 2007). Activation of 47kDa protein (Novikova et al., 2000) later identified as MPK6 (Ouaked et al., 2003) occurred after application of the ethylene precursor ACC. Recently, a novel MKK9-MPK3/6 cascade was identified that phosphorylates and stabilizes EIN3 during ethylene signaling (Yoo et al., 2008). It was shown that treatment with ACC inactivated the CTR1 pathway but activated the MKK9-MPK3/6 pathway. After activation, MKK9 relocated from the cytoplasm to the nucleus and activated nuclear MPK3 and MPK6. These two MAPKs are able to phosphorylate and thus stabilize EIN3 and the downstream transcription machinery (Chao et al., 1997; Yoo et al., 2008). MPK3 and MPK6 were not activated in *mkk9* loss-of-function mutant plants of Arabidopsis showing ethylene insensitivity (Yoo et al., 2008). This implies that positive-acting and negative-acting MAPK pathways operate simultaneously and are integrated into regulation of EIN3 (through phosphorylation and protein stabilization) and downstream transcription events (Yoo et al., 2008). Additionally, MPK6 might regulate synthesis of ethylene via phosphorylation and stabilization of ethylene biosynthetic enzymes such as ACS 2 and 6 (Liu and Zhang, 2004).

JA, as an important player in plant response to environmental stresses and developmental cues, activated the Arabidopsis MKK3-MPK6 cascade. Genetic analyses using loss-of-function and gain-of-function mutants of the MKK3-MPK6 cascade showed that the JA-induced activation of this cascade negatively regulates the *AtMYC2*

(transcription factor/a positive regulator of JA-inducible gene expression), thus affecting both JA-dependent gene expression and inhibition of root growth (Takahashi et al., 2007). In Arabidopsis, a MAPK phosphatase AP2C1 was found to regulate MAPK activities and the amount of JA. AP2C1 regulated early transmission of wound-induced signals through dephosphorylation (inactivation) of MPK4 and MPK6 (Schweighofer et al., 2007).

Several studies revealed that signaling by auxin, as an essential plant hormone, is mediated by MAPK pathways including NPK1 (a MAP3K) in tobacco (Kovtun et al., 1998), and ANP1 (a NPK1 ortholog) in Arabidopsis protoplasts (Kovtun et al., 2000). Rapid activation of unidentified MAPKs in Arabidopsis root with different auxins was observed by Mockaitis and Howell (2000). Genetic studies revealed a role of Arabidopsis MKK7 as a negative regulator of polar auxin transport (Dai et al., 2006). Recently, Arabidopsis MPK12 was identified as a new negative regulator of auxin signaling and as a substrate of MAPK phosphatase called IBR5 (indole-3-butyric acid response 5) (Lee et al., 2008). It was shown that MPK12 specifically interacts with IBR5 phosphatase while activated MPK12 can be dephosphorylated and inactivated by this phosphatase (Lee et al., 2008). Transgenic plants with reduced expression of the *MPK12* gene showed increased auxin sensitivity, but normal ABA sensitivity. However, *ibr5* mutant plants displayed defective responses to both auxin and ABA. Suppression of MPK12 in an *ibr5* background partially rescued the *ibr5* auxin-insensitive phenotype.

5. MAPK modules involved both in plant development and in stress response

Previous and recent studies revealed that several stress-induced MAPKs and their upstream activators such as MAP2Ks and MAP3Ks are also involved in the regulation of diverse plant developmental processes. In our previous study we have found that SIMK (stress-induced MAPK) from *Medicago sativa* is not only activated by diverse abiotic and biotic stresses (Jonak et al., 1996; Bögre et al., 1997) but together with the actin cytoskeleton it is also involved in the root hair formation and development (Šamaj et al., 2002). This was the first study combining genetic and cell biological approaches to reveal the function of plant MAPK in the developmental process. Other alfalfa and tobacco MAPKs were differentially expressed during the cell cycle and proposed to be involved in the regulation of cell division (Calderini et al., 1998; Bögre et al., 1999; Nishihama et al.,

2001; Soyano et al., 2003). In Arabidopsis, MAP3Ks called ANP2/3 were reported to be involved in the last stage of cell division, cytokinesis (Krysan et al., 2002). Recently, MPK4, downstream of ANP2/3, was found to be essential for plant cytokinesis (Kosetsu et al., 2010; Beck et al., 2011). Molecular interactions of plant MAPKs with proteins belonging to the microtubular cytoskeleton such as microtubular motors kinesins and microtubule bundling proteins of the MAP65 family were identified and characterized in more detail (Nishihama et al., 2002; Sasabe et al., 2006, 2011; Smertenko et al., 2006; Takahashi et al., 2010). Moreover, cytokinetic *anp2/anp3* mutants also showed aberrant mitotic microtubules (Beck et al., 2011). Additionally, some MAPKs such as MPK18, MPK6 and MPK4 were proposed to regulate and/or interact with cortical microtubules, thus participating in the determination of plant cell shapes (Walia et al., 2009; Beck et al., 2010; Müller et al., 2010).

Stomata development represents a very good example of developmentally regulated process controlled by both stress-induced and developmentally-triggered MAPK modules. In the case of stomata development, the whole module is relatively well characterized especially by using genetic means. In Arabidopsis, this module is composed of YODA, MKK4/MKK5 and MPK3/MPK6 (Bergmann et al., 2004; Wang et al., 2007; Lampard et al., 2009). Downstream target of this pathway is a transcription factor SPEECHLESS (Lampard et al., 2008) while the upstream activator is most probably a protein kinase named SHORT SUSPENSOR (Bayer et al., 2009). Additionally, it was proposed in these studies that the same MAPK modules can integrate environmental and developmental cues to achieve proper stomata development and functioning (Wang et al., 2007; Lampard et al., 2008). In spite of the fact that this signaling module is relatively well characterized, little is known about the subcellular localization and mechanisms of MAPK signaling during stomata development.

A very similar MAPK-dependent signaling cascade regulating asymmetric cell division might operate during embryo development in Arabidopsis (Lukowitz et al., 2004; Bayer et al., 2009).

In addition to vital function during stomata and embryo development, the Arabidopsis MPK6 is also involved in the regulation of cell division polarity during post-

embryogenic development of seedling roots (Müller et al., 2010) as well as in anther and inflorescence development (Bush et al., 2007).

Finally, transcriptomic studies revealed differential regulation of stress-induced genes in MAP3K mutants *anp2/3* (Krysan et al., 2002), *yoda* (Bergmann et al., 2004) and *mekk1* as well as in *mkk1/2* and *mpk4* mutants (Pitzschke et al., 2009). These examples together with others summarized in Table 2 illustrate that several developmentally regulated MAPKs and MAPK modules are also involved in plant stress responses.

6. Omics approaches to study stress-induced MAPKs and related plant stress tolerance

In principle, several 'omic' strategies exist to tackle the function of plant MAPKs and their respective roles in stress tolerance, including transcriptomics, proteomics and phosphoproteomics (i.e. the post-translational modification (PTM) of proteins). Large scale whole genome transcriptomic databases are publically available for Arabidopsis (<https://www.genevestigator.com/gv/index.jsp> or <http://urgv.evry.inra.fr/CATdb>) but they possess also information about other plant species such as rice, soybean, wheat, barley, maize, tomato, tobacco or poplar. These databases contain a large datasets of genes which show altered expression under diverse stress and hormonal conditions and after chemical treatments at different levels up to the certain growth zones or tissues of diverse organs. Identified individual genes can be grouped to hierarchical categories by MapMan program (<http://gabi.rzpd.de/projects/MapMan>) according to cellular processes and/or up- or down-regulation of group of genes. Additionally, co-expressions of genes in Arabidopsis and rice can be analyzed and correlated by using ATTEDII web-based tool (<http://atted.jp/>) in order to estimate possible gene functions. Recently, also several signaling mutants, such as *mekk1*, *mkk1/2* and *mpk4*, were analyzed by using transcriptomic approach (Qiu et al., 2008a; Pitzschke et al., 2009a). Resulting transcriptomic profiles of such signaling mutants can be also correlated and hypotheses about putative signaling cascades can be raised for those mutants which show significant overlap in their transcriptomic datasets. Hypotheses about pathway organization should be validated by other experimental approaches such as phenotyping, genetic crossing, bimolecular fluorescence complementation and physiological experiments including measurements of stress-related hormones. This was

nically demonstrated for MEKK1-MKK1/2-MPK4 pathway which plays a negative role in plant innate immunity against fungal pathogen *Pseudomonas syringae* (Gao et al., 2008). Corresponding mutants of this pathway are dwarfed, contain elevated levels of salicylic acid (SA) and show differential expression of SA- and redox-responsive genes.

Moreover, transcriptional regulatory regions in co-expressed genes (e.g. those regulated by common transcription factors) can be identified by programs PLACE (www.dna.affrc.go.jp/PLACE/) or PlantCARE (<http://sphinx.rug.ac.be:8080/PlantCARE/>) which detect known cis-elements within a set of diverse promoters. Next, motif abundance in a given promoter can be compared to the genomic background frequency and the statistical significance of the enrichment of candidate motifs in promoters assessed using POBO tool (<http://ekhidna.biocenter.helsinki.fi/poxo/pobo/pobo>). Identified DNA motifs should be experimentally tested using overexpression of synthetic promoter-reporter constructs (Rushton et al., 2002) and candidate transcription factors (Pitzsche et al., 2009b) in transformed protoplasts or plants. In such case, induction or repression of genes containing candidate DNA target motifs recognized by transcription factors is evaluated, as it was recently demonstrated in the case of VIP1 transcription factor (Pitzschke et al., 2009b). Transcription factors can be considered as the end point of a signaling cascade and a phylogenetic analysis may provide first indications about their behavior or potential DNA target motifs. Some transcription factors such as WRKY show preference for certain spacing between adjacent DNA motifs (W boxes) which is important for their transcriptional activity (Ciolkowski et al., 2008).

