Abscisic Acid–Cytokinin Antagonism Modulates Resistance Against *Pseudomonas syringae* in Tobacco

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ABSTRACT

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Phytohormones are known as essential regulators of plant defenses, with ethylene, jasmonic acid, and salicylic acid as the central immunity backbone, while other phytohormones have been demonstrated to interact with this. Only recently, a function of the classic phytohormone cytokinin in plant immunity has been described in *Arabidopsis*, rice, and tobacco. Although interactions of cytokinins with salicylic acid and auxin have been indicated, the complete network of cytokinin interactions with other immunity-relevant phytohormones is not yet understood. Therefore, we studied the interaction of kinetin and abscisic acid as a negative regulator of plant immunity to modulate resistance in tobacco against *Pseudomo-*

nas syringae. By analyzing infection symptoms, pathogen proliferation, and accumulation of the phytoalexin scopoletin as a key mediator of kinetin-induced resistance in tobacco, antagonistic interaction of these phytohormones in plant immunity was identified. Kinetin reduced abscisic acid levels in tobacco, while increased abscisic acid levels by exogenous application or inhibition of abscisic acid catabolism by diniconazole neutralized kinetin-induced resistance. Based on these results, we conclude that reduction of abscisic acid levels by enhanced abscisic acid catabolism strongly contributes to cytokinin-mediated resistance effects. Thus, the identified cytokinin–abscisic acid antagonism is a novel regulatory mechanism in plant immunity.

Additional keywords: *Nicotiana tabacum*, plant–pathogen interaction.

Plant immunity is essentially regulated by the phytohormones ethylene (ET), jasmonic acid (JA), and salicylic acid (SA), which are classically regarded as the central regulatory network of plant immunity. Detailed studies on hormonal regulation of plant immunity meanwhile extended this network to interactions with other phytohormones (e.g., abscisic acid [ABA], auxin, brassinosteroids, or gibberellins). While ET and JA mainly regulate defense responses against necrotrophic pathogens, SA is essential for the defense against (hemi)biotrophs such as *Pseudomonas syringae* (21). Other phytohormones such as ABA or auxin can negatively interact with SA responses to modulate defense mechanisms (21,23). By specific effectors, *P. syringae* can directly manipulate ABA and auxin to suppress SA-related defense responses (2,6), indicating the strong interregulatory network of phytohormones in plant immunity.

Recent advances in hormonal regulation of plant immunity revealed an important role of the classic phytohormone cytokinin (CK) to induce resistance against mainly (hemi)biotrophic pathogens (1,3,9,10,20,22) but also necrotrophs (3,25). The underlying mechanisms contributing to CK-induced resistance, depending on the plant–pathogen interaction and the analyzed CKs, are not fully elucidated yet. However, known mechanisms involved in CK-mediated resistance include phytoalexin formation (10,11) or induction of SA-related responses (1,3,9,10,14). In

addition to SA interactions, an antagonistic relation between CKs and auxin in the resistance of *Arabidopsis* against *P. syringae* has been shown (20), an antagonism also found in developmental processes. These findings of synergistic and antagonistic interactions of CKs with SA and auxin, respectively, clearly indicate that interactions with other phytohormones for the regulation of plant immunity through CKs also have to be considered.

ABA is well-known as “abiotic stress-related” phytohormone but also has been described as an important modulator of various defense reactions (27); in particular, as negative regulator of SA-dependent defenses (e.g., against *P. syringae*) (5,7,19,28). Interestingly, ABA and CKs seem to have opposite impacts on certain processes relevant for pathogen resistance such as stomatal closure (26) or callose deposition (3,6). Despite this information and findings in *Persea americana* that CKs can regulate the degradation of ABA eventually to phaseic acid (4), which mainly depends on ABA-8′-hydroxylase activity (16,24), no direct interactions of these two phytohormones with respect to plant immunity have been addressed thus far.

