DL- β -Aminobutyric Acid–Induced Resistance of Potato Against *Phytophthora infestans* Requires Salicylic Acid but Not Oxylipins

Lennart Eschen-Lippold, Simone Altmann, and Sabine Rosahl

Leibniz Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle (Saale), Germany

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Inducing systemic resistance responses in crop plants is a promising alternative way of disease management. To understand the underlying signaling events leading to induced resistance, functional analyses of plants defective in defined signaling pathway steps are required. We used potato, one of the economically most-important crop plants worldwide, to examine systemic resistance against the devastating late blight pathogen *Phytophthora infestans*, induced by treatment with DL- β -aminobutyric acid (BABA). Transgenic plants impaired in either the 9-lipoxygenase pathway, which produces defense-related compounds, or the 13-lipoxygenase pathway, which generates jasmonic acid–derived signals, expressed wild-type levels of BABA-induced resistance. Plants incapable of accumulating salicylic acid (SA), on the other hand, failed to mount this type of induced resistance. Consistently, treatment of these plants with the SA analog 2,6-dichloroisonicotinic acid restored BABA-induced resistance. Together, these results demonstrate the indispensability of a functional SA pathway for systemic resistance in potato induced by BABA.

New methods or agents for controlling plant diseases are of great agricultural interest due to recurring severe crop losses caused by various plant pathogens, including bacteria, viruses, oomycetes, and fungi. In the case of potato (*Solanum tuberosum* L.), one of the most important crop plants world wide (according to the United Nations Food and Agriculture Organization), infections with *Phytophthora infestans*, the causal agent of late blight disease, lead to great annual yield losses despite the intense use of protectants (Dowley et al. 2008; Fry and Goodwin 1997; Garelik 2002). To produce long-term resistance in potato, plant breeders generated cultivars harboring resistance (*R*) genes from the wild relative *Solanum demissum* (Park et al. 2009; Turner 2005). According to the gene-for-gene model (Flor 1971), these mediate specific recognition of those *P. infestans* races that express the corresponding avirulence proteins (Armstrong et al. 2005; Oh et al. 2009; van Poppel et al. 2008). Generally, upon *R* protein–induced signaling, strong defense responses are mounted, which may include an oxidative burst, defense-gene activation and local hypersen-

sitive cell death, leading to defeat of the attacker (Dangl and Jones 2001). Up to now, attempts to generate durable *R* gene–mediated resistance in potato against *P. infestans* failed, due to the high adaptability of the pathogen (Fry 2008; Kamoun 2003). The speed of adaptation might be explained by the presence of fast-evolving families of effector proteins in highly dynamic regions of the *P. infestans* genome (Haas et al. 2009). In the last years, promising resistance genes from different wild *Solanum* species that confer broad-spectrum resistance against various *P. infestans* races were identified (Park et al. 2009). Introgression into domesticated potato varieties and cultivation under field conditions will test the durability of resistance mediated by these *R* genes.

Other approaches conducted in various plant species aimed at the induction of general systemic defense responses enabling the plants to defend themselves against a broad spectrum of pathogens. These systemic defense responses have a multigenic background, making the adaptation for pathogens more difficult. They result from an initial recognition of pathogens or rhizobacteria (systemic acquired resistance [SAR] [Durrant and Dong 2004] or induced systemic resistance [ISR] [Pieterse et al. 2001], respectively) or are induced by chemical inducers. Establishment of SAR is known to be dependent on the accumulation of salicylic acid (SA) in most plants analyzed (Loake and Grant 2007). ISR of *Arabidopsis*, on the other hand, relies on functional jasmonic acid (JA) and ethylene (ET) signaling (Pieterse et al. 1998, 2001). Among the chemical inducers, 2,6-dichloroisonicotinic acid (INA) and benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) were shown to positively act in the SAR pathway downstream of SA (Friedrich et al. 1996; Vernooij et al. 1995). Both conferred higher levels of resistance in several plant-pathogen interactions, such as INA in tobacco against *Tobacco mosaic virus* (TMV) (Ward et al. 1991) and BTH in wheat against *Blumeria graminis* f. sp. *tritici* (Görlach et al. 1996).

