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Salicylic Acid Mediates Elicitin-Induced Systemic Acquired Resistance, but Not Necrosis in Tobacco

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Elicitins are proteinaceous elicitors which are secreted by all *Phytophthora* species analyzed so far. When applied on tobacco they induce necrosis and systemic acquired resistance (SAR). Elicitin-treated tobacco plants that express the bacterial *nahG* gene coding for salicylate hydroxylase did not show SAR against infection with the black shank fungus, *Phytophthora parasitica* var. *nicotianae*. However, *nahG*-expressing plants still responded to a treatment with the basic elicitin cryptogein by formation of necrosis. Elicitin-induced expression of genes encoding PR-1a, PR-2, PR-5, and basic class III chitinase was suppressed in NahG tobacco, while the induced mRNA accumulation of basic *PR-1*, *PR-3*, *str-246* and *str-319* appeared to be unchanged. NahG plants showed enhanced susceptibility to several *Phytophthora* species and isolates that produce elicitins, but were not affected in the necrosis response during interaction. While SA is involved in SAR and disease resistance to *Phytophthora*, our results suggest that it does not mediate the hypersensitive-like necrosis response.

Additional keywords: capsicein, hypersensitive response, *SAR* genes, 2,6-dichloroisonicotinic acid.

The incompatible interaction between plants and pathogens is characterized by the induction of various defense mechanisms and local restriction of infection (for review see Collinge and Slusarenko 1987; Hahlbrock and Scheel 1987; Paxton and Groth 1994). One common feature of many incompatible interactions is the development of a hypersensitive response (HR) on the plant. Symptoms of the HR are collapse and death of plant cells in and around infection sites (Klement 1982). The HR coincides with numerous metabolic changes of the affected plant cells such as strengthening of structural barriers (Ride 1983), synthesis of lytic enzymes (Linthorst et al. 1990), or production of phytoalexins (Paxton and Groth 1994). Furthermore, many plants which have undergone an HR show increased resistance against subsequent microbial attacks. This non-localized and long-lasting induced protection, which is active against a broad spectrum of pathogens is called systemic acquired resistance (SAR) (Ross 1961).

To gain a better understanding of the processes triggering HR, extensive work was carried out on the identification of pathogen-derived molecules which determine early recognition of the microorganisms by a given plant. Elicitors of plant defense responses could be isolated from either viruses, bacteria, or fungi (for review see Ebel and Cosio 1994). Well-characterized pathogen-derived elicitors which trigger a hypersensitive-like necrosis and SAR are the elicitins (Bonnet et al. 1986; Ricci et al. 1989), a family of homologous holoproteins that are secreted into the culture medium by all *Phytophthora* species analyzed so far (for review see Ricci et al. 1993). Only those isolates of *Phytophthora parasitica* which are highly virulent on tobacco (*P. parasitica* Dastur var. *nicotianae* (Breda de Haan) Tucker), do not produce these proteins (Bonnet et al. 1994). Therefore, elicitins act as avirulence factors in the tobacco/*Phytophthora* interaction (Ricci et al. 1992). When applied to tobacco, purified elicitins induce different degrees of leaf necrosis, an effect which seems to be modulated by a few amino acid substitutions in an otherwise strongly conserved sequence, rendering the proteins of different *Phytophthora* species either basic (strong necrotizers) or acidic (no, or weak necrotizers) (Ricci et al. 1993). All analyzed elicitins are able to induce resistance to subsequent infections with *P. parasitica* var. *nicotianae*, the causal agent of the tobacco black shank disease (Ricci et al. 1989; Bonnet et al. 1996), although 10- to 50-fold higher concentrations of the acidic elicitins are required to induce similar levels of resistance as with basic elicitins (Bonnet et al. 1996). This resistance is systemic and works not only against other pathogens in tobacco, but also in some crucifers like radish (Kamoun et al. 1993).

Following pathogen infection of tobacco or Arabidopsis, a number of genes are expressed in both infected and non-infected tissue. The transcriptional activation of these genes is closely correlated to the establishment of SAR (Ryals et al. 1994; Uknes et al. 1992, 1993; Ward et al. 1991b). Due to this association, the genes were termed *SAR* genes, encoding, among others, several pathogenesis-related (PR) proteins. Transcriptional activation of *SAR* genes has become a valuable marker for SAR (Bowling et al. 1994; Cao et al. 1994; Delaney et al. 1994, 1995).

