JPP JOURNAL OF PROTEINS AND PROTEOMICS
9(1), 2018, pp. 57-72

Review Article

MITOGEN ACTIVATED PROTEIN KINASE: A VERSATILE SIGNALING CASCADE IN PLANTS

Kushi Yadav[1], Saroj K. Jha[1], Manisha Sharma and Girdhar K. Pandey*

Department of Plant Molecular Biology, University of Delhi South Campus, Benito Juarez Road, Dhaula Kuan, New Delhi-110021, India
[1] *Equal contribution*

Abstract: Mitogen Activated Protein Kinase (MAPK) cascade is evolutionarily conserved, universal signaling module that plays significant role invarious cellular signaling pathways in plants. MAPK cascade relies on transfer of information from sensor to three types of reversibly phosphorylated kinases,which lead to phosphorylation of substrate proteins due to which wide range of responses take place. These responses help plants to develop adaptive mechanisms to cope up with a number of abiotic and biotic stresses. These MAPKs are classified on the basis of their structure, functions and working mechanism.The interaction between different tiers of MAPK cascade is highly specific due to their canonical structure and widerange of subfamilies. Activation of a MAPK cascade exhibits specific as well as interrelated behavior.MAPK cascade work in a stipulated manner and generate specific responses towards certain information identified by sensors.In this review, weare discussing evolutionary and canonical structure, classification, activation mechanism and importance of MAPK in protecting plants from various stresses.

Keywords: Constitutive triple response 1(CTR1); Dual specificity MAPK phosphatase (DSP);Enhanced disease resistance 1(EDR1); Extracellular signal-regulated kinase (ERK); Mitogen-activated protein kinase (MAPK or MPK); MAPK kinase (MAPKK).

1. Introduction

In eukaryotes one of the highly conserved signaling module is Mitogen Activated Protein Kinase (MAPK) cascade (Widmann *et al.*, 1999). Almost all fundamental processes of universal signaling mechanism involve ligand (stimuli) binding to a receptor, which triggers downstream signaling cascade including protein phosphorylation (Pawson and Scott, 2005). The general mechanism of MAPK activation involves activation of MAP kinase kinase kinases (MAP3Ks; also called MAPKKKs or MEKKs)(Chen and Thorner, 2007). Activation of upstream MAP3Ks ensue sequential activation of the downstream MAP kinase kinases and finally MAPKs that target various effector proteins present in the cytoplasm or in the nucleus (Zhang *et al.*, 2001).These effector proteins include other kinases, enzymes or transcription factors (Khokhlatchev *et al.*, 1998). MAPKs were first identified in alfalfa as *MsERK1* and in pea as D5kinase (Duerr *et al.*, 1993). In the same year, other MAPKs were also discovered and cloned from *Arabidopsis* as well as from tobacco (Mizoguchi *et al.*, 1993). Simultaneously, four MAPK pathways were also outlined in mammalian cell, which included two extracellular signal regulated kinase (ERK) and two pathways of MAPKs c-Jun N-terminal kinase/stress activated protein kinase (JUN/SAPK) and p38/Hog(Chang and Karin, 2001).

Corresponding Author: **Girdhar K. Pandey**
E-mail: gkapndey@south.du.ac.in

Received: September 22, 2017
Accepted: February 9, 2018
Published: February 11, 2018

In *Arabidopsis,*20 MAPKs, 10 MAPKKs and approximately 60 putative MAPKKKs have been identified (Ichimura *et al.*, 2002). These 60 putative MAPKKKs are again sub-grouped in MEKK (MAPK/ERK kinase kinase) group, which includes 12 putative MAPKKK and CTR1 (Constitutive Triple Response 1) sub-group that includes all other putative MAPKKK. Based on substantial evidence as demonstrated by biochemical assays and genetics, MEKK members were reported as *bona fide* MAPKKK (Bergmann *et al.*, 2004; del Pozo *et al.*, 2004; Wang *et al.*, 2007b; Qiu *et al.*, 2008; Meng *et al.*, 2012). Contrary to this, recent study on CTR1 group in prokaryotes show that CTR1 and EDR1 (Enhanced disease resistance1) function together (Ju *et al.*, 2012; Qiao *et al.*, 2012; Wen *et al.*, 2012; Zhao *et al.*, 2014). Earlier studies were majorly focused on identification of functional role of 3 MAPKs; MAPK3,MAPK4 and MAPK6 in *Arabidopsis* during plant immunity and stress response due to their readily detectable activation by kinase assay (Zhang and Klessig, 2001; Ichimura *et al.*, 2002; Meng and Zhang, 2013). Several efforts to screen MAPK components on the basis of forward genetic screening could identify only some MAPK components (Petersen *et al.*, 2000; Bergmann *et al.*, 2004; Dai *et al.*, 2006; Meng and Zhang, 2013; Duan *et al.*, 2014). The key functions of MAPK components in plant growth and development revealed by reverse genetic studies helped in the establishment of several MAPK cascades (Takahashi *et al.*, 2004; Wang *et al.*, 2007a; Meng *et al.*, 2012; Meng and Zhang, 2013). In this review, we are presenting an overview of MAPK cascade, their history, classification, structural and functional properties.

2. History of MAPK

In 1986, Sturgill and Ray for the first time discovered MAPK from an animal cell (Sturgill and Ray, 1986). They coined the term 'microtubule-associated protein kinases i.e., MAPK for the identified protein.Further research revealed that these kinase proteins are phosphorylated in response to mitogens at tyrosine residue hence termed as 'mitogen activated protein kinase'(Rossomando *et al.*, 1987). In 1993, MAPK protein was first recognized in pea using antiserum specific to mammalian MAPK (ERKs) (Stafstrom *et al.*, 1993). New advancement in the research techniques facilitated identification of MAPKs in many more plant species. For example, a MAPK gene *NTF3* was identified through PCR amplification from cell suspension in tobacco (Wilson *et al.*, 1993). In 1995, tobacco *NPK1* was identified which shows similarity to MEK protein (Pearceet *et al*, 1993). In 1998, MEK1 gene of MAPKK group was identified in *Arabidopsis* (Ichimura *et al.*, 1998). The MEK1 gets activated and shows transcriptional regulation during development and wounding (Ichimura *et al.*, 1998). In 2001, a MAPKK gene in maize, *ZmMEK* was identified and in the same year EDR1 MAPKKK gene in *Arabidopsis* was also identified that belongs to *RAF* (Rapidly Accelerated Fibrosarcoma) group of MAPKKK (Nakashima *et al.*, 1998; Hardin and Wolniak, 2001; Frye *et al.*, 2001). Subsequently, *LeMPK1-LeMPK3* MAPK genes were identified in tomato (Mayrose *et al.*, 2004). Initial studies have identified more of *MAPK* genes in comparison to *MAPKK* and *MAPKKK*. MAPKK gene, *MKK1-3* was identified in alfalfa but the inducibility of this gene was not clear under any stress (Mishra *et al.*, 2006). The first component of MAPK cascade, MAPKKK was first identified in 1993 in *Arabidopsis* in the form of *CTR1*. The CTR1 belongs to RAF subgroup of MAPKKK (Nühse *et al.*, 2000). In 2001, *ETR1* was discovered in *Arabidopsis,* which again belongs to RAF subgroup of MAPKKK (Frye *et al.*, 2001). Many subsequent researches could identify multiple MAPKKK, MAPKK and MAPK and some of them are shown in Table 1.

Table 1
Number of MAPK, MAPKK and MAPKKK identified in different plant species

Species	*MAPK*	*MAPKK*	*MAPKKK*
Arabidopsis	20	10	80
Oryza sativa	17	8	75
Zea mays		19	974
Lycopersiconesculentum	16	6	89
Glucine max	38	11	150
Cucumissativus	14	6	59

3. Classification of MAPKs

The plant MAPKs are classified into different classes on the basis of their evolutionary origin, structure and function. On the basis of structural and functional analyses, MAPK cascade is classified into 3 components that are further divided into subfamilies (Figure 1).

There are three main components of MAPK cascade namely MAPKKK, MAPKK and MAPK, which are further divided in the following

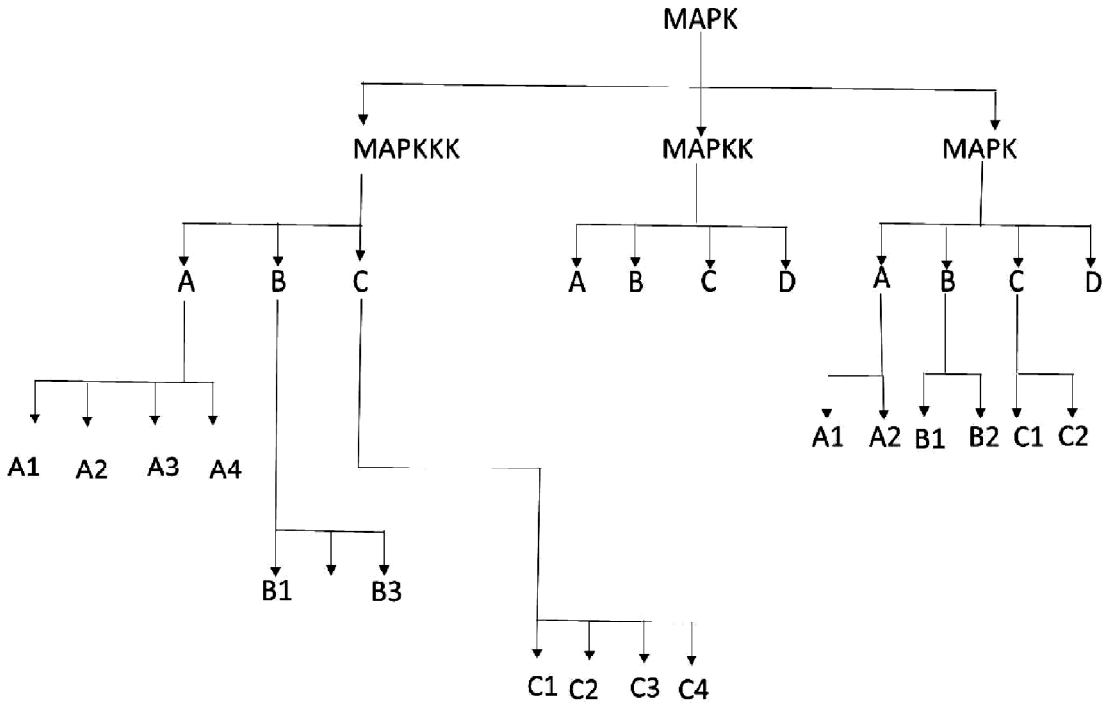

Figure 1: **Classification of three tiers of MAPK cascade.** Detailed classification of MAPK cascade and their components.

subfamilies on the basis of their structural and functional differences (Jonak *et al.*, 2002). The first component of MAPK cascade is **MAPKKK,** which is divided into 3 major sub families A, B and C (Ichimura *et al.*, 2002). Few examples based on this classification are presented in Table 3.

