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Minireview

Molecules and Cells

Plant Stress Surveillance Monitored by ABA and Disease Signaling Interactions

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Abiotic and biotic stresses are the major factors that negatively impact plant growth. In response to abiotic environmental stresses such as drought, plants generate resistance responses through abscisic acid (ABA) signal transduction. In addition to the major role of ABA in abiotic stress signaling, ABA signaling was reported to downregulate biotic stress signaling. Conversely recent findings provide evidence that initial activation of plant immune signaling inhibits subsequent ABA signal transduction. Stimulation of effector-triggered disease response can interfere with ABA signal transduction *via* modulation of internal calcium-dependent signaling pathways. This review overviews the interactions of abiotic and biotic stress signal transduction and the mechanism through which stress surveillance system operates to generate the most efficient resistant traits against various stress condition.

INTRODUCTION

As sessile organisms facing diverse levels of stresses including abiotic environmental harsh condition and biotic pathogen attacks, plants are required to build up elaborate stress surveillance system and corresponding resistant mechanisms. Plant abiotic stress responses are largely controlled by phytohormone ABA through the regulation of its synthesis, transport, and onset of multi layers of signal transduction (Cutler et al., 2010; Kim et al., 2010). In addition to ABA's major roles in abiotic stress resistant signal transduction, ABA has been shown to function during pathogen infection and the following plant immune response pathways (de Torres-Zabala et al., 2007; Fan et al., 2009). Although, in many cases, ABA affects negatively on the generation of immune responses there have been reports that ABA synthesis after pathogen recognition assists proliferation of the infected pathogen depending on the types of particular pathogen and host pairs (Ton et al., 2009).

This review focuses on an opposite way of regulation that is how biotic stress resistant responses can regulate abiotic stress signal transduction. Recent findings demonstrated that modulation of ABA signal transduction would occur *via* components previously known as major regulators of pathogen signaling. Especially control of abiotic responses by *NB-LRR* (*Nucleotide Binding-Leucine Rich Repeat*) *R* (*Resistant*) gene immune receptors and Ca^{2+} signaling as an integrator combining abiotic and biotic stress surveillance systems are particularly interesting and discussed more in detail.

INTERACTION OF ABA SIGNALING WITH BIOTIC STRESS RESPONSE PATHWAYS

In addition to the genetic interactions of ABA signaling with ethylene, gibberellins, and brassinosteroids signal transduction in embryonic and early postembryonic development (Beaudoin et al., 2000; Gazzarrini et al., 2004; Ghassemian et al., 2000; Zhang et al., 2009), novel roles of ABA signaling during pathogen infection and following defense responses against biotic stresses have been suggested (Asselbergh et al., 2008; Cao et al., 2011; Fujita et al., 2006; Ton et al., 2009). Jasmonic acid (JA) and salicylic acid (SA) are two major plant hormones regulating plant biotic stress signal transduction. Interactions between ABA and JA/SA signaling may coordinate to produce combined optimal resistant responses when plants face both abiotic and biotic stresses simultaneously (Fig. 1).

Both wound and pathogen *Botrytis cinerea* treatment induce biosynthesis of ABA, JA, and SA (Pan et al., 2008) suggesting synergistic or antagonistic interactions among these induced hormone signaling to generate defense responses. MeJA (methyl jasmonate) treatment induces stomatal closing through a *CORONATINE INSENSITIVE1* (*COI1*)- and *JASMO-NATE RESISTANT1* (*JAR1*)-dependent signaling pathway (Munemasa et al., 2007; Suhita et al., 2004). JA-triggered activation of S-type anion channel and I_{Ca}-channel activities is probably under the control of the same second messengers elicited by ABA because MeJA does not induce stomatal closures in *ABA insensitive2-1* (*abi2-1*) as well as in *coi1* (Fig. 1). In regulation of merging signals for ABA- and JA-triggered stomatal closures, guard cell abundant myrosinase THIOGLUCOSIDE GLUCOHYDROLASE1 (TGG1) may have a role. *tgg1* showed defects in ABA-inhibition of inward K^+-channel activity and stomatal opening (Zhao et al., 2008) and *ttg1 ttg2* produced reduced responses in the ABA- and JA-induced stomatal closures (Islam et al., 2009) suggesting a role of glucosinolate metabolism in the guard cell ABA signaling.

