Diverse Responses Are Involved in the Defence of *Arabidopsis thaliana* against *Turnip Crinkle Virus*

Hui Yang[a,*], Shu Yuan[b], Yi Luo[a], Ji Huang[a], Yang-Er Chen[c], Xin Sun[a], Yun Huang[a], De-Hui Xi[d], and Hong-Hui Lin[d,*]

[a] Agricultural College, Sichuan Agricultural University, Chengdu 611130, China. Fax: 86-28-61386802. E-mail: yanghui981@sohu.com
[b] College of Resources and Environmental Sciences, Sichuan Agricultural University, Chengdu 611130, China
[c] College of Biology and Sciences, Sichuan Agricultural University, Ya'an 625014, China
[d] Key Laboratory of Bio-resources and Eco-environment (Ministry of Education), College of Life Science, Sichuan University, Chengdu 610064, China. Fax: 86-28-85415300. E-mail: honghuilin@hotmail.com

* Authors for correspondence and reprint requests

Z. Naturforsch. **68 c**, 148–154 (2013); received February 29, 2012/March 1, 2013

Plant hormones play pivotal roles as signals of plant-pathogen interactions. Here, we report that exogenous application of salicylic acid (SA), jasmonic acid (JA), ethephon (ETH), and abscisic acid (ABA) can reduce *Turnip crinkle virus* (TCV) accumulation in systemic leaves of *Arabidopsis thaliana* during early infection. SA and ABA are more efficient and confer a longer-lasting resistance against TCV than JA and ETH, and the plant hormones interact in effecting the plant defence. Synergistic actions of SA and JA, and SA and ET, and an antagonistic action of SA and ABA have been observed in the *Arabidopsis*-TCV interaction. ABA can down-regulate the expression of the pathogenesis-related genes *PR1* and *PDF1.2*, and compared to the wild type, it drastically reduces TCV accumulation in *NahG* transgenic plants and the *eds5-p1* mutant, both of which do not accumulate SA. This indicates that SA signaling negatively regulates the ABA-mediated defence. ABA-induced resistance against TCV is independent of SA. We also found that mitogen-activated protein kinase 5 (MPK5) may be involved in ABA-mediated defence. These results indicate that *Arabidopsis* can activate distinct signals to inhibit virus accumulation. Cooperative or antagonistic crosstalk between them is pivotal for establishing disease resistance. These results show potential to enhance the plant defence against viruses by manipulating diverse hormones.

Key words: Turnip Crinkle Virus, Salicylic Acid, Abscisic Acid, Defence Response

Introduction

Crucial roles of salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) signal pathways, respectively, in plant defence are well documented. However, it has become increasingly clear that a plant's resistance to pathogens is not brought about by the isolated activation of parallel or linear hormonal circuits, but rather is the consequence of a complex regulatory network, enabling each to collaborate with or antagonize the others (Pieterse *et al.,* 2009; De Vleesschauwer *et al.*, 2010). Furthermore, there is extensive crosstalk between plant hormone signaling pathways, such as a large number of reports suggested that the SA and JA/ET defence pathways are mutually antagonistic. However, evidence of synergistic interactions has also been reported (Van Wees *et al*., 2000; Clarke *et al*., 2009). In addition abscisic acid (ABA) has recently emerged as a key determinant in the outcome of plant-pathogen interactions. In most cases, ABA behaves as a negative regulator of disease resistance. Exogenous application of ABA increases the susceptibility to some bacterial and fungal pathogens (De Vleesschauwer *et al.*, 2010). ABA likely counteracts SA- and JA/ET-dependent basal defences (Robert-Seilaniantz *et al*., 2007). In contrast, some studies describe a positive role of ABA in the activation of defence responses. ABA primes the plant callose accumulation and enhances basal defence against *Blumeria graminis* f. sp. *hordei* and activates induced resistance in response to the necrotrophic fungus *Alternaria brassicicola* (Flors *et al*., 2008; Ton *et al*., 2009; De Vleesschauwer *et al.*, 2010). ABA can crosscommunicate with other signal pathways to fine-tune the defence response, depending on the type of attacker encountered.