Another compound inducing systemic resistance is DL- β -aminobutyric acid (BABA). This amino-acid derivative proved to be effective in various pathosystems and different types of application (Cohen 2002; Jakab et al. 2001). The determination of the underlying regulatory signaling pathways in some systems revealed that it is not possible to generalize the requirements of specific signaling pathways for BABA-induced resistance. For example, detailed mutant analyses in *Arabidopsis* showed that BABA-induced resistance against *Plectosphaerella cucumerina* requires both functional abscissic-acid signaling and callose deposition (Ton and Mauch-Mani 2004), whereas SA signaling via functional NPR1 is indispensable for the establishment of BABA-induced resistance against *Botrytis cinerea* (Zimmerli et al. 2001). On the other hand,

L. Eschen-Lippold and S. Altmann contributed equally to the work.

Current address for S. Altmann: Department of Disease and Stress Biology, John Innes Centre, Norwich NR4 7UH, U.K.

Corresponding author: S. Rosahl; Telephone: +49 345 5582 1440; Fax: +49 345 5582 1409; E-mail: srosahl@ipb-halle.de

neither SA, JA, nor ET pathways were involved in BABA-induced resistance against the obligate biotrophic oomycete *Hyaloperonospora arabidopsidis* (formerly *Peronospora parasitica* [Zimmerli et al. 2000]).

In the potato-*P. infestans* pathosystem, BABA also induces high levels of resistance (Altamiranda et al. 2008; Cohen 2000; Si-Ammour et al. 2003). Germination of *P. infestans* is not altered on BABA-treated compared with mock-treated potato plants, but a slight reduction in appressoria formation was observed in BABA-treated plants (Kim and Jeun 2007). However, only little is known about biochemical changes during establishment of induced resistance in response to BABA treatment. Compared with mock-treated tubers, tubers pretreated with BABA and subsequently infected with *P. infestans* contained increased levels of phenols and phytoalexins as well as the defense-related proteins β -1,3-glucanase and aspartyl protease (Andreu et al. 2006; Olivieri et al. 2009). cDNA-amplification fragment length polymorphism analyses revealed the BABA- and *P. infestans*-induced accumulation of 9-lipoxygenase (9-LOX) transcripts (Li et al. 2009).

Since these correlative data do not reveal a role of specific compounds for BABA-induced resistance in potato, we addressed this point by performing functional analyses. Transgenic potato plants with reduced levels of SA (Halim et al. 2004) and JA (Halim et al. 2009) as well as 9-LOX-derived oxylipins (Eschen-Lippold et al. 2007; Göbel et al. 2003) were analyzed to assess the role of these compounds in mediating or establishing BABA-induced resistance. 9-LOX-derived oxylipins were included, based on the observation from other pathosystems that these oxylipins i) have functions in establishing resistance (Cowley and Walters 2005; Gao et al. 2008, 2009; Rancé et al. 1998), ii) have direct antimicrobial effects (Prost et al. 2005; Weber et al. 1999), or iii) are needed by the attacking pathogen for differentiation and virulence (Gao et al. 2007; Isakeit et al. 2007; Sagaram et al. 2006). Potato plants unable to accumulate SA but not those with reduced levels of JA or 9-LOX-derived oxylipins were compromised in establishing BABA-induced resistance. Since this defect is rescued by exogenous application of the SA analog INA, it can be concluded that the ability to accumulate SA is required for BABA-induced resistance in potato.

RESULTS

Effects of BABA on infections with *P. infestans*.