However an antagonistic effect of ABA in JA signaling was also presented based on the observation that exogenous ABA treatment repressed the expression of JA-dependent defense

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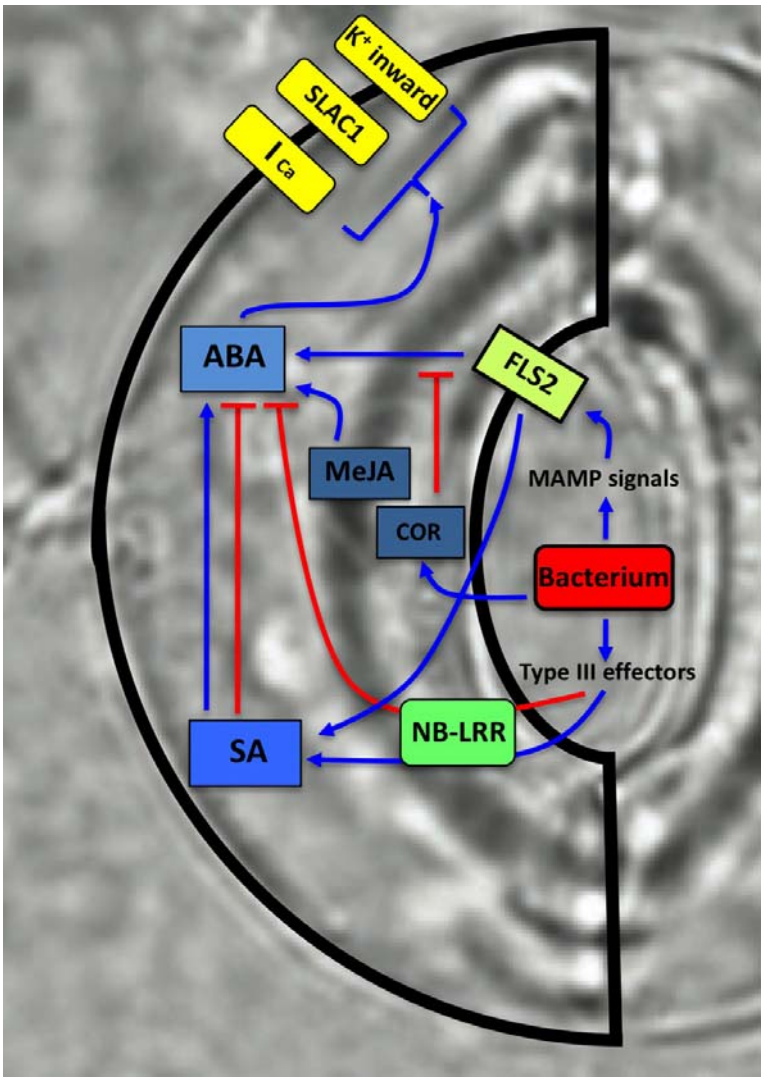

Fig. 1. Summary of guard cell signaling displaying cross-talks of ABA and biotic stress responses. Depending on the types of pathogen-plant host pairs SA signaling can affect either negatively or positively guard cell ABA signal transduction. Negative regulation of ABA signaling by NB-LRR can be independent of SA signaling. Whereas coronatine from pathogen was shown to inhibit ABA responses, MeJA treatment was reported to induce stomatal closing. See the text for more detailed discussion.