Arabidopsis thaliana is the host of the *Turnip crinkle virus* (TCV). Ecotype Col-0 develops systemic symptoms, supporting TCV replication and spreading. In this study we initiated changes of SA, JA, ET, and ABA in wild-type and mutant plants by exogenous application of these phytohormones. We found that a complex network regulates the plant defence response to TCV, and diverse signals are involved in this interaction. Therefore, targeting hormone pathways and alteration of hormone levels may be an effective strategy to alter the host immunity.

Material and Methods

Plant materials and growth conditions

All *Arabidopsis* plants used in this report were in the Col-0 background. Seedlings were grown in a growth chamber at 22 °C and under 16 h light/8 h dark condition. ETH (ethephon), SA, JA, and ABA were purchased from Sigma (St. Louis, MO, USA). SA was dissolved in water. JA was dissolved in a few drops of ethanol prior to diluting in water. ABA was dissolved to 20 mM in ethanol; this stock solution was then diluted with water. For ETH treatment, the plants were sprayed with ETH dissolved in water. The concentrations used were as follows: 200 μM SA, 50 μM JA, 60 μM ETH, and 80 μM ABA. In Fig. 1, also lower concentrations were applied, *i.e.* 50 μM SA, 20 μM JA, 20 μM ETH and 25 μM ABA, respectively. The plants were sprayed daily for 3 d before TCV inoculation. Treatment with the hormones at the above concentrations did not visibly affect the growth of the plants during the whole experiment. Twelve plants were used for each treatment. The experiments were repeated three times.

Virus infection

TCV inoculum was prepared by grinding infected *A. thaliana* tissues with 2 mM potassium phosphate buffer (pH 7.0, 1:10, w/v). Two rosette leaves of comparable age of 4- to 5-week-old *A. thaliana* plants were inoculated with TCV by rubbing (Yang *et al.*, 2010a). Four uninoculated leaves were taken from each of 6 plants, mixed, and used for the analyses.

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Total RNA was extracted as described by Xi *et al.* (2007). RNA concentrations were determined spectrophotometrically at 260 nm. Total RNA (5 μg) was reverse transcribed in a 20-μl volume with 1 μl SuperScript® reverse transcriptase (Invitrogen, Carlsbad, CA, USA), then the first-strand cDNA was diluted to 40 μl. Three μl of first-strand cDNA were used as the template for PCR amplification with rTaq (TakaRa Biotech. Co. Ltd., Dalian, China) on a MyCycler gradient PCR amplifier (Bio-Rad, Richmond, CA, USA). The PCR cycles used were 35, 35, 24, and 33 for *PR1*, *PDF1.2*, *EF1α*, and *TCV-CP*, respectively. The primers for *TCV-CP* were: TCV-F, CCAGCAGACAGAAACAGACC; TCV-R, GAATGCCAGAGCCA CCTT. The primer sequences and conditions of amplification for *PR1*, *PDF1.2*, and *EF1α* were the same as previously reported (He *et al.*, 2007).

Protein extraction and Western blot analysis

Systemic leaves at different days post inoculation (dpi) were ground in liquid nitrogen and mixed with 10 volumes of extraction buffer (50 mM Tris-HCl, pH 6.8, 5% mercaptoethanol, 10% glycerol, 4% sodium dodecyl sulfate, 4 M urea) in an ice bath. The resulting slurries were centrifuged at 10,000 x *g* for 10 min at 4 °C, and the supernatants were stored at –80 °C (Xi *et al.*, 2007; Yang *et al.*, 2010b). Protein concentrations were determined as described by the Bradford method (1976).

For sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analyses, 20 μg protein from each sample were electrophoresed in 12.5% polyacrylamide gels, and then transferred electrophoretically to a nitrocellulose membrane according to Sambrook *et al.* (1989). After that, the nitrocellulose membranes were blocked with 5% non-fat milk in TBST (20 mM Tris, pH 7.5, 150 mM NaCl, 0.05% Tween-20) for 2 h and then incubated with the respective primary antibody (rabbit polyclonal antisera) for 2 h. Antisera against TCV coat protein were obtained from Prof. Anne Simon (Department of Cell Biology and Molecular Genetics, University of Maryland College Park, College Park, MD 20742, USA). After washing with TBST for three times, the nitrocellulose membranes were incubated with goat anti-rabbit IgG alkaline phosphatase conjugate (dilution 1:1,000) for 2 h. Then the secondary antibody was detected using nitro blue tetrazolium (NBT) and 5-bromo-

4-chloro-3-indolyl phosphate (BCIP) (Xi *et al.*, 2010). The intensities of the signals of Western blotting and PCR products were analysed densitometrically by a thin-layer scanner.

Statistical analysis

An independent (unpaired) Student's t-test (two-tailed) was chosen to test the significance of differences among means of small '*n*' sample sets. A difference was considered to be statistically significant when $p < 0.05$.

Results

Treatment with exogenous SA, JA, ETH, and ABA induces resistance against TCV

To analyse the effect of the plant hormones on plant defence, *Arabidopsis* plants were pretreated with SA, JA, ETH, and ABA, respectively. We determined the TCV coat protein accumulation, as a measure of the virus load, in systemic leaves at 6 dpi and 9 dpi. As seen in Fig. 1, the plants pretreated with SA, JA, ETH, and ABA were significantly protected against TCV at 6 dpi ($p < 0.05$). However, higher or lower concentrations of JA and ETH had no obvious effects on TCV accumulation at 9 dpi. Pretreatment with 200 μM SA rendered plants more resistant to TCV than 50 μM SA. Thus, a 50-μM concentration of SA was not high enough to trigger optimal resistance. On the other hand, supplying plants with 25 μM or 80 μM ABA induced relatively high levels of protection. These results showed that SA and ABA acted as powerful activators of induced resistance against TCV.

Influence of SA, JA, and ETH co-pretreatment on plant resistance against TCV

SA and JA co-pretreatment significantly inhibited TCV accumulation at 9 dpi compared to SA or JA single pretreatment (Fig. 2) ($p < 0.05$). A similar result was obtained for SA and ETH co-pretreatment, suggesting that there was a synergistic interaction between SA and JA, and between SA and ET, which subsequently induced defence against TCV. However, JA and ETH co-pretreatment increased TCV accumulation in systemic leaves. Apparently JA and ET strongly antagonized each other during defence signal transduction.

ABA-induced resistance against TCV acts through SA- and JA-independent pathways

The role of ABA in plant defence is less understood, and is sometimes controversially discussed. Since ABA significantly inhibited TCV accumulation, we determined whether the enhanced resistance was associated with defence-related gene expression. We collected systemic leaves at 9 dpi from TCV-infected plants, which had been pretreated with different phytohormones. The transcript levels of selected defence

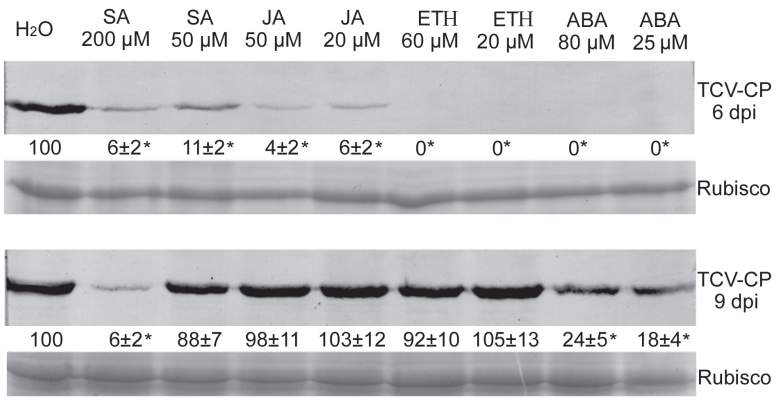

Fig. 1. TCV coat protein (TCV-CP) levels of systemic leaves pretreated with different phytohormones. TCV-CP was detected by Western blotting at 6 and 9 dpi. Coomassie blue stain of Rubisco protein was used as a loading control. All experiments were repeated three times, and typical results are presented. Quantities of infected control seedlings (H_2O) were normalized to 100. The data are presented as means ± SD ($n = 3$). The asterisks indicate statistically significant differences from the water-treated sample ($p < 0.05$).