Induced resistance requires a systemically activated signal transduction pathway to connect events from the primary infection site to distal tissues during the establishment of SAR. Although there is still a controversy about the nature of the long-distance signal (Shulaev et al. 1995; Vernooij et al. 1994)

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it is evident that salicylic acid (SA) is involved in the transduction pathway and is required for the establishment of SAR. Its accumulation correlates with the onset of resistance in tobacco (Malamy et al. 1990; Silverman et al. 1993), cucumber (Métraux et al. 1990), and Arabidopsis (Uknes et al. 1992) and application of exogenous SA leads to expression of *SAR* genes (Brederode et al. 1991; Uknes et al. 1992, 1993; van de Rhee and Bol 1993; Ward et al. 1991b), accumulation of PR proteins (Yalpani et al. 1991), and enhanced pathogen resistance (Kessmann et al. 1994; White 1979). The most convincing demonstration of the involvement of SA in SAR signal transduction came from experiments with transgenic tobacco plants (NahG tobacco), harboring a bacterial gene for salicylate hydroxylase, an enzyme which catabolizes SA to catechol (Gaffney et al. 1993). Plants expressing this gene do not accumulate SA and do not establish the SAR response after inoculation with tobacco mosaic virus (TMV).

In this paper we examine whether elicitins trigger SAR via a SA-dependent pathway, by comparing elicitin-induced responses in tobacco plants expressing or not expressing the *nahG* gene. We find that accumulation of SA is required for the establishment of elicitin-induced SAR, but is not required for necrosis. Analyses of mRNA accumulation revealed that the expression of a subset of elicitin-responsive *SAR* genes (Keller et al. 1996) is suppressed in NahG tobacco. In contrast, transcripts from other *SAR* genes and from the so-called *str* genes (Gough et al. 1995; Marco et al. 1990) accumulate, regardless of whether the *nahG* gene is expressed or not expressed. We show results from inoculation experiments on NahG plants, which were performed to examine whether SA modulates the responses of tobacco during the incompatible interaction with several elicitin-producing *Phytophthora* species and isolates.

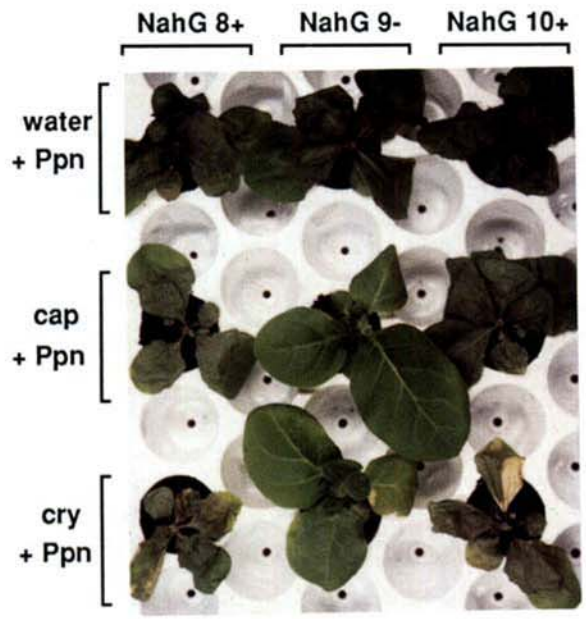

Fig. 1. Elicitin-induced systemic acquired resistance is suppressed in *nahG*-expressing tobacco plants. Three-week old tobacco plants expressing (NahG 8+ and 10+) or not expressing (NahG 9–) the *nahG* gene were treated with 0.15 nmol cryptogein (cry), 2 nmol capsicein (cap), or water by application to the apical stem cuts 4 h prior to root inoculation with zoospore suspensions of *Phytophthora parasitica* var. *nicotianae*. All elicitin-treated plants of line NahG 9– were protected, while NahG 8+ and NahG 10+ plants were fully susceptible to *P. parasitica* var. *nicotianae* infection. The photograph shows representative plants from an assay performed with five plants per transgenic line and treatment.

RESULTS

Tobacco requires SA for elicitin-induced resistance against *P. parasitica* var. *nicotianae* infection.

