Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks

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Plants have evolved a wide range of mechanisms to cope with biotic and abiotic stresses. To date, the molecular mechanisms that are involved in each stress has been revealed comparatively independently, and so our understanding of convergence points between biotic and abiotic stress signaling pathways remain rudimentary. However, recent studies have revealed several molecules, including transcription factors and kinases, as promising candidates for common players that are involved in crosstalk between stress signaling pathways. Emerging evidence suggests that hormone signaling pathways regulated by abscisic acid, salicylic acid, jasmonic acid and ethylene, as well as ROS signaling pathways, play key roles in the crosstalk between biotic and abiotic stress signaling.

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Introduction
Plants undergo continuous exposure to various biotic and abiotic stresses in their natural environment. To survive under such conditions, plants have evolved intricate mechanisms to perceive external signals, allowing optimal response to environmental conditions. Phytohormones such as salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and abscisic acid (ABA) are endogenous, low-molecular-weight molecules that primarily regulate the protective responses of plants against both biotic and abiotic stresses via synergistic and antagonistic actions, which are referred to as signaling crosstalk [1,2,3]. Moreover, the generation of reactive oxygen species (ROS) has been proposed as a key process that is shared between biotic and abiotic stress responses [4,5]. Rapidly accumulating data, resulting from large-scale transcriptome analyses with DNA microarray technology, strongly support the existence of such crosstalk between signaling networks [6–9,10]. Biotic and abiotic stresses regulate the expression of different but overlapping suites of genes. For example, the correlation of transcriptional regulation with environmental challenges, such as heavy metal (CuSO4) stress, and with incompatible necrotrophic pathogen infection reveals significant overlap between responses to biotic and abiotic stresses. These data suggest that ROS are a common signal that trigger downstream stress responses [11]. Thus, a growing body of evidence supports the notion that plant signaling pathways consist of elaborate networks with frequent crosstalk, whereby allowing plants to regulate both abiotic stress tolerance and disease resistance. This review focuses upon recent studies that have identified and characterized the function of genes that are involved in this crosstalk, subsequently shedding light on the molecular convergence points between biotic and abiotic stress signaling pathways (Figure 1).

Hormone signaling pathways govern biotic and abiotic stress responses
ABA is a phytoregulator that is extensively involved in responses to abiotic stresses such as drought, low temperature, and osmotic stress. ABA also governs a variety of growth and developmental processes, including seed development, dormancy, germination, and stomatal movement. By contrast, the phytohormones SA, JA, and ET play central roles in biotic stress signaling upon
pathogen infection. In many cases, ABA acts as a negative regulator of disease resistance [3**]. For example, the ABA-deficient tomato mutant *sitisens* has increased resistance to pathogens and application of exogenous ABA restored the susceptibility of *sitisens* [12,13]. The *sitisens* mutant has greater SA-mediated responses, suggesting that high ABA concentrations inhibit the SA-dependent defense response in tomato. ABA and ET are well known to interact, mostly antagonistically, in a number of development processes and in vegetative tissues [14,15]. Genetic analysis of enhanced response to ABA3 (era3) alleles revealed that ERA3 is allelic to ETHYLENE INSENSITIVE2 (EIN2), which encodes a membrane-bound putative divalent cation sensor that may represent a crosstalk point that intersects the ABA and ET signaling pathways [15]. Furthermore, jasmonic acid resistance1 (jar1) and jasmonic acid insensitive4 (jin4) mutants, which are hypersensitive to ABA-mediated inhibition of germination, exhibit antagonistic effects of ABA and JA [2,16**]. Additionally, exogenous application of ABA resulted in the downregulation of JA- or ET-responsive defense gene expression in wildtype plants, whereas higher expression levels of these defense genes were observed in ABA-deficient mutants without any treatments [16**]. Taken together with the findings that exogenous application of methyl-JA and ET cannot restore the defense gene expression that is suppressed by exogenous ABA application, these data suggest that the ABA-mediated abiotic stress response is a dominant process [16**].

Recent studies demonstrate that the basic helix-loop-helix (bHLH) transcription factor *AtMYC2* plays a role in multiple hormone signaling pathways. Genetic analysis of the jasmonate-insensitive *jin1* mutant revealed that *JIN1* is allelic to *AtMYC2* [17]. *AtMYC2*, which was first identified as a transcriptional activator that is involved in the ABA-mediated drought stress signaling pathway [18], upregulates the expression of genes that are involved in the JA-mediated wounding response and negatively regulates the expression of JA/ET-mediated pathogen defense genes [16**,17,19]. *AtMYC2* knockout mutants are less sensitive to ABA and exhibit significantly reduced ABA-responsive gene expression [18]. In addition, *AtMYC2* deficiency results in increased JA- or ET-regulated defense gene expression and in enhanced resistance to a necrotrophic pathogen [16**,17]. Although ABA-mediated *AtMYC2* activation cannot solely account for the inhibitory effect of ABA on JA-regulated expression of defense genes, *AtMYC2* might be a key regulator of crosstalk via hormone signaling between biotic and abiotic stress responses.