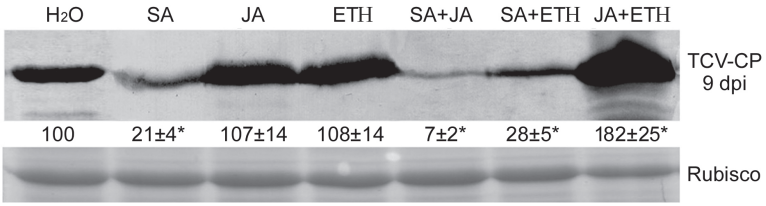

Fig. 2. TCV coat protein (TCV-CP) levels of systemic leaves co-pretreated with different phytohormones. TCV-CP was detected by Western blotting at 9 dpi. SA+JA, SA+ETH, JA+ETH mean SA and JA, SA and ETH, JA and ETH simultaneous co-pretreatment, respectively. The concentrations of SA, JA, ETH, and ABA were 200 μM, 50 μM, 60 μM, and 80 μM, respectively. Coomassie blue stain of Rubisco protein was used as a loading control. All experiments were repeated three times, and typical results are presented. Quantities of infected control seedlings (H_2O) were normalized to 100. The data are presented as means ± SD (n = 3). The asterisks indicate statistically significant differences from the water-treated sample ($p < 0.05$).

response genes were examined by RT-PCR: *PR1* for the SA signaling pathway (Uknes *et al.*, 1992) and *PDF1.2* for the JA/ET signaling pathway (Penninckx *et al.*, 1996). We found that both *PR1* and *PDF1.2* transcript levels decreased in ABA-pretreated plants (Fig. 3), suggesting that ABA-induced defence was independent of SA or JA. Otherwise, in the SA signaling mutant *eds5-1* and SA-deficient transgenic plants carrying the *NahG* gene, JA and ETH treatments had no obvious effects on TCV accumulation, implying that JA/ET-induced defence depended on SA signaling. However, ABA powerfully inhibited TCV accumulation in *NahG* and *eds5-1* plants (Fig. 4), as well as in the wild-type plants (Fig. 1), again indicating that ABA-induced resistance was independent of SA.

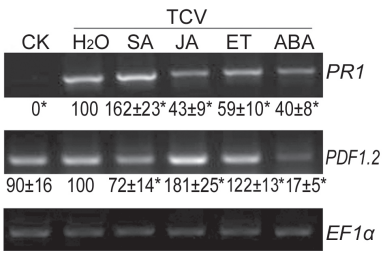

Fig. 3. RT-PCR analysis of *PR1* and *PDF1.2* transcripts on systemic leaves infected with TCV at 9 dpi. *EF1α* transcripts were used as loading controls. CK means mock-inoculation. The concentrations of SA, JA, ETH, and ABA were 200 μM, 50 μM, 60 μM, and 80 μM, respectively. All experiments were repeated three times, and typical results are presented. Quantities of infected control seedlings (H_2O) were normalized to 100. The data are presented as means ± SD (n = 3). The asterisks indicate statistically significant differences from the water-treated sample ($p < 0.05$).