To establish reproducible conditions for infection experiments after BABA pretreatment, potato wild-type plants were soil-drenched with different concentrations of BABA prior to inoculation with *P. infestans*. Subsequent pathogen biomass determinations were carried out with a realtime polymerase chain reaction (PCR)-based approach by quantifying *P. infestans* DNA (data not shown). Applications of 1 mM BABA proved to induce a resistance state strongly inhibiting *P. infestans* growth compared with that found in mock-treated plants (Fig. 1A). Under these experimental conditions, the expression of the defense-related genes *StPR1*, *StPR5* (*pathogenesis-related 1* and *5*), and *St4CL* (*4-coumarate:CoA ligase*) as well as *StPIN2* (*proteinase-inhibitor 2*) as a JA-dependent gene was analyzed by Northern hybridizations and was compared with mock-treated plants (Fig. 1B). Transcripts of all genes analyzed accumulated in *P. infestans*-treated leaves. Higher transcript levels of *StPR1* and *StPR5* were detectable 1 day after infection, while expression of *St4CL* and *StPIN2* occurred after 2 and 3 days, respectively. Interestingly, none of the genes tested showed stronger expression in samples derived from BABA-treated plants compared with samples from mock-treated plants. Indeed, despite the elevated resistance after BABA

treatment, the defense-related marker genes showed less expression in these plants. This is in contrast to other plants in which BABA treatment can induce a 'primed' state of the plant that results in faster and stronger defense-gene expression after pathogen infection in BABA-treated plants compared with that in untreated plants (Cohen 2002). Possibly, the lower levels of *PR* gene expression in BABA-treated potato plants result from the reduced pathogen spread, which leads to a lower number of infected and, thus, responding cells.

To show that *StPR1* and *StPR5* expression in potato is dependent on the SA pathway, plants were sprayed with SA or its analog, INA. RNA analyses revealed SA- and INA-dependent accumulation of *StPR1* and *StPR5* transcripts (Fig. 1C). Thus, both SA- and JA-inducible genes are activated upon *P. infestans* infection in both untreated and BABA-treated plants. Interestingly, BABA treatment of potato plants did not induce the accumulation of JA, JA-Ile, nor its biosynthetic precursor 12-oxo-phytodienoic acid (OPDA) (Fig. 1D). Moreover, no significant differences in SA or SA glucoside (SAG) levels for up to 48 h were observed after BABA-treatment (Fig. 1D).

The role of the 9-LOX pathway in BABA-induced resistance.

For 9- and 13-LOX pathway-generated oxylipins, defense-related functions have been described either as signaling or direct antimicrobial compounds (Rosahl and Feussner 2004; Wasternack 2007). To analyze whether BABA treatment had an effect on the expression of these oxylipin biosynthetic genes, their transcript levels were determined in plants after BABA-treatment alone or after subsequent *P. infestans* inoculation. Under the experimental conditions, BABA alone did not induce transcript accumulation of the 9-LOX branch genes encoding 9-LOX (*StLOX1*) and 9-divinyl ether synthase (*St9DES*) (Fig. 2A). Following infection with *P. infestans*, transcripts of both genes accumulated in plants with or without pretreatment with BABA. As seen for the defense-related genes in Figure 1B, expression was higher in infected plants without BABA treatment, which became evident especially in case of *St9DES* (Fig. 2B). Transgenic plants expressing RNA-interference constructs against *StLOX1* (Göbel et al. 2003) and the downstream *St9DES* (Eschen-Lippold et al. 2007) were subsequently tested for their ability to mount BABA-induced resistance. We already reported that, without any additional treatments, *St9DES*-RNAi plants display the same level of susceptibility towards *P. infestans* as control plants (Eschen-Lippold et al. 2007). Here, we demonstrate also that, in untreated *StLOX1*-RNAi plants, growth of *P. infestans* is equal to that in control plants (Fig. 2C). Together, these results indicate that the entire 9-LOX branch of the oxylipin pathway is dispensable for basal resistance in this interaction. Interestingly, pretreatment of *StLOX1*-RNAi and *St9DES*-RNAi plants with BABA induced resistance against *P. infestans* to the same extent of control plants (Fig. 2C). Therefore, 9-LOX pathway-derived antimicrobial compounds are also dispensable for BABA-induced resistance in this interaction.