genes (Anderson et al., 2004). Additionally, mutations in the positive ABA signaling bHLH (basic Helix-Loop-Helix) transcription factor *AtMYC2* and the ABA biosynthesis gene *ABA DEFICIENT2* (*ABA2*) produced increased resistance to pathogen (Anderson et al., 2004). As a response to the flg22 perception by FLAGELLIN SENSITIVE2 (FLS2), plants close the possible bacterial entrance sites, guard cells by inducing stomatal closures (Melotto et al., 2006) and inhibiting stomatal openings (Zhang et al., 2008). The MAMP (microbe-associated molecular pattern)-triggered stomatal response requires components of ABA signal transduction *OPEN STOMATA1/SnRK2.6* (*OST1*) and G-protein subunit *GPA1* for stomatal closures and openings, respectively (Fig. 1). Stomatal closures by flg22 can be repressed by the compound coronatine secreted by *Pst*DC 3000 and also by mutations in ABA and SA biosynthesis genes (Melotto et al., 2006; Zeng et al., 2010) suggesting interactions between ABA and SA/JA signaling in guard cells.

SA is a central biotic stress hormone inducing systemic resistance to bacterial and fungus pathogens. Whereas pathogen infection induces biosynthesis of ABA and SA, an antagonistic effect of ABA on both SA biosynthesis and signaling via control of transcription was observed (de Torres Zabala et al., 2009; Fan et al., 2009; Mosher et al., 2010; Yasuda et al., 2008) (Fig. 1). Although the detailed mechanism through which ABA-SA antagonism balances the downstream responses remain under investigation, *ABA-RESPONSIVE1* (*ABR1*) expression by *Xanthomonas campestris* infection was shown to control antagonistic biosynthesis of ABA and SA and produce the disease resistance (Choi and Hwang, 2011).

Moreover findings a regulatory function of race-specific immune receptor NB-LRR proteins in drought/humidity responses support further complex interactions between ABA and SA signaling components. The CC (coiled-coil)-NB-LRR mutant *activated disease resistance1* (*adr1*) was originally isolated as a disease resistant mutant. Overexpression of *ADR1* conferred a specific drought resistant phenotype *via ENHANCED DISEASE SUSCEPTIBILITY1* (*EDS1*) and *ABA INSENSI-TIVE1* (*ABI1*) pathways (Chini et al., 2004). The Toll and interleukin-1 receptor homolog (TIR)-NB-LRR-WRKY mutant *sensitive low humidity1* (*slh1*) is hypersensitive to low humidity by induction of hyperactive disease responses (Noutoshi et al., 2005). Constitutive lesion phenotypes of the TIR-NB-LRR mutant *ssi4* (*suppressor of salicylic acid insensitivity of npr1-5*) is also suppressed by high humidity treatment perhaps *via* down-regula-

tion of the mitogen activated protein (MAP) kinases MPK3/6 (Zhou et al., 2004). Involvement of MAPK pathways during interactions between abiotic and biotic stress signaling is additionally supported by the identification of a novel allele in *KEEP ON GOING* (*KEG*) as a suppressor of enhanced disease resistance and ABA hypersensitive phenotypes of *enhanced disease resistance1* (*edr1*) (Wawrzynska et al., 2008). *EDR1* encodes a putative MAP Kinase Kinase Kinase. Humidity control of disease responses and ABA-insensitivity produced by induction of disease signaling genes was also observed in the *constitutive expression of PR genes22* (*cpr22*) mutant, of which mutation causes to express a mutant chimeric cyclic nucleotide-gated ion channel11/12 (CNGC11/12) protein (Mosher et al., 2010). Since CNGCs are known to function as ligand-gated channels maintaining cation homeostasis and Ca^{2+} transport (Kudla et al., 2010), it is plausible to hypothesize that Ca^{2+} regulation is important for cross-talks of abiotic and biotic stress signaling.