Despite unquestionable usefulness of full genome transcriptomic approaches they have also disadvantages such as high cost of experiments, necessity to handle a large datasets which needs bioinformatic analysis and lack of information about post-transcriptional modifications of gene products, namely proteins which are often crucial for their function.

Signaling pathways and networks can be constructed also with the help of proteomic approaches. Set of interacting proteins can be identified by yeast two-hybrid (Y2H) screens or by mass spectrometry analysis of purified protein complexes. Further, protein microchips can be used for screening of protein interactions. Publically available in silico analysis (<http://bar.utoronto.ca/interactions/cgi->

bin/arabidopsis_interactions_viewer.cgi) provides valuable information about protein-protein interactions. More importantly in respect to kinase-mediated signaling, phosphoprotein microarray chips were used for identification of putative MAPK candidate substrates in Arabidopsis (Feilner et al., 2005; Popescu et al., 2009). Establishment of novel protein microarray-based proteomic method using threshold-based quantification allowed identification of 48 potential substrates for MPK3 and 39 for MPK6. A large number of these substrates (26) was common for both kinases (Feilner et al., 2005). Furthermore, several novel signaling modules comprising diverse MPKK/MPK pairs and 570 phosphorylated substrates of these modules (including several WRKY and TGA transcription factors) were identified using high-density Arabidopsis microarrays containing 2158 proteins (Popescu et al., 2009).

Because a Y2H or protein microarray predicted interaction does not necessarily mean that two proteins truly interact in planta, candidate interacting proteins must be scrutinized by additional selection criteria, including their spatio-temporal expression pattern and their subcellular localization. The integration of transcriptomic and proteomic data clearly facilitates the identification of top candidate genes and proteins involved in transduction of diverse stress signals. Some of these candidates might be stress-responsive genes encoding for proteins regulated by MAPK-dependent phosphorylation. Phosphopeptide motifs typical for MAPKs can be identified by detailed analysis of available phosphopeptide sequences. It is desirable to verify candidate phosphorylation motifs of individual proteins using *in vitro* and/or *in vivo* phosphorylation assays. Furthermore, a web-based TAIR patmach tool can be used to screen for all Arabidopsis proteins harboring the same peptide motif. In summary, the usefulness, robustness, and limitations of applying various transcriptomics and proteomics-based technologies for deciphering signaling pathways is still in its infancy. Clearly, their literally unlimited number of elaborate combinations harbors high potential to significantly speed up the progress in signaling research by allowing experiments to be designed in a highly targeted manner and replacing bench work to a large extent.

7. Strategies for genetic manipulations of kinases and their targets with biotechnological potential

Considering the fact that MAPK signaling cascades are activated within minutes and they may affect transcription through regulation of transcription factors, candidate genes and target proteins of these pathways may be identified by simultaneous searching in transcriptomic, proteomic and phosphoproteomic data sets of early stress responses in diverse plant species. Functional characterization and genetic manipulation of identified targets might be used not only for basic science and plant species such as *Arabidopsis* but identified functional homologs will be of great value also for improvement of desirable traits such as multiple stress resistance in crops. Promoters inducible by chemicals and/or active only in certain plant tissues (tissue-specific promoters) might be used to overcome undesirable effects on plant growth and development resulting from constitutive overexpression of genes. This is a very promising integrative approach. Such strategy was used to genetically manipulate VIP1 transcription factor regulating stress-related gene such as *PR1* (Djamei et al., 2007). Lethal constitutive overexpression system of VIP1 was replaced by expression from estradiol inducible promoter which allowed functional localization studies of YFP-tagged VIP1 showing relocation to the nucleus upon *Agrobacteria* and *flg22* treatments as well as induction of *PR1* gene.

An alternative approach to targeting transcription factors is the identification of the upstream protein kinases themselves that are mediating the stress signals and ultimately regulate the activity of key transcription factors. Knowledge on the activation mechanism of the protein kinases is, however, essential for a success in this strategy. For example, the MKK2 kinase is involved in cold and salt stress and *mkk2* plants are hypersensitive to these stresses (Teige et al., 2004). Simple overexpression of the wild type MKK2 gene has no beneficial effect on stress tolerance. However, the replacement of the threonine and serine residues of MKK2 that are normally phosphorylated by the upstream regulator MEKK1 by acidic amino acids yields an autoactive protein kinase. Transgenic plants that express constitutively active MKK2 are phenotypically normal but are now highly stress resistant (Teige et al., 2004).

To translate the generated knowledge from model plants such as *Arabidopsis* to crop plants is of course a major aim in agriculture. The first step in this process involves the

identification of the homologous factor in the crop plants. Although this problem sounds like an easy task, genome evolution of large gene families can make this endeavor rather complicated. A helpful tool for these approaches is a bioinformatic tool at <http://bioinfoserver.rsbs.anu.edu.au/utills/affytrees/>, which provides information about the homologs of a protein of interest in other plant species. Upon successful identification of a target gene, an overexpression strategy is the most direct way to obtain the desired phenotype. However, there also exist other methods such as TILLING or oligonucleotide-directed mutagenesis to obtain crops with a gene of interest containing a modified amino acid sequence.

Finally, hybrid/artificial kinases can be created that modify proteins other than their true targets or that prevent phosphorylation of a protein by outcompeting the true modifying upstream kinase. Given that phosphorylation events are a common feature in the signaling of almost all responses and biological processes, this approach has high potential for synthetic biology but also crop improvement.

8. Stress tolerance in Arabidopsis with genetically modified MAPKs

As described above, plants utilize two defensive mechanisms that enable them to efficiently cope with various stress conditions. Biotic stress mediated by pathogen-derived compounds is perceived by transmembrane PRRs as well as by other receptor proteins, called resistance proteins, implicated in rapid defense mechanisms. While both biotic and abiotic stress responses often share similar signaling modules, proper understanding of abiotic stress-involved responses is hindered by complexity of these processes which are not limited to MAPK signaling pathways. Nonetheless, the signaling pathway represented by MEKK1-MKK1/2-MPK4 is the backbone of pathogen-induced responses, and also plays an important role in mediating homeostasis of ROS which is vital for maintaining biotic and abiotic stress tolerance.

MPK4 is a key regulator of plant defense mechanisms based primarily on negative regulation of SA signaling. The role of MPK4 in PTI through receptors such as FLS2 is thoroughly discussed above. Apart from the key role in plant innate immunity, MPK4 is also involved in the regulation of other types of stress signaling. In addition to MPK3 and MPK6, MPK4 was confirmed as a third MAPK susceptible to the activation by

hypoosmotic stress (Droillard et al., 2004). Moreover, possible involvement of MPK4 in hyperosmotic stress tolerance was also hypothesized. Hyperosmotic stress responses in *mpk4* suggested that MPK4 may play yet another negative regulatory function in addition to its role in the negative regulation in FLS2-MEKK1-MKK1/MKK2-MPK4 signaling pathway (Droillard and Boudsocq, 2004). The same pathway also represents an important regulatory mechanism in the homeostasis of ROS. Transcriptomic analyses of *mekk1*, *mkk1/2*, and *mpk4* have revealed a network of ROS-dependent genes and confirmed the role of this MAPK cascade as an integrating element in ROS- and SA-initiated stress pathways (Pitzschke et al., 2009a).

Moreover, several members of MAPK modules are involved in abiotic stress responses and are indispensable for conferring tolerance to stress conditions such as salt, drought or cold. Namely, overexpression of MKK2 that targeted both MPK4 and MPK6 resulted in constitutive upregulation of several stress-induced genes, and the plants exhibited increased freezing and salt tolerance (Teige et al., 2004). The same signaling system also seems to control hormone levels in response to pathogens such as *Pseudomonas* sp. and helps to maintain resistance against several bacterial pathogens (Brader et al., 2007). MPK3 and MPK6 play a vital role in the control of another important physiological process, namely stomatal opening/closure. Additionally, these two kinases together with their upstream activators MKK4 and MKK5 are key regulators of stomatal development and patterning (Wang et al., 2007). They operate in close cooperation with hydrogen peroxide and abscisic acid (ABA) and together they control stomatal movements (Gudesblat et al., 2007a,2007b). Further, MPK3-linked regulation of stomatal movement represents an important defense mechanism, which is able to effectively prevent bacterial invasion through stomata (Gudesblat et al., 2007a). All these results provide clear evidence that defensive strategies in biotic and abiotic stress conditions often go hand in hand, and rely on similar signaling mechanisms.

Other abiotic stress conditions include touch, wounding, salinity, drought or UV light (Holley et al., 2003; Ichimura et al., 2000; Mizoguchi et al., 1996). MEKK1 is activated by most of these abiotic stress conditions (Mizoguchi and Irie, 1996) and it activates downstream signaling modules MKK1, MKK2 and MPK4 (Ichimura et al., 1998). Also, as mentioned above, the MEKK1-MPK4 pathway is a key regulator in ROS

metabolism and signaling (Nakagami et al., 2006; Pitzschke et al., 2009a). However, similarly to flagellin-induced pathogen responses, also homeostasis of ROS is a complex process with several signaling modules working independently on each other. MPK3 and MPK6 are another players in ROS-induced signaling since MPK3/MPK6 downregulations by RNAi technology produced plants hypersensitive to ozone and activation of the respective kinase in the single knockdowns was significantly impaired (Miles et al., 2005). Interestingly, the network of ROS-signaling pathways appears to be even more intriguing with other controlling mechanisms adding to its complexity. A study of Arabidopsis kinase of the NDP family, AtNDPK2, pointed to H₂O₂-mediated activation of this kinase while overexpression studies revealed its involvement in the regulation of ROS metabolism (Moon et al., 2003). Moreover, NDPK2 specifically interacts with MPK3 and MPK6. This opens up a possibility that NDPK2 may present an upstream element involved in fine-tuning of MPK3/MPK6 plant defense pathways conferring the stress tolerance.