The role of the 13-LOX pathway in BABA-induced resistance.

Treatment of potato wild-type plants with BABA did not induce transcript accumulation of the 13-LOX branch genes *StLOX3*, *StAOC* (*allene oxide cyclase*), and *StOPR3* (*12-OPDA-reductase 3*). For *StOPR3*, no basal transcript levels could be detected, while for the two upstream genes *StLOX3* and *StAOC*, certain levels of basal expression were present (Fig. 3A). These varied slightly at the different timepoints but were not reproducibly elevated by BABA in different experiments. In accordance with this lack of induced expression,

Fig. 1. DL-β-aminobutyric acid (BABA)-induced resistance against *Phytophthora infestans* in wild-type potato plants. **A**, Untreated potato control plants and plants pretreated with BABA were inoculated with *P. infestans*. Samples for *P. infestans* biomass determinations were harvested 3 days later and were analyzed by quantitative real time polymerase chain reaction. Plants pretreated with BABA were significantly more resistant (Mann-Whitney test, $P < 0.01$). **B**, RNA was isolated from leaves of untreated or BABA-treated plants after infection with *P. infestans* at the timepoints indicated. Northern-hybridizations were carried out with radioactively labeled probes of defense-related genes *StPR1*, *StPR5*, *St4CL*, and *StPIN2*. rRNA was stained with ethidium bromide to visualize equal loading. **C**, RNA was isolated from leaves of untreated plants or plants treated with ethanol (EtOH), SA, or 2,6-dichloroisonicotinic acid (INA) for 1 or 2 days. Northern analyses were carried out using radioactively labeled probes of *StPR1* and *StPR5*. rRNA was stained with ethidium bromide to visualize equal loading. **D**, Levels of 12-oxo-phytodienoic acid (OPDA), JA, JA-Ile, SA, and SA glucoside (SAG) were determined in leaves of BABA-treated plants at the timepoints indicated.

measurements of OPDA, JA, and JA-Ile did not reveal any changes in overall contents (Fig. 1 D).

Upon infection with *P. infestans*, transcripts of *StLOX3* accumulated 3 to 4 days postinoculation. This result was seen in both BABA-treated and untreated control plants, occurring to the same extent (Fig. 3B). For *StAOC* and *StOPR3*, no increase of the constitutive transcript levels was found upon *P. infestans* infection (Fig. 3B). To assess the influence of the 13-LOX branch on establishing BABA-induced resistance in potato, plants impaired in JA biosynthesis (*StAOC*-RNAi and *StOPR3*-RNAi) and JA perception (*StCOI1*-RNAi) (Halim et al. 2009) were analyzed. Interestingly, watering the plants with BABA induced the same level of resistance against *P. infestans* in transgenic RNAi-silenced plants and control plants (Fig. 3C), proving the JA-independence of BABA-induced resistance in the potato-*P. infestans* pathosystem.

The role of SA in BABA-induced resistance.

In previous work, we demonstrated the importance of SA for basal resistance of potato against *P. infestans* (Halim et al. 2007, 2009). In this study, we used transgenic plants expressing a bacterial salicylate hydroxylase gene (*NahG*), which converts SA to catechol (Gaffney et al. 1993). These plants are impaired in SA accumulation and were used to determine the impact of SA on BABA-induced resistance against *P. infestans*. In the first place, experiments with potato wild-type plants revealed that BABA-treatment does not induce the accumulation of SA or SAG (Fig. 1 D). In infection experiments with *P. infestans*, BABA-treated wild-type plants macroscopically developed smaller lesions than untreated plants. *NahG* plants, however, did not show any treatment-dependent differences in lesion formation (Fig. 4A). Strikingly, *P. infestans* biomass determinations revealed that *NahG* plants were not able to mount BABA-induced resistance to a significant extent (Fig. 4B). As previously published, *NahG*-expressing plants allowed

significantly elevated colonization by *P. infestans* compared with that in control plants, despite no significant differences in lesion size (Fig. 4A and B) (Halim et al. 2007).