MAPKKK^{A}/**MEKK** subfamily has conserved protein structure with kinase domain(Cristina *et al.*, 2010). These domains may be located either at N-terminal or C-terminal or sometimes in the central part of the protein (Rao *et al.*, 2010). They participate in canonical MAPK cascade by activating or phosphorylating MAPKK (Cristina *et al.*, 2010). MEKKshows similarity to mammalian MEKK1 and yeast STE11 and BCK1 (Ichimura *et al.*, 2002). This MAPKKK^{A}group is further divided into 4 subgroups- A1 comprises 4 kinase proteins (Mizoguchi *et al.*, 1996). These proteins are known to get activated by several abiotic stimuli such as increased salinity, touch and drought (Mizoguchi *et al.*, 1996). Among all 4, AtMAPKKK4 is unique in structure and its N-terminus has several functional domains such as WRKY domain (Eulgem *et al.*, 2000). WRKY domains have special property of direct DNA binding and this domain play important role during defense response (Ichimura *et al.*, 2002). Qualities of MAPKKK^{A}A2 are not much known but MAPKKK^{A} A3 is believed to be involved in regulation of cytokinesis and also acts as negative regulator in stress responses(Nishihama *et al.*, 2001). Last minor group of MAPKKK^{A} is A4, which is functional majorly during cell division(Jouannic *et al.*, 2001).

MAPKKK^{B}/**RAF/MLK** subfamily shows more similarity to mammalian RAF1 than MEKK (Ichimura *et al.*, 2002). They possess C-terminal kinase domain and extended N-terminal regulatory domain (Rao *et al.*, 2010). The RAF-like protein in *Arabidopsis* that do not take part in MAPK cascade majorly is CTR1 (Constitutive Triple Response1) and EDR1 (Enhanced Disease Resistance1) (Kieber *et al.*, 1993; Frye *et al.*, 2001; Huang *et al.*, 2003; Dóczi *et al.*, 2007). This group is further divided into 3 minor groups. MAPKKK^{B} B1 includes CTR1 and EDR1, which get activated during ethylene and disease resistance signaling pathways (Kieber *et al.*, 1993). MAPKKK^{B} B2 has peculiar feature of PAS (Per, Arnt and Sim) domain in N-terminal region (Zhulin *et al.*, 1997). This PAS domain acts as a sensor domain in many signaling pathways (Zhulin *et al.*, 1997). MAPKKK^{B}B3 is distinguished by the presence of central alanine rich region (Ichimura *et al.*, 2002). Lastly, MAPKKK^{C}**/ZIK** is the smallest sub family of MAPKKK. These have N-terminal kinase domain but are unable to phosphorylate MAPKK in plants (Jonak *et al.*, 2002). In *Arabidopsis,* out of

Table 2
MAPK components identified from different plant species. The overexpression or knock-outstrategy reveals the MAPK component biological functions in the respective plant species

Sl. No.	*Species*	*MAPK component*	*Overexpression/ Knock-out*	*Response*	*Reference*
1	*Arabidopsis*	MKK5	Overexpression	Promote CSD1/CSD2expression under high light stress	Xing *et al.* (2013)
2	*Nicotianatabacum*	SIPK	Overexpression	Reduce H_2O_2 accumulation in response to harpin	Samuel *et al.* (2005)
3	*Solanum lycopersicum*	SIMPK1/ SIMPK2	Knock-out	Reduce H_2O_2 accumulation in response to Brassino steroids	Nie *et al.* (2013)
4	*Oryza sativa*	OsMAPK33	Overexpression	Reduce expression of ion transporter gene under salt stress	Lee *et al.* (2011)
5	*Zea mays*	ZmSIMK1	Overexpression	Upregulate *RD29A* and *P5CS1 under salt stress*	Lingkun *et al.* (2010)
6	*Medicago sativa*	MsSAMK	Overexpression	Act as a regulator in pheromone responses under cold, drought, touch wounding and fungal elicitor signaling	Cardinale *et al.* (2000) Bögre *et al.* (1996) Jonak *et al.* (1996)
7	*Vitisvinifera*	VviMAPKKK	Overexpression and Knock-out	Putative involvement of VviMAPKKK genes in growth and development during the life cycle corresponding to various developmental stages (including flower, berry, bud, leaf, rachis, root, seed, seedling, stem, and tendril)	Wang *et al.* (2014) Cakir *et al.* (2015)
8	*Pisumsativum*	*PsMPK2*	Overexpression	Due to mechanical injury and other stress signals as abscisic acid, jasmonic acid and hydrogen peroxide increase kinase activity of PsMPK2	Dolores *et al.*, (2008)
9	*Solanum tuberosum*	StMPK1	Overexpression	Under wounding, fungal elicitor signaling jasmonic acid (JA) and abscicic acid (ABA), but not in response to ethylene or salicylic acid	Flavio *et al.* (2006)
10	*Cucumissativus*	*TIPK*	Overexpression	Respond against pathogenic effect and involved in defense response pathway	Shoresh *et al.* (2006)

80 *MAPKKK*, 21 belong to *MEKK*, 48 belong to *RAF* and 11 belong to *ZIK* subfamily (Jonak *et al.*, 2002). 60% of MAPKKK family members belong to RAF sub-family (Jonak *et al.*, 2002; Rao *et al.*, 2010; Kong *et al.*, 2013; Sun *et al.*, 2014). It was also found that ZIK and RAF/MLK are sister clades (Champion *et al.*, 2004). This group is further divided into 4 minor subgroups (Tregear *et al.*, 1996; Ichimura *et al.*, 1997). The MAPKKK^{C}C1 has RAF related sequence and ankyrin repeats in their N-terminus. The subgroup C2 has anaspartokinase, chorismatemutase and Tyr A (ACT) domain known to be regulating the activity of metabolic enzymes (Aravind and Koonin, 1999). MAPKKK^{C}C4 has lysine-rich regionin their C-

Table 3
Examples of MAPK classification components presented in the text from *Arabidopsis*, *Nicotiana* and rice plant species

Species	*Group*	*Sub group*	*Example of MAPK component*
Arabidopsis	MAPKKK	A	AtMEKK1
	MAPKK	A	AtMKK1
	MAPK	A	AtMPK3
	MAPKKK	B	AtMAP3K01
	MAPKK	B	AtMKK3
	MAPK	B	AtMPK4
	MAPKKK	C	AtMRK1
	MAPKK	C	AtMKK4
	MAPK	C	AtMPK1
	MAPKK	D	AtMKK9
	MAPK	D	AtMPK20
Nicotianatabacum	MAPKKK	A	NtNPK1
	MAPKK	A	NtSIPKK
	MAPK	A	NtWIPK
	MAPKK	B	NtNPK2
	MAPK	B	NtNTF6
	MAPKK	C	NtMEK2
	MAPK	C	NtNTF3
Oryza sativa	MAPKK	A	OsMEK1
	MAPK	A	OsMAPK2
	MAPKKK	B	OsEDR1
	MAPK	C	OsMAPK3
	MAPKK	D	OsMAPKG1

terminus and a serine-rich N-terminus region (Ichimura *et al.*, 2002).

The second important component of MAPK cascade is **MAPKK,** which connects upstream MAPKKK to downstream MAPK(Hamel *et al.*, 2006). Phosphorylation motif of MAPKK is S/TxxxxxS/T in plants and slightly different in mammals i.e., S/TxxxS/T. Docking site of plant MAPKK located at N-terminal extension is similar to MAPKK of animals. MAPKK is also divided into 4 subfamilies A, B, C, and D as shown in above (Figure 1) (Mizoguchi *et al.*, 1996).MAPKK^{A} play important role in innate immunity of plants and cell division (Teige *et al.*, 2004; Mészáros *et al.*, 2006; Qiu *et al.*, 2008). MAPKK^{A} participates in canonical MAPK cascade by activating downstream MAPK. MAPKK^{A} is also involved in various responses for abiotic and biotic stresses. It also plays major role in plant cell division as it is seen that in tobacco plant, *NtMAPKK1* plays important role in cytokinesis because it connects upstream signals from kinase related protein *NtNACK1* and *NtNACK2* to the downstream target(Nishihama *et al.*, 2002).

MAPKK^{B} is usually found in higher plants and play important roles against pathogen attacks(Dóczi *et al.*, 2007; Takahashi *et al.*, 2007). These MAPKK^{B} have the ability to interact with a range of MAPK(Dóczi *et al.*, 2007; Lee *et al.*, 2008b). The most important and peculiar feature of this group is that it has nuclear transfer factor (NTF) domain (Quimby *et al.*, 2000). NTF domain increases nuclear import of cargo proteins and is involved in cytoplasmic nuclear trafficking (Hamel *et al.*, 2006). NTF of this group shows higher similarity to eukaryotic nuclear transfer factor. MAPKK^{C} are basically involved in stress signaling and during developmental growth of plants (Steggerda and Paschal, 2002). They also control floral abscission and are involved in cell fate determination during stomatal differentiation (Wang *et al.*, 2007b). The last group of this component is MAPKK^{D}, which plays significant role in both development and defense mechanism of plants (Rodriguez *et al.*, 2010). Their role in development majorly includes senescence. Interestingly, both MAPKK^{C} and MAPKK^{D} proteins do not contain introns in their sequence(Bardwell and Thorner, 1996).

The third component of MAPK cascade is MAPK, which is comprised of 4 subgroups *i.e.* A, B, C and D (Ichimura *et al.*, 2002). This classification is based on the evolutionary relationships of MAPK in different plant species and sequence comparisons of conserved amino acid motif TXY (Ichimura *et al.*, 2002). It is well established that TXY phosphorylation motifs are subdivided into TEY phosphorylation motif and TDY phosphorylation motif, so on the basis of this fact MAPK^{A}, MAPK^{B} and MAPK^{C} belong to TEY phosphorylation motif and MAPK^{D} belongs to TDY phosphorylation motif (Taj *et al.*, 2010). However, on the basis of functional analyses, MAPK major groups are also divided into 8 minor groups i.e.,MAPK^{A}A1, MAPK^{A}A2, MAPK^{B}B1, MAPK^{B}B2, MAPK^{C} C1,MAPK^{C} C2,MAPK^{D}D1 and MAPK^{D}D2(Ichimura *et al.*, 2002). These groups are formed on the basis of specific features and functions. MAPK^{A} A1 basically functions in oxidative stresses (Seo *et al.*, 1995; Mizoguchi *et al.*, 1996; Ichimura *et al.*, 2000; Kovtun *et al.*, 2000; Nühse *et al.*, 2000; Desikan *et al.*, 2001; Yuasa *et al.*, 2001). MAPK^{B} are involved in signaling

mechanisms to counter environmental stresses and also during developmental stages especially cell division (Petersen *et al.*, 2000). MAPK^{B}B1 is activated in both abiotic and biotic stresses. For instance, AtMAPK4 of B2 group MAPK are activated in cell cycle dependent manner with predicted role in cytogenesis (Calderini *et al.*, 1998; Bögre *et al.*, 1999; Ichimura *et al.*, 2000; Desikan *et al.*, 2001). MAPK^{C} are also responsive to circadian rhythm and regulate expression accordingly (Schaffer *et al.*, 2001). MAPK^{D} has CD domain and TDY phosphorylation motif, which is one of the peculiar features of this group (Schoenbeck *et al.*, 1999; He *et al.*, 1999).