In addition to the above signaling pathways involved in the ROS homeostasis, plants have evolved yet another defensive mechanism helping them to get rid of the dangerous forms of molecular oxygen resulting primarily from various abiotic and also biotic stress conditions. These so called scavenger enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, or glutathione reductase are regulated by ABA and they respond to H₂O₂ treatment (Zhang et al., 2007). In addition, cross-talks between Ca²⁺ and ROS signaling were suggested to induce antioxidant defense enzymes (Jiang and Zhang, 2003). This is in agreement with another observation that Ca²⁺ channel blocker inhibited H₂O₂/ozone activation of SIPK (orthologous to Arabidopsis MPK3 and MPK6) suggesting that this upstream event was required in the ROS-mediated signaling pathway (Samuel et al., 2000). Interestingly, Arabidopsis catalase CAT1 was shown to be linked to MKK1-MPK6 signaling pathway (Xing et al., 2008). Neither *mkk1* nor *mpk6* mutants were able to produce CAT1 in response to ABA treatment. Conversely, overexpression of these two signaling modules resulted in enhanced ABA-dependent expression of CAT1 and production of H₂O₂.

9. Stress tolerance in crop species with genetically modified MAPKs

In addition to Arabidopsis, pathogen-induced immune responses have been extensively studied in several other plant species. Studies on tobacco MAPKs WIPK and SIPK (orthologs of MPK3 and MPK6) brought evidence these two kinases were implicated in the regulation of systemic resistance against tobacco mosaic virus mediated by SA (Zhang and Klessig, 1998a, 1998b). Both WIPK and SIPK are also sensitive to various mechanical and osmotic stresses. Another tobacco MAPK, Ntf4, with close similarities to WIPK/SIPK has been described (Ren et al., 2006). All these signaling modules share a common upstream MAPKK, NtMEK2, and they are all sensitive to elicitor treatments. It was shown that both elicitor treatment and overexpression of Ntf4 resulted in localized cell death in rapid HR (Ren et al., 2006). HR was also described in disease-resistant plants using tomato and tobacco as models. Using Pto-AvrPto resistance system the authors demonstrated that tomato MAPKKK, MAPKKK α , was required in resistance and HR response leading to pathogen-independent cell death (del Pozo et al., 2004).

Wheat homologs of MPK3/MPK6, called TaMPK3 and TaMPK6, were also studied during compatible interaction of wheat with necrotrophic fungal pathogen *Mycosphaerella graminicola* (Rudd et al., 2008). Interestingly, this signaling system proves to be different in several aspects from what we know from other species. In Arabidopsis, tobacco and others these two signaling modules are activated simultaneously in response to pathogen elicitation. In contrast, only TaMPK3 was transcriptionally activated before pathogen induced programmed cell death (PCD) response. Surprisingly, protein levels of TaMPK6 were reduced at this stage of infection, which can be explained by specific or unspecific protein degradation of this kinase (Rudd et al., 2008). Interestingly, wheat phosphatase TMKP1 has been described recently, and its specific association with TaMPK3 and TaMPK6 was confirmed (Zaïdi et al., 2010). Expression profiling revealed the enzyme is induced under salt and osmotic stress. Moreover, localization experiment with all signaling modules suggested the TMKP1 can control subcellular localization of its interacting partners.

ROS signaling and its implication in various abiotic stress signaling is a key factor in biotechnological applications in economically important plants. In crops, much attention has been paid to mechanisms controlling tolerance to drought, oxidative and cold stress. Tobacco NPK1, an ortholog of Arabidopsis MAPKKK, was shown to confer resistance to

multiple environmental stress conditions when constitutively expressed in tobacco (Kovtun et al., 2000). It has been long known that cold acclimation leads to mild oxidative stress and enhanced freezing tolerance (Prasad et al., 1994). Interestingly, constitutively active NPK1 can mimic ROS signaling with similar MAPK modules operating in the process. In agreement, low level constitutive expression of NPK1 in maize led to enhanced freezing tolerance in the transgenic plants (Shou et al., 2004a). Additionally, these transgenic plants also exhibited enhanced drought tolerance, which may be linked to a putative protecting mechanism preserving the photosynthetic machinery from dehydration damage (Shou et al., 2004b).

In ROS homeostasis, new signaling mechanisms are still being proposed and debated that contribute to our understanding of many levels of control in this sophisticated equilibrium. Recently, a member of the group C of MAPKs, the GhMPK2 from cotton, has been characterized (Zhang et al., 2011). Overexpression of GhMPK2 in tobacco rendered the plants resistant to fungal and viral pathogens, which was accompanied by upregulation of several pathogen-related genes. Additionally, upregulation of scavenger antioxidant enzymes in the transgenic plants resulted in enhanced oxidative stress tolerance. The antioxidant enzymes represent a major instrument for the plant to rapidly metabolize ROS and avoid oxidative damage. Interestingly, antioxidant defensive MAPK cascade involving scavenger enzymes is also sensitive to nitric oxide (NO) treatment (Zhang et al., 2007). It has been proposed that NO works in response to ABA-mediated H₂O₂ production, and subsequently, NO signaling is involved in the upregulation of expression of antioxidant enzymes in ABA signaling.

In maize, ABA-induced production of H₂O₂ activates two other MAPKs, ZmMPK3 and ZmMPK5. ZmMPK3 is sensitive to various signaling molecules such as jasmonic acid or salicylic acid, and it is also responsive to abiotic stress conditions including wounding, cold, drought, salinity or UV light (Wang et al., 2010). ZmHK5 is involved in a positive feedback regulation mechanism comprising ABA-mediated production and ROS-producing NADPH oxidase genes *ZmRbohA-D* (Lin et al., 2009). Production of NADPH oxidase is a biphasic process that can be partially controlled with MAPK inhibitors and H₂O₂ scavengers. ZmHK5 is involved in the activation of the second phase of biphasic induction of NADPH oxidase which in turn regulates H₂O₂ production.

MAPK pathways and stress tolerance in response to various environmental stimuli have also been extensively studied in rice. Prolonged incubations at moderately low temperatures (12°C) are potentially harmful for rice plants and may lead to male sterility and various growth arrest phenotypes. Therefore, involvement of several MAPKs in the process has been examined including MAPKK OsMEK1 and MAPK OsMAP1 (Wen et al., 2002). The two signaling modules physically interact on the protein level and therefore, they may be part of the same signaling pathway. Similarly, OsWJUMK1 is also inducible by moderate cold-stress conditions (Agrawal et al., 2003) and OsMAPK4 is specifically responsive to the cold-stress (Fu et al., 2002). In contrast, multiple stress responsive kinases OsMSRMK2 and OsMSRMK3 have been described as inducible by plethora of abiotic stress conditions such as wounding, salinity, drought, heavy metals, fungal elicitors or UV irradiation (Agrawal et al., 2003; Agrawal et al., 2002).

Another example of MAPK with multiple roles in both biotic and abiotic stress responses is OsMAPK5. The negative role of OsMAPK5 in the set of defense responses (Xiong and Yang, 2003) is similar to that of MPK4 as described above. Down-regulation of this kinase resulted in constitutive expression of several pathogen-related genes and enhanced resistance to both fungal and bacterial pathogens. However, these plants also showed reductions in tolerance against cold, salt or drought. Conversely, overexpression of OsMAPK5 in transgenic plants can lead to the increased multiple-stress tolerance.

Drought resistance in rice plants was addressed in the case of MAKKK of the B3 subgroup, namely for DSM1 (Ning et al., 2010). The *dsm1* mutants were hypersensitive to drought and lost water more rapidly as controls, while overexpression of *DSM1* conferred increased dehydration stress tolerance. Possible link to two peroxidase genes based on expression analysis and increased sensitivity in *dsm1* mutant to oxidative damage suggested the kinase may be involved in ROS signaling. Finally, OsMAPK33 is also induced by drought stress (Lee et al., 2011). Surprisingly, neither suppression nor overexpression of OsMAPK33 displayed any significant differences in drought tolerance. Instead, role in osmotic homeostasis was hypothesized based on enhanced sensitivity to salt stress in the overexpressing lines. This negative role in salt tolerance may present another regulatory mechanism in response to environmental stimuli.

10. Concluding remarks and future perspectives

MAPK signal transduction pathways relay information of the extracellular environment to the cellular interior, most often resulting in changes in the gene expression programs and in the plant development. In all eukaryotes, MAPK signaling pathways are highly conserved modules that are most commonly composed of a number of protein kinases that phosphorylate and thereby change the activity of their respective target proteins. Because the activation of a signaling pathway generally changes expression of a large number of genes, failure or modification of the activity of signaling pathways are often related to pathologic conditions in man, animals and plants. However, careful modification of MAPKs can also have beneficial effects for the organisms as evidenced by the enhanced tolerance against environmental conditions or pathogen attack. Therefore MAPKs and also MAPK-related phosphatases are ideal targets of genetic modification. The usefulness and biotechnological potential of targeted MAPK approaches is discussed in this review with respect to the potential to improve plant stress performance. Additionally, simultaneous functions of some members of MAPK modules both in the stress signaling and in the plant development are also discussed. A deeper knowledge about regulation of MAPK cascades, e.g. through integrated omics (transcriptomics, proteomics, phosphoproteomics, metabolomics, cellomics) approaches using publicly available software tools for in silico database analyses, might help to develop novel strategies to improve stress tolerance in plants.