Defects of *NahG* potato plants can be rescued by the exogenous application of the SA analog INA, which, by itself, does not influence growth of *P. infestans* on potato plants (Halim et al. 2007, 2009). Spraying *NahG* plants with 0.33 mM INA restored BABA-induced resistance to levels observed in BABA-treated wild-type plants (Fig. 4B). Thus, our results suggest that the ability to accumulate SA is essential for this type of chemically induced resistance against *P. infestans* in potato.

DISCUSSION

In the context of an increasing requirement for food security, the importance of potato as one of the five major crop plants world wide and the devastating crop losses due to *P. infestans* infection have prompted research into alternative ways to manage disease. In potato, resistance against *P. infestans* can be induced by infection with pathogens (Kombrink et al. 1996) and by exogenous application of compounds such as JA, arachidonic acid, or BABA (Cohen 2002; Cohen et al. 1993; Coquoz et al. 1995).

Since the mechanisms of BABA-induced resistance in potato are not understood, we analyzed the role of the 9-LOX pathway as well as the signaling compounds JA and SA for resistance in potato, using transgenic plants with reduced amounts of these compounds.

The rationale to analyze the role of 9-LOX-derived oxylipins for BABA-induced resistance was based on the observation that these compounds accumulate in potato plants in response to infection with *P. infestans* or *Pseudomonas syringae* pv. *maculicola* (Göbel et al. 2002). Data from other pathosystems, for instance almond-*Aspergillus carbonarius* (Mita et al. 2007) or cotton-*Xanthomonas campestris* (Marmey et al. 2007), also

Fig. 2. The role of the 9-lipoxygenase (9-LOX) pathway for DL-β-aminobutyric acid (BABA)-induced resistance. **A**, RNA from leaves of wild-type potato plants treated with BABA was subjected to Northern analyses. Hybridization was carried out with radioactively labeled probes of *StLOX1* and *St9DES*. rRNA was stained with ethidium bromide to visualize equal loading. **B**, RNA was isolated from leaves of untreated or BABA-treated plants after infection with *Phytophthora infestans* at the timepoints indicated (dpi = days postinfection). Northern hybridizations were carried out with radioactively labeled probes of *StLOX1* and *St9DES*. The graphs show signal intensities calculated from three independent experiments. **C**, Control plants (ctrl. = wild-type and empty vector-containing plants) as well as transgenic plants expressing RNAi constructs against *StLOX1* and *St9DES* were inoculated with *P. infestans*. Pathogen biomass was determined by quantitative real time polymerase chain reaction 3 days after infection (with [+] and without [-] BABA pretreatment). Different letters indicate statistically significant differences (Kruskal-Wallis test, *P* values as indicated).

point to an involvement of the 9-LOX pathway in pathogen defense. Moreover, a crucial role for the pathogen-induced 9-LOX of tobacco was demonstrated for race-cultivar-specific resistance against *Phytophthora parasitica* (Rancé et al. 1998). In maize, 9-LOX is required for defense against root knot nematodes (Gao et al. 2008). Finally, a possible role of 9-LOX products for systemic signaling was suggested. Lipid transfer proteins such as DIR1 (Maldonado et al. 2002) or AZI1 (Jung et al. 2009) are important for systemic signaling in *Arabidopsis*; however, the nature of the lipid bound by these proteins has not been elucidated. In barley seeds, a lipid transfer protein (LTP) is covalently modified by the α -ketol 9-hydroxy-10-oxo-12(Z)-octadecenoic acid, a product of the 9-LOX and 9-allene oxide synthase (AOS) branch of the LOX pathway (Bakan et al. 2006). This result, together with the determination of the LTP-oxylipin conjugate's crystal structure (Bakan et al. 2009), allows speculation about a possible role of 9-LOX products in systemic signaling. The compromised accumulation of 9-LOX-derived oxylipins in *StLOX1*-RNAi and *St9DES*-RNAi potato plants (Eschen-Lippold et al. 2007; Göbel et al. 2003), therefore, presented a suitable system to evaluate possible signaling functions of 9-LOX-derived oxylipins. However, BABA-induced resistance was not altered in plants with down-regulated 9-LOX-pathway genes (Fig. 2C), demonstrating that these compounds do not play a role or are dispensable for BABA-induced resistance in potato.