Experiments were undertaken to determine the role of SA in the elicitin-induced resistance of tobacco to *P. parasitica* var. *nicotianae*, using two lines of transgenic tobacco expressing the *nahG* gene (NahG 8+ and NahG 10+). As a control, a transgenic line that contains but does not express this gene (NahG 9-) was used. When 3-week-old NahG 9– plants were pretreated with either 0.15 nmol of cryptogein (from *P. cryptogea*) or 2 nmol of capsicein (from *P. capsici*), a complete resistance to root inoculation with zoospore suspensions of *P. parasitica* var. *nicotianae* was obtained (Fig. 1), similar to re-

Fig. 2. Degradation of salicylic acid suppresses elicitin-induced systemic acquired resistance, but not elicitin-induced necrosis. Six-week-old tobacco plants were treated with water, 0.1 nmol cryptogein, or 5 nmol capsicein via the decapitated stem 48 h prior to petiolar inoculations with mycelium plugs of *Phytophthora parasitica* var. *nicotianae*. Arrows indicate the inoculation sites for *P. parasitica* var. *nicotianae*. Pretreatment of plants with capsicein led to restriction of fungal invasion in lines Xanthi.nc and NahG 9–. Plants expressing the *nahG* gene (NahG 8+ and 10+) were not able to acquire resistance. Capsicein-treatment did not lead to leaf defects on the treated plants. Application of cryptogein, in contrast, led to the formation of large necrotic lesions, regardless of whether or not the plants acquired resistance to *P. parasitica* var. *nicotianae* infection. Disease on NahG 8+ and NahG 10+ plants appeared as a brownish, shrunken area on the stem, starting at the inoculation site and extending about 10 cm in 1 week. The photograph shows representative plants 7 days after inoculation.

Table 1. Leaf necrosis and stem protection induced by elicitin treatments on tobacco plants expressing or not expressing the *nahG* gene[a]

Tobacco line	Water invasion[b]	Cryptogein			Capsicein[e]	
		Leaf necrosis[c]	Invasion	Protection[d]	Invasion	Protection
Xanthi.nc	81.7 ± 25.2	23.2 ± 7.6	2.0 ± 3.5	98	38.0 ± 27.0	54
NahG 9–	60.7 ± 7.1	24.0 ± 5.9	2.7 ± 2.1	96	16.7 ± 17.6	73
NahG 8+	107.3 ± 17.8	20.3 ± 3.5	96.7 ± 12.6	10	118.3 ± 10.4	0
NahG 10+	95.3 ± 4.5	35.1 ± 4.6	100.7 ± 6.0	0	116.3 ± 7.8	0

[a] Fifty-day-old plants were decapitated and treated with 20 µl of water or a solution containing either 0.1 nmol of cryptogein or 5 nmol of capsicein. Plants were inoculated 48 h later with mycelia of *Pythophthora parastica* var. *nicotianae* on the petiole of the second upper remaining leaf. All measurements were performed 7 days postinoculation.

[b] Extent of stem invasion by mycelia of *Pythophthora parastica* var. *nicotianae* (in mm); means and standard deviations of measurements on three individual plants per treatment.

[c] Extent of necrosis in percent of total leaf area, as calculated from the nine upper leaves of a plant; means and standard deviations of measurements on six (Xanthi.nc and NahG 8+), five (NahG 9–), and three (NahG 1O+) individual plants.

[d] Relative reduction of stem invasion (in %) compared to water-treated plants of the corresponding tobacco line.

[e] No leaf necrosis was observed on Xanthi.nc or NahG plants after treatment with capsicein.

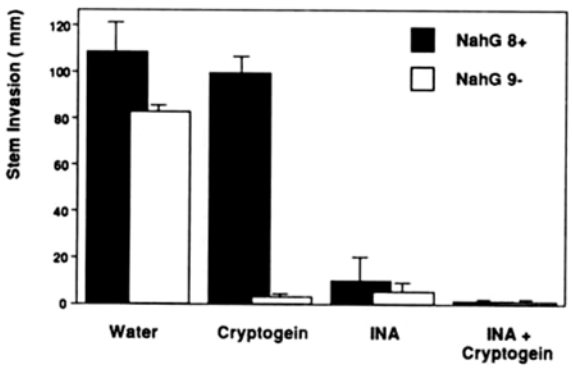

Fig. 3. Systemic acquired resistance to *Phytophthora parasitica* var. *nicotianae* in NahG plants can be restored by the application of INA. Three plants of tobacco lines NahG 8+ and NahG 9– were pretreated with water, cryptogein (0.1 nmol), INA (2.6 µmol), or a mixture of INA and cryptogein 48 h prior to petiolar inoculation with *P. parasitica* var. *nicotianae*. Stem invasion was determined 7 days after inoculation with fungal mycelia.