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References

- Agrawal GK, Agrawal SK, Shibato J, Iwahashi H, Rakwal R. Novel rice MAP kinases OsMSRMK3 and OsWJUMK1 involved in encountering diverse environmental stresses and developmental regulation. *Biochem Biophys Res Commun* 2003;300:775-83.
- Agrawal GK, Rakwal R, Iwahashi H. 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* 2002;294:1009-16.
- Ahlfors R, Macioszek V, Rudd J, Brosché M, Schlichting R, Scheel D et al. Stress hormone-independent activation and nuclear translocation of mitogen-activated protein kinases in *Arabidopsis thaliana* during ozone exposure. *Plant J* 2004;40:512-22.
- Andreasson E, Jenkins T, Brodersen P, Thorgrimsen S, Petersen NHT, Zhu S, et al. The MAP kinase substrate MKS1 is a regulator of plant defense responses. *EMBO J* 2005;24:2579-89.
- Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL, Gomez-Gomez L, et al. MAP kinase signalling cascade in *Arabidopsis* innate immunity. *Nature* 2002;415:977-83.
- Asai S, Ohta K, Yoshioka H. MAPK signaling regulates nitric oxide and NADPH oxidase-dependent oxidative bursts in *Nicotiana benthamiana*. *Plant Cell* 2008;20:1390-406.
- Bayer M, Nawy T, Giglione C, Galli M, Meinel T, Lukowitz W. Paternal control of embryonic patterning in *Arabidopsis thaliana*. *Science* 2009;323(5920):1485-8.
- Beck M, Komis G, Müller J, Menzel D, Šamaj J. *Arabidopsis* homologs of nucleus- and phragmoplast-localized kinase 2 and 3 and mitogen-activated protein kinase 4 are essential for microtubule organization. *Plant Cell* 2010;22:755-71.
- Beck M, Komis G, Ziemann A, Menzel D, Šamaj J. Mitogen-activated protein kinase 4 is involved in the regulation of mitotic and cytokinetic microtubule transitions in *Arabidopsis thaliana*. *New Phytol* 2011;189:1069-83.
- Bergmann DC, Lukowitz W, Somerville CR. Stomatal development and pattern controlled by a MAPKK kinase. *Science* 2004;304:1494-7.
- Binder BM, Walker JM, Gagne JM, Emborg TJ, Hemmann G, Bleecker AB, et al. The *Arabidopsis* EIN3 binding F-Box proteins EBF1 and EBF2 have distinct but overlapping roles in ethylene signaling. *Plant Cell* 2007;19:509-23.

- Bögre L, Ligterink W, Meskiene I, Barker PJ, Heberle-Bors E, Huskisson NS, et al. Wounding Induces the Rapid and Transient Activation of a Specific MAP Kinase Pathway. *Plant Cell* 1997;9:75-83.
- Bögre L, Calderini O, Binarova P, Mattauch M, Till S, Kiegerl S, et al. A MAP kinase is activated late in plant mitosis and becomes localized to the plane of cell division. *Plant Cell* 1999;11:101-13.
- Brader G, Djamei A, Teige M, Palva ET, Hirt H. The MAP kinase kinase MKK2 affects disease resistance in Arabidopsis. *Mol Plant Microbe Interact* 2007;20:589-96.
- Bush SM, Krysan PJ. Mutational evidence that the Arabidopsis MAP kinase MPK6 is involved in anther, inflorescence, and embryo development. *J Exp Bot* 2007;58:2181-91.
- Calderini O, Bögre L, Vicente O, Binarova P, Heberle-Bors E, Wilson C. A cell cycle regulated MAP kinase with a possible role in cytokinesis in tobacco cells. *J Cell Sci* 1998;111:3091-100.
- Cardinale F, Jonak C, Ligterink W, Niehaus K, Boller T, Hirt H. Differential activation of four specific MAPK pathways by distinct elicitors. *J Biol Chem* 2000;275:36734-40.
- Cardinale F, Meskiene I, Ouaked F, Hirt H. Convergence and divergence of stress-induced mitogen-activated protein kinase signaling pathways at the level of two distinct mitogen-activated protein kinase kinases. *Plant Cell* 2002;14:703-11.
- Chao Q, Rothenberg M, Solano R, Roman G, Terzaghi W, Ecker JR. Activation of the ethylene gas response pathway in Arabidopsis by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. *Cell* 1997;89:1133-44.
- Chinchilla, D., Zipfel, C., Robatzek, S., Kemmerling, B., Nurnberger, T., Jones, J. et al. A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature* 2007;448:497–500.
- Ciolkowski I, Wanke D, Birkenbihl RP, Somssich IE. Studies on DNA-binding selectivity of WRKY transcription factors lend structural clues into WRKY-domain function. *Plant Mol Biol* 2008;68:81–92.
- Clark KL, Larsen PB, Wang X, Chang C. Association of the Arabidopsis CTR1 Raf-like kinase with the ETR1 and ERS ET receptors. *Proc Natl Acad Sci USA* 1998;95:5401–06.

- Colcombet J, Hirt H. Arabidopsis MAPKs: a complex signalling network involved in multiple biological processes. *Biochem J* 2008;413:217-26.
- Dai Y, Wang H, Li B, Huang J, Liu X, Zhou Y, et al. Increased expression of MAP KINASE KINASE7 causes deficiency in polar auxin transport and leads to plant architectural abnormality in Arabidopsis. *Plant Cell* 2006;18:308–20.
- del Pozo O, Pedley KF, Martin GB. MAPKKK α is a positive regulator of cell death associated with both plant immunity and disease. *EMBO J* 2004;23:3072-82.
- Desikan R, Clarke A, Atherfold P, Hancock JT, Neill SJ. Harpin induces mitogen-activated protein kinase activity during defence responses in Arabidopsis thaliana suspension cultures. *Planta* 1999;210:97–103.
- Desikan R, Hancock JT, Ichimura K, Shinozaki K, Neill SJ. Harpin induces activation of the Arabidopsis mitogen-activated protein kinases AtMPK4 and AtMPK6. *Plant Physiol* 2001;126:1579-87.
- Djamei A, Pitzschke A, Nakagami H, Rajh I, Hirt H. Trojan horse strategy in Agrobacterium transformation: abusing MAPK defense signaling. *Science* 2007;318:453–6.
- Doczi R, Brader G, Pettko-Szandtner A, Rajh I, Djamei A, Pitzschke A, et al. The Arabidopsis mitogen-activated protein kinase kinase MKK3 is upstream of group C mitogen-activated protein kinases and participates in pathogen signaling. *Plant Cell* 2007;19:3266–79.
- Droillard M, Boudsocq M, Barbier-Brygoo H, Lauriere C. Different protein kinase families are activated by osmotic stresses in Arabidopsis thaliana cell suspensions. Involvement of the MAP kinases AtMPK3 and AtMPK6. *FEBS Lett.* 2002;527:43-50.
- Droillard MJ, Boudsocq M, Barbier-Brygoo H, Lauriere C. Involvement of MPK4 in osmotic stress response pathways in cell suspensions and plantlets of *A. thaliana thaliana*: activation by hypoosmolarity and negative role in hyperosmolarity tolerance. *FEBS Lett* 2004;574:42–8.
- Feilner T, Hultschig C, Lee J, Meyer S, Immink RG, Koenig A, et al. High throughput identification of potential Arabidopsis mitogen-activated protein kinases substrates. *Mol Cell Proteomics* 2005;4:1558–68.

- Felix G, Duran JD, Volko S, Boller T. Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant J* 1999;18:265–76.
- Fu SF, Chou WC, Huang DD, Huang HJ. Transcriptional regulation of a rice mitogen-activated protein kinase gene, OsMAPK4, in response to environmental stresses. *Plant Cell Physiol* 2002;43:958-63.
- Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K. et al. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. *Curr Opin Plant Biol.* 2006;9:436-42.
- Gao Z, Chen YF, Randlett MD, Zhao XC, Findell JL, Kieber JJ, et al. Localization of the Raf-like kinase CTR1 to the endoplasmic reticulum of Arabidopsis through participation in ethylene receptor signaling complexes. *J Biol Chem* 2003;278:34725-32.
- Gao M, Liu J, Bi D, Zhang Z, Cheng F, Chen S, et al. MEKK1, MKK1/MKK2 and MPK4 function together in a mitogen-activated protein kinase cascade to regulate innate immunity in plants. *Cell Res* 2008;18:1190-8.
- Gomez-Gomez L, Boller T. FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. *Mol Cell* 2000;5:1003–11.
- Gomi, K, Ogawa, D, Katou S, Kamada H, Nakajima N, Saji H, et al. A mitogen-activated protein kinase NtMPK4 activated by SIPKK is required for jasmonic acid signaling and involved in ozone tolerance via stomatal movement in tobacco. *Plant Cell Physiol* 2005;46:1902-14.
- Gudesblat GE, Iusem ND, Morris PC. Arabidopsis MPK3, a Key Signalling Intermediate in Stomatal Function. *Plant Signal Behav* 2007a;2:271-2.
- Gudesblat GE, Iusem ND, Morris PC. Guard cell-specific inhibition of Arabidopsis MPK3 expression causes abnormal stomatal responses to abscisic acid and hydrogen peroxide. *New Phytol* 2007b;173:713-21.
- Holley SR, Yalamanchili RD, Moura DS, Ryan CA, Stratmann JW. Convergence of signaling pathways induced by systemin, oligosaccharide elicitors, and ultraviolet-B radiation at the level of mitogen-activated protein kinases in *Lycopersicon peruvianum* suspension-cultured cells. *Plant Physiol* 2003;132:1728-38.