Callose deposition is associated with plant defence (Ton *et al.*, 2009). Some evidence implicated ABA as a positive signal in priming callose biosynthesis or that ABA can repress bacterial induction of callose (Flors *et al.*, 2008; Ton *et al.*, 2009; De Vleesschauwer *et al.*, 2010). Therefore, we selected the ABA-insensitive *abi2-1* mutant [which deposits augmented levels of callose upon infection with *Pseudomonas syringae* (de Torres-Zabala *et al.*, 2007)] and the *abi4-1* mutant [which deposits less callose (Ton *et al.*, 2009)] for further investigations. No obvious differences in the levels of TCV coat protein could be observed among these plants (Fig. 5). The reason may be that insufficient callose deposition did not enforce cell walls sufficiently to interfere with TCV spreading in the mutants.

ABA-induced resistance against TCV requires MPK5

Mitogen-activated protein kinase 5 (MPK5) is involved in ABA-induced antioxidant defence and functions irreplaceably in ABA signaling in maize (Zhang *et al.*, 2010). In this study, we found that pretreatment of the wild type and the *mpk5* mutant with ABA produced different resistance responses to TCV. The accumulation level of TCV coat protein was much higher in *mpk5* mutants than that in the wild type at 9 dpi ($p < 0.05$) (Fig. 6). This result indicated that the ABA-inducible resistance involves MPK5.

Discussion

We found that multiple crosscommunicating signaling pathways are involved in plant defence against TCV. SA and ABA provided long-lasting resistance to TCV while JA and ETH co-pretreat-

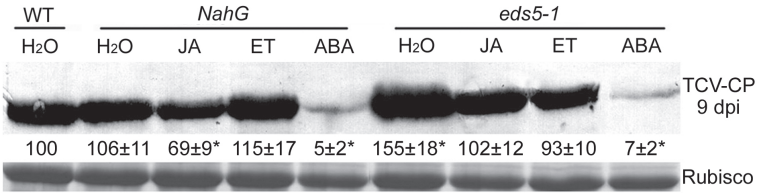

Fig. 4. TCV coat protein (TCV-CP) levels of systemic leaves in the *eds5-1* mutant and *NahG* transgenic plants co-pretreated with different phytohormones. TCV-CP was detected by Western blotting at 9 dpi. The concentrations of JA, ETH, and ABA were 50 μM, 60 μM, and 80 μM, respectively. Coomassie blue stain of Rubisco protein was used as a loading control. All experiments were repeated three times, and typical results are presented. Quantities of wild-type infected control seedlings (WT H_2O) were normalized to 100. The data are presented as means ± SD (n = 3). The asterisks indicate statistically significant differences from the water-treated wild-type sample ($p < 0.05$).

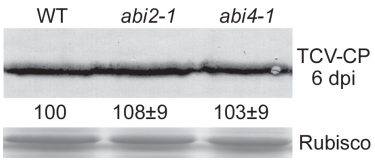

Fig. 5. TCV coat protein (TCV-CP) levels of systemic leaves in *abi2-1* and *abi4-1* mutants at 6 dpi. Coomassie blue stain of Rubisco protein was used as a loading control. All experiments were repeated three times, and typical results are presented. Quantities of wild-type infected seedlings (WT) were normalized to 100. The data are presented as means ± SD (n = 3). The asterisks indicate statistically significant differences from the wild-type sample ($p < 0.05$).

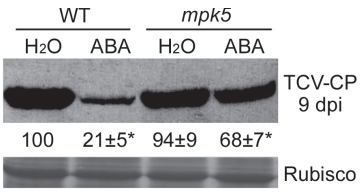

Fig. 6. TCV coat protein (TCV-CP) levels of systemic leaves in wild-type plant Col-0 and *mpk5* mutant pretreated with ABA. The ABA concentration was 80 μM. Water treatment was used as a control. Coomassie blue stain of Rubisco protein was used as a loading control. All experiments were repeated three times, and typical results are presented. Quantities of wild-type infected control seedlings (WT H_2O) were normalized to 100. The data are presented as means ± SD (n = 3). The asterisks indicate statistically significant differences from the water-treated wild-type sample ($p < 0.05$).

ment increased TCV accumulation. This is likely due to an antagonistic effect of JA and ET on the SA pathways. SA-dependent defences are primarily directed against biotrophic pathogens (which include biotrophic fungi, bacteria, and viruses), while JA-dependent and ET-dependent defences are generally effective against necrotrophic pathogens (Thomma *et al*., 2001; Rojo *et al*., 2003). However, synergistic actions of SA and JA, as well as of SA and ET were observed in response to TCV infection in our experiments. This is consistent with previous reports (Shang *et al*., 2011) suggesting that SA and JA co-pretreatment is highly inhibitory to *Cucumber mosaic virus* (CMV) replication.