4. Structure of MAPK

All MAPKs share a canonical structure that is highly conserved in eukaryotes (Zhang *et al.*, 1994; Goldsmith and and Cobb, 1994; Wilson *et al.*, 1996). All MAPKsare either bilobed (N-terminal lobe and C-terminal lobe) or are divided into two subtypes based on the presence of two different TEY and TDY phosphorylation subtypes within their activation loop (Figure 2) (Goldsmith and Cobb, 1994; Menges *et al.*, 2008b). The N-terminal lobe is nearly 135 residues long and consists of β-sheets and a glycine-rich loop acting as ATP binding pocket (Wilhelm *et al.*, 2008). On the other hand, C-terminal lobe comprising 225 amino acid residues contains α helices, a catalytic base, magnesium binding sites and the phosphate binding activation loop also known as T-loop or phosphorylation loop (Knighton *et al.*, 1991; Wilson *et al.*, 1996).

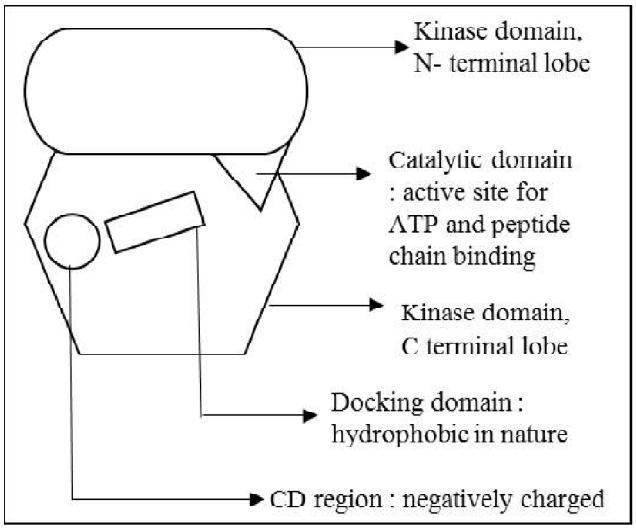

Figure 2: Some common features of canonical structure of all MAPKs (MAPK canonical structure showing bilobed structure with the ATP binding pocket attached to small lobe and docking domain at the larger lobe).

Phosphorylation site is present in the activation loop hence it is also called as phosphorylation lip/loop (Goldsmith and Cobb, 1994; Wilson *et al.*, 1996; Menges *et al.*, 2008a). In TXY phosphorylation motif, 'T' and 'Y'represent threonine and tyrosine respectively (Lewis *et al.*, 1998). 'X' represents either glutamic acid or aspartic acid. If 'X' is glutamic acid then it's called as 'TEY' motif and if 'X' is aspartic acid then it's called 'TDY' motif (Ichimura *et al.*, 2002). On the basis of these motifs,an additional classification of MAPK has been recognized. TXY motif is denoted as pTXpY because it has phosphor threonine and phosphor tyrosine residues. Catalytic domain is the site where protein-protein interactions occur (Seeliger *et al.*, 2009). This catalytic domain is present in the cleft between small C-terminal lobe and large N-terminal lobe (Seeliger *et al.*, 2009). Movement of these 2 lobes of protein makes cleft open or close (Seeliger *et al.*, 2009). Open cleft releases ADP from active site and allows access of ATP; on the other hand residues brings into catalytically active state when cleft is closed (Seeliger *et al.*, 2009).

On the basis of site of phosphorylation, MAPKs are divided into three categories *i.e.*, MAPKs catalyzing phosphorylation of tyrosine, threonine and both threonine as well as tyrosine (Pulidoetal., 1998, Farooq *et al.*, 2003). Short docking motif (D-site) identify the complementary region and directly binds to its target catalytic domain such assignaling enzyme (Garai *et al.*, 2012). Presence of docking domain increases the specificity of promiscuous active site (Ubersax and Ferrell Jr, 2007). The binding surfaces for kinase and phosphatase are distinct from the active site (Reményi *et al.*, 2006).

5. Kinase activity

MAPK signal transduction pathway converts different extracellular stimuli into specific cellular responses by the phosphorylation of some specific substrate proteins. MAPKs show preferential phosphorylation of substrates having serine/threonine sites. To detect active and inactive kinase, various *in-vivo* and *in-vitro* methods are used. The *in-vivo* integrity of a kinase cascade requires specificity of protein interactions (Chang and Karin, 2001).

There are few specific chemical inhibitors that targets MAPK signaling components. Most commonly known compounds used to block MAPKK activation are PD098059 and U0126

(Favata *et al.*, 1998). They inhibit MAPKK by stabilizing low-activity form of MAPKK without competing for MAPK-binding site and ATP binding site (Favata *et al.*, 1998). Some other inhibitors of MAPKs are SB203580, PD169316 and SB202190. Additionally, site directed mutagenesis is another preferred way to deter the catalytic activity of MAPK components. Mutagenesis causes a change in the conformation of ATP binding pocket of kinase allowing it to accept cell-permeable inhibitors (Bishop *et al.*, 2000; Asai *et al.*, 2002; Suarez-Rodriguez *et al.*, 2007). Some of the examples based on site directed mutagenesis are:

a) MAPKKKs and MAPKKs get inactive when there is a substitution of a conserved lysine residue for methionine in the ATP binding pocket (Asai *et al.*, 2002; Suarez-Rodriguez *et al.*, 2007).

b) Similarly, if there is a substitution of threonine to alanine and tyrosine to phenylalaninein the TXY phosphorylation site or T-loop, it results in non-phosphorylative, inactive kinase (Bishop *et al.*, 2000).

c) In MAPKs phosphotransferase site, there is a substitution of a lysine to arginine which results in catalytically inactive MAPK that however, is capable of binding to ATP (Bishop *et al.*, 2000).

6. Bio-signaling of MAPK in plants

Plants show complicated signaling mechanism in response to various external stimuli (Jalmi and Sinha, 2015). MAPK cascade activated by these external stimuli is usually generated due to two major stresses i.e., abiotic stress and biotic stress (Khokhlatchev *et al.*, 1998).In plants, MAPK signaling pathway is a canonical signaling pathway and is organized in three tiers i.e., MAPKKK, MAPKK and MAPK (Cardinale *et al.*, 2002) (Figure 3).

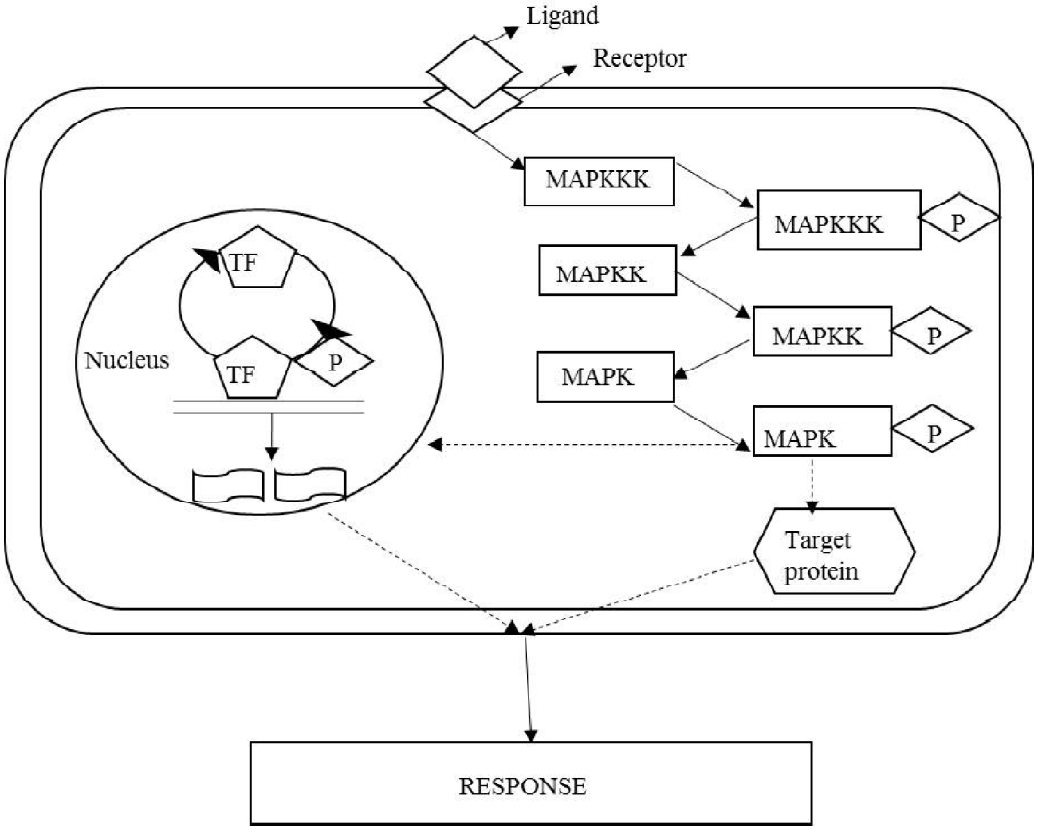

Figure 3: **Biosignaling pathway of MAPK cascade.** Ligand generated in response to a particular stress binds to the membrane bounded receptor and leads to activation of MAPKKK by phosphorylation, which in turn phosphorylate MAPKK and finally lead to activation of MAPK through addition of phosphate group. Activated MAPK can act on both cytoplasmic and nuclear site depending upon the kind of the stress stimuli. Activated MAPK can further activate downstream responsive proteins such as transcription factor or some other target protein in the cytoplasmic region, which leads to specific response against that particular stress.

