Research review paper

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

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A B S T R A C T

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.

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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; IBI5, 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; 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 (Fig. 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/compartmentalization 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 (Fig. 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 an 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 also be 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 localized programmed cell death (PCD). Ozone activated MPK3 and MPK6 and it caused the nuclear translocation of these MAPKs in Arabidopsis (Athfors 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 MK2 (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 MK2 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 (Doci 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
At 120 of plant immunity is called effector-triggered immunity (ETI) and is cell death restricting the spread of the pathogen. This second level and effectively stop PTI. At a second level of defense, plants can recognize proteins that can overcome the activities of PTI signaling components of a plant by a pathogen. To suppress PTI, pathogens produce effector of PAMP-triggered immunity (PTI) that can stop further colonization production of antimicrobial compounds. This leads to the activation changes of enzymatic activity, gene expression reprogramming and geres defense responses of plant cells. Such defense responses include with transmembrane pattern recognition receptors (PRRs) and trig-
tion of conserved pathogen-associated molecular patterns (PAMPs) Ta
responses against pathogens. The plant innate immune system involves two levels of defense responses involving MAP3Ks, MAP2Ks and MAPKs that MAPKs play important roles in innate immune response and resistance (Jones and Dangl, 2006). In recent years, it has been established that MAPKs are involved in responses to microbial pathogens and their effector proteins as well as in plethora of abiotic stress responses. 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 a plant by a 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 (Desikan et al., 2001; Nuhse et al., 2000). 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.