Both *AtMYC2* and the *R2R3MYB*-type transcription factor *AtMYB2* bind cis-elements in the dehydration-inducible *RD22* gene and cooperatively activate its expression [18]. Interestingly, transgenic plants that overexpress both *AtMYC2* and *AtMYB2* display greater sensitivity to ABA and enhanced osmotic stress tolerance when compared to wildtype plants [18]. In addition, *Botrytis* infection induces the expression of *Botrytis SUSCEPTIBLE 1* (*BOS1*), which shares high sequence similarity with *AtMYB2*, through a JA-mediated defense signaling pathway [20]. Disruption of *BOS1* results in increased sensitivity to necrotrophic pathogens and impaired drought, salinity, and oxidative stress tolerance. Furthermore, other data suggested that *BOS1* mediates both biotic and abiotic stress signaling via ROS. Therefore, *R2R3MYB* transcription factors might serve as important mediators of stress responses that have complex activities spanning multiple stress signaling pathways.

**Figure 1**

Convergence points in abiotic and biotic stress signaling networks.
the repression of pathogenesis-related proteins and in elevated susceptibility to soil-borne fungal pathogens.

Additionally, recent studies identified other molecular entities that significantly impact crosstalk among stress-response pathways via hormone signaling. For instance, both nitric oxide [24] and Ca²⁺ signaling play an important role in plant defense responses, ABA-dependent stomatal movements, and drought stress responses [3**]. Calcium-dependent protein kinases in tobacco might control biotic and abiotic stress responses via signaling pathways that are mediated by hormones such as SA, ET, JA, and ABA [25–27]. In addition, fungal elicitors can activate a branch of the ABA signaling pathway in guard cells that regulates plasma membrane Ca²⁺ channels [28]. Moreover, a battery of studies examining the induction of resistance by the non-protein amino acid β-aminobutyric acid revealed that ABA considerably enhances plant resistance to fungal pathogens through its positive effect on callose deposition [3**,29,30].

**MAP-kinase cascades mediate stress signaling crosstalk**

Protein phosphorylation and dephosphorylation significantly influence both the regulation of physiological morphology and gene expression that is associated with basic cellular activities. In all eukaryotes, mitogen-activated protein (MAP) kinase (MAPK/MPK) cascades are highly conserved central regulators of diverse cellular processes, such as differentiation, proliferation, growth, death, and stress responses. In plants, the MAPK cascade plays a crucial role in various biotic and abiotic stress responses and in hormone responses that include ROS signaling [31,32*].

The *Arabidopsis* MAP kinase 1 (MEKK1) is transcriptionally induced by cold, salt, drought, touch, and wounding [33]. In particular, MEKK1–MAPK kinase 2 (MKK2)–MPK4/MPK6 cascade has been shown to function as part of cold and salt stress signaling [34**,35]. By contrast, MEKK1–MKK4/MKK5–MPK3/MPK6 cascades have been reported to regulate the pathogen defense response pathway via the expression of WRKY22 and WRKY29 [36,37]. MPK3 and MPK6 are also activated by abiotic stresses [35,38] and involved in hormone signaling pathways. MPK3 has been shown to function in ABA signaling at the post-germination stage [39]. MPK6 is thought to be involved in ET production through phosphorylation of the rate-limiting enzymes of ET biosynthesis, the 1-aminoacyclopropane-1-carboxylic acid (ACC) synthases ACS2 and ACS6 [40**], in JA-dependent root growth and in AtMYC2 gene expression (F Takahashi, unpublished). The rice *OsMPK5* gene is an ortholog of *Arabidopsis* MPK3. The gene expression and kinase activity of OsMPK5 is also induced by ABA, various abiotic stresses and pathogen infection [41]. Gain- and loss-of-function analyses revealed that OsMPK5 positively regulates tolerance of drought, salt, and cold stresses and negatively regulates *PR* gene expression and broad-spectrum disease resistance in rice.

MPK6 is activated by oxidative stress in *Arabidopsis* cultured cells [42]. MPK3 and MPK6 activities in ROS signaling are affected by an *Arabidopsis* serine/threonine kinase, OXI1, whose kinase activity is induced by [H₂O₂][43]. Also, ANP1, an *Arabidopsis* MAPKKK, activates MPK3 and MPK6 via H₂O₂. Tobacco plants that over-expressed constitutively active tobacco NPK1 (*Nicotiana* protein kinase 1), a homolog of ANP1, exhibited improved tolerance of freezing, heat, drought and high-salt conditions [44]. *A. thaliana NUCLEOSIDE-DIPHOSPHATE KINASE 2 (AtNDPK2)*, whose expression is induced by H₂O₂, specifically interacts with MPK3 and MPK6, thereby activating MPK3 *in vitro* [45]. Overexpression of *AtNDPK2* enhances tolerance of cold, salt, and oxidative stresses. Thus, MAPK cascades apparently mediate ROS signaling, and consequently control tolerance of environmental stresses in each transgenic plant by improving ROS scavenging capacity.