As for 9-LOX-derived oxylipins, the inability of potato plants to synthesize or perceive JA is not relevant for the induction of resistance against *P. infestans* by BABA (Fig. 3C).

Similarly, JA synthesis or signaling was not required for protection of *Arabidopsis* plants by BABA against *H. arabidopsidis* or *P. syringae* pv. *tomato* DC3000 (Zimmerli et al. 2000). In addition, the *Arabidopsis coi1* mutant still exhibits BABA-induced resistance against the necrotrophic fungi *Alternaria brassicicola* and *Plectosphaerella cucumerina* (Ton and Mauch-Mani 2004). Inhibitor studies in grapevine, on the other hand, point to an involvement of the JA pathway in BABA-induced resistance against *Plasmopara viticola* (Hamiduzzaman et al. 2005). Also, the tomato *def* mutant, which has an unknown defect in JA accumulation (Howe et al. 1996), does not mount resistance against *P. infestans* upon BABA treatment, as determined by evaluation of disease symptoms (Yan et al. 2002). These studies nicely emphasize that the requirement of specific signaling compounds for BABA-induced resistance, such as JA, depend on both plant and pathogen species.

In contrast to 9-LOX-derived oxylipins and JA, SA is indispensable for the establishment of BABA-induced resistance in potato against *P. infestans* (Fig. 4). Concerning the induction of SA accumulation, different types of BABA application were analyzed in other solanaceous species. Spraying BABA induced transient increases of SA levels in leaves of tobacco (Siegrist et al. 2000) and pepper (Hwang et al. 1997), whereas in tomato, 1 mM BABA applied as soil-drench did not induce SA accumulation in aboveground parts (Jeun et al. 2000). Similarly, our own results from potato plants watered with 1 mM BABA did not reveal SA accumulation in leaves (Fig. 1D). In another study from tomato, watering with 10-fold higher concentrations of BABA (10 mM) led to a transient SA

Fig. 3. The role of the 13-lipoxygenase (13-LOX) pathway for DL-β-aminobutyric acid (BABA)-induced resistance. **A**, RNA from leaves of wild-type potato plants treated with BABA was subjected to Northern analyses. Hybridization was carried out with radioactively labeled probes of *StLOX3*, *StAOC*, and *StOPR3*. rRNA was stained with ethidium bromide to visualize equal loading. **B**, RNA was isolated from leaves of untreated (white bars) or BABA-treated plants (black bars) after infection with *Phytophthora infestans* at the timepoints indicated (dpi = days postinfection). Northern hybridizations were carried out with radioactively labeled probes of *StLOX3*, *StAOC*, and *StOPR3*. The graphs show signal intensities calculated from two independent experiments. **C**, Control plants (ctrl. = wild-type and empty vector-containing plants) as well as transgenic plants expressing RNAi constructs against *StLOX3*, *StAOC*, and *StOPR3* were inoculated with *P. infestans*. Pathogen biomass was determined by quantitative real time polymerase chain reaction 3 days after infection (with [+] or without [-] BABA pretreatment). Different letters indicate statistically significant differences (Kruskal-Wallis test, *P* values as indicated).

accumulation in roots correlating with cell death, pointing to an overload of the experimental system (Chamsai et al. 2004). The same effect was seen in our own experiments, leading even to death of the plants one day after watering with very high concentrations of BABA (1 M; data not shown).