Here, we demonstrate that kinetin treatment reduces ABA levels in tobacco, which contributes to CK-induced resistance against *Pseudomonas syringae*. By simultaneous application of ABA or inhibition of ABA catabolism, this kinetin-dependent resistance was neutralized, resulting in enhanced symptom development and *P. syringae* proliferation as well as reduced accumulation of the phytoalexin scopoletin. These findings support the impact of this novel mechanism contributing to CK-induced resistance in tobacco and, based on a strong CK–ABA antagonism, allow further integration of CKs into the phytohormone network as key interface which regulates plant immunity.

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* The e-Xtra logo stands for “electronic extra” and indicates that Figures 2, 3, and 5 appear in color online.

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MATERIALS AND METHODS

Plant material. Tobacco (*Nicotiana tabacum* L. 'Petit Havana SR1') plants were grown under greenhouse conditions at 20 to 24°C and a day and night cycle of 16 and 8 h, respectively, with supplemented lighting (Envirolite 200-W 6400K fluorescent lamps), as described before (10). Plants were cultivated on Naturahum potting soil (Ostendorf Gärtnereierden GmbH) supplied once per week with Hakaphos Grün fertilizer (COMPO EXPERT GmbH). After germination, seedlings were grown in standard pots of 9 cm in diameter and were transferred to pots of 13 cm in diameter $\approx$ 1 month later. Water was supplied by table flooding once per day. Relative humidity was not specifically regulated.

Fig. 1. Accumulation of abscisic acid (ABA) in tobacco leaves post ABA or kinetin (Kin) modulation or *Pseudomonas syringae* pv. *tabaci* infection. ABA levels (ng g⁻¹ fresh weight [FW]) in tobacco leaves treated with **A**, 10 μ M Kin or mock and infection with *P. syringae* pv. *tabaci* at 10⁶ CFU ml⁻¹ or mock treatment; **B**, 10 μ M ABA, 10 μ M Kin, 10 μ M Kin + 10 μ M ABA, or mock treatment; and **C**, 5 μ M Din, 10 μ M Kin, 10 μ M Kin + 5 μ M Din, or mock. Mean values $\pm$ standard error of minimum three independent biological experiments consisting of two technical replicates each. Letters indicate different significance groups based on paired Student's *t* test (*P* < 0.05).

Leaves of comparable developmental status were randomly selected from the middle part of 6- to 8-week-old plants and used for the experiments. A maximum of two leaves per plant was detached and used for petiole feeding experiments. The leaves were randomly placed in 200-ml glasses filled with phytohormone suspensions, mock control, or tap water, respectively. The glasses with the leaves were placed on a light shelf equipped with common light tubes at similar conditions as applied in the greenhouse (day and night cycle of 16 and 8 h, respectively, at $\approx$ 24°C).

Pathogen experiments. A tetracycline-resistant strain of the virulent hemibiotrophic pathogen *P. syringae* pv. *tabaci* (10) was used for this study. An exponentially growing culture of *P. syringae* pv. *tabaci* in Luria-Bertani (LB) medium containing tetracycline at 20 mg liter⁻¹ was harvested and adjusted to 10⁶ CFU ml⁻¹ in 10 mM MgCl₂. The prepared suspension was infiltrated with a needleless syringe from the abaxial side of 6- to 8-week-old detached tobacco leaves at three individual sites per leaf half. Symptom development was determined up to 12 days postinfection (dpi) and was classified as described before (10). The seven categories represent different degrees of visible infection symptoms (0 = no symptoms, 0.5 = $\leq$ 75% chlorosis, 1 = >75% chlorosis, 2 = $\leq$ 10% necrosis, 3 = 10 to 50% necrosis, 4 = 51 to 75% necrosis, and 5 = 76 to 100% necrosis). Differences in symptom development based on this scale were used to determine effects of applied treatments on *P. syringae* pv. *tabaci* infection. As mock controls, leaves were infiltrated with 10 mM MgCl₂.