Another evidence revealing NB-LRR receptors as an important regulator of ABA signal transduction was presented based on the finding of novel small compound DFPM and the following chemical genetics approach (Kim et al., 2011) (Fig. 1). DFPM was isolated from a screen of small compound library as an ABA signaling inhibitor. Gene expression and genetic analyses of DFPM indicated that ABA signaling interference by DFPM was caused by activation of *NB-LRR* and subsequent signaling pathways. As induction of disease response pathways by DFPM efficiently interfered with guard cell ABA signal transduction, plant infection by *Pseudomonas syringae* affected negatively ABA-induction of gene expression as well as ABA-induced stomatal closures (Kim et al., 2011). Notably, analyses of pathogen signaling mutants showed that major early regulators of effector-triggered immunity pathways, including *EDS1*, *PHYTOALEXIN DEFICIENT4* (*PAD4*), *REQUIRED FOR Mla12 RESISTANCE* (*RAR1*), and *SUPPRESSOR OF G2 ALLELE OF SKP1B* (*SGT1B*), are required for the DFPM- as well as for biological *Pseudomonas*-inhibition of ABA signal transduction. SA signaling and the *EDS16* and *NONEXPRESSER OF PR GENES1* (*NPR1*) loci were not necessary for this interference of ABA signaling, demonstrating a difference to the converse ABA interference of biotic stress signaling. DFPM interference of ABA signaling occurs at the events downstream of intracellular Ca^{2+} and Ca^{2+}-activation of anion channels, where-as upstream ABA signaling events involving PYR/PYL/RCAR receptors and SnRK2 kinase activation were not affected by DFPM treatment (Kim et al., 2011) (Fig. 2). Therefore regulation of Ca^{2+}-dependent downstream signaling steps probably at the bottleneck position may adjust stress responses after simultaneous or serial perception of abiotic and biotic stimuli.

CALCIUM SIGNAL REGULATION AT THE POINT OF ABA AND DISEASE SIGNALING INTERACTIONS

By changing amplitude, duration, and frequency of Ca^{2+} transients accordingly to environmental and developmental stimuli, plant cells interpret the source of input signals and then produce proper responses (Dodd et al., 2010; Sanders et al., 2002). Considering various responses controlled by Ca^{2+} signals, plant genome is expected to encode diverse Ca^{2+} sensors and mechanisms to transmit the general secondary signals to specific target components. The model plant *Arabidopsis thaliana* contains at least 250 proteins containing EF-hand motifs that can bind Ca^{2+} ions (Day et al., 2002). Calcium-dependent protein kinases (CDPKs or gene name CPKs) receive calcium stimuli using EF-hand motifs within the calmodulin-like domain at the C-terminal. Ca^{2+}-bindings to CDPKs activate the serine-threonine kinase domain at the N-terminal and phosphorylate downstream targets to relay the signal. At low levels of calcium, the middle autoinhibitory domain, which is intervening between kinase and calmodulin-like domains, suppresses the kinase activity of CDPKs. With previous reports on the roles of CDPKs in abiotic signal transduction as well as during biotic disease stress responses (Ludwig et al., 2004), CDPK is proposed as a candidate regulator to function through Ca^{2+} signals at the point of ABA and disease signaling interactions (Kim et al., 2011). The *Arabidopsis* genome has 34 *CDPK* genes. With different affinities to Ca^{2+} ions and with diverse subcellular and tissue-specific localizations, particular groups of *CDPK*s may define a specific signaling pathway from upstream Ca^{2+} signals to downstream targets. Alternatively, *CDPK*s may play a role as a converging point of multiple signal transductions by combining different sources of Ca^{2+} signals from various stimuli into a regulation of common downstream factors. Otherwise *CDPK*s could function as nexus regulators where both Ca^{2+} signal convergence and specification occur.

CPK3 and CPK6 are the major CDPKs expressed in guard cells and regulate ABA induction of S-type anion channel and Ca^{2+}-permeable channels (Mori et al., 2006). Single cell type-based guard cell microarray analyses identified that *CPK3* and *CPK6* expressions were mainly localized in guard cells. Disruption of these two genes produced defects in ABA- and Ca^{2+}-activation of S-type anion channel. Additionally, ROS (reactive oxygen species)-activation of I_{Ca}-channel was also reduced in the *cpk3 cpk6* mutant suggesting CPK3 and CPK6 might control downstream of ROS and Ca^{2+} signaling steps in ABA signal transduction (Mori et al., 2006) (Fig. 2). Furthermore, with the pronounced phenotypes of the double mutant *cpk3 cpk6* compared to the individual single mutants, functional overlaps of *CPK3* and *CPK6* activity in guard cell ABA signal transduction were expected (Mori et al., 2006).