Initially, plant receives some external stimuli in the form of ligand, which binds to the membrane receptor (Chang and Karin, 2001). Due to this ligand-receptorcomplex formation, there is a change in redox state which ultimately results in the production and accumulation of reactive oxygen species (ROS) in some specific parts of plant cells i.e., chloroplast, mitochondria, cell wall bounded NADPH oxidase, peroxidases and microsomes (Apel and Hirt, 2004). Among these, cell wall bounded NADPH oxidase is a major source for production of ROS after perceiving external stress stimuli (Mittler *et al*., 2004). The activation of MAPK cascade occurs due to increase in ROS level (Jalmi and Sinha, 2015). This increase in ROS level results in the phosphorylation/activation of MAPKKK (Dan *et al.*, 2001). Activated MAPKKK further phosphorylates the phosphorylation motif of MAPKK i.e. S/TxxxxxS/T(Chang and Karin, 2001).After S/TxxxxxS/T phosphorylation, MAPKK becomes active (Chang and Karin, 2001). Now the next step of this cascade is to activate MAPK. Therefore, activated MAPKK phosphorylates the phosphorylation motif of MAPK *i.e.,* TXY(TDY/TEY) resulting in its activation (Figure 4) (Janitza *et al.*, 2012).

In MAPK cascade, interaction between MAPKK and MAPK is highly specific because of their docking domains and catalytic domain (Yoshioka, 2004). These two domains are closely placed and play important role in recognition and binding of target proteins (Yoshioka, 2004). Increase in docking interaction affinity leads to increase in specificity as well as efficiency of protein kinase interactions with substrate proteins (Chang *et al.*, 2002). For example, the interaction of core and structural motifs of Fus3 and Hog1 MAPK in yeast (Figure 5) (Mody *et al.*, 2009). This confirms the role of docking domain in substrate binding (Mody *et al.*, 2009).

In addition to mediating substrate specificity, the docking sites are also the chief sites where in various conformational changes resulted into the evolutionary origin of paralogs with variable substrate specificity (Mody *et al.*, 2009). This high specificity and accuracy is attained with the help of three mechanisms which include: i) Cross inhibition, because it was observed that in yeast cells if individual yeast cell receives both hyperosmotic and pheromone stimuli simultaneously, then it responds in a switch-like mode by activating signaling cascade. In this case cross inhibition is a mutational inhibition between hyperosmotic and pheromone pathway (McClean *et al.*, 2007).ii) Feedback control, it can be negative or positive feedback control. For example in Hog1 cascade a membrane-bounded receptor, Sho1 activates

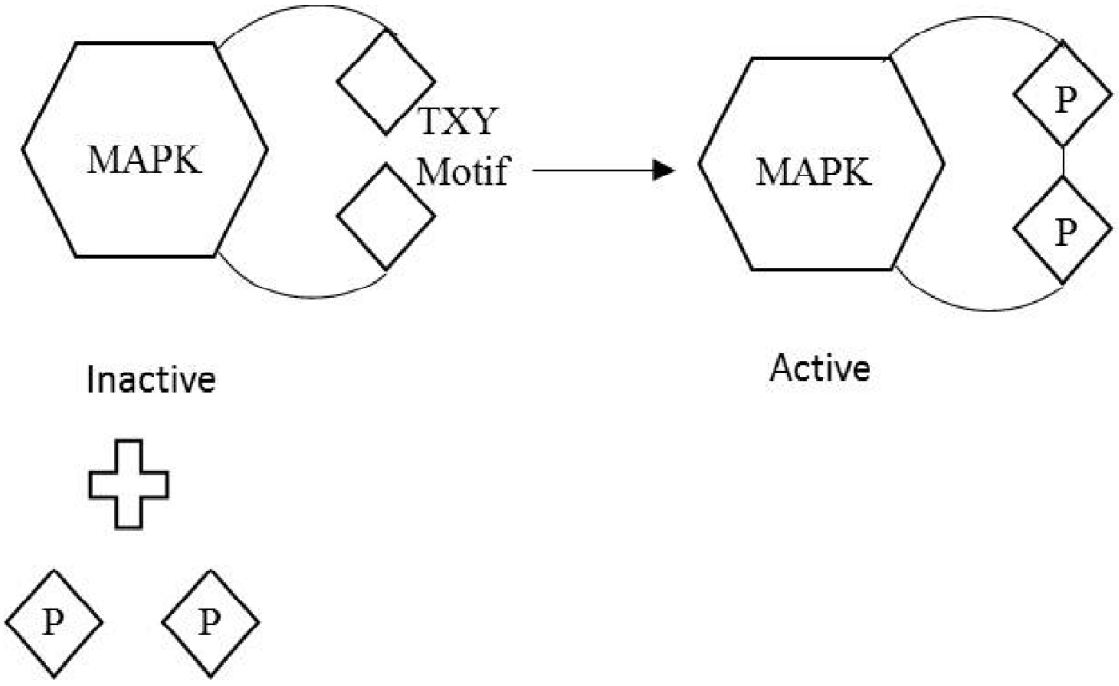

Figure 4: **Activation mechanism of MAPK.** Activation mechanism of MAPK protein by addition of phosphate groups at TXY phosphorylation motif by MAPKK.

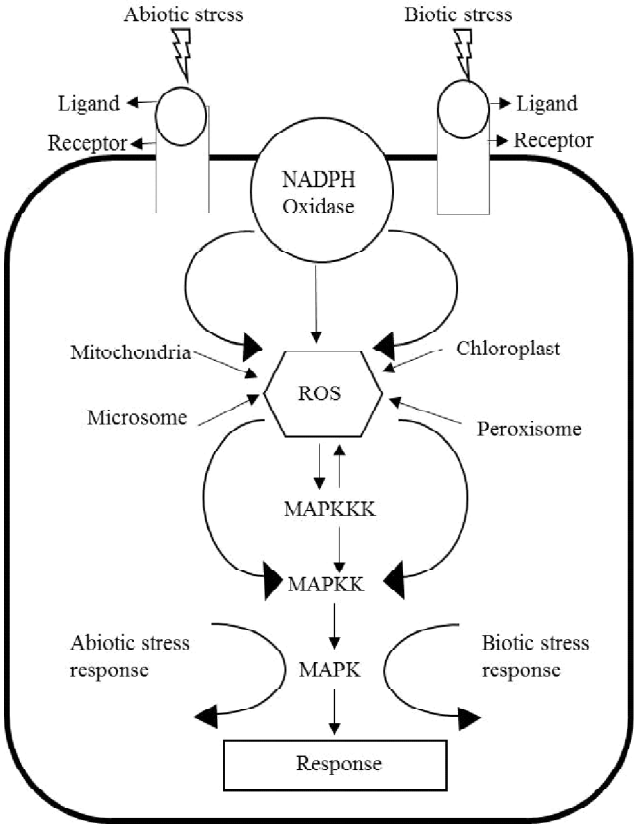

Figure 5: **Abiotic and biotic stress mediated sequential MAPK cascade.** Diagrammatic representation of activation of MAPK cascade in response to both abiotic and biotic stresses; both abiotic and biotic stresses lead to production of ROS in the cell. ROS accumulation results into the activation of MAPK signaling cascade to generate tolerance response against these stress conditions.

MAPKKK Ste11. Hog1 can phosphorylate Sho1. As a result, Sho1 gets inactivated which attenuate Hog1 signals. Hence, it works as a negative feedback loop (Zou *et al.*, 2008).

Scaffolding helps in propagation of signals in correct direction of cellular localization. This includes various scaffolding proteins, which have many domains and binds two or more components of a signaling cascade simultaneously (Taj *et al.*, 2010). These proteins have increased specificity and accuracy of MAPK components by bringing them together which also result in acceleration of their activation and increase rate of reaction (Cristina *et al.*, 2010). Various techniques are used to map the interaction of MAPK with scaffolding protein such as mutagenesis, hydrogen exchange-mass spectroscopy and X-ray crystallography (Tanoue *et al.*, 2000; Chang *et al.*, 2002). Major role of scaffolding is to bring the compatible signaling components close to each other to facilitate functional interaction by binding them in a multi-enzyme complex and this complex formation helps to protect MAPK components from phosphatases, which deactivate them and direct or indirect interference of many other signaling cascades. For example, in alfalfa due to oxidative stress, MAP3K (OMTK1) gets activated and interacts with MMK3 in protoplasts (Nakagami *et al.*, 2004). In *Arabidopsis*, MEKK1 interacts with MKK2 and MAPK4 (Suarez-Rodriguez *et al.*, 2007). MEKK1 shows independent kinase activity. It has been proved that *mekk1* mutant phenotype is rescued by complementation of MEKK kinase inactive version (Karandikar *et al.*, 2000). MEKK1 is also known to be involved in MAPK cascade with MAPK3, MAPK4 and MAPK6 (Karandikar *et al.*, 2000; Cristina *et al.*, 2010). Phosphorylation of downstream factors in MAPK cascade is responsible for the regulation of several genes. In *Arabidopsis*,39 substrates of MAPK and 48 substrates of MAPK3 have been identified. In *Arabidopsis*,salicylic acid dependent resistance is activated by phosphorylation of MAPK4 by MKS1(MAPK Substrate 1). In case of tobacco MAPK, NRK/NTF6 phosphorylates NtMAP65-1 (microtubule binding protein), which results in reduced microtubule binding activity (Sasabe *et al.*, 2006). In metaphase and telophase, MAPK also control MAPK65-1 (Harding *et al.*, 2005).

The downstream effectors of MAPK cascade are various transcription factors and other proteins and kinases, which get activated when phosphorylated MAPK interact with these substrates. The substrates may be present in the cytoplasm or in nucleus. MAPKs mediate interaction with substrates in the cytoplasm and if required may also translocate to the nucleus (Harding *et al.*, 2005). In *Arabidopsis*, MAPK3, MAPK4 and MAPK6 are activated by MAPKK. However, MAPK3 and MAPK6 move inside the nucleus to activate transcription factors and MAPK4 activate its target proteins in the cytoplasm (Harding *et al.*, 2005). These different MAPKs are activated by the multiple external stimuli. For instance, AtMAPK3 is activated due to oxidative stress, AtMAPK6 is activated due to both abiotic and biotic stresses and AtMAPK4 is activated due to many environmental stresses (Yoo *et al.*, 2008). MEKK1 interacts with WRKY53 senescence related transcription factor (Miao *et al.*, 2004). These interactions take place in the nucleus. Both *Arabidopsis* and tobacco show nuclear localization of MAPKKKs during interphase.These MAPKKKs have bipartite nuclear localization signal

(NLS) located at the carboxyl terminal region, which proves that this NPK1-NLS is responsible for nuclear localization (Ishikawa *et al.*, 2002; Heazlewood *et al.*, 2007).

Bioinformatics analysis also played an important role in organizing information from microarray experiments and results in gene expression for approximately 114 MAPK cascade components (Menges *et al.*, 2008a). The responses generated against particular stress provide resistance or ability to tolerate the environmental stresses.