Exogenous phytohormone and diniconazole application. Phytohormones were applied in 10 μ M concentration to tobacco leaves via petiole feeding, as described before (10). Kinetin (100 mM stock solution in 0.5 M NaOH; Duchefa) and *cis-trans*-ABA (100 mM stock solution in ethanol; Duchefa) were diluted with tap water to final concentrations. Treatments of kinetin or ABA, including mock treatments as control, were performed 24 h prior to infection with *P. syringae* pv. *tabaci*. After infection, leaves were transferred to fresh tap water.

For parallel kinetin and diniconazole treatments, *P. syringae* pv. *tabaci* suspensions or MgCl₂ were supplemented with 5 μ M diniconazole (10 mM stock solution in methanol; Fluka) immediately before infiltration. Control infections were performed using the same *P. syringae* pv. *tabaci* suspensions supplemented with corresponding amounts of methanol as mock treatment. Diniconazole-supplemented and control infections were performed on separate leaf halves of the same leaves, fed with kinetin or mock treatment.

Instrumental analyses. Phytohormones and phytoalexins were determined from pooled samples of a minimum of three leaves treated in parallel. In the case of infiltrations (diniconazole or *P. syringae* pv. *tabaci* infection), only treated zones were harvested. ABA and scopoletin were extracted and analyzed as reported before (8).

Statistics. Statistical analyses were performed for datasets deriving from a minimum of three independent biological experiments that were performed at different times using individual plant batches and bacterial cultures. Symptom development was scored in a minimum of 25 individual leaves per indicated treatment, bacterial growth was determined in three individual sets of three technical replicates per biologically independent experiment, and individual samples for instrumental analyses were derived from three leaves treated in parallel. A paired Student's *t* test was used to compare group differences. *P* values of <0.05 were considered significant.

RESULTS AND DISCUSSION

ABA antagonizes CK-mediated resistance. We demonstrated that kinetin induces resistance in tobacco against *P. syringae* pv. *tabaci* mediated by increased levels of the phytoalexins capsidiol

and scopoletin initially independent from SA accumulation (10). Interestingly, ABA levels in the identical experimental samples are significantly reduced by kinetin pretreatment, which is already evident at 24 h, when the kinetin treatment ends and *P. syringae* pv. *tabaci* infection is performed (Fig. 1A). Although ABA levels seem to be generally increased during the experiment (48 h), the reduction of ABA levels by kinetin is robust in control as well as in *P. syringae* pv. *tabaci*-infected samples. Because ABA is well known as a negative regulator of defense responses against *P. syringae* (5,7,19,27,28), these reduced levels post kinetin treatment could additionally contribute to CK-mediated resistance effects, next to the induction of phytoalexins or SA (1,3,9–11).

To prove that reduced ABA levels directly contribute to kinetin-induced resistance, we aimed to antagonize the kinetin effect by parallel application of ABA. Therefore, tobacco leaves were treated with kinetin, ABA, or both phytohormones simultaneously. ABA levels showed a strong transient increase in the tissue by petiole feeding at 24 h, the time point of infection (Fig. 1B), while the ABA levels declined to physiological levels at 48 h (24 h after end of petiole feeding). The differential impact of kinetin and ABA on plant immunity becomes obvious by symptom development of *P. syringae* pv. *tabaci* infection at 6 and 12 dpi (Fig. 2A and B). Kinetin (Kin) treatment strongly suppressed symptom development as expected, while ABA enhanced it. The combined treatment of kinetin and ABA (Kin+ABA)

Fig. 2. Abscissic acid (ABA) and kinetin (Kin) antagonistically modulate resistance against *Pseudomonas syringae* pv. *tabaci* in tobacco. Exemplary symptom development of *P. syringae* pv. *tabaci* infection at **A**, 6 and **B**, 12 days postinfection (dpi) in tobacco leaves treated with 10 μ M ABA, 10 μ M Kin, 10 μ M Kin + 10 μ M ABA, or mock. Average symptom development of *P. syringae* pv. *tabaci* infection at **C**, 6 and **D**, 12 dpi in tobacco leaves treated with 10 μ M ABA, 10 μ M Kin, 10 μ M Kin + 10 μ M ABA, or mock. Mean values $\pm$ standard error of a minimum of three independent biological experiments representing a minimum of 75 infections in total. Letters indicate different significance groups based on paired Student's *t* test ($P < 0.05$). **E**, *P. syringae* pv. *tabaci* proliferation at 0 to 96 h postinfection (hpi) in tobacco leaves treated with 10 μ M ABA, 10 μ M Kin, 10 μ M Kin + 10 μ M ABA, or mock. Mean values $\pm$ standard error of three independent biological experiments consisting of three sets of three technical replicates each. Letters indicate different significance groups per time-point based on paired Student's *t* test ($P < 0.05$).