ABA is generally regarded an important global abiotic stress regulator, but recently, it has also been considered a key regulator of disease resistance (Asselbergh *et al*., 2008; Ton *et al*., 2009). The role of ABA varies among different plant-pathogen interactions. ABA can act as either a positive or negative regulator in plant disease resistance. One of the reasons for this dual role appears to be the antagonistic effects of ABA on the SA, JA, and ET signaling or other biotic stress signaling (Asselbergh *et al*., 2008; Ton *et al*., 2009). For example, ABA can suppress SA-inducible defence against *Pseudomonas syringae* DC3000 (de Torres-Zabala *et al*., 2007). ABA promotes susceptibility to *Fusarium oxysporum* by suppression of JA- and ET-dependent defences (Anderson *et al*., 2004). However, perhaps the most controversial function of ABA comes from its dual role in pathogen-induced callose deposition (Ton *et al*., 2009). Some studies indicated that ABA suppresses callose deposition in *Arabidopsis* cotyledons upon treatment with bacterial flagellin (Clay *et al*., 2009; Ton *et al*., 2009). Some other studies demonstrated that ABA induces callose deposition following fungal infection (Iriti and Faoro, 2008; Kaliff *et al*., 2007). On the other hand, there are reports of a positive correlation between ABA levels and virus resistance owing to the inhibition

of the transcription of a basic β-1,3-glucanase that can degrade callose (β-1,3-glucan) (Whenham *et al.*, 1986). In our study, ABA treatments also significantly inhibited virus accumulation. ABA-inducible defence against TCV may be also partly dependent on callose deposition but not on the SA or JA/ET pathways. In the ABA signal transduction pathway, the ABI4 transcription factor acts as a positive regulator of ABA-dependent callose deposition against fungal pathogens (Kaliff *et al.*, 2007; Ton *et al.*, 2009), and ABI2 functions as a negative regulator of the ABA pathway (Merlot *et al.*, 2001; Ton *et al.*, 2009). We wanted to know whether ABI2 and ABI4 regulate callose deposition after virus infection. However, in both the *abi2-1* and the *abi4-1* mutant, no difference was found compared to the wild type. This indicates that ABI2 and ABI4 may be not involved in defence responses against TCV. Virus, bacteria, and fungi are known to activate different pathways triggering callose formation (Ton *et al.*, 2009). In addition, we found that ABA-induced resistance against TCV involved MPK5. A loss-of-function mutant of *MPK5* failed to respond to ABA with a defence reaction, compared to the wild-type plant. Thus, the ABA signaling pathway is complex, which requires further investigations.

Otherwise, recent studies demonstrated that, in addition to known defence pathways (such as SA, JA, and ET), ABA and other hormones, such as brassinosteriods (BR), auxins, gibberellins (GA), and cytokinins (CK), also play important roles in plant defence responses (López *et al.*, 2008). For example, BR treatment enhanced the resistance to Tobacco mosaic virus (TMV) in tobacco (Nakashita *et al.*, 2003). The key regulator of BR signal transduction, BAK1, is also involved in defence responses against TCV (Yang *et al.*, 2010a). Whether ABA interacts with these hormones requires further investigations.

In summary, our results and previous reports indicate that diverse phytohormones contribute to plant defence responses. Therefore, targeting hormone pathways and finding the appropriate hormone concentrations and rational blending ratios could be effective strategies to enhance host immunity. The method would also be non-toxic, broad-spectrum, and easy to perform.