Inactivation of MAPK cascade requires dephosphorylation of threonine and tyrosine residue on TXY motif present within activation or T- loop (Cristina *et al.*, 2010). For this highly organized inactivation, there are specific enzyme i.e., phosphatases,which inactivate different isoforms of MAPKs also known as MAPK phosphatase (Figure 6) (Cristina *et al.*, 2010). These MAPK phosphatases are divided into two major groupsi.e.PIP- protein tyrosine phosphatase and SIP –serine threonine phosphatase. The PIP are further divided into two subgroups tyrosine specific phosphatase (TSP) and dual specific phosphatase (DSP) (Luan, 2003). The phosphatase regulates the duration and intensity of signals through MAPK cascade. In *Arabidopsis*, activation of MAPK4 requires phosphorylation at the threonine residue but this essential phosphorylation is inactivated by PTP1 (Huang *et al.*, 2000).Dual Specificity Phosphatase (DSP) MPK1, act as negative regulator of MAPK cascade, which function in response to ultra-violet (UV), genotoxic stress and many other stress conditions (Ulm *et al.*, 2002). The MAPK and MAPK phosphatases (MKP) are known to function together. In case of microtubule stabilization in *Arabidopsis*, interaction between MAPK18 and PHS1 (PROPYZA-MIDE HYPERSENSITIVE) (Walia *et al.*, 2009) is essential to bring about the necessary response (Walia *et al.*, 2009). It has been suggested that MAPK phosphatase activity is enhanced in Ca^{2+} dependent manner (Lee *et al.*, 2008a). In alfalfa, MP2C inactivates the stress induced MAPK, SIMK in wound signaling pathway under negative feedback loop (Meskiene *et al.*, 2003). In *Arabidopsis*, during wounding Ser/Thr phosphatase of type 2C, AP2C1 inactivates MAPK4 and MAPK6 to regulate the JA level (Schweighofer *et al.*, 2007). After wounding AP2C1 shows enhanced affinity for MAPK6, hence there is a probability that AP2C1 may regulate both MAPK4 and MAPK6 (Schweighofer *et al.*, 2007).

Similar to *Arabidopsis* AP2C1 (homolog of MAP2C), in alfalfa,wound signaling pathway is regulated by MP2 (phosphatase from negative feedback loop) by activation of SIMK (stress induced MAPK).

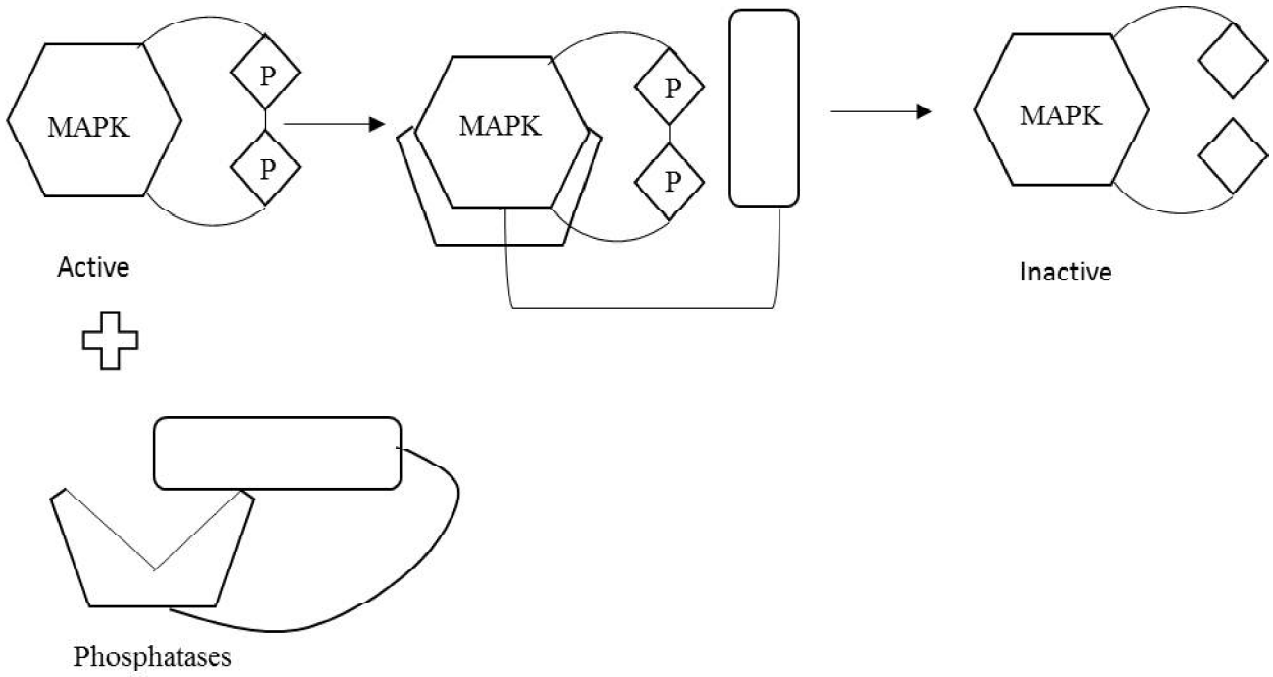

Figure 6: **Inactivation mechanism of MAPK by phosphatase.** The molecular mechanisms of dephosphorylation of activated MAPK.Phosphatases are molecules responsible for removal of phosphate group and result in inactivation /dephosphorylation of MAPK.

6. Conclusion and future perspectives

In the field of plant stress signaling,MAPKsact as the crucial regulator of tolerance response during stress conditions (Table 2). MAPK acts as critical regulator because the protein phosphorylated through MAPK have great potential for improving stress tolerance in plants.In recent years, on the basis of combination of physiological, biochemical and genetic approaches, understanding of MAPK signaling has been attempted. These studies have identified that MAPKs are involved in various signaling responses. Various studies on MAPK are now converged to classify complete cascade of MAPK in response to abiotic and biotic stresses. To further define specific functions and validation of functional plant MAPK components and mechanisms of MAPK cascade, there is a need to design new approaches and strategies. Identification of different stress induced signaling components and substrates through application of protein-protein interactions can be performed. Through the studies on particular protein structure, it will become easier to understand the docking interactions of MAPK components with other substrates. Use of bioinformatics analysis has helped to predict novel functions for different kinases at the developmental level. These studies also helped to elucidate the feedback mechanism, their controland interactions with other cellular pathways.

Acknowledgement

The research work in the GKP lab is funded by University Grant Commission (UGC-SAP/DRS-III project), Department of Science and Technology (DST) and Department of Biotechnology (DBT), India. SKJ would like to acknowledge Department of Science and Technology (DST), India for INSPIRE fellowship.

Abbreviations

At, *Arabidopsis;***CTR1,** Constitutive triple response 1; **DSP,** dual specificity MAPK phosphatase; **EDR1,** enhanced disease resistance 1; **ERK,** extracellular signal-regulated kinase; **MAPK (or MPK),** mitogen-activated protein kinase; **MAPKK,** MAPK kinase; **MAPKKK,** MAPKK kinase; **MEK/MKK,** MAPK kinase; **MEKK,** MAPKK kinase; **MKP,** MAPK phosphatase; **MMK,** *Medicago* MAPK; **Ms,** *Medicago sativa;* **NPK1,** *Nicotiana* protein kinase; **Nt,** *Nicotianatabaccum;* **PTP,** phosphotyrosine phosphatase; **SA,** salicylic acid; **SAMK,** stress-activated MAPK; **SAR,** systemic acquired resistance; **SIMK,** salt-induced MAPK; **SIMKK,** SIMK kinase; **SIPK,** salicylic-acid-inducible protein kinase; **WIPK,** wound-inducible protein kinase

Conflict of Interest

The authors do not have any conflict of interest regarding contents of this manuscript.

References

Apel, K. and Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. *55,* 373–99.

Aravind, L. and Koonin, E. V. (1999). Gleaning non-trivial structural, functional and evolutionary information about proteins by iterative database searches. J. Mol. Biol. *287,* 1023–1040.

Asai, T., Tena, G., Plotnikova, J., Willmann, M. R., Chiu, W-L., Gomez-Gomez, L., Boller, T., Ausubel, F.M., and Sheen, J. (2002). MAP kinase signalling cascade in *Arabidopsis* innate immunity. Nature *415,* 977–83.

Bardwell, L. and Thorner, J. (1996). A conserved motif at the amino termini of MEKs might mediate high-affinity interaction with the cognate MAPKs. Trends Biochem Sci*21,* 373–4.

Bergmann, D.C., Lukowitz, W. and Somerville, C. R. (2004). Stomatal development and pattern controlled by a MAPKK kinase. Science (New York, N.Y.)*304,* 1494–1497.

Biondi, R.M. and Nebreda, A. R. (2003). Signalling specificity of Ser/Thr protein kinases through docking-site-mediated interactions.Biochem J. *372,* 1–13.

Bishop, A. C., Ubersax, Ja., Petsch, D.T., Matheos, D.P., Gray, N.S., Blethrow, J., Shimizu, E., Tsien, J.Z., Schultz, P.G., Rose, M.D., *et al*. (2000). A chemical switch for inhibitor-sensitive alleles of any protein kinase. Nature *407,* 395–401.

Blanco, F.A., Zanetti, M.E., Casalongué, C.A. and Daleo, G.R. (2006). Molecular characterization of a potato MAP kinase transcriptionally regulated by multiple environmental stresses. Plant Physiol Biochem. 44, 315-322.

Bögre, L., Calderini, O., Binarova, P., Mattauch, M., Till, S., Kiegerl, S., Jonak, C., Pollaschek, C., Barker, P., Huskisson, N.S., *et al.* (1999). A MAP kinase is activated late in plant mitosis and becomes localized to the plane of cell division. Plant Cell *11*, 101–113.

Bögre, L., Ligterink, W., Meskiene, I., Barker, P. J., Heberle-Bors, E., Huskisson, N.S. and Hirt, H. (1997). Wounding induces the rapid and transientactivation of a specific MAP kinase pathway. Plant Cell *9*, 75-83.

Bögre, L., Ligterink, W., Heberle-Bors, E. and Hirt, H. (1996). Mechanosensors in plants. Nature. *383*, 489-490.

Brodersen, P., Petersen, M., Nielsen, H.B., Zhu, S., Newman, M.A., Shokat, K.M., Rietz, S., Parker, J. and Mundy, J. (2006). *Arabidopsis* MAP kinase 4 regulates salicylic acid- and jasmonic acid/ethylene-dependent responses via EDS1 and PAD4. Plant J. *7,* 532–546.