In previous work, we demonstrated that SA is necessary for basal resistance of potato against *P. infestans* (Halim et al. 2007). In this study, our finding that *NahG*-expressing potato plants are unable to mount BABA-induced resistance, clearly demonstrates the crucial involvement of SA also in this type of chemically induced resistance against *P. infestans* (Fig. 4B). The SA analog INA, which itself does not inhibit *P. infestans* growth on wild-type plants (Halim et al. 2007), restored the ability to establish BABA-induced resistance in *NahG* potato lines (Fig. 4B). This corroborates our finding that INA also complements basal resistance against *P. infestans* in these plants (Halim et al. 2007). Studies of BABA-induced resistance in other solanaceous species showed the involvement of SA in the tomato–*P. infestans* (Yan et al. 2002) and tobacco–TMV (Siegrist et al. 2000) pathosystems. In contrast, in *Arabidopsis*, BABA-induced resistance against *P. cucumerina* was unaffected both in *NahG*-expressing plants and the SA signaling mutant *npr1* (Ton and Mauch-Mani 2004).

So far, no detailed analyses with potato plants impaired in the accumulation and perception of signaling pathway components had been conducted. Thus, our results contribute to the understanding of chemically induced resistance in the important potato–*P. infestans* pathosystem. BABA may serve as an

inducer of resistance with great potential for potato cultivation, since it is transported in potato plants upon leaf treatment (Cohen 2002) and applications throughout the crop cycle increase tuber yield (Olivieri et al. 2009) and resistance against *P. infestans* in stored tubers (Andreu et al. 2006).

MATERIALS AND METHODS

Cultivation and treatment of plants.

In this study, potato wild-type plants (*S. tuberosum* cv. Désirée) were used as well as transgenic Désirée plants harboring RNA-interference constructs targeted against genes of the LOX pathway, *StLOX1*-RNAi (Göbel et al. 2003), *St9DES*-RNAi (Eschen-Lippold et al. 2007), *StAOC*-RNAi, *StOPR3*-RNAi, and *StCOI1*-RNAi (Halim et al. 2009). To target the SA pathway, Désirée plants expressing a salicylate hydroxylase (*NahG* plants) were used (Halim et al. 2004). Potato plants were grown as described by Halim and associates (2007), and infection experiments with *P. infestans* were carried out in a phytochamber as previously described (Eschen-Lippold et al. 2009). BABA (Sigma-Aldrich, St. Louis) was applied by watering the plants with 100 ml BABA (1 mM, dissolved in water) 2 days prior to inoculation with *P. infestans*. To rescue the SA-deficient phenotype of *NahG* plants, 0.33 mM INA (Sigma-Aldrich) in 1% ethanol or 1% ethanol alone was sprayed on the adaxial leaf surface of the plants 1 day prior to *P. infestans* inoculation on the abaxial side of the leaf.

RNA analyses.

Northern analyses were performed with radioactively labeled cDNA probes from *StLOX1* (Geerts et al. 1994), *St9DES* (Göbel et al. 2001), *StLOX3* (Royo et al. 1996), *StAOC* (Halim et al. 2009), *StOPR3* (Halim et al. 2009), *StPIN2* (X04118), *St4CL* (Becker-André et al. 1991), *StPR1* (TC126356), and *StPR5* (TC112036). Experimental conditions were previously described (Halim et al. 2004). To calculate relative expression data, signal intensities were standardized to ethidium bromide-stained rRNA.

Determination of SA and JA.

To extract SA and SAG, the method of Verberne and associates (2002) was applied by either using a high-performance liquid chromatography system (Halim et al. 2004) or an Acquity UPLC (BEH C18 1.7 μ m 2.1 $\times$ 50 mm column; Waters Corp., Milford, MA, U.S.A.). Details about the extraction are given by Halim and associates (2004). SA was detected using an excitation wavelength of 300 nm and an emission wavelength of 410 nm. JA was measured according to Göbel and associates (2002).

Determination of *P. infestans* biomass.

Pathogen biomass was determined by real time PCR as described (Eschen-Lippold et al. 2007).

Statistical analyses.

Statistical data analyses were carried out using the software Prism 5 (GraphPad Software, Inc., La Jolla, CA, U.S.A.).

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