resulted in a similar symptom development compared with the mock-treated leaves (Fig. 2A and B). This is also evident from the average symptom development of leaves following these treatments, which show statistically significant inhibition of symptoms by kinetin and increase through ABA at 6 and 12 dpi, respectively, while the combined treatment results in minor changes at 6 dpi and even no significant changes compared with the control at 12 dpi (Fig. 2C and D). These data clearly indicate an antagonistic effect of kinetin and ABA which, as single treatments, inhibit or enhance symptom development, respectively, whereas the combined treatment shows symptom development on the level of mock-treated leaves. This antagonism is strongly supported by analysis of pathogen proliferation (Fig. 2E). Similar to symptom development, pathogen growth is considerably inhibited by kinetin treatment, whereas ABA enhances *P. syringae* pv. *tabaci* proliferation. At all analyzed time points, the combined treatment (Kin+ABA) caused no significant differences in *P. syringae* pv. *tabaci* proliferation compared with the mock treatment (Fig. 2E). Thus, on the level of pathogen growth, kinetin and ABA appear to neutralize each other, eventually causing similar effects on infection symptoms. This strongly indicates the significant contribution of reduced ABA levels to CK-mediated resistance effects.

Inhibition of ABA catabolism counteracts CK-mediated resistance. ABA levels were reduced by kinetin already at 24 h (directly before *P. syringae* pv. *tabaci* infection), whereas mock treatment at this time point was comparable with the 0-h control,

and kinetin treatment was efficiently counteracting the general ABA increase at later time points (Fig. 1A). This suggests that kinetin induces ABA degradation rather than inhibiting ABA synthesis. To confirm this hypothesis, which would be in agreement with findings in *Persea americana* (4), inhibition of ABA catabolism also should have a neutralizing effect on kinetin-induced resistance. Therefore, the activity of the key ABA catabolic enzyme ABA-8'-hydroxylase (16,24) was inhibited by application of diniconazole (15,18). While kinetin significantly reduced ABA levels compared with the mock control, diniconazole (Din) significantly increased ABA levels at 24 and 48 h postapplication (Fig. 1C). The combined treatment of kinetin and diniconazole (Kin+Din) showed no significant difference in ABA accumulation compared with the mock treatment at 24 h, which corresponds to the time frame of kinetin pretreatment and establishment of kinetin-mediated resistance effects in previously described experiments (see above). By applying diniconazole simultaneously to the infection, its direct impact on symptom development and pathogen proliferation during kinetin-induced resistance was analyzed. Diniconazole treatment clearly caused enhanced symptom development in tobacco leaves challenged with *Pseudomonas syringae* pv. *tabaci* at 6 and 12 dpi for both mock- and kinetin-treated leaves (Fig. 3A and B, right leaf halves) compared with the mock controls (Fig. 3A and B, left leaf halves). Also, the average symptom development of all leaves showed significantly enhanced symptom development caused by diniconazole compared with the corresponding mock controls at