**Roles of ROS at points of convergence between biotic and abiotic stress response pathways**

The tight regulation of the steady-state levels of ROS is involved in multiple cellular processes in plants [4]. Some ROS species are toxic byproducts of aerobic metabolism, whereas ROS also function as signaling molecules [4]. Rapid ROS production plays a pivotal role in both ABA signaling and disease resistance responses [46,47]. Several lines of evidence suggest that the NADPH-dependent respiratory burst oxidase homolog genes (*AtbohD* and *AtbohF*) are required for ROS generation, leading to ABA-induced stomatal closure and to hypersensitive cell death in response to avirulent pathogen attack [5*,48,49].

ROS scavengers are thought to detoxify the cytotoxic effects of ROS under various stress conditions [4,50]. Large-scale transcriptome analyses of plants that had been subjected to various abiotic and biotic stress treatments revealed the induction of a large set of genes that encode ROS-scavenging enzymes under these conditions [6,8,50]. Moreover, scavenging enzymes (e.g., superoxide dismutase, glutathione peroxidase and ascorbate peroxidase) have been utilized to engineer plants that are tolerant of abiotic stresses [51,52]. Microarray analysis using *Arabidopsis* cultured cells reveal that many ABA-inducible genes are induced by oxidative stress [53]. Recently, it has been suggested that a C₂H₂-type zinc-finger transcription factor, Zat12, might be a regulator in the ROS scavenging mechanism that is involved in biotic and abiotic stress responses. Deficiency in Zat12, which is highly responsive to multiple stresses including wounding, pathogen infection and abiotic stresses [6,22,54,55], suppresses the expression of the ASCORBATE
Effects of high humidity and high temperature upon biotic stress responses

High humidity and high temperature both affect plant disease development, attenuating plant disease resistance while promoting pathogen growth. Consistently, phenotypes of several lesion-mimic mutants, which are caused by misregulated R genes (e.g., chimeric Mi, overexpression of the powdery mildew R gene RPW8, SA-insensitivity4 [ssi4], bonzai1/copine1 [bon1/cpl1] and slh1), are also suppressed by environmental cues [58–63], suggesting the existence of cross-talk between R-gene-mediated disease resistance responses and abiotic stress responses. For example, the cell death phenotypes of the overexpressor of RPW8 [60] and slh1 [61] mutants of Arabidopsis are suppressed by both high temperature and high humidity. In the ssi4 mutant, high humidity conditions inhibited the constitutive activation of MPK3 and MPK6 as well as H$_2$O$_2$ production, suggesting that a high humidity sensor acts early in the signaling cascade [59]. Recently, it has been revealed that abundance of the barley R proteins MLA1 and MLA6 decreased dramatically within several hours after subjection to a temperature shift from 18°C to 37°C, without reduction of the MLA1 and MLA6 transcripts [64*]. Thus, R protein instability that is imposed by environmental stresses might represent a solid cross point between disease resistance and abiotic stress response. High humidity and high temperature both affect disease resistance that is mediated by receptor-like kinase type R genes [65]; therefore, abiotic stresses might also destabilize receptor-like kinase (RLK)-type R proteins and/or signaling components that are common to cytosolic- and membrane-anchored-type R proteins.

Conclusions

Current evidence supports the concept that ROS represent a significant point of convergence between pathways that respond to biotic and abiotic stresses. Nevertheless, our current understanding of ROS participation in crosstalk between these pathways is very limited. Thus, dissecting the genetic network that regulates ROS signaling in response to biotic and abiotic stresses merits extensive future study. When combined, the results of large-scale transcriptome, proteome, and metabolome analyses in plants will enable the elucidation of the ROS network components that govern multiple stress signaling pathways. In particular, the Genevestigator software should yield powerful clues, allowing us to connect these key molecular players and to discover novel cross-talk networks [66].