In contrast to the CPK3 and CPK6's function as positive regulators, another CDPK, CPK10 was reported as a negative regulator of ABA and Ca^{2+}-dependent stomatal closing responses in guard cells. The mutant *cpk10* was more sensitive to ABA and drought treatment, whereas the overexpression of *CPK10* displayed insensitive responses to both treatments (Zou et al., 2010), suggesting a complex network of CDPK's activity determines downstream of ABA signaling pathways.

While genetic evidence pointed out *CPK3, CPK6,* and *CPK10* as important regulators of S-type anion channel currents, cell biological and biochemical evidence showed that CPK21 and CPK23 directly interact with SLAC1 S-type anion channel. Phosphorylation of SLAC1 may be required to activate anion channel currents (Geiger et al., 2010). Interestingly, SLAC1 activation by CPK21 was Ca^{2+}-dependent but CPK23 activated SLAC1 in a Ca^{2+}-insensitive manner indicating a bifurcating point of Ca^{2+} signaling pathways resides here. Another anion channel SLAH3 (SLAC1 homolog3) was shown to mediate nitrate anion conductance in guard cells (Geiger et al., 2011). Patch clamp analyses of the mutant *slah3-1* showed a defect in generation of nitrate-induced anion currents. CPK21 was shown to interact with SLAH3 directly and activate SLAH3 by phosphorylation (Geiger et al., 2011). Moreover, in response to osmotic stress, CPK21 is activated for production of resistant responses against osmotic stress. The mutant *cpk21* was more resistant to osmotic stress condition and exhibited transcriptional activation of stress-related marker genes even without stresses (Franz et al., 2011).

CPK4, CPK11, and CPK32 were shown to be involved in regulation of general ABA signal transduction in tissues other