Fig. 3. Diniconazole (Din) counteracts kinetin (Kin)-induced resistance against *Pseudomonas syringae* pv. *tabaci* in tobacco. Exemplary symptom development of *P. syringae* pv. *tabaci* infection at **A**, 6 and **B**, 12 days postinfection (dpi) in tobacco leaves treated with 5 μ M Din, 10 μ M Kin, 10 μ M Kin + 5 μ M Din, or mock. Average symptom development of *P. syringae* pv. *tabaci* infection at **C**, 6 and **D**, 12 dpi in tobacco leaves treated with 5 μ M Din, 10 μ M Kin, 10 μ M Kin + 5 μ M Din, or mock. Mean values $\pm$ standard error of three independent biological experiments representing 105 infections in total. Letters indicate different significance groups based on paired Student's *t* test ($P < 0.05$). **E**, *P. syringae* pv. *tabaci* proliferation at 0 to 96 h postinfection (hpi) in tobacco leaves treated with 5 μ M Din, 10 μ M Kin, 10 μ M Kin + 5 μ M Din, or mock. Mean values $\pm$ standard error of three independent biological experiments consisting of three sets of three technical replicates each. Letters indicate different significance groups per time-point based on paired Student's *t* test ($P < 0.05$).

6 and 12 dpi (Fig. 3C and D). Concomitantly, *P. syringae* pv. *tabaci* growth reveals similar effects of kinetin and diniconazole to limit or enhance pathogen proliferation, respectively (Fig. 3E). However, *P. syringae* pv. *tabaci* proliferation is more strongly enhanced by diniconazole in kinetin-treated samples at 48 to 96 h postinfection (hpi) compared with the mock-treated controls, in which the diniconazole effect only becomes visible at late time points (72 and 96 hpi).

Consistent with the theory of kinetin-induced ABA-8'-hydroxylase activity, the effect of diniconazole in kinetin samples seems to be higher compared with mock controls, as is evident by significantly increased ratios (Kin+Din/Kin+Mock higher than Mock+Din/Mock+Mock) of average symptom development and pathogen proliferation at specific time points (Table 1). No significant difference of diniconazole impact on symptom development was observed at 6 dpi because symptoms were increased by diniconazole 2.58-fold (± 0.23) after kinetin treatment and 2.66-fold (± 0.22) in mock controls ($P = 0.45$). In contrast, diniconazole had a significantly stronger impact on symptom development in kinetin-treated leaves. After kinetin treatment, symptoms at 12 dpi were increased by diniconazole 2.25-fold (± 0.24) but only a slight increase of 1.07-fold (± 0.03) was observed in mock controls ($P < 0.001$). In addition to the diniconazole effect on symptom development, pathogen proliferation was significantly enhanced by diniconazole in kinetin samples. Diniconazole enhanced *P. syringae* pv. *tabaci* growth 2.81-fold at 48 hpi and 3.37-fold at 72 hpi after kinetin treatment but, after mock treatment, only 1.15-fold ($P = 0.02$) and 1.91-fold ($P = 0.04$), respectively. Corresponding ratios (Din/Mock) at other experimental time points showed no significant differences in infection symptoms or *P. syringae* pv. *tabaci* proliferation (Table 1). This increased effect of diniconazole as inhibitor of ABA catabolism post kinetin treatment strongly supports the induction of ABA catabolism through kinetin, which is prevented by diniconazole treatment.

Slightly different dynamics of symptom development and pathogen proliferation between kinetin versus ABA and kinetin versus diniconazole experiments may result from differences in ABA levels in the tissue through ABA or diniconazole treatment (Fig. 1B and C), or from the infection dynamics because kinetin versus ABA treatments are pretreatments 24 h prior to infection and kinetin versus diniconazole treatments started parallel to the infection.

ABA-CK antagonism as a regulatory mechanism of plant immunity. In agreement with our finding that kinetin and ABA antagonistically affect *P. syringae* pv. *tabaci* infection, levels of capsidiol, which is one of two important tobacco phytoalexins mediating kinetin-induced resistance against *P. syringae* pv. *tabaci* (10), was shown to be reduced by ABA in wild tobacco (17). Thus, it was interesting whether this CK-ABA antagonism is also observable at the level of the second phytoalexin involved