- Hua J, Meyerowitz EM. Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. *Cell* 1998;94:261-71.
- Huang Y, Li H, Hutchison CE, Laskey J, Kieber JJ. Biochemical and functional analysis of CTR1, a protein kinase that negatively regulates ethylene signaling in *Arabidopsis*. *Plant J* 2003;33:221–33.
- Ichimura K, Casais C, Peck SC, Shinozaki K, Shirasu K. MEKK1 is required for MPK4 activation and regulates tissue-specific and temperature-dependent cell death in *Arabidopsis*. *J Biol Chem* 2006;281:36969-76.
- Ichimura K, Mizoguchi T, Irie K, Morris P, Giraudat J, Matsumoto K, et al. Isolation of ATMEKK1 (a MAP kinase kinase kinase)-interacting proteins and analysis of a MAP kinase cascade in *Arabidopsis*. *Biochem Biophys Res Commun* 1998;253:532-43.
- Ichimura K, Mizoguchi T, Yoshida R, Yuasa T, Shinozaki K. Various abiotic stresses rapidly activate *Arabidopsis* MAP kinases ATMPK4 and ATMPK6. *Plant J* 2000;24:655-65.
- Ikeda Y, Men S, Fischer U, Stepanova AN, Alonso JM, Ljung K, et al. Local auxin biosynthesis modulates gradient-directed planar polarity in *Arabidopsis*. *Nat Cell Biol* 11;2009:731-40.
- Jiang M, Zhang J. Cross-talk between calcium and reactive oxygen species originated from NADPH oxidase in abscisic acid-induced antioxidant defence in leaves of maize seedlings. *Plant Cell Environ* 2003;26:929-39.
- Jonak C, Kiegerl S, Ligterink W, Barker PJ, Huskisson NS, Hirt H. Stress signaling in plants: a mitogen-activated protein kinase pathway is activated by cold and drought. *Proc Natl Acad Sci USA* 1996;93:11274-9.
- Jonak C, Nakagami H and Hirt H. Heavy metal stress. Activation of distinct mitogen-activated protein kinase pathways by copper and cadmium. *Plant Physiol* 2004;136:3276–83.
- Jones JD, Dangl JL. The plant immune system. *Nature* 2006;444:323-9.
- Joo S, Liu Y, Lueth A, Zhang S. MAPK phosphorylation-induced stabilization of ACS6 protein is mediated by the non-catalytic C-terminal domain, which also contains the cis-determinant for rapid degradation by the 26 S proteasome pathway. *Plant J* 2008;54:129–40.

- Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR. CTR1, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the raf family of protein kinases. *Cell* 1993;72:427–41.
- Kiegerl S, Cardinale F, Siligan C, Gross A, Baudouin E, Liwosz A, et al. SIMKK, a mitogen-activated protein kinase (MAPK) kinase, is a specific activator of the salt stress-induced MAPK, SIMK. *Plant Cell*. 2000;12:2247-58.
- Kim JA, Agrawal GK, Rakwal R, Han KS, Kim KN, Yun CH, et al. Molecular cloning and mRNA expression analysis of a novel rice (*Oryza sativa* L.) MAPK kinase kinase, OsEDR1, an ortholog of Arabidopsis AtEDR1, reveal its role in defense/stress signalling pathways and development. *BiochemBiophys Res Commun* 2003;300:868-76.
- Kosetsu K, Matsunaga S, Nakagami H, Colcombet J, Sasabe M, Soyano T, et al. The MAP kinase MPK4 is required for cytokinesis in Arabidopsis thaliana. *Plant Cell* 2010;22:3778-90.
- Kovtun Y, Chiu WL, Tena G, Sheen J. Functional analysis of oxidative stress-activated mitogen activated protein kinase cascade in plants. *Proc Natl Acad Sci USA* 2000;97:2940–5.
- Kovtun Y, Chiu WL, Zeng W, Sheen J. Suppression of auxin signal transduction by a MAPK cascade in higher plants. *Nature* 1998;395:716–20.
- Krysan PJ, Jester PJ, Gottwald JR, Sussman MR. An Arabidopsis mitogen-activated protein kinase kinase gene family encodes essential positive regulators of cytokinesis. *Plant Cell* 2002;14:1109-20.
- Kunze G, Zipfel C, Robatzek S, Niehaus K, Boller T, Felix G. The N terminus of bacterial elongation factor Tu elicits innate immunity in Arabidopsis plants. *Plant Cell* 2004;16:3496–507.
- Lampard GR, Macalister CA, Bergmann DC. Arabidopsis stomatal initiation is controlled by MAPK-mediated regulation of the bHLH SPEECHLESS. *Science* 2008;322:1113-6.
- Lampard GR, Lukowitz W, Ellis BE, Bergmann DC. Novel and expanded roles for MAPK signaling in Arabidopsis stomatal cell fate revealed by cell type-specific manipulations. *Plant Cell* 2009;21:3506-17.

- Lee J, Rudd JJ, Macioszek VK, Scheel D. Dynamic changes in the localization of MAPK cascade components controlling pathogenesis-related (PR) gene expression during innate immunity in parsley. *J Biol Chem*. 2004;279:22440-8.
- Lee JS, Ellis BE. Arabidopsis MAPK phosphatase 2 (MKP2) positively regulates oxidative stress tolerance and inactivates the MPK3 and MPK6 MAPKs. *J Biol Chem* 2007;282:25020-9.
- Lee JS, Wang S, Sritubtim S, Chen JG, Ellis BE. Arabidopsis mitogen-activated protein kinase MPK12 interacts with the MAPK phosphatase IBR5 and regulates auxin signaling. *Plant J* 2009;57:975-85.
- Lee SK, Kim BG, Kwon TR, Jeong MJ, Park SR, Lee JW, et al. Overexpression of the mitogen-activated protein kinase gene OsMAPK33 enhances sensitivity to salt stress in rice (*Oryza sativa* L.). *J Biosci* 2011;36:139-51.
- Limmongkon A, Giuliani C, Valenta R, Mittermann I, Heberle-Bors E, Wilson C. MAP kinase phosphorylation of plant profilin. *Biochem Biophys Res Commun* 2004;324:382-6.
- Lin F, Ding H, Wang J, Zhang H, Zhang A, Zhang Y, et al. Positive feedback regulation of maize NADPH oxidase by mitogen-activated protein kinase cascade in abscisic acid signalling. *J Exp Bot* 2009;60:3221-38.
- Liu Y, Schiff M, Dinesh-Kumar SP. Involvement of MEK1 MAPKK, NTF6 MAPK, WRKY/MYB transcription factors, COI1 and CTR1 in N-mediated resistance to tobacco mosaic virus. *Plant J* 2004;38:800-9.
- Liu Y, Zhang S. Phosphorylation of 1-aminocyclopropane-1-carboxylic acid synthase by MPK6, a stress-responsive mitogen-activated protein kinase, induces ET biosynthesis in Arabidopsis. *Plant Cell* 2004;16:3386-99.
- Lukowitz W, Roeder A, Parmenter D, Somerville C. A MAPKK kinase gene regulates extra-embryonic cell fate in Arabidopsis. *Cell* 2004;116:109-19.
- Mao G, Meng X, Liu Y, Zheng Z, Chen Z, Zhang S. Phosphorylation of a WRKY transcription factor by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in Arabidopsis. *Plant Cell* 2011;23:1639-53.
- Matsuoka D, Nanmori T, Sato K, Fukami Y, Kikkawa U, Yasuda T. Activation of AtMEK1, an Arabidopsis mitogen-activated protein kinase kinase, in vitro and in vivo:

- analysis of active mutants expressed in *E. coli* and generation of the active form in stress response in seedlings. *Plant J.* 2002;29:637-47.
- Mayrose M, Bonshtien A, Sessa G. LeMPK3 is a mitogen-activated protein kinase with dual specificity induced during tomato defense and wounding responses. *J Biol Chem.* 2004;279:14819-27.
- Menges M, Dóczy R, Okrészl L, Morandini P, Mizzi L, Soloviev M, et al. Comprehensive gene expression atlas for the Arabidopsis MAP kinase signalling pathways. *New Phytol* 2008;179:643-62.
- Menke FL, van Pelt JA, Pieterse CM, Klessig DF. Silencing of the mitogen-activated protein kinase MPK6 compromises disease resistance in Arabidopsis. *Plant Cell* 2004;16:897-907.
- Mészáros T, Helfer A, Hatzimasoura E, Magyar Z, Serazetdinova L, Rios G, et al. The Arabidopsis MAP kinase kinase MKK1 participates in defence responses to the bacterial elicitor flagellin. *Plant J* 2006;48:485–98.
- Miles GP, Samuel MA, Zhang Y, Ellis BE. RNA interference-based (RNAi) suppression of AtMPK6, an Arabidopsis mitogen-activated protein kinase, results in hypersensitivity to ozone and misregulation of AtMPK3. *Environ Pollut* 2005;138:230-7.
- Miya A, Albert P, Shinya T, Desaki Y, Ichimura K, Shirasu K, et al. CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in Arabidopsis. *Proc Natl Acad Sci USA* 2007;104:19613–8.
- Mizoguchi T, Irie K, Hirayama T, Hayashida N, Yamaguchi-Shinozaki K, Matsumoto K et al. A gene encoding a mitogen-activated protein kinase 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* 1996;93:765-9.
- Mockaitis K, Howell SH. Auxin induces mitogenic activated protein kinase (MAPK) activation in roots of Arabidopsis seedlings. *Plant J* 2000;24:785-96.
- Moon H, Lee B, Choi G, Shin D, Prasad DT, Lee O, et al. NDP kinase 2 interacts with two oxidative stress-activated MAPKs to regulate cellular redox state and enhances multiple stress tolerance in transgenic plants. *Proc Natl Acad Sci USA* 2003;100:358-63.