sults already obtained with untransformed tobacco (Keller et al. 1996). In contrast, plants from the *nahG*-expressing lines were not able to induce SAR against *P. parasitica* var. *nicotianae* infection and were invaded by the fungus to an equal extent as water-treated control plants (Fig. 1).

NahG plants develop elicitin-induced necrosis.

When 6-week-old plants of the different tobacco lines were treated with capsicein (5 nmol/plant) and inoculated 48 h later with *P. parasitica* var. *nicotianae* by application of mycelium to the petiole, the wild type and NahG 9– line showed induced resistance to infection, whereas lines NahG 8+ and NahG 10+ became invaded by the pathogen (Fig. 2; Table 1). The treatment with capsicein did not lead to visible necrosis on the plants. Application of cryptogein prior to infection also did not lead to the establishment of acquired resistance against *P. parasitica* var. *nicotianae* in NahG 8+ and NahG 10+ tobacco (Fig. 2; Table 1). All plants, however, responded to the application of 0.1 nmol cryptogein per plant by the formation of strong necrosis (20 to 35% of total leaf surface of a plant; Table 1), regardless of whether the tobacco line acquired elicitin-induced SAR or not.

The chemical 2,6-dichloroisonicotinic acid (INA) induces resistance in tobacco and other plants against pathogen infection (Kogel et al. 1994; Métraux et al. 1991, Vernooij et al. 1995). Treatment of decapitated tobacco with 2.6 µmol (500 µg) of INA led to acquired resistance towards *P. parasitica* var. *nicotianae* infection in NahG 8+ and NahG 9– plants (Fig. 3). INA application did not induce visible damage.

The accumulation of several, but not all, elicitin-induced plant mRNAs is mediated by SA.

Elicitin treatment of tobacco leads to the transcriptional activation of *SAR* genes and other defense-related genes (Keller et al. 1996). If the expression of these genes is tightly correlated with elicitin-induced SAR, the accumulation of their mRNAs should be suppressed in *nahG*-expressing plants. Therefore, we analyzed the accumulation of mRNAs of nine defense-related genes in NahG 8+ and NahG 9– plants after treatment with either cryptogein, capsicein, or SA.

NahG 9– plants reacted to treatment with cryptogein by accumulating transcripts of the *SAR* genes, and transcripts of *str 246* and *str 319* (Fig. 4). Decapitation in control plants did not lead to detectable mRNA accumulation. The highest level of gene induction in response to cryptogein was observed for *basic PR-1*, *PR-3*, *PR-4*, *PR-5*, *str-246*, and *str-319* (Fig. 4). Weaker mRNA inductions were observed for *PR-1a*, *PR-2*, and the *basic class III chitinase* gene.

In NahG 8+ plants, the induction of *basic PR-1*, *PR-3*, *PR-4*, *str-246*, and *str-319* mRNA in response to cryptogein followed a similar time course with similar intensity (Fig. 4). In contrast, little or no induction of the *PR-1a*, *PR-2*, or *basic class III chitinase* gene occurred. In addition, accumulation of the *PR-5* transcripts was reduced and delayed after cryptogein application. Thus, the breakdown of elicitin-induced resistance in NahG 8+ tobacco is correlated with suppressed induction of a subset of inducible genes, namely *PR-1a*, *PR-2*, *PR-5*, and *basic class III chitinase*.

The overall induction in NahG 9– plants was stronger after treatment with cryptogein than that observed in capsicein-treated plants. The higher level of mRNA accumulation correlated to the higher necrosis-inducing activity of the basic elicitin. However, capsicein-induced accumulation of the *PR-1a*, *PR-2*, *basic class III chitinase*, and *PR-5* mRNA occurred predominantly in NahG 9– plants. Suppressed induction of those mRNAs in NahG 8+ tobacco after capsicein-treatment correlated to the loss of SAR and was consistant with the observations after cryptogein application (Fig. 5).