Çakır, B. and Kılıçkaya, O. (2015). Mitogen-activated protein kinase cascades in Vitis vinifera. Front in Plant Sci, 2015, 6, 556.

Calderini, O., Bögre, L., Vicente, O., Binarova, P., Heberle-Bors, E. and Wilson, C. (1998). A cell cycle regulated MAP kinase with a possible role in cytokinesis in tobacco cells. J. Cell Sci. *111*, 3091–100.

Cardinale, F., Meskiene, I., Ouaked, F. and Hirt, H. (2002). Convergence and divergence of stress-induced MAPK signaling pathways at the level of two distinct MAPKK. Plant Cell1 *4*, 1–10.

Cardinale, F., Jonak, C., Ligterink, W., Niehaus, K., Boller, T. and Hirt, H. (2000). Differential activation of four specific MAPK pathways by distinctelicitors. J Biol Chem *275*, 36734-36740.

Champion, A., Picaud, A. and Henry, Y. (2004). Reassessing the MAP3K and MAP4K relationships. Trends Plant Sci. *9,* 123–129.

Chang, L. and Karin, M. (2001). Mammalian MAP kinase signalling cascades. Nature *410*: 37–40.

Chang, C. I., Xu, B., Akella, R., Cobb, M.H. and Goldsmith, E.J. (2002). Crystal structures of MAP kinase p38 complexed to the docking sites on its nuclear substrate MEF2A and activator MKK3b. Mol Cell*9*, 1241–9.

Chen, R.E. and Thorner, J. (2007). Function and regulation in MAPK signaling pathways: lessons learned from the yeast *Saccharomyces cerevisiae*. Biochim Biophys Acta *1773*, 1311–40.

Cristina, M., Rodriguez, S., Petersen, M., Mundy, J., Rodriguez, M.C.S., Petersen, M. and Mundy, J. (2010). Mitogen-activated protein kinase signaling in plants. Annu. Rev. Plant Biol. *61*, 621–49.

Dai, Y., Wang, H., Li, B., Huang, J., Liu, X., Zhou, Y., Mou, Z. and Li, J. (2006). Increased expression of MAP KINASE KINASE7 causes deficiency in polar auxin transport and leads to plant architectural abnormality in *Arabidopsis*. Plant Cell *18,* 308–320.

Dan, I., Watanabe, N.M. and Kusumi, A. (2001). The Ste20 group kinases as regulators of MAP kinase cascades. Trends Cell Biol *11*, 220–230.

Desikan, R., Hancock, J.T., Ichimura, K., Shinozaki, K. and Neill, S.J. (2001). Harpin induces activation of the *Arabidopsis* mitogen-activated protein kinases AtMPK4 and AtMPK6. Plant Physiol. *126,* 1579–1587.

Dóczi, R., Brader, G., Pettkó-Szandtner, A., Rajh, I., Djamei, A., Pitzschke, A., Teige, M. and Hirt, H. (2007). The *Arabidopsis* mitogen-activated protein kinase kinase MKK3 is upstream of group C mitogen-activated protein kinases and participates in pathogen signaling. Plant Cell *19*, 3266–3279.

Duan, P., Rao, Y., Zeng, D., Yang, Y., Xu, R., Zhang, B., Dong, G., Qian, Q. and Li, Y. (2014). SMALL GRAIN 1, which encodes a mitogen-activated protein kinase kinase 4, influences grain size in rice. Plant J. 77, 547–557.

Duerr, B., Gawienowski, M., Ropp, T. and Jacobs, T. (1993). MsERK1: a mitogen-activated protein kinase from a flowering plant. Plant Cell*5,* 87–96.

Eulgem, T., Rushton, P.J., Robatzek, S. and Somssich, I.E. (2000). The WRKY superfamily of plant transcription factors. Trends Plant Sci. *5*, 199–206.

Favata, M. F., Horiuchi, K.Y., Manos, E.J., Daulerio, A.J., Stradley, D.A., Feeser, W.S., Van Dyk, D.E., Pitts, W.J., Earl, R.A., Hobbs, F.et al.(1998). Identification of a novel inhibitor of mitogen-activated protein kinase kinase. J BiolChem. *273,* 18623–18632.

Frye, C.A., Tang, D. and Innes, R.W. (2001). Negative regulation of defense responses in plants by a conserved MAPKK kinase.Proc. Natl. Acad. Sci. U.S.A. *98*, 373–378.

Garai, A., Zeke, A., Gogl, G., Toro, I., Fordos, F., Blankenburg, H., Barkai, T., Varga, J., Alexa, A., Emig, D., *et al.* (2012). Specificity of Linear Motifs That Bind to a Common Mitogen-Activated Protein Kinase Docking Groove. Sci. Signal 5, ra74-ra74.

Goldsmith, E.J. and Cobb, M.H. (1994). Protein kinases.Curr Opin Struct Biol*4*, 833–840.

Gu, L., Liu,Y., Zong, X., Liu, L., Li, D.P. and Li, D.Q. (2010). Overexpression of maize mitogen-activated protein kinase gene, ZmSIMK1 in Arabidopsis increases tolerance to salt stress. Mol Bio Rep. *37*, 4067–4073.

Hamel, L.P., Nicole, M.C., Sritubtim, S., Morency, M.J., Ellis, M., Ehlting, J., Beaudoin, N., Barbazuk, B., Klessig, D., Lee, J., *et al.* (2006). Ancient signals: comparative genomics of plant MAPK and MAPKK gene families. Trends Plant Sci. *11,* 192–198.

Hardin, S.C. and Wolniak, S.M. (2001). Expression of the mitogen-activated protein kinase kinase ZmMEK1 in the primary root of maize. Planta *213,* 916–926.

Harding, A., Tian, T., Westbury, E., Frische, E. and Hancock, J. F. (2005). Subcellular localization determines MAP kinase signal output. Curr Biol. *15,* 869–873.

He, C., Fong, S.H.T., Yang, D. and Wang, G-L. (1999). BWMK1, a Novel MAP Kinase Induced by Fungal Infection and Mechanical Wounding in Rice.Mol Plant Microbe Interact *12,* 1064–1073.

Heazlewood, J.L., Verboom, R.E., Tonti-Filippini. J., Small, I. and Millar, A. H. (2007). SUBA: the *Arabidopsis* Subcellular Database. Nucleic Acids Res*35,* 213-218.

Huang, Y., Li, H., Gupta, R., Morris, P.C., Luan, S. and Kieber J.J. (2000). ATMPK4, an Arabidopsis homolog of mitogen-activated protein kinase, is activated in vitro by AtMEK1 through threonine phosphorylation. Plant Physiol. *122,* 1301–1310.

Huang, Y., Li, H., Hutchison, C.E., Laskey, J. and Kieber, J. J. (2003). Biochemical and functional analysis of CTR1, a protein kinase that negatively regulates ethylene signaling in *Arabidopsis*. Plant J. *33,* 221–233.

Ichimura, K., Mizoguchi, T., Irie, K., Morris, P., Giraudat, J., Matsumoto, K. and Shinozaki, K. (1998). Isolation of ATMEKK1 (a MAP kinase kinase kinase)-interacting proteins and analysis of a MAP kinase cascade in *Arabidopsis*. Biochem Biophys Res Commun. *253,* 532–543.

Ichimura, K., Mizoguchi, T. and Shinozaki, K. (1997). ATMRK1, an *Arabidopsis* protein kinase related to mammal mixed-lineage kinases and Raf protein kinases. Plant Sci. *130,* 171–179.

Ichimura, K., Mizoguchi, T., Yoshida, R., Yuasa, T. and Shinozaki, K. (2000). Various abiotic stresses rapidly activate *Arabidopsis* MAP kinases ATMPK4 and ATMPK6. Plant J. *24*, 655–665.

Ichimura, K., Shinozaki, K., Tena, G., Sheen, J., Henry, Y., Champion, A., Kreis, M., Zhang, S., Hirt, H., Wilson, C., *et al.* (2002). Mitogen-activated protein kinase cascades in plants: a new nomenclature. Trends Plant Sci. *7*, 301–308.

Ishikawa, M., Soyano, T., Nishihama, R. and Machida, Y. (2002). The NPK1 mitogen-activated protein kinase kinase kinase contains a functional nuclear localization signal at the binding site for the NACK1 kinesin-like protein. Plant J. *32*, 789–798.

Ichimura, K., Mizoguchi, T., Irie, K., Morris, P., Giraudat, J., Matsumoto, K. and Shinozaki, K. (1998). Isolation of ATMEKK1 (a MAP kinase kinase kinase)-interacting proteins and analysis of a MAP kinase cascade in *Arabidopsis*. Biochem. Biophys. Res. Commun. *253*,532–543.

Jalmi, S.K. and Sinha, A.K. (2015). ROS mediated MAPK signaling in abiotic and biotic stress- striking similarities and differences. Front Plant Sci *6*, 769-last page n0.

Janitza, P., Ullrich, K.K. and Quint, M. (2012). Toward a Comprehensive Phylogenetic Reconstruction of the Evolutionary History of Mitogen-Activated Protein Kinases in the Plant Kingdom. Front Plant Sci *3*, 271-last page no.

Jiang, M., Wen, F., Cao, J., Li, P., She, J. and Chu, Z. (2015). Genome-wide exploration of the molecular evolution and regulatory network of mitogen-activated protein kinase cascades upon multiple stresses in Brachypodium distachyon. BMC Genomics*16*, 228-last page no.

Jonak, C., Ökrész, L., Bögre, L. and Hirt, H. (2002). Complexity, cross talk and integration of plant MAP kinase signalling. Curr OpinPlant Biol. *5*, 415–424.

Jonak, C., Kiegerl, S., Ligterink, W., Barker, P.J., Huskisson, N.S. and Hirt, H. (1996). Stress signaling in pathway is activated by cold and drought. Proc Natl Acad Sci USA *93*, 11274-11279.

Jouannic, S., Champion, A., Segui-Simarro, J.M., Salimova, E., Picaud, A., Tregear, J., Testillano, P., Risueno, M.C., Simanis, V., Kreis, M., *et al.* (2001). The protein kinases AtMAP3Kepsilon1 and BnMAP3Kepsilon1 are functional homologues of S. pombe cdc7p and may be involved in cell division. Plant J*26*, 637–649.

Ju, C., Yoon, G.M., Shemansky, J.M., Lin, D.Y., Ying, Z.I., Chang, J., Garrett, W.M., Kessenbrock, M., Groth, G., Tucker, M.L.,Cooper, B., Kieber, J.J. and Chang, C. (2012). CTR1 phosphorylates the central regulator EIN2 to control ethylene hormone signaling from the ER membrane to the nucleus in Arabidopsis. Proc. Natl. Acad. Sci. U.S.A.*109*, 19486–91.