A significant body of research suggests an antagonistic interaction between ABA-mediated abiotic stress signaling and disease resistance. This relationship may simply suggest that plants have developed strategies to avoid simultaneously producing proteins that are involved in abiotic stress and disease resistance responses [16**]. In nature, simultaneous exposure of plants to drought and necrotrophic pathogen attack is actually rare, as successful pathogen infection requires relatively humid conditions. The finding that high humidity and high temperature weaken plant resistance to pathogen attack is consistent with this concept. Moreover, the view that the ABA-mediated abiotic stress signaling potentially takes precedence over biotic stress signaling [16**] also supports the notion that water stress more significantly threatens plant survival than does pathogen infection. To date, the biological significance of cross-talk between signaling pathways that operate under stress conditions and the mechanisms that underlie this cross-talk remain obscure. We are just beginning to dissect key factors governing the crosstalk between these signaling pathways under various stress conditions.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


An up-to-date, comprehensive review covering the various functions of plant roothe genes, including their relationships with other signaling molecules such as calcium ions and MAP kinases.


The findings published in this paper establish the cytosolic ascorbate peroxidase APX1 as a key component of the ROS gene network in Arabidopsis. Using a dominant-negative approach, the authors demonstrate that HSP21, a redox-sensitive transcription factor, regulates the expression of ZAT12, a multiple-stress-responsive transcription factor. The data also suggest that HSP21 is probably an initial sensor for H$_2$O$_2$, that is accumulated in response to various stresses.


On the basis of genetic analysis of JA-, ET- and ABA-insensitive mutants, this work demonstrates that the ABA and JA/ET-signaling pathways are mutually antagonistic, and modulate defense and stress-responsive gene expression in response to biotic and abiotic stresses. The authors also show that AtMYC2, a positive regulator of ABA signaling, negatively regulates JA/ET signaling. Both mutations that cause ABA deficiencies and mutations of AtMYC2 increase the expression of JA/ET-regulated defense genes and resistance against Fusarium oxysporum.


This current review summarizes the role of the MAPK cascade in plant stress signaling. The MAPK cascade might play a crucial role in multiple cellular events, such as development, cell proliferation, hormone signaling, and biotic or abiotic stress signaling.


This work identifies and functionally analyzes the roles of the MEKK1-MKK2-MPK4/MPK6 cascade in cold- and salt-stress signaling, mkk2-null mutant plants display hypersensitivity to cold and salt stress, whereas MKK2 overexpressor plants have enhanced tolerance of cold and salt stresses, and show changes in the expression of 152 genes. Consequently, the authors propose that the MKK2 pathway mediates cold- and salt-stress responses and modulates stress tolerance through the expression of specific genes.


40. Liu Y, Zhang S: Phosphorylation of 1-aminocyclopropane-1-carboxylic acid synthase by MPK6, a stress-responsive mitogen-activated protein kinase, induces ethylene biosynthesis in Arabidopsis. Plant Cell 2004, 16:3386-3399. Using a sophisticated approach that encompasses biochemical and genetic methods, the authors describe a biological function for the MKK4-MPK6 cascade in a pathogen response that regulates ethylene biosynthesis. They found that two ACS molecules act as substrates of MPK6, which is activated by MKK4. Phosphorylation by MPK6 leads to the accumulation of ACS protein and to elevated levels of ACS activity in vivo, consequently increasing ethylene production. This represents the first evidence of a direct path plant MAPK phosphorylation target, and demonstrates that ethylene is biosynthesized in response to stress signaling.


43. Liu Y, Zhang S: Phosphorylation of 1-aminocyclopropane-1-carboxylic acid synthase by MPK6, a stress-responsive mitogen-activated protein kinase, induces ethylene biosynthesis in Arabidopsis. Plant Cell 2004, 16:3386-3399. Using a sophisticated approach that encompasses biochemical and genetic methods, the authors describe a biological function for the MKK4-MPK6 cascade in a pathogen response that regulates ethylene biosynthesis. They found that two ACS molecules act as substrates of MPK6, which is activated by MKK4. Phosphorylation by MPK6 leads to the accumulation of ACS protein and to elevated levels of ACS activity in vivo, consequently increasing ethylene production. This represents the first evidence of a direct path plant MAPK phosphorylation target, and demonstrates that ethylene is biosynthesized in response to stress signaling.


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59. Bieri S, Mauch S, Shen OH, Peart J, Devoto A, Casais C, Cerón F, Schulze S, Steinbiss HH, Shirasu K et al.: RAR1 positively controls steady state levels of barley MLA resistance proteins and enables sufficient MLA6 accumulation for effective resistance. Plant Cell 2004, 16:3480-3495. Transformation plants expressing epitope-tagged MLA variants are used to describe the effects of the rarr (required for MLA resistance!) mutation upon...
the abundance of the barley powdery mildew resistance R proteins MLA1 and MLA6. This analysis of rar1 mutants led the authors to propose that an effective resistance response requires a threshold level of R protein. In addition, the authors demonstrate that MLA protein stability decreased under high-temperature conditions. Transgene transcription levels and the amounts of RAR1 and SGT1 (SUPPRESSOR OF THE G2 ALLELE OF skp1-4) proteins remained unchanged during stress treatment.