### Table 1

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Stress response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP3Ks</td>
<td>Fungal pathogens resistance</td>
<td>Tang and Innes (2002)</td>
</tr>
<tr>
<td>AEDR1</td>
<td>Oxidative stress</td>
<td>Kottun et al. (2000)</td>
</tr>
<tr>
<td>ATAP1</td>
<td>Touch, cold, salinity, oxidative stress, bacterial pathogens resistance</td>
<td>Mizoguchi et al., 1996; Teige et al., 2004; Ichimura et al., 2006; Gao et al., 2008; Pitzschke et al., 2009a, 2009b</td>
</tr>
<tr>
<td>ATMEKK1</td>
<td>Bacterial pathogens resistance</td>
<td>del Pozo et al. (2004)</td>
</tr>
<tr>
<td>MSOMTK1</td>
<td>Oxidative stress</td>
<td>Nakagami et al. (2004)</td>
</tr>
<tr>
<td>NNPK1</td>
<td>Cold, drought, hyperosmotic stress</td>
<td>Kottun et al., 2000; Shou et al., 2004a; Shou et al., 2004b</td>
</tr>
<tr>
<td>OSEDR1</td>
<td>Fungal pathogens, drought, high salt and sugar, heavy metals</td>
<td>Kim et al. (2003)</td>
</tr>
<tr>
<td>MAP2Ks</td>
<td>Wounding, cold, drought, and high salt</td>
<td>Matsuoka et al., 2002; Teige et al., 2004</td>
</tr>
<tr>
<td>AMMK1</td>
<td>Cold and high salt stress</td>
<td>Teige et al. (2004)</td>
</tr>
<tr>
<td>AMMK2</td>
<td>Bacterial and fungal pathogens resistance</td>
<td>Asai et al. (2002)</td>
</tr>
<tr>
<td>AMMK5</td>
<td>Bacterial elicitor stress signaling</td>
<td>Pedley and Martin (2004)</td>
</tr>
<tr>
<td>LeMKK1</td>
<td>Cold stress</td>
<td>Wen et al. (2002)</td>
</tr>
<tr>
<td>MsMOKX</td>
<td>Pathogen elicitor stress signaling, heavy metals and high salt stress</td>
<td>Kieber et al., 2000; Cardinale et al., 2002; Jonak et al., 2004</td>
</tr>
<tr>
<td>NfMEK2</td>
<td>Multiple defense responses against pathogens</td>
<td>Yang et al., 2001; Ren et al., 2002; del Pozo et al., 2004</td>
</tr>
<tr>
<td>PfMKK5</td>
<td>Fungal and bacterial elicitor stress signaling</td>
<td>Lee et al. (2004)</td>
</tr>
<tr>
<td>MAPKs</td>
<td>Oxidative and osmotic stress, bacterial elicitor stress signaling</td>
<td>Desikan et al., 1999; Kottun et al., 2000; Asai et al., 2002; Droillard et al., 2002; Ahfors et al., 2004</td>
</tr>
<tr>
<td>ATMPK4</td>
<td>Cold, drought, hyper-osmolality, touch, wounding and oxidative stress, pathogen resistance</td>
<td>Desikan et al., 1999; Ichimura et al., 2000; Petersen et al., 2000; Droillard et al., 2004; Teige et al., 2004; Nakagami et al., 2006</td>
</tr>
<tr>
<td>ATMPK6</td>
<td>Cold, drought, hyper-osmolality, touch, wounding and oxidative stress, pathogen resistance</td>
<td>Desikan et al., 1999; Ichimura et al., 2000; Kottun et al., 2000; Nuhse et al., 2000; Asai et al., 2002; Droillard et al., 2002; Ahfors et al., 2004; Menke et al., 2004</td>
</tr>
<tr>
<td>LeMPK1</td>
<td>Bacterial effectors, UV-B radiation</td>
<td>Holley et al. (2003)</td>
</tr>
<tr>
<td>LeMPK2</td>
<td>Bacterial and fungal pathogens, mechanical stress and wounding, UV-B radiation</td>
<td>Holley et al., 2003; Mayrose et al., 2004; Pedley and Martin, 2004</td>
</tr>
<tr>
<td>LeMPK3</td>
<td>Heavy metal stress</td>
<td>Jonak et al. (2004)</td>
</tr>
<tr>
<td>MsMMK2</td>
<td>Mechanical stimulation, wounding, drought, and cold, heavy metals, pathogen resistance</td>
<td>Jonak et al., 1996; Bögre et al., 1997; Cardinale et al., 2000; Jonak et al., 2004</td>
</tr>
<tr>
<td>MsSAMK</td>
<td>Wounding, bacterial elicitor or avirulent pathogen responses</td>
<td>Sharma et al. (2003)</td>
</tr>
<tr>
<td>MsSMPK</td>
<td>Osmotic stress, wounding, fungal, bacterial and viral pathogen resistance</td>
<td>Zhang and Klessig, 1998a; Zhang et al., 2000; del Pozo et al., 2004</td>
</tr>
<tr>
<td>MsNPK</td>
<td>Cold temperature stress</td>
<td>Wen et al. (2002)</td>
</tr>
<tr>
<td>MsMAPK4</td>
<td>Sugar starvation, high salinity, cold</td>
<td>Fu et al. (2002)</td>
</tr>
<tr>
<td>MsMAPK5</td>
<td>Pathogen resistance, wounding, drought, salt, and cold stress</td>
<td>Xiong and Yang (2003)</td>
</tr>
<tr>
<td>MsMAPK33</td>
<td>Drought, osmotic stress</td>
<td>Lee et al. (2011)</td>
</tr>
<tr>
<td>OsMSRMK2</td>
<td>Wounding, drought, heavy metals, fungal elicitors, UV irradiation,</td>
<td>Agrawal et al., 2002; Agrawal et al., 2003</td>
</tr>
<tr>
<td>OsMSRMK3</td>
<td>Heavy metals, high salt and sucrose</td>
<td>Agrawal et al. (2003)</td>
</tr>
<tr>
<td>OsWIPK1</td>
<td>Oxidative stress, cold, heavy metals</td>
<td>Rudd et al. (2008)</td>
</tr>
<tr>
<td>TmMPK3</td>
<td>Fungal pathogens resistance</td>
<td></td>
</tr>
<tr>
<td>TmMPK6</td>
<td>Cold, drought, ultraviolet light, salinity, heavy metal and mechanical wounding</td>
<td>Wang et al. (2010)</td>
</tr>
<tr>
<td>ZmMPK3</td>
<td>Oxidative stress</td>
<td>Lin et al. (2009)</td>
</tr>
</tbody>
</table>

At = Arabidopsis thaliana, Le = Lycopersicum esculentum, Ms = Medicago sativa, Ns = Nicotiana tabacum, Os = Oryza sativa, Pc = Petroselinum crispum, Nb = Nicotiana benthamiana, Ta = Triticum aestivum, Zm = Zea mays.
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 (BR1-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 MK4 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 (Joo et al., 2008; Liu et al., 2004). 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 VirF1-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 PR1 (Djamei et al., 2007).