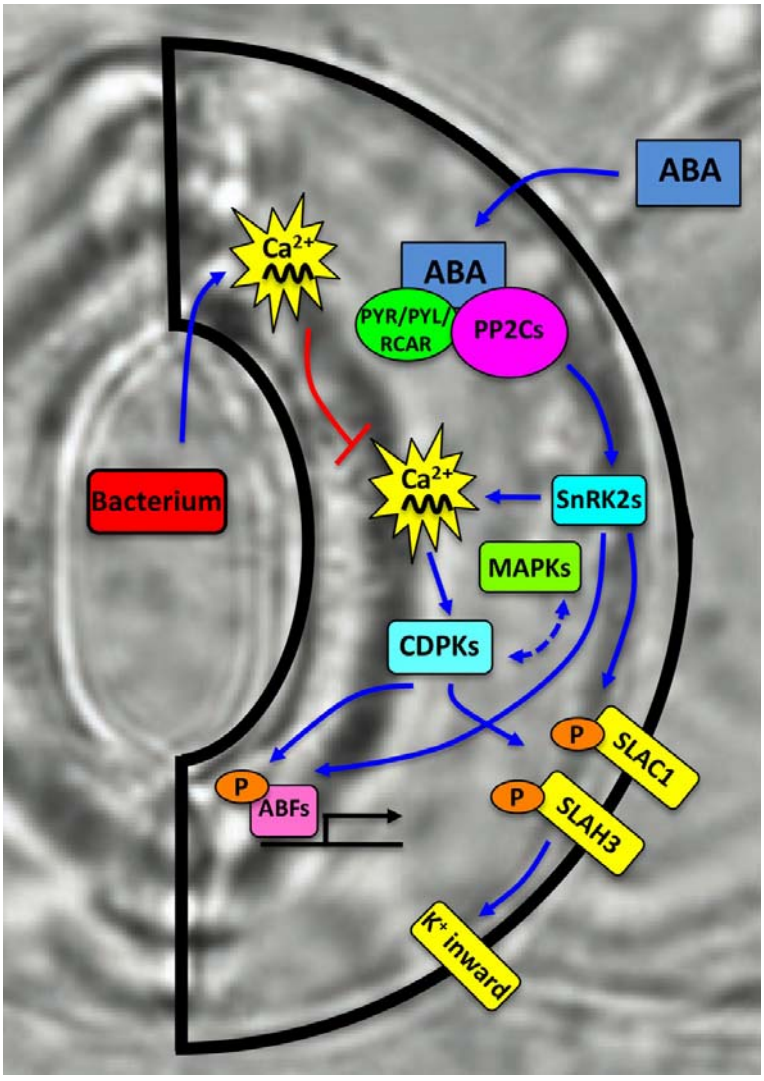

Fig. 2. A guard cell model illustrating ABA signaling from ABA perception to downstream ion channel regulation and gene expression. This model focuses on the role of internal Ca^{2+} signals and cross-regulation of protein kinases including SnRK2s, CDPKs, and MAPKs. Ca^{2+}-activation of protein kinases elicits phosphorylation of downstream targets resulting ion channel activation or induction of gene expression. Ca^{2+} signals triggered by pathogen infection affect ABA signal transduction either through cross regulation of common downstream factors or by consuming Ca^{2+} messengers of which homeostasis to be maintained for priming of guard cell ABA responses.

than guard cells. Consistent to their roles in mediating ABA signaling in many different types of tissues, CPK32 was reported to interact directly with ABA responsive bZIP transcription factor4 (ABF4) and upregulate ABA-induced gene expression (Choi et al., 2005). Similarly CPK4 and CPK11 were shown to biochemically target ABF1 and ABF4 (Zhu et al., 2007). The lack of phosphorylation of ABF1 and ABF4, reduced ABA-induction of gene expression including *ABI4, ABI5, RAB18,* and *KIN1*, and more susceptible phenotypes to drought stress in *cpk4-1 cpk11-2* (Zhu et al., 2007) well correlate with the proposed functions of CDPKs as an important positive regulator of ABA signal transduction. It is noteworthy to compare the above observation with recent results showing *cpk4-2 cpk11-2* showed a defect in Ca^{2+} oscillation-induced stomatal closures, whereas no effects on the ABA-induced stomatal closures (Hubbard et al., 2011). This discrepancy may be because different mutant alleles of *cpk4* were used for each genetic analysis. It is also intriguing that ABA-activated OST1/SnRK2.6 can phosphorylate ABF3, which is then predicted to stabilize ABF3 (Sirichandra et al., 2010). However, the presence of multiple protein kinase bands nearby CPK4 in in-gel-kinase assays (Zhu et al., 2007) indicate that a protein kinase(s) other than CPK4 or SnRK2s could also be involved in parallel to consummate the fine-tuning of ABA and biotic signal transduction.

Elevated Ca^{2+} influx and transients followed by pathogen recognition comprises a major part of disease signal transduction (Du et al., 2009). One of the major responses by infection-induced cytosolic Ca^{2+} influx is the generation of hypersensitive response (HR) and ROS production. Moreover pharmacological experiments with Calmodulin (CaM) antagonist and the *calmodulin-like24-4* (*cml24-4*) mutant demonstrated that Ca^{2+} influx mediates generation of Nitric oxide (NO) signals through activation of CaM or CML (Ma et al., 2008). For the decoding of pathogen-induced Ca^{2+} signature, AtSR1 also known as Ca^{2+}/calmodulin-binding transcription factor3 (CAMTA3) acts as a negative regulator of SA signaling-mediated HRs by binding to the promoter of the *EDS1* gene (Du et al., 2009). In addition, CDPKs could also play a role as positive regulators in disease responses by transferring Ca^{2+} signals to downstream target elements. A series of genetic and cell biological evidence proposed that CPK4 and CPK11 along with CPK5 and CPK6 might be activated by a MAMP (microbe-associated molecular patterns) signal flg22 (Boudsocq et al., 2010). Activation of CDPKs by flg22, which was dependent on the flg22 receptor