Acknowledgements

We thank Dr. Dawei Zhang (Iowa State University, Ames, IA, USA) for language improvement. This work was supported by the Educational Commission of Sichuan Province (11ZA091), the National Nature Science Foundation of China (91017004, 31070210, 31000682), the Sichuan and Chengdu Nature Science Foundation (2010JQ0080, 11DXYB097JH-027), and the Doctoral Foundation of the Ministry of Education (20110181110059 and 20120181130008).

Anderson J. P., Badruzsaufari E., Schenk P. M., Manners J. M., Desmond O. J., Ehlert C., Maclean D. J., Ebert P. R., and Kazan K. (2004), Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defence gene expression and disease resistance in *Arabidopsis*. Plant Cell **16**, 3460–3479.

Asselbergh B., De Vleesschauwer D., and Höfte M. (2008), Global switches and fine-tuning ABA modulates plant pathogen defence. Mol. Plant-Microbe Interact. **21**, 709–719.

Bradford M. N. (1976), A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**, 248–254.

Clarke S. M., Cristescu S. M., Miersch O., Harren F. J. M., Wasternack C., and Mur L. A. J. (2009), Jasmonates act with salicylic acid to confer basal thermotolerance in *Arabidopsis thaliana.* New Phytol. **182**, 175–187.

Clay N. K., Adio A. M., Denoux C., Jander G., and Ausubel F. M. (2009), Glucosinolate metabolites required for an *Arabidopsis* innate immune response. Science **323**, 95–101.

de Torres-Zabala M., Truman W., Bennett M. H., Lafforgue G., Mansfield J. W., Rodriguez Egea P., Bögre L., and Grant M. (2007), *Pseudomonas syringae* pv. Tomato hijacks the *Arabidopsis* abscisic acid signaling pathway to cause disease. EMBO J. **26**, 1434–1443.

De Vleesschauwer D., Yang Y., Cruz C. V., and Höfte M. (2010), Abscisic acid-induced resistance against the brown spot pathogen *Cochliobolus miyabeanus* in rice involves MAP kinase-mediated repression of ethylene signaling. Plant Physiol. **152**, 2036–2052.

Flors V., Ton J., van Doorn R., Jakab G., Garcia-Agustin P., and Mauch-Mani B. (2008), Interplay between JA, SA and ABA signalling during basal and induced resistance against *Pseudomonas syringae* and *Alternaria brassicicola*. Plant J. **54**, 81–92.

He K., Gou X., Yuan T., Lin H. H., Asami T., Yoshida S., and Li J. (2007), BAK1 and BKK1 regulate brassinosteroid-dependent growth and brassinosteroid-independent cell death pathways. Curr. Biol. **17**, 1109–1115.

Iriti M. and Faoro F. (2008), Abscisic acid is involved in chitosan induced resistance to *Tobacco necrosis virus* (TNV). Plant Physiol. Biochem. **46**, 1106–1111.

Kaliff M., Staal J., Myrenås M., and Dixelius C. (2007), ABA is required for *Leptosphaeria maculans* resistance via ABI1 and ABI4 dependent signalling. Mol. Plant-Microbe Interact. **20**, 335–345.

López M. A., Bannenberg G., and Castresana C. (2008), Controlling hormone signaling is a plant and pathogen challenge for growth and survival. Curr. Opin. Plant Biol. **11**, 420–427.

Merlot S., Gosti F., Guerrier D., Vavasseur A., and Giraudat J. (2001), The ABI1 and ABI2 protein phosphatases 2C act in a negative feedback regulatory loop of the abscisic acid signaling pathway. Plant J. **25**, 295–303.

Nakashita H., Yasuda M., Nitta T., Asami T., Fujioka S., Arai Y., Sekimata K., Takatsuto S., Yamaguchi I., and Yoshida S. (2003), Brassinosteroid functions in a broad range of disease resistance in tobacco and rice. Plant J. **33**, 887–898.