The second MAPK cascade, comprised of MEKK1, MKK1/MKK2 and MPK4, negatively regulates defense responses. In this defense re- sponse, 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), and 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 to- bacco, there are at least two MAPK pathways activated by inoculation with tobacco mosaic virus (TMV). The first one is composed of MEKK2-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–NTP6 and attenuates resistance to TMV (Liu et al., 2004). 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 the MAPK 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 (Clark et al., 1998; Gao et al., 2003; Huang et al., 2003; Kieber et al., 1993). In the absence of ethylene, ETR1 suppressed signal transduction pathway by activating the negative regulator CTR1 (Hua and Meyerowitz, 1998). Therefore, 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 (Binder et al., 2007; Chao et al., 1997; Yanagisawa et al., 2003). Activation of 47KD 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 simultaneouly 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 served 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., 2009). It was shown that MPK12 specifically interacts with IBR5 phosphatase while activated MPK12 can be dephosphorylated and inactivated by this phosphatase (Lee et al., 2009). 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

5.1 Arabidopsis

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 (Bögø et al., 1997; Jonák et al., 1996) 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 (Bögø et al., 1999; Calderini et al., 1998; Nishihama et al., 2001; Soyan 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 (Beck et al., 2011; Kossetsu et al., 2010). 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 (Beck et al., 2010; Müller et al., 2010; Walia et al., 2009).

Stomata development represents a very good example of a 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; Lampard et al., 2009; Wang et al., 2007). 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 (Lampard et al., 2008; Wang et al., 2007). 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 (Bayer et al., 2009; Lukowicz et al., 2004).

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 and Krysan, 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., 2009a). 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://urvg.evy.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 (Pitzschke et al., 2009a; Qiu et al., 2008a). 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 nicely demonstrated for MEEKK1–MKK1/2–MPK4 pathway which plays a negative role in plant innate

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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.aaffrc.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/pobo/pobo/pobo). Identified DNA motifs should be experimentally tested using overexpression of synthetic promoter constructs (Rushton et al., 2002) and candidate transcription factors (Pitzschke 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 large datasets which need 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 patmatch 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

### Table 2

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Developmental process</th>
<th>Stress response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP3Ks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AtMKP2/3</td>
<td>Cytokinesis</td>
<td>Oxidative stress?, pathogen response?, heat?</td>
<td>Krysan et al., 2002</td>
</tr>
<tr>
<td>AYODA</td>
<td>Stomata and embryo development</td>
<td>Plant defense?</td>
<td>Bergmann et al., 2004; Lukowitz et al., 2004</td>
</tr>
<tr>
<td>NINPK1</td>
<td>Cytokinesis</td>
<td>Oxidative stress</td>
<td>Kottun et al., 2000; Nishihama et al., 2001</td>
</tr>
<tr>
<td>AIMERK1</td>
<td>Root hair and lateral root development</td>
<td>Oxidative stress, salt, drought, wounding, bacterial elicitor flg22, cell death</td>
<td>Asai et al., 2002; Ichimura et al., 2006; Nakagami et al., 2006; Suarez-Rodriguez et al., 2007; Muller et al., 2010</td>
</tr>
<tr>
<td>AtRC1</td>
<td>Root planar polarity</td>
<td>Ethylene</td>
<td>Kieber et al., 1993; Bieda et al., 2009</td>
</tr>
<tr>
<td>MAP2Ks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NINQK1</td>
<td>Cytokinesis</td>
<td>Cell death, fungal elicitor</td>
<td>Sozano et al., 2003; del Pozo et al., 2004</td>
</tr>
<tr>
<td>AtMKK4</td>
<td>Stomata development</td>
<td>Bacterial elicitor flg22</td>
<td>Wang et al., 2007</td>
</tr>
<tr>
<td>AtMKK5</td>
<td>Stomata development</td>
<td>Bacterial elicitor flg22</td>
<td>Wang et al., 2007</td>
</tr>
<tr>
<td>MAPks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MsSIMK</td>
<td>Root hair development</td>
<td>Salt, fungal elicitor pep13, wounded, cold, drought, heavy metals</td>
<td>Jonak et al., 1996; Bögre et al., 1997; Cardinale et al., 2002; Samaj et al., 2002; Jonak et al., 2004</td>
</tr>
<tr>
<td>MsMMK3</td>
<td>Cell division</td>
<td>Fungal elicitor pep13, ethylene, heavy metals, oxidative stress, cell death</td>
<td>Bögre et al., 1999; Cardinale et al., 2002; Ouaked et al., 2003; Jonak et al., 2004; Nakagami et al., 2004</td>
</tr>
<tr>
<td>NINNtK6</td>
<td>Cytokinesis</td>
<td>Virus resistance, oxidative stress</td>
<td>Calderini et al., 1998; Liu et al., 2004; Asai et al., 2008</td>
</tr>
<tr>
<td>ARMKK3</td>
<td>Stomata development</td>
<td>Ozone, oxidative stress, hypoosmolarity, bacterial elicitor flg22, fungal resistance</td>
<td>Kottun et al., 2000; Asai et al., 2002; Ahlfors et al., 2004; Droillard et al., 2004; Lee and Ellis, 2007; Wang et al., 2007; Ren et al., 2008</td>
</tr>
<tr>
<td>ARPK4</td>
<td>Cytokinesis, seedling and root hair development, cell shape control</td>
<td>Oxidative stress, cold, salt, hypo- and hyperosmolarity, touch, wounding, bacterial elicitor flg22</td>
<td>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</td>
</tr>
<tr>
<td>ARPK6</td>
<td>Cell division, stomata, anther and inflorescence development</td>
<td>Ozone, oxidative stress, cold, salt, hypo- and hyperosmolarity, touch, bacterial elicitor flg22</td>
<td>Ichimura et al., 2000; Kottun et al., 2000; Yuasa et al. 2001; Asai et al., 2002; Ahlfors et al., 2004; Droillard et al., 2004; Teige et al., 2004; Bush and Krysan, 2007; Lee and Ellis, 2007; Takahashi et al., 2007; Wang et al., 2007; Ren et al., 2008; Muller et al., 2010</td>
</tr>
</tbody>
</table>