in kinetin-induced resistance; namely, scopoletin. While kinetin treatment showed the known induction of scopoletin accumulation, parallel treatment of ABA or diniconazole reversed this effect completely, because both parallel treatments caused scopoletin levels comparable with the mock control or even below that (Fig. 4A and B). This opposite impact of kinetin and ABA on phytoalexin accumulation strongly supports and at least partially explains the antagonism of these two phytohormones in plant immunity; in particular, for resistance against *P. syringae* pv. *tabaci*. The described antagonistic effect of diniconazole as inhibitor of ABA catabolism additionally strengthens the theory of kinetin-induced ABA-8'-hydroxylase activity to cause resistance through decreased ABA levels. Our data clearly demonstrate an antagonistic interaction between CKs and ABA in plant immunity, which has not been described thus far. Our data suggest that kinetin acts directly on ABA catabolism, causing reduced levels of this phytohormone, known to negatively regulate the resistance against *P. syringae*. The impact of ABA catabolism in this aspect also confirms the importance of its regulation during stress situations, which has been described mainly for abiotic interactions (13,24). Together with interactions of CKs and the phytohormones SA (1,3,9,10,14) and auxin (20), the identified antagonism with ABA extends the concept of phytohormonal regulation of the defense against (hemi)biotrophic pathogens by an additional component (Fig. 5). This antagonism between CKs and ABA seems to be very important, because both phytohormones affect several defense mechanisms such as callose deposition (3,6), stomatal closure (26), or the accumulation of phytoalexins (10,17, this study). Interestingly, the strong inter-

TABLE 1. Impact of diniconazole (Din) on *Pseudomonas syringae* pv. *tabaci* proliferation^a

Time (hpi)	Ratio	
	Mock+Din/Mock+Mock	Kin+Din/Kin+Mock
0	1.14 $\pm$ 0.13	1.17 $\pm$ 0.10
24	1.48 $\pm$ 0.34	0.95 $\pm$ 0.16
48	1.15 $\pm$ 0.21	2.81 $\pm$ 0.74*
72	1.91 $\pm$ 0.67	3.37 $\pm$ 0.60*
96	2.37 $\pm$ 0.83	1.97 $\pm$ 0.32

^a Ratios of average *P. syringae* pv. *tabaci* proliferation of Din to mock-treated leaves post kinetin (Kin) or mock treatment at 0 to 96 h postinfection (hpi) as mean values $\pm$ standard error of three independent biological experiments representing in a total of nine individual ratios between mean values of sets of three technical replicates; * indicates significant difference based on paired, one-sided Student's *t* test ($P < 0.05$).

Fig. 4. Scopoletin accumulation in tobacco is antagonistically regulated by kinetin (Kin) and abscisic acid (ABA). Scopoletin accumulation (nmol g⁻¹ fresh weight [FW]) in tobacco leaves treated with **A**, 10 μM ABA or 10 μM Kin and **B**, 5 μM diniconazole (Din) or 10 μM Kin. Mean values $\pm$ standard error of three independent biological experiments consisting of two technical replicates each. Letters indicate different significance groups based on paired Student's *t* test ($P < 0.05$).

Fig. 5. Integration of the abscisic acid–cytokinin (ABA–CK) antagonism into the phytohormone network of plant immunity regulation. The model includes recent information of direct and indirect interactions of cytokinins in plant immunity. Aspects of the novel ABA–CK antagonism addressed in this study are indicated in gray. SA = salicylic acid.

connection of these phytohormones and its importance, particularly the action of ABA, auxin, and CKs on SA-related reactions, becomes also evident by the function of *P. syringae* type III effectors (Fig. 5). In addition to auxin (AvrRpt2) (2) or ABA (AvrPtoB) (6) as well-known targets of such effectors, only recently, the action of the *P. syringae* effector HopQ1 on CK signaling to interfere with plant immunity has been observed (12). Finally, our findings extend the knowledge on and underline the significance of CK function in plant–pathogen interactions. Furthermore, we identified additional integration sites for this novel player in the regulatory network of phytohormones in plant immunity.