Karandikar, M., Xu, S. and Cobb, M.H. (2000). MEKK1 binds Raf-1 and the ERK2 cascade components. J BiolChem. *275*, 40120–40127.

Khokhlatchev, A.V., Canagarajah, B., Wilsbacher, J., Robinson, M., Atkinson, M., Goldsmith, E. and Cobb, M. H. (1998). Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation. Cell*93*, 605–615.

Kieber, J.J., Rothenberg, M., Roman, G., Feldmann, K.A. and Ecker, J.R. (1993). CTR1, a negative regulator of the ethylene response pathway in arabidopsis, encodes a member of the Raf family of protein kinases. Cell*72*, 427–441.

Knighton, D.R., Zheng, J.H., Ten, E.L.F., Ashford, V.A., Xuong, N.H., Taylor, S.S. and Sowadski, J.M. (1991). Crystal structure of the catalytic subunit of cyclic adenosine monophosphate-dependent protein kinase. Science *253*, 407–414.

Kong, X., Lv, W., Zhang, D., Jiang, S., Zhang, S. and Li, D. (2013). Genome-Wide Identification and Analysis of Expression Profiles of Maize Mitogen-Activated Protein Kinase Kinase Kinase. PLoS ONE *8(2)*, e57714.

Kovtun Y, Chiu WL, Tena G, Sheen J. (2000). Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proc. Natl. Acad. Sci. U.S.A.*97*, 2940–2945.

Lee, K., Eun, H.S., Ho, S.K., Jae, H.Y., Hay, J.H., Mi, S.J., Sang, M.L., Kyung, E.K., Min, C.K., Moo, J.C., *et al.* (2008)a. Regulation of MAPK phosphatase 1 (AtMKP1) by calmodulin in arabidopsis. J BiolChem*283*, 23581–23588.

Lee, S.K., Kim, B.G., Kwon, T.R., Jeong, M.J., Park, S.R., Lee, J.W., Byun, M.O., Kwon, H.B., Matthews, B.F., Hong, C.B. and Park, S.C. (2011). Overexpression of the mitogen-activated protein kinase gene OsMAPK33 enhances sensitivity to salt stress in rice (Oryza sativa L.). J. Biosci. *36*, 139–151.

Lee, J.S., Huh, K.W., Bhargava, A. and Ellis, B.E. (2008)b. Comprehensive analysis of protein-protein interactions between *Arabidopsis* MAPKs and MAPK kinases helps define potential MAPK signalling modules. Plant Signa Behav *3*, 1037–1041.

Lewis, T.S., Shapiro, P.S. and Ahn, N.G. (1998). Signal transduction through MAP kinase cascades. Adv Cancer Res. *74*, 49–139.

Luan S. (2003). Protein Phosphatases in Plants. Annu. Rev. Plant Biol.*54*, 63–92.

MAPK Group (Ichimura, K. *et al.*), Ichimura, K., Shinozaki, K., Tena, G., Sheen, J., Henry, Y., Champion, A., Kreis, M., Zhang, S., Hirt, H., Wilson, C., Heberle-Bors, E., Ellis, B. E., Morris, P. C., Innes, R. W., Ecker, J.R., Scheel, D., Klessig, D.F., Machida, Y., Mundy, J., Ohashi, Y. and Walker, J.C. (2002). Mitogen-activated protein kinase cascades in plants: a new nomenclature. Trends Plant Sci, *7*, 301-308.

Mayrose, M., Bonshtien, A. and Sessa, G. (2004). LeMPK3 Is a Mitogen-activated Protein Kinase with Dual Specificity Induced during Tomato Defense and Wounding Responses. J BiolChem. *279*, 14819–14827.

McClean, M.N., Mody, A., Broach, J.R. and Ramanathan, S. (2007). Cross-talk and decision making in MAP kinase pathways. Nature Genet*39*, 409–414.

Meng, X., Wang, H., He, Y., Liu, Y., Walker, J.C., Torii, K.U. and Zhang, S. (2012). A MAPK cascade downstream of ERECTA receptor-like protein kinase regulates *Arabidopsis* inflorescence architecture by promoting localized cell proliferation. Plant Cell*24,* 4948–60.

Meng, X. and Zhang S. (2013). MAPK Cascades in Plant Disease Resistance Signaling. Annu RevPhytopathol *51,* 245–266.

Menges, M., Dóczi, R., Okrész, L., Morandini, P., Mizzi, L., Soloviev, M., Murray, J.A.and Bögre L.(2008)a. Comprehensive gene expression atlas for the *Arabidopsis* MAP kinase signalling pathways. New Phytol*179*, 643–662.

Menges, M., Dóczi, R., Okrész, L., Morandini, P., Mizzi, L., Soloviev, M., Murray, J.A. and Bögre L.(2008)b. Comprehensive gene expression atlas for the *Arabidopsis* MAP kinase signalling pathways. New Phytol*179,* 643–662.

Meskiene, I., Baudouin, E., Schweighofer, A., Liwosz, A., Jonak, C., Rodriguez, P.L., Jelinek, H. and Hirt, H. (2003). Stress-induced protein phosphatase 2C is a negative regulator of a mitogen-activated protein kinase. JBiolChem*278,* 18945–18952.

Mészáros, T., Helfer, A., Hatzimasoura, E., Magyar, Z., Serazetdinova, L., Rios, G., Bardóczy, V., Teige, M., Koncz, C., Peck, S., *et al.* (2006). The *Arabidopsis* MAP kinase kinase MKK1 participates in defence responses to the bacterial elicitor flagellin. Plant J*48,* 485–498.

Miao, Y., Laun, T., Zimmermann, P. and Zentgraf, U. (2004). Targets of the WRKY53 transcription factor and its role during leaf senescence in *Arabidopsis*. Plant Mol Biol. *55*, 853–867.

Mishra, N.S., Tuteja, R. and Tuteja, N. (2006). Signaling through MAP kinase networks in plants. Arch BiochemBiophys*452,* 55–68.

Mizoguchi, T., Hayashida, N., Yamaguchi-Shinozaki, K., Kamada, H. and Shinozaki, K. (1993). ATMPKs: a gene family of plant MAP kinases in *Arabidopsis*. FEBS Lett*336,* 440–444.

Mizoguchi, T., Irie, K., Hirayama, T., Hayashida, N., Yamaguchi-Shinozaki, K., Matsumoto, K. and Shinozaki, K. (1996). 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*. Proc. Natl. Acad. Sci. U.S.A.*93*, 765–769.

Mody, A., Weiner, J. and Ramanathan, S. (2009). Modularity of MAP kinases allows deformation of their signalling pathways. Nature Cell Biol. *11,* 484–91.

Nakagami, H., Kiegerl, S. and Hirt, H. (2004). OMTK1, a novel MAPKKK, channels oxidative stress signaling through direct MAPK interaction.J Biol Chem*279,* 26959–66.

Nakashima, M., Hirano, K., Nakashima, S., Banno, H., Nishihama, R. and Machida, Y. (1998). The expression pattern of the gene for NPK1 protein kinase related to mitogen-activated protein kinase kinase kinase (MAPKKK) in a tobacco plant: correlation with cell proliferation. Plant Cell Physiol*39,* 690–700.

Nishihama, R., Ishikawa, M., Araki, S., Soyano, T., Asada, T. and Machida, Y. (2001). The NPK1 mitogen-activated protein kinase kinase kinase is a regulator of cell-plate formation in plant cytokinesis. Genes Dev. *15,* 352–363.

Nie, W.F., Wang, M.M., Xia, X.J., Zhou, Y.H., Shi, K., Chen, Z. and Yu, J.Q. (2013). Silencing of tomato RBOH1 and MPK2 abolishes brassinosteroid-induced H_2O_2 generation and stress tolerance. Plant Cell Environ. *36,* 789–803.

Nishihama, R., Soyano, T., Ishikawa, M., Araki, S., Tanaka, H., Asada, T., Irie, K., Ito, M., Terada, M., Banno, H., Yamazaki, Y. and Machida, Y.(2002). Expansion of the cell plate in plant cytokinesis requires a kinesin-like protein/MAPKKK complex. Cell*109,* 87–99.

Nühse, T.S., Peck, S.C., Hirt, H. and Boller, T. (2000). Microbial elicitors induce activation and dual phosphorylation of the*Arabidopsis* MAPK 6. J BiolChem*275*, 7521–7526.

Ortiz-Masia, D., Perez-Amador, M.A., Carbonell, P., Aniento, F., Carbonell, J. and Marcote, M.J. (2008). Characterization of PsMPK2, the first C1 subgroup MAP kinase from pea (*Pisum sativum* L.). Planta, *227,* 1333–1342.

Pawson, T. and Scott, J.D. (2005). Protein phosphorylation in signaling - 50 Years and counting. Trends Biochem Sci. *30,* 286–290.

Pearce, G., Johnson, S., Ryan, C.A. (1993). Structure-Activity of Deleted and Substituted Systemin, an 18-Amino Acid Polypeptide Inducer of Plant Defensive Genes. J. Biol. Chem. *268,* 212–216.

Petersen, M., Brodersen, P.,Naested, H., Andreasson, E., Lindhart, U., Johansen, B., Nielsen, H.B., Lacy, M., Austin, M.J.,Parker, J.E., *et al.* (2000). Arabidopsis map kinase 4 negatively regulates systemic acquired resistance. Cell *103*, 1111–1120.

del Pozo, O., Pedley, K.F. and Martin, G.B. (2004). MAPKKKalpha is a positive regulator of cell death associated with both plant immunity and disease. EMBO J.*23*, 3072–3082.

Qiao, H., Shen, Z., Huang, S.C., Schmitz, R.J., Urich, M., Briggs, S.P. and Ecker, J.R. (2012). Processing and Subcellular Trafficking of ER-Tethered EIN2 Control Response to Ethylene Gas. Science 338, 390–393.

Qiu, J-L., Fiil, B.K., Petersen, K., Nielsen, H.B.B., Botanga, C.J., Thorgrimsen, S., Palma, K., Suarez-Rodriguez, M.C., Sandbech-Clausen, S., Lichota, J., *et al.* (2008). *Arabidopsis* MAP kinase 4 regulates gene expression through transcription factor release in the nucleus. EMBO J *27*, 2214–2221.

Quimby, B.B., Wilson, C.A. and Corbett, A.H. (2000). The interaction between ran and NTF2 is required for cell cycle progression. Mol Biol Cell *11,* 2617–2629.

Rao, K.P., Richa, T., Kumar, K., Raghuram, B. and Sinha, A.K. (2010). In silico analysis reveals 75 members of mitogen-activated protein kinase kinase kinase gene family in rice. DNA Res. *17,* 139–153.