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

7 = function in stress response was suggested from transcriptomic data but not directly experimentally determined.
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/ utils/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 hyposmotic stress tolerance was also hypothesized. Hypoosmotic 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 et al., 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 et al., 1996) and it activates downstream signaling modules MKK1, MKK2 and MPK4 (Ichimura et al., 1998). Moreover, 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, homeostasis of ROS is also a complex process with several signaling modules working independently on each other. MPK3 and MPK6 are other 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 NDF family, AtNDFPK2, pointed to H2O2-mediated
In response to ABA treatment. Conversely, overexpression of these genes and enhanced resistance to both fungal and bacterial pathogens. However, these plans also showed reductions in tolerance to multiple environmental stress conditions when constitutive-ly 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 H$_2$O$_2$ production, and subsequently, NO signaling is involved in the upregulation of expression of antioxidant enzymes in ABA signaling.

In maize, ABA-induced production of H$_2$O$_2$ 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 bi- phasic process that can be partially controlled with MAPK inhibitors and H$_2$O$_2$ scavengers. ZmHK5 is involved in the activation of the second phase of biphasic induction of NADPH oxidase which in turn regulates H$_2$O$_2$ 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 a plethora of abiotic stress conditions such as wounding, salinity, drought, heavy metals, fungal elicitors or UV irradiation (Agrawal et al., 2002; Agrawal et al., 2003).

Another example of MAPK with multiple roles in both biotic and abiotic stress responses is OsMPK5. The negative role of OsMPK5 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 plans also showed reductions in tolerance against cold, salt or drought. Conversely, overexpression of OsMPK5 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 *dsml* 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 peroxi-
dase genes based on expression analysis and increased sensitivity in *dsml* mutant to oxidative damage suggested that 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 differ-
ences 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 ex-
cellular environment to the cellular interior, most often resulting in
changes in the gene expression programs and in the plant develop-
ment. In all eukaryotes, MAPK signaling pathways are highly con-
served 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 signal-
ing 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 environ-
mental conditions or pathogen attack. Therefore MAPKs and also
MAPK-related phosphatases are ideal targets of genetic modi-

fication. The usefulness and biotechnological potential of targeted MAPK
approaches is discussed in this review with respect to the potential to
improve stress tolerance in plants.

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