FLS2, produced innate immunity against bacterial attacks. Interestingly, *cpk5 cpk6 cpk11* loss-of-function mutants and C-terminally truncated autoactivated form of CPK5 and CPK11 demonstrated that CDPKs and MAPKs signaling pathways are independently regulated after the flg22 activation. Moreover none of the ABA-regulated genes were recovered as co-regulated genes with the CPK11-regulated genes. These data suggest that stimulus-specific signaling pathways separately exist as well as cross-talks of signaling networks impose stress responses. How the activation of same CPKs by different sources of stimuli can distinguish the proper downstream targets remains to be investigated. One possibility is that different stimuli generate different characteristics of Ca^{2+} signatures and that encodes the specificity of CPK activation and downstream target selection. Alternatively, pathway-specific regulators in the process are stimulated independent of Ca^{2+} signals and that contributes to selective activation of relevant CDPKs in specific signal transduction.

Additional function of CDPKs reported is for regulation of MeJA-induced stomatal closing. The mutant studies using *cpk3-1*, *cpk6-1*, *cpk4-1*, and *cpk11-2* indicated that only CPK6 among them specifically controls MeJA-induced stomatal movement regulation (Munemasa et al., 2011). The fact that MeJA-triggered production of ROS and NO was not affected in *cpk6-1* while Ca^{2+}-permeable cation channel and S-type anion channel were not activated by MeJA in *cpk6-1* suggested again that the position of Ca^{2+} signaling step is at downstream of ROS production and upstream of ion channel regulation.

It has been presented that activation of CDPKs as well as MAPK pathways comprise of critical steps of plant immune responses (Boudsocq et al., 2010; Wurzinger et al., 2011). Cross-talks between these two kinase pathways participate in generating diverse resistant mechanisms. Moreover MAPK pathways have been reported to be a part of abiotic stress or ABA signal transduction. Besides a role in disease signaling, the MAP kinase kinase, MKK2 was reported to function as a negative regulator of cold and salt stress signal transduction (Teige et al., 2004). In contrast, guard cell preferentially expressed *MPK9* and *MPK12* act as positive regulators of ABA signaling at downstream of ROS and Ca^{2+} signals and upstream of S-type anion channel regulation (Jammes et al., 2009). The mutant *mpk9-2 mpk12-1* provided genetic evidence displaying disruption of ABA and Ca^{2+}-stimulated stomatal closures. Furthermore *mpk9-2 mpk12-1* exhibited more susceptibility to *Pseudomonas* infections (Jammes et al., 2011) suggesting a cross-talk between ABA and disease response signaling operates through MAPK pathways. It will be informative to test a hypothesis whether MAPK pathways can be affected by CPDK activation or *vice versa* (Fig. 2).

CONCLUSIONS

Recent results demonstrate that plant biotic stress signaling rapidly interferes with ABA signal transduction through modulation of downstream Ca^{2+} signaling. Cytosolic Ca^{2+}-transient activation of ABA signal transduction can be primed by prior exposure to Ca^{2+} concentrations or to ABA treatment. For example, pre-exposure to high Ca^{2+} concentration is required to produce activation of cytosolic Ca^{2+}-induced S-type anion channels (Allen et al., 2002). ABA pre-treatment to guard cells augments the cytosolic Ca^{2+}-induced S-type anion channel activation (Chen et al., 2010; Siegel et al., 2009). This calcium sensitivity priming hypothesis can offer an explanation how different sources of cytosolic Ca^{2+} transients can antagonistically control ABA and disease signal transduction. When pathogen-induced cytosolic Ca^{2+} influx activates disease response pathways, consumption of intracellular Ca^{2+} bursts may deprime calcium sensitivity mechanisms for ABA signal transduction (Fig. 2). In an opposite way, ABA or abiotic stress-triggered signal transduction passes through intracellular Ca^{2+} messengers and this affects negatively or in some specific cases positively priming the mode of disease signal transduction. Further investigation will shed light on how Ca^{2+} signals offer cross-talks when plants face various stresses. Calcium-binding proteins especially including CDPKs could mediate network interactions and thereby maintain stress surveillance system in plants. These findings will be instrumental for dissection of abiotic and biotic stress signaling interactions and characterization of the underlying molecular mechanisms adjusting plant adaptive responses against combined abiotic and biotic stress exposures.

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