Penninckx I. A., Eggermont K., Terras F. R., Thomma B. P., Samblanx G. W., Buchala A., Métraus J. P., Manners J. M., and Broekaert W. F. (1996), Pathogen induced systemic activation of a plant defence gene in *Arabidopsis* follows a salicylic acid-independent pathway. Plant Cell **8**, 2309–2323.

Pieterse C. M. J., Leon-Reyes A., Van der Ent S., and Van Wees S. (2009), Networking by small molecule hormones in plant immunity. Nat. Chem. Biol. **5**, 308–316.

Robert-Seilaniantz A., Navarro L., Bari R., and Jones J. D. (2007), Pathological hormone imbalances. Curr. Opin. Plant Biol. **10**, 372–379.

Rojo E., Solano R., and Sanchez-Serrano J. J. (2003), Interactions between signaling compounds involved in plant defence. J. Plant Growth Regul. **22**, 82–98.

Sambrook J., Fritsch E. F., and Maniatis T. (1989), Molecular Cloning: a Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

Shang J., Xi D. H., Xu F., Wang S. D., Cao S., Xu M. Y., Zhao P. P., Wang J. H., Jia S. D., Zhang X. W., Yuan S., and Lin H. H. (2011), A broad-spectrum, effcient and nontransgenic approach to control plant viruses by application of salicylic acid and jasmonic acid. Planta **233**, 299–308.

Thomma B., Tierens K. F. M., Penninckx I., Mauch-Mani B., Broekaert W. F., and Cammue B. P. A. (2001), Different micro-organisms differentially induce *Arabidopsis* disease response pathways. Plant Physiol. Biochem. **39**, 673–680.

Ton J., Flors V., and Mauch-Mani B. (2009), The multifaceted role of ABA in disease resistance. Trends Plant Sci. **14**, 310–317.

Uknes S., Mauch-Mani B., Moyer M., Potter S., Williams S., Dincher S., Chandler D., Slusarenko A., Ward E., and Ryal J. (1992), Acquired resistance in *Arabidopsis*. Plant Cell **4**, 645–656.

Van Wees S. C., de Swart E. A., van Pelt J. A., van Loon L. C., and Pieterse C. M. (2000), Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defence pathways in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **97**, 8711–8716.

Whenham R. J., Fraser R. S. S., Brown L. P., and Payne J. A. (1986), Tobacco mosaic virus-induced increase in abscisic acid concentration in tobacco leaves: intracellular location in light and dark green areas, and relationship to symptom development. Planta **168**, 592–598.

Xi D. H., Feng H., Lan L., Du J., Wang J., Zhang Z., Xue L., Xu W., and Lin H. H. (2007), Characterization of synergism between *Cucumber mosaic virus* and *Tobacco necrosis virus* in *Nicotiana benthamiana*. J. Phytopathol. **155**, 570–573.

Xi D. H., Yang H., Jiang Y., Xu M. Y., Shang J., Zhang Z. W., Cheng S. Y., Sang L. S., and Lin H. H. (2010), Interference between *Tobacco necrosis virus* and *Turnip crinkle virus* in *Nicotiana benthamiana*. J. Phytopathol. **158**, 263–269.

Yang H., Gou X. P., He K., Xi D. H., Du J. B., Lin H. H., and Li J. (2010a), BAK1 and BKK1 in *Arabidopsis thaliana* confer reduced susceptibility to *Turnip crinkle virus*. Eur. J. Plant Pathol. **127**, 149–156.

Yang H., Wang S. D., Xi D. H., Yuan S., Wang J. H., Xu M. Y., and Lin H. H. (2010b), Interaction between *Cucumber mosaic virus* and *Turnip crinkle virus* in *Arabidopsis thaliana.* J. Phytopathol. **158**, 833–836.

Zhang A., Zhang J., Ye N., Cao J., Tan M., Zhang J., and Jiang M. (2010), ZmMPK5 is required for the NADPH oxidase-mediated self-propagation of apoplastic H_2O_2 in brassinosteroid-induced antioxidant defence in leaves of maize. J. Exp. Bot. **61**, 4399–4411.