- Müller J, Beck M, Mettbach U, Komis G, Hause G, Menzel D, et al. Arabidopsis MPK6 is involved in cell division plane control during early root development, and localizes to the pre-prophase band, phragmoplast, trans-Golgi network and plasma membrane. *Plant J* 2010;61:234-48.
- Nakagami H, Kiegerl S, Hirt H. OMTK1, a novel MAPKKK, channels oxidative stress signaling through direct MAPK interaction. *J Biol Chem* 2004;279:26959-66.
- Nakagami H, Soukupová H, Schikora A, Zárský V, Hirt H. A Mitogen-activated protein kinase kinase kinase mediates reactive oxygen species homeostasis in Arabidopsis. *J Biol Chem* 2006;281:38697-704.
- Naoi K, Hashimoto T. A semidominant mutation in an Arabidopsis mitogen-activated protein kinase phosphatase-like gene compromises cortical microtubule organization. *Plant Cell* 2004;16:1841-53.
- Ning J, Li X, Hicks LM, Xiong L. A Raf-like MAPKKK gene DSM1 mediates drought resistance through reactive oxygen species scavenging in rice. *Plant Physiol* 2010;152:876-90.
- Nishihama R, Ishikawa M, Araki S, Soyano T, Asada T, Machida Y. The NPK1 mitogen-activated protein kinase kinase kinase is a regulator of cell-plate formation in plant cytokinesis. *Genes Dev* 2001;15:352-63.
- Nishihama R, Soyano T, Ishikawa M, Araki S, Tanaka H, Asada T, et al. Expansion of the cell plate in plant cytokinesis requires a kinesin-like protein/MAPKKK complex. *Cell* 2002;109:87-99.
- Novikova GV, Moshkov IE, Smith AR, Hall MA. The effect of ET on MAPKinase-like activity in Arabidopsis thaliana. *FEBS Lett* 2000;474:29-32.
- Nuhse TS, Peck SC, Hirt H, Boller T. Microbial elicitors induce activation and dual phosphorylation of the Arabidopsis thaliana MAPK 6. *J Biol Chem* 2000;275:7521-6.
- Ortiz-Masia D, Perez-Amador MA, Carbonell J, Marcote MJ. Diverse stress signals activate the C1 subgroup MAP kinases of Arabidopsis. *FEBS Lett*. 2007;581:1834-40.
- Ouaked F, Rozhon W, Lecourieux D, Hirt H. A MAPK pathway mediates ET signaling in plants. *EMBO J* 2003;22:1282-8.

- Pedley KF, Martin GB. Identification of MAPKs and their possible MAPK kinase activators involved in the Pto-mediated defense response of tomato. *J Biol Chem*. 2004;279:49229-35.
- Petersen M, Brodersen P, Naested H, Andreasson E, Lindhart U, Johansen B, et al. Arabidopsis map kinase 4 negatively regulates systemic acquired resistance. *Cell* 2000;103:1111-20.
- Petersen K, Qiu JL, Lutje J, Fiil BK, Hansen S, Mundy J, et al. Arabidopsis MKS1 is involved in basal immunity and requires an intact N-terminal domain for proper function. *PLoS ONE* 2010;5:e14364.
- Pitzschke A, Djamei A, Bitton F, Hirt H. A major role of the MEKK1-MKK1/2-MPK4 pathway in ROS signalling. *Mol Plant* 2009a;2:120-37.
- Pitzschke A, Djamei A, Teige M, Hirt H. VIP1 response elements mediate mitogen-activated protein kinase 3-induced stress gene expression. *Proc Natl Acad Sci USA* 2009b;106:18414-9.
- Popescu SC, Popescu GV, Bachan S, Zhang Z, Gerstein M, Snyder M, et al. MAPK target networks in Arabidopsis thaliana revealed using functional protein microarrays. *Genes Dev* 2009;23:80-92.
- Prasad TK, Anderson MD, Martin BA, Stewart CR. Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. *Plant Cell* 1994;6:65-74.
- Qiu JL, Fiil BK, Petersen K, Nielsen HB, Botanga CJ, Thorgrimsen S, et al. Arabidopsis MAP kinase 4 regulates gene expression through transcription factor release in the nucleus. *Embo J* 2008a;27:2214-21.
- Qiu JL, Zhou L, Yun BW, Nielsen HB, Fiil BK, Petersen K, et al. Arabidopsis mitogen-activated protein kinase kinases MKK1 and MKK2 have overlapping functions in defense signaling mediated by MEKK1, MPK4, and MKS1. *Plant Physiol* 2008b;148:212-22.
- Qutob D, Kemmerling B, Brunner F, Kufner I, Engelhardt S, Gust AA, et al. Phytotoxicity and innate immune responses induced by Nep1-like proteins. *Plant Cell* 2006;18:3721-44.

- Ren D, Liu Y, Yang KY, Han L, Mao G, Glazebrook J, et al. A fungal-responsive MAPK cascade regulates phytoalexin biosynthesis in Arabidopsis. *Proc Natl Acad Sci USA* 2008;105:5638-43.
- Ren D, Yang KY, Li GJ, Liu Y, Zhang S. Activation of Ntf4, a tobacco mitogen-activated protein kinase, during plant defense response and its involvement in hypersensitive response-like cell death. *Plant Physiol* 2006;141:1482-93.
- Ren D, Yang H, Zhang S. Cell death mediated by MAPK is associated with hydrogen peroxide production in Arabidopsis. *J Biol Chem*. 2002;277:559-65.
- Robatzek S, Chinchilla D, Boller T. Ligand-induced endocytosis of the pattern recognition receptor FLS2 in Arabidopsis. *Genes Dev* 2006;20:537-42.
- Rudd JJ, Keon J, Hammond-Kosack KE. The wheat mitogen-activated protein kinases TaMPK3 and TaMPK6 are differentially regulated at multiple levels during compatible disease interactions with *Mycosphaerella graminicola*. *Plant Physiol*. 2008;147:802-15.
- Rushton PJ, Reinstadler A, Lipka V, Lippok B, Somssich IE. Synthetic plant promoters containing defined regulatory elements provide novel insights into pathogen- and wound-induced signaling. *Plant Cell* 2002;14:749-62.
- Samuel MA, Miles GP, Ellis BE. Ozone treatment rapidly activates MAP kinase signalling in plants. *Plant J* 2000;22:367-76.
- Sasabe M, Machida Y. MAP65: a bridge linking a MAP kinase to microtubule turnover. *Curr Opin Plant Biol* 2006a;9:563-70.
- Sasabe M, Soyano T, Takahashi Y, Sonobe S, Igarashi H, Itoh TJ, et al. Phosphorylation of NtMAP65-1 by a MAP kinase down-regulates its activity of microtubule bundling and stimulates progression of cytokinesis of tobacco cells. *Genes Dev* 2006b;20:1004-14.
- Sasabe M, Kosetsu K, Hidaka M, Murase A, Machida Y. Arabidopsis thaliana MAP65-1 and MAP65-2 function redundantly with MAP65-3/PLEIADE in cytokinesis downstream of MPK4. *Plant Signal Behav* 2011;6:743-7.
- Šamaj J, Ovecka M, Hlavacka A, Lecourieux F, Meskiene I, Lichtscheidl I, et al. Involvement of the mitogen-activated protein kinase SIMK in regulation of root hair tip growth. *EMBO J* 2002;21:3296-306.
- Schweighofer A, Kazanaviciute V, Scheikl E, Teige M, Doczi R, Hirt H, et al. The PP2C-type phosphatase AP2C1, which negatively regulates MPK4 and MPK6, modulates

- innate immunity, jasmonic acid, and ethylene levels in Arabidopsis. *Plant Cell* 2007;19:2213–24.
- Sharma PC, Ito A, Shimizu T, Terauchi R, Kamoun S, Saitoh H. Virus-induced silencing of WIPK and SIPK genes reduces resistance to a bacterial pathogen, but has no effect on the INF1-induced hypersensitive response (HR) in *Nicotiana benthamiana*. *Mol Genet Genomics*. 2003;269:583-91.
- Shou H, Bordallo P, Fan JB, Yeakley JM, Bibikova M, Sheen J, et al. Expression of an active tobacco mitogen-activated protein kinase kinase kinase enhances freezing tolerance in transgenic maize. *Proc Natl Acad Sci U S A*. 2004a;101:3298-303.
- Shou H, Bordallo P, Wang K. Expression of the *Nicotiana* protein kinase (NPK1) enhanced drought tolerance in transgenic maize. *J Exp Bot*. 2004b;55:1013-9.
- Soyano T, Nishihama R, Morikiyo K, Ishikawa M, Machida Y. NQK1/NtMEK1 is a MAPKK that acts in the NPK1 MAPKKK-mediated MAPK cascade and is required for plant cytokinesis. *Genes Dev* 2003;17:1055-67.
- Smertenko AP, Chang HY, Sonobe S, Fenyk SI, Weingartner M, Bögre L, et al. Control of the AtMAP65-1 interaction with microtubules through the cell cycle. *J Cell Sci* 2006;119:3227-37.
- Spoel SH, Mou Z, Tada Y, Spivey NW, Genschik P, Dong X. Proteasome-mediated turnover of the transcription coactivator NPR1 plays dual roles in regulating plant immunity. *Cell* 2009;137:860–72.
- Suarez-Rodriguez MC, Adams-Phillips L, Liu Y, Wang H, Su SH, Jester PJ, et al. MEKK1 is required for flg22-induced MPK4 activation in Arabidopsis plants. *Plant Physiol* 2007;143:661–9.
- Takahashi F, Yoshida R, Ichimura K, Mizoguchi T, Seo S, Yonezawa M, et al. The mitogen-activated protein kinase cascade MKK3-MPK6 is an important part of the jasmonate signal transduction pathway in Arabidopsis. *Plant Cell* 2007;19:805-18.
- Takahashi Y, Soyano T, Kosetsu K, Sasabe M, Machida Y. HINKEL kinesin, ANP MAPKKs and MKK6/ANQ MAPKK, which phosphorylates and activates MPK4 MAPK, constitute a pathway that is required for cytokinesis in *Arabidopsis thaliana*. *Plant Cell Physiol* 2010;51:1766-76.