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LITERATURE CITED

- Argueso, C. T., Ferreira, F. J., Eppe, P., To, J. P. C., Hutchison, C. E., Schaller, G. E., Dangel, J. L., and Kieber, J. J. 2012. Two-component elements mediate interactions between cytokinin and salicylic acid in plant immunity. *PLoS Genet.* 8:e1002448.
- Chen, Z., Agnew, J. L., Cohen, J. D., He, P., Shan, L., Sheen, J., and Kunkel, B. N. 2007. *Pseudomonas syringae* type III effector AvrRpt2 alters *Arabidopsis thaliana* auxin physiology. *Proc. Natl. Acad. Sci. USA* 104:20131–20136.
- Choi, J., Huh, S. U., Kojima, M., Sakakibara, H., Paek, K. H., and Hwang, I. 2010. The cytokinin-activated transcription factor ARR2 promotes plant immunity via TGA3/NPR1-dependent salicylic acid signaling in *Arabidopsis*. *Dev. Cell* 19:284–295.
- Cowan, A. K., Cairns, A. L. P., and Bartels-Rahm, B. 1999. Regulation of abscisic acid metabolism: Towards a metabolic basis for abscisic acid-cytokinin antagonism. *J. Exp. Bot.* 50:595–603.
- de Torres Zabala, M., Bennett, M. H., Truman, W. H., and Grant, M. R. 2009. Antagonism between salicylic and abscisic acid reflects early host-pathogen conflict and moulds plant defence responses. *Plant J.* 59:375–386.
- 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.
- Fan, J., Hill, L., Crooks, C., Doerner, P., and Lamb, C. 2009. Absciscic acid has a key role in modulating diverse plant–pathogen interactions. *Plant Physiol.* 150:1750–1761.
- Großkinsky, D. K., Albacete, A., Jammer, A., Krbez, P., van der Graaff, E., Pfeifhofer, H., and Roitsch, T. 2014. A rapid phytohormone and phytoalexin screening method for physiological phenotyping. *Mol. Plant.*