Reményi, A., Good, M.C. and Lim, W.A. (2006). Docking interactions in protein kinase and phosphatase networks. Curr Opin Struct Biol*16,* 676–685.

Rodriguez, M.C.S., Petersen, M. and Mundy, J. (2010). Mitogen-activated protein kinase signaling in plants. Annu Rev Plant Biol. *61,* 621–49.

Rossomando, E.F., Hadjimichael, J., Varnum-Finney, B. and Soll, D.R. (1987). HLAMP-a conjugate of hippuryllysine and AMP which contains a phosphoamide bond-stimulates chemotaxis in Dictyostelium discoideum. Differentiation *35*, 88–93.

Samuel, M.A., Hall, H., Krzymowska, M., Drzewiecka, K., Hennig, J. and Ellis, B.E. (2005). SIPK signaling controls multiple components of harpin-induced cell death in tobacco. Plant J. *42*, 406–416.

Sasabe, M., Soyano, T., Takahashi, Y., Sonobe, S., Igarashi, H., Itoh, T.J., Hidaka, M. and Machida, Y. (2006). 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. *20*, 1004–1014.

Schaffer, R., Landgraf, J., Accerbi, M., Simon, V., Larson, M. and Wisman, E. (2001). Microarray analysis of diurnal and circadian-regulated genes in *Arabidopsis*. Plant Cell *13,*113–123.

Schoenbeck, M.A., Samac, D.A., Fedorova, M., Gregerson, R.G., Gantt, J.S. and Vance, C.P. (1999). The alfalfa (*Medicago sativa*) TDY1 gene encodes a mitogen-activated protein kinase homolog. Mol Plant Microbe Interact *12,* 882–893.

Schweighofer, A., Kazanaviciute, V., Scheikl, E., Teige, M., Doczi, R., Hirt, H., Schwanninger, M., Kant, M., Schuurink, R., Mauch, F.,Buchala, A.,Cardinale, F. and Meskienea, I (2007). The PP2C-Type Phosphatase AP2C1, Which Negatively Regulates MPK4 and MPK6, Modulates Innate Immunity, Jasmonic Acid, and Ethylene Levels in *Arabidopsis*. Plant Cell *19,* 2213–2224.

Seeliger, M.A., Ranjitkar, P., Kasap, C., Shan, Y., Shaw, D.E., Shah, N.P., Kuriyan, J. and Maly, D.J. (2009). Equally potent inhibition of c-Src and Abl by compounds that recognize inactive kinase conformations. Cancer Res. *69,*2384–2392.

Seo, S., Okamoto, M., Seto, H., Ishizuka, K., Sano, H. and Ohashi, Y. (1995). Tobacco MAP kinase: a possible mediator in wound signal transduction pathways. Science *270*, 1988–92.

Shoresh, M., Gal-On, A., Leibman, D. and Chet I. Characterization of a mitogen-activated protein kinase gene from cucumber required for trichoderma-conferred plant resistance. (2006). Plant Physiol, 142, 1169-79.

Zhang, S. (2008). Mitogen-Activated Protein Kinase Cascades in Plant Intracellular Signaling, in Annu Plant Rev. Intracellular Signaling in Plants, ed. Z. Yang, *33*, 100-136 Wiley-Blackwell, Oxford, UK.

Stafstrom, J.P., Altschuler, M. and Anderson, D.H. (1993). Molecular cloning and expression of a MAP kinase homologue from pea. Plant Mol. Biol. *22,* 83–90.

Steggerda, S.M. and Paschal, B.M. (2002). Regulation of nuclear import and export by the GTPase Ran. Int Rev Cytol *217,* 41–91.

Sturgill, T.W. and Ray, L.B. (1986). Muscle proteins related to microtubule associated protein-2 are substrates for an insulin-stimulatable kinase. Biochem Biophy Res Commun. *134,* 565–71.

Suarez-Rodriguez, M.C., Adams-Phillips, L., Liu, Y., Wang, H., Su, S.H., Jester, P.J., Zhang, S., Bent, F. and Krysan, P.J. (2007). MEKK1 Is Required for flg22-Induced MPK4 Activation in *Arabidopsis* Plants. Plant Physiol.*143,*661–669.

Sun, Y., Wang, C., Yang, B., Wu, F., Hao, X., Liang, W., Niu, F., Yan, J., Zhang, H., Wang, B., Wang, B.,Deyholos, M. K. and Jiang, Y.Q. (2014). Identification and functional analysis of mitogen-activated protein kinase kinase kinase (MAPKKK) genes in canola *(Brassica napus L.*). J ExpBot. *65,* 2171–2188.

Taj, G., Agarwal, P., Grant, M. and Kumar, A. (2010). MAPK machinery in plants: recognition and response to different stresses through multiple signal transduction pathways. Plant Signal Behav. *5,* 1370–1378.

Takahashi, Y., Soyano, T., Sasabe, M. and Machida, Y. (2004). A MAP kinase cascade that controls plant cytokinesis. J Bioche *136,* 127–132.

Takahashi, F., Yoshida, R., Ichimura, K., Mizoguchi, T., Seo, S., Yonezawa, M., Maruyama, K., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2007). The mitogen-activated protein kinase cascade MKK3-MPK6 is an important part of the jasmonate signal transduction pathway in *Arabidopsis*. Plant Cell.*19,* 805–818.

Tanoue, T., Adachi, M., Moriguchi, T. and Nishida, E. (2000). A conserved docking motif in MAP kinases common to substrates, activators and regulators. Nature Cell Biol*2,* 110–116.

Teige, M., Scheikl, E., Eulgem, T., Doczi, R., Ichimura, K., Shinozaki, K., Dangl, J.L. and Hirt, H. (2004). The MKK2 pathway mediates cold and salt stress signaling in *Arabidopsis*. Mol Cell. *15,* 141–152.

Tregear, J.W., Jouannic, S., Schwebel-Dugué, N. and Kreis, M. (1996). An unusual protein kinase displaying characteristics of both the serine/threonine and tyrosine families is encoded by the *Arabidopsis* gene ATN1. Plant Sci. *117*, 107–119.

Ubersax, J.A. and Ferrell, Jr. J.E. (2007). Mechanisms of specificity in protein phosphorylation. Nature Rev Mol. Cell Biol *8,* 530–541.

Ulm, R., Ichimura, K., Mizoguchi, T., Peck, S.C., Zhu, T., Wang, X., Shinozaki, K. and Paszkowski, J. (2002). Distinct regulation of salinity and genotoxic stress responses by *Arabidopsis* MAP kinase phosphatase 1. EMBO J. *21*, 6483–6493.

Walia, A., Lee, J.S., Wasteneys, G. and Ellis, B. (2009). *Arabidopsis* mitogen-activated protein kinase MPK18 mediates cortical microtubule functions in plant cells. Plant J. *59*, 565–575.

Wang, H., Chevalier, D., Larue, C., Cho, S.K. and Walker, J.C. (2007)a. The Protein Phosphatases and Protein Kinases of *Arabidopsis*. Arabidopsis *Book* 5:e0106.

Wang, G., Lovato, A., Polverari, A.,Wang,M., Liang, Y.H., Ma, Y.C., and Cheng, Z.M. (2014). Genome-wide identification and analysis of mitogen activated protein kinase kinase kinase gene family in grapevine (Vitis vinifera). BMC Plant Biol, *14*, 219.

Wang, H., Ngwenyama, N., Liu, Y., Walker, J.C. and Zhang, S. (2007)b. Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in *Arabidopsis*. Plant Cell*19*, 63–73.

Wen, X., Zhang, C., Ji, Y., Zhao, Q., He, W., An, F., Jiang, L. and Guo, H. (2012). Activation of ethylene signaling is mediated by nuclear translocation of the cleaved EIN2 carboxyl terminus. Cell Res. *22,* 1613–1616.

Widmann, C., Gibson, S., Jarpe, M.B. and Johnson, G.L. (1999). Mitogen-Activated Protein Kinase: Conservation of a Three-Kinase Module From Yeast to Human. Physiol Rev. *79*, 143–180.

Wilhelm, S.M., Adnane, L., Newell, P., Villanueva, A., Llovet, J.M. and Lynch, M. (2008). Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. Mol. Cancer Ther. *7*, 3129–3140.

Wilson, C., Eller, N., Gartner, A., Vicente, O. and Heberle-Bors, E. (1993). Isolation and characterization of a tobacco cDNA clone encoding a putative MAP kinase. Plant Mol Biol. *23,* 543–551.

Wilson, K.P., Fitzgibbon, M.J., Caron, P.R., Griffith, J.P., Chen, W., McCaffrey, P.G., Chambers, S.P. and Su, M.S. (1996). Crystal structure of p38 mitogen-activated protein kinase. J Biol Chem. *271,* 27696–27700.

Xing, Y., Cao, Q., Zhang, Q., Qin, L., Jia, W. and Zhang, J. (2013). MKK5 regulates high light induced gene expression of Cu/Zn superoxide dismutase 1 and 2 in Arabidopsis. Plant Cell Physiol. *54,* 1217–1227.

Yoo, S-D., Cho, Y-H., Tena, G., Xiong, Y. and Sheen, J. (2008). Dual control of nuclear EIN3 by bifurcate MAPK cascades in C2H4 signalling. Nature *451,* 789–95.

Yoshioka, K. (2004). Scaffold proteins in mammalian MAP kinase cascades. J. Biochem.*135,* 657–661.

Yuasa, T., Ichimura, K., Mizoguchi, T. and Shinozaki, K. (2001). Oxidative stress activates ATMPK6, an *Arabidopsis* homologue of MAP kinase. Plant Cell Physiol. *42,* 1012–1016.

Zhang, S. and Klessig, D. F. (2001). MAPK cascades in plant defense signaling. Trends in Plant Science*6,* 520–527.

Zhang, F., Strand, A., Robbins, D., Cobb, M.H. and Goldsmith, E. J. (1994). Atomic structure of the MAP kinase ERK2 at 2.3 A resolution. Nature *367*, 704–711.

Zhao, C., Nie, H., Shen, Q., Zhang, S., Lukowitz, W. and Tang, D. (2014). EDR1 Physically Interacts with MKK4/MKK5 and Negatively Regulates a MAP Kinase Cascade to Modulate Plant Innate Immunity. PLoS Genet. *10(5),* e1004389.

Zhulin, I.B., Taylor, B.L. and Dixon, R. (1997). PAS domain S-boxes in Archaea, Bacteria and sensors for oxygen and redox. Trends Biochem Sci*22,* 331–333.

Zou, X., Peng, T. and Pan, Z. (2008). Modeling specificity in the yeast MAPK signaling networks. J Theor Biol. *250,* 139–155.