- Tang D, Innes RW. Overexpression of a kinase-deficient form of the EDR1 gene enhances powdery mildew resistance and ethylene-induced senescence in Arabidopsis. *Plant J* 2002;32:975-83.
- Teige M, Scheikl E, Eulgem T, Dóczy R, Ichimura K, Shinozaki K, et al. The MKK2 pathway mediates cold and salt stress signaling in Arabidopsis. *Mol Cell* 2004;15:141-52.
- Walia A, Lee JS, Wasteneys G, Ellis B. Arabidopsis mitogen-activated protein kinase MPK18 mediates cortical microtubule functions in plant cells. *Plant J* 2009;59:565-75.
- Wang H, Ngwenyama N, Liu Y, Walker JC, Zhang S. Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in Arabidopsis. *Plant Cell* 2007;19:63-73.
- Wang J, Ding H, Zhang A, Ma F, Cao J, Jiang M. A novel mitogen-activated protein kinase gene in maize (*Zea mays*), ZmMPK3, is involved in response to diverse environmental cues. *J Integr Plant Biol.* 2010;52:442-52.
- Wen JQ, Oono K, Imai R. Two novel mitogen-activated protein signaling components, OsMEK1 and OsMAP1, are involved in a moderate low-temperature signaling pathway in rice. *Plant Physiol* 2002;129:1880-91.
- Xing Y, Jia W, Zhang J. AtMEK1 mediates stress-induced gene expression of CAT1 catalase by triggering H₂O₂ production in Arabidopsis. *J Exp Bot* 2007;58:2969-81.
- Xing Y, Jia W, Zhang J. AtMKK1 mediates ABA-induced CAT1 expression and H₂O₂ production via AtMPK6-coupled signaling in Arabidopsis. *Plant J* 2008;54:440-51.
- Xing Y, Jia W, Zhang J. AtMKK1 and AtMPK6 are involved in abscisic acid and sugar signaling in Arabidopsis seed germination. *Plant Mol Biol* 2009;70:725-36.
- Xiong L, Yang Y. Disease resistance and abiotic stress tolerance in rice are inversely modulated by an abscisic acid-inducible mitogen-activated protein kinase. *Plant Cell* 2003;15:745-59.
- Yanagisawa S, Yoo SD, Sheen J. Differential regulation of EIN3 stability by glucose and ethylene signalling in plants. *Nature* 2003; 425:521-5.
- Yang KY, Liu Y, Zhang S. Activation of a mitogen-activated protein kinase pathway is involved in disease resistance in tobacco. *Proc Natl Acad Sci U S A.* 2001;98:741-6.

- Yeh CM, Chien PS, Huang HJ. Distinct signalling pathways for induction of MAP kinase activities by cadmium and copper in rice roots. *J Exp Bot* 2007;58:659–71.
- Yoo SD, Cho YH, Tena G, Xiong Y, Sheen J. Dual control of nuclear EIN3 by bifurcate MAPK cascades in C2H4 signalling. *Nature* 2008;451:789–95.
- Zaïdi I, Ebel C, Touzri M, Herzog E, Evrard JL, Schmit AC, et al. TMKP1 is a novel wheat stress responsive MAP Kinase phosphatase localized in the nucleus. *Plant Mol Biol* 2010;73:325-38.
- Zhang A, Jiang M, Zhang J, Ding H, Xu S, Hu X, et al. Nitric oxide induced by hydrogen peroxide mediates abscisic acid-induced activation of the mitogen-activated protein kinase cascade involved in antioxidant defense in maize leaves. *New Phytol.* 2007;175:36-50.
- Zhang S, Klessig DF. Resistance gene N-mediated de novo synthesis and activation of a tobacco mitogen-activated protein kinase by tobacco mosaic virus infection. *Proc Natl Acad Sci USA* 1998a;95:7433-8.
- Zhang S, Klessig DF. The tobacco wounding-activated mitogen-activated protein kinase is encoded by SIPK. *Proc Natl Acad Sci U S A.* 1998b;95:7225-30.
- Zhang S, Liu Y, Klessig DF. Multiple levels of tobacco WIPK activation during the induction of cell death by fungal elicitors. *Plant J.* 2000;23:339-47.
- Zhang L, Xi D, Luo L, Meng F, Li Y, Wu CA, et al. Cotton GhMPK2 is involved in multiple signaling pathways and mediates defense responses to pathogen infection and oxidative stress. *Febs J.* 2011;278:1367-78.
- Zipfel C, Robatzek S, Navarro L, Oakeley EJ, Jones JD, Felix G, et al. Bacterial disease resistance in Arabidopsis through flagellin perception. *Nature* 2004;428:764-7.

Figure legend**Figure 1 Model depicting the subcellular organization of mitogen-activated protein kinase (MAPK) modules and their targets.**

MAPKs are phosphorylating enzymes representing main signal transducers in plants. They are organized in protein complexes, so called MAPK modules, which are held together by scaffold protein. These scaffold proteins have binding sites for MAP3K, MAP2K and MAPK and usually possess specific PH and/or FYVE domains which can bind to phospholipids in endomembranes. Eventually, also some MAP3Ks and MAP2Ks may also function as scaffold proteins. MAPK signaling can be triggered by diverse stimuli such as stress and developmental cues. During signal transduction, MAP3Ks and MAP2Ks are stepwise phosphorylated and activated on serine (S) or threonine (T) residues while MAPKs must be double phosphorylated on both threonine (T) and tyrosine (Y) residues. Activated MAPKs are released from MAPK modules, they relocate within the cell and regulate broad range of substrates (e.g. transcription factors, protein kinases, enzymes and cytoskeletal proteins) which are localized in/at diverse subcellular compartments (nucleus, cytosol, cytoskeleton). This subcellular compartmentalization of MAPK modules (e.g. association with endomembranes or with cytosol) and targets of activated MAPKs (e.g. nucleus, cytosol, cytoskeleton) is believed to bring some specificity to the MAPK signaling.

Figure 1

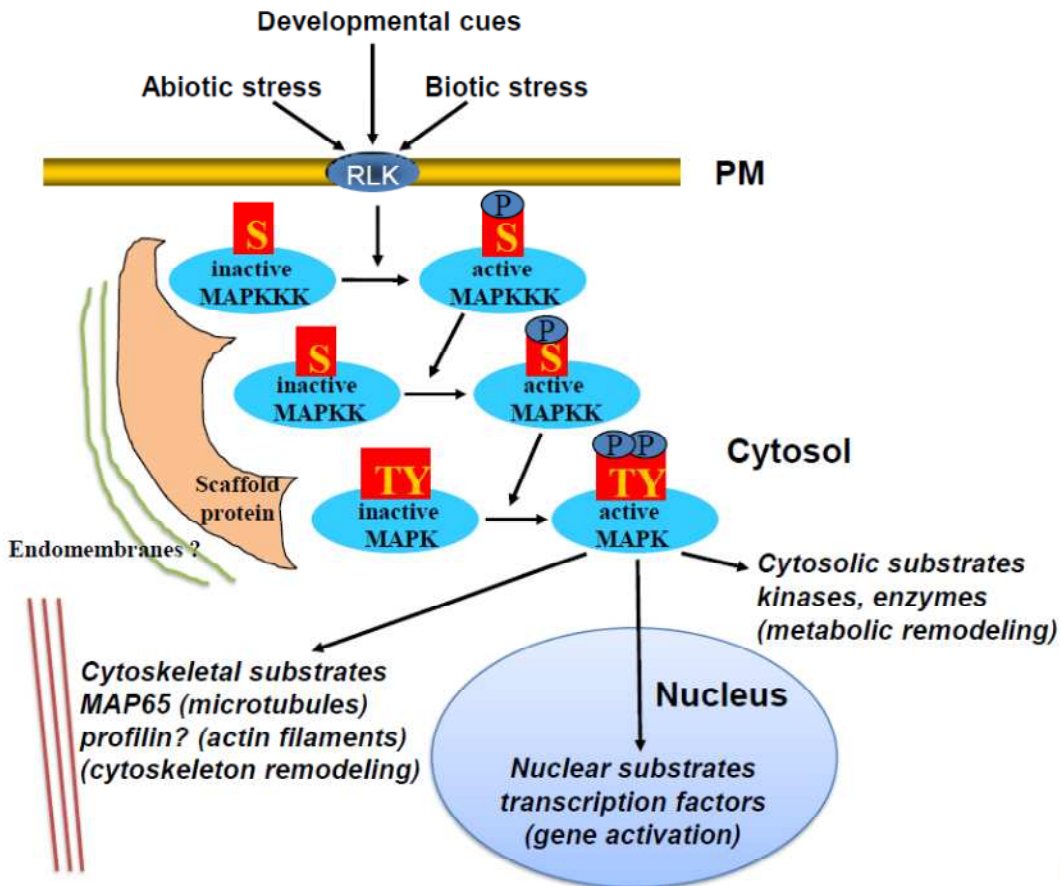


Figure 1

Table 1. Overview of MAP3Ks, MAP2Ks and MAPKs involved in plant stress responses.