- 7:1053–1056.
- Großkinsky, D. K., Edelsbrunner, K., Pfeifhofer, H., van der Graaff, E., and Roitsch, T. 2013. Cis- and trans-zeatin differentially modulate plant immunity. *Plant Signal. Behav.* 8:e24798.
- Großkinsky, D. K., Naseem, M., Abdelmohsen, U. R., Plickert, N., Engelke, T., Griebel, T., Zeier, J., Novák, O., Strnad, M., Pfeifhofer, H., van der Graaff, E., Simon, U., and Roitsch, T. 2011. Cytokinins mediate resistance against *Pseudomonas syringae* in tobacco through increased antimicrobial phytoalexin synthesis independent of salicylic acid signaling. *Plant Physiol.* 157:815–830.
- Großkinsky, D. K., van der Graaff, E., and Roitsch, T. 2012. Phytoalexin transgenics in crop protection—Fairy tale with a happy end? *Plant Sci.* 195:54–70.
- Hann, D. R., Domínguez-Ferreras, A., Motyka, V., Dobrev, P. I., Schornack, S., Jehle, A., Felix, G., Chinchilla, D., Rathjen, J. P., and Boller, T. 2014. The *Pseudomonas* type III effector HopQ1 activates cytokinin signaling and interferes with plant innate immunity. *New Phytol.* 201:585–598.
- Ji, X., Dong, B., Shiran, B., Talbot, M. J., Edlington, J. E., Hughes, T., White, R. G., Gubler, F., and Dolferus, R. 2011. Control of abscisic acid catabolism and abscisic acid homeostasis is important for reproductive stage stress tolerance in cereals. *Plant Physiol.* 156:647–662.
- Jiang, C. J., Shimono, M., Sugano, S., Kojima, M., Liu, X., Inoue, H., Sakakibara, H., and Takatsui, H. 2013. Cytokinins act synergistically with salicylic acid to activate defense gene expression in rice. *Mol. Plant-Microbe Interact.* 26:287–296.
- Kitahata, N., Saito, S., Miyazawa, Y., Umezawa, T., Shimada, Y., Min, Y. K., Mizutani, M., Hirai, N., Shinozaki, K., Yoshida, S., and Asami, T. 2005. Chemical regulation of abscisic acid catabolism in plants by cytochrome P450 inhibitors. *Bioorg. Med. Chem.* 13:4491–4498.
- Kushiro, T., Okamoto, M., Nakabayashi, K., Yamagishi, K., Kitamura, S., Asami, T., Hirai, N., Koshiba, T., Kamiya, Y., and Nambara, E. 2004. The *Arabidopsis* cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: Key enzymes in ABA catabolism. *EMBO J.* 23:1647–1656.
- Mialoundama, A. S., Heintz, D., Debayle, D., Rahier, A., Camara, B., and Bouvier, F. 2009. Absciscic acid negatively regulates elicitor-induced synthesis of capsidiol in wild tobacco. *Plant Physiol.* 150:1556–1566.
- Mizutani, M., and Todoroki, Y. 2006. ABA 8′-hydroxylase and its chemical inhibitors. *Phytochem. Rev.* 5:385–404.
- Mohr, P. G., and Cahill, D. M. 2007. Suppression by ABA of salicylic acid and lignin accumulation and the expression of multiple genes, in *Arabidopsis* infected with *Pseudomonas syringae* pv. *tomato*. *Funct. Integr. Genomics* 7:181–191.
- Naseem, M., Philippi, N., Hussain, A., Wangrosch, G., Ahmed, N., and Dandekar, T. 2012. Integrated systems view on networking by hormones in *Arabidopsis* immunity reveals multiple crosstalk for cytokinin. *Plant Cell* 24:1793–1814.
- Pieterse, C. M. J., Leon-Reyes, A., van der Ent, S., and van Wees, S. C. M. 2009. Networking by small-molecule hormones in plant immunity. *Nat. Chem. Biol.* 5:308–316.
- Reusche, M., Klásková, J., Thole, K., Truskina, J., Novák, O., Janz, D., Strnad, M., Spíchal, L., Lipka, V., and Teichmann, T. 2013. Stabilization of cytokinin levels enhances *Arabidopsis* resistance against *Verticillium longisporum*. *Mol. Plant-Microbe Interact.* 26:850–860.
- Robert-Seilanianz, A., Grant, M., and Jones, J. D. G. 2011. Hormone crosstalk in plant disease and defense: More than just a jasmonate-salicylate antagonism. *Annu. Rev. Phytopathol.* 49:317–343.
- Seiler, C., Harshavardhan, V. T., Rajesh, K., Reddy, P. S., Strickert, M., Rolletschek, H., Scholz, U., Wobus, U., and Sreenivasulu, N. 2011. ABA biosynthesis and degradation contributing to ABA homeostasis during barley seed development under control and terminal drought-stress conditions. *J. Exp. Bot.* 62:2615–2632.
- Swartzberg, D., Kirshner, B., Rav-David, D., Elad, Y., and Granot, D. 2008. *Botrytis cinerea* induces senescence and is inhibited by auto-regulated expression of the *IPT* gene. *Eur. J. Plant Pathol.* 120:289–297.
- Tanaka, Y., Sano, T., Tamaoki, M., Nakajima, N., Kondo, N., and Hasegawa, S. 2006. Cytokinin and auxin inhibit abscisic acid induced stomatal closure by enhancing ethylene production in *Arabidopsis*. *J. Exp. Bot.* 57:2259–2266.
- Ton, J., Flors, V., and Mauch-Mani, B. 2009. The multifaceted role of ABA in disease resistance. *Trends Plant Sci.* 14:310–317.
- Yasuda, M., Ishikawa, A., Jikumaru, Y., Seki, M., Umezawa, T., Asami, T., Maruyama-Nakashita, A., Kudo, T., Shinozaki, K., Yoshida, S., and Nakashita, H. 2008. Antagonistic interaction between systemic acquired resistance and the abscisic acid-mediated abiotic stress response in *Arabidopsis*. *Plant Cell* 20:1678–1692.