At = *Arabidopsis thaliana*, *Le* = *Lycopersicon esculentum*, *Ms* = *Medicago sativa*, *Nt* = *Nicotiana tabacum*, *Os* = *Oryza sativa*, *Pc* = *Petroselinum crispum*, *Nb* = *Nicotiana benthamiana*, *Ta* = *Triticum aestivum*, *Zm* = *Zea mays*

Name	Stress Response	Reference
MAP3Ks		
<i>At</i> EDR1	Fungal pathogens resistance	Tang and Innes, 2002
<i>At</i> ANP1	Oxidative stress	Kovtun et al., 2000
<i>At</i> MEKKK1	Touch, cold, salinity, oxidative stress, bacterial pathogens resistance	Mizoguchi et al., 1996; Teige et al., 2004; Ichimura et al., 2006; Gao et al., 2008 Pitzschke et al., 2009
<i>Le</i> MAPKKK α	Bacterial pathogens resistance	del Pozo et al., 2004
<i>Ms</i> OMTK1	Oxidative stress	Nakagami et al., 2004
<i>Nt</i> NPK1	Cold, drought, hyperosmotic stress	Kovtun et al., 2000; Shou et al., 2004a; Shou et al., 2004b
<i>Os</i> EDR1	Fungal pathogens, drought, high salt and sugar, heavy metals	Kim et al., 2003
MAP2Ks		
<i>At</i> MKK1	Wounding, cold, drought, and high salt	Matsuoka et al, 2002; Teige et al., 2004
<i>At</i> MKK2	Cold and high salt stress	Teige et al., 2004
<i>At</i> MKK4 <i>At</i> MKK5	Bacterial and fungal pathogens resistance	Asai et al., 2002
<i>Le</i> MKK2 <i>Le</i> MKK4	Bacterial elicitor stress signaling	Pedley et al., 2004
<i>Os</i> MEK1	Cold stress	Wen et al., 2002
<i>Ms</i> SIMKK	Pathogen elicitor stress signaling, heavy metals and high salt stress	Kiegerl et al., 2000; Cardinale et al., 2002; Jonak et al., 2004
<i>Nt</i> MEK2	Multiple defense responses against pathogens	Yang et al., 2001; Ren et al., 2002; del Pozo et al., 2004
<i>Pc</i> MKK5	Fungal and bacterial elicitor stress signaling	Lee et al., 2004
MAPKs		
<i>At</i> MPK3	Oxidative and osmotic stress, bacterial elicitor stress signaling	Desikan et al., 1999; Kovtun et al., 2000; Asai et al., 2002; Droillard et al., 2002; Ahlfors et al., 2004
<i>At</i> MPK4	Cold, drought, hyper-osmolarity, touch, wounding and oxidative stress, pathogen resistance	Desikan et al., 1999; Ichimura et al., 2000; Petersen et al., 2000; Droillard et al., 2004; Teige et al. 2004; Nakagami et al., 2006
<i>At</i> MPK6	Cold, drought, hyper-osmolarity, touch, wounding and oxidative stress, pathogen resistance	Desikan et al., 1999; Ichimura et al., 2000; Kovtun et al., 2000; Nuhse et al., 2000; Asai et al., 2002; Droillard et al., 2002; Ahlfors et al., 2004; Menke et al., 2004
<i>Le</i> MPK1 <i>Le</i> MPK2	Bacterial elicitors, UV-B radiation	Holley et al., 2003

<i>Le</i> MPK3	Bacterial and fungal pathogens, mechanical stress and wounding, UV-B radiation	Holley et al., 2003; Mayrose et al., 2004; Pedley and Martin, 2004
<i>Ms</i> MMK2 <i>Ms</i> MMK3	Heavy metal stress	Jonak et al., 2004
<i>Ms</i> SAMK <i>Ms</i> SIMK	Mechanical stimulation, wounding, drought, and cold, heavy metals, pathogen resistance	Jonak et al., 1996; Bogre et al., 1997; Cardinale et al., 2000; Jonak et al., 2004
<i>Nb</i> SIPK <i>Nb</i> WIPK	Wounding, bacterial elicitor or avirulent pathogen responses	Sharma et al., 2003
<i>Nt</i> SIPK <i>Nt</i> WIPK	Osmotic stress, wounding, fungal, bacterial and viral pathogen resistance	Zhang and Klessig, 1998a; Zhang et al., 2000; del Pozo et al., 2004
<i>Os</i> MAP1	Cold temperature stress	Wen et al., 2002
<i>Os</i> MAPK4	Sugar starvation, high salinity, cold	Fu et al., 2002
<i>Os</i> MAPK5	Pathogen resistance, wounding, drought, salt, and cold stress	Xiong and Yang, 2003
<i>Os</i> MAPK33	Drought, osmotic stress	Lee et al., 2011
<i>Os</i> MSRMK2 <i>Os</i> MSRMK3	Wounding, drought, heavy metals, fungal elicitors, UV irradiation, heavy metals, high salt and sucrose	Agrawal et al., 2002; Agrawal et al., 2003
<i>Os</i> WJUMK1	Oxidative stress, cold, heavy metals	Agrawal et al., 2003
<i>Ta</i> MPK3 <i>Ta</i> MPK6	Fungal pathogens resistance	Rudd et al., 2008
<i>Zm</i> MPK3	Cold, drought, ultraviolet light, salinity, heavy metal and mechanical wounding	Wang et al., 2010
<i>Zm</i> MPK5	Oxidative stress	Lin et al., 2009

Table 2 Overview of MAP3Ks, MAP2Ks and MAPKs involved both in plant development and stress responses.

At = *Arabidopsis thaliana*, *Ms* = *Medicago sativa*, *Nb* = *Nicotiana benthamiana*, *Nt* = *Nicotiana tabacum*

? = function in stress response was suggested from transcriptomic data but not directly experimentally determined

Name	Developmental Process	Stress Response	Reference
MAP3Ks			
<i>At</i> ANP2/3	Cytokinesis	Oxidative stress?, pathogen response?, heat?	Krysan et al., 2002
<i>At</i> YODA	Stomata and embryo development	Plant defense?	Bergmann et al. 2004; Lukowitz et al. 2004
<i>Nt</i> NPK1	Cytokinesis	Oxidative stress	Kovtun et al., 2000; Nishihama et al. 2001
<i>At</i> MEKK1	Root hair and lateral root development	Oxidative stress, salt, drought, wounding, bacterial elicitor flg22, cell death	Asai et al. 2002; Ichimura et al., 2006; Nakagami et al. 2006; Suarez-Rodriguez et al., 2007;
<i>At</i> CTR1	Root planar polarity	Ethylene	Kieber et al. 1993; Ikeda et al. 2009
MAP2Ks			
<i>Nt</i> NQK1	Cytokinesis	Cell death, fungal elicitor	Soyano et al. 2003; del Pozo et al. 2004
<i>At</i> MKK4	Stomata development	Bacterial elicitor flg22	Tena et al. 2001; Wang et al. 2007
<i>At</i> MKK5	Stomata development	Bacterial elicitor flg22	Tena et al. 2001; Wang et al. 2007;
MAPKs			
<i>Ms</i> SIMK	Root hair development	Salt, fungal elicitor pep13, wounding, cold, drought, heavy metals	Jonak et al. 1996; Bögre et al. 1997; Cardinale et al. 2002; Šamaj et al., 2002; Jonak et al. 2004
<i>Ms</i> MMK3	Cell division	Fungal elicitor pep13, ethylene, heavy metals, oxidative stress, cell death	Bögre et al. 1999; Cardinale et al. 2002; Ouaked et al. 2003; Jonak et al. 2004; Nakagami et al. 2004
<i>Nb</i> Ntf6	Cytokinesis	Virus resistance, oxidative stress	Calderini et al. 1998; Liu et al. 2004; Asai et al. 2008
<i>At</i> MPK3	Stomata development	Ozone, oxidative stress, hypoosmolarity, bacterial	Kovtun et al. 2000; Asai et al. 2002; Ahlfors et al. 2004;

		elicitor flg22, fungal resistance	Droillard et al. 2004; Lee and Ellis 2007; Wang et al. 2007; Ren et al. 2008
<i>At</i> MPK4	Cytokinesis, seedling and root hair development, cell shape control	Oxidative stress, cold, salt, hypo- and hyperosmolarity, touch, wounding, bacterial elicitor flg22	Ichimura et al. 2000; Asai et al. 2002; Droillard et al. 2004; Teige et al. 2004; Nakagami et al. 2006; Beck et al. 2010; Kosetsu et al. 2010; Beck et al. 2011
<i>At</i> MPK6	Cell division, stomata, anther and inflorescence development	Ozone, oxidative stress, cold, salt, hypo- and hyperosmolarity, touch, wounding, bacterial elicitor flg22, fungal resistance, jasmonic acid	Ichimura et al. 2000; Kovtun et al. 2000; Yuasa et al. 2001; Asai et al. 2002; Ahlfors et al. 2004; Droillard et al. 2004; Teige et al. 2004; Bush et al. 2007; Lee and Ellis 2007; Takahashi et al. 2007; Wang et al. 2007; Ren et al. 2008; Müller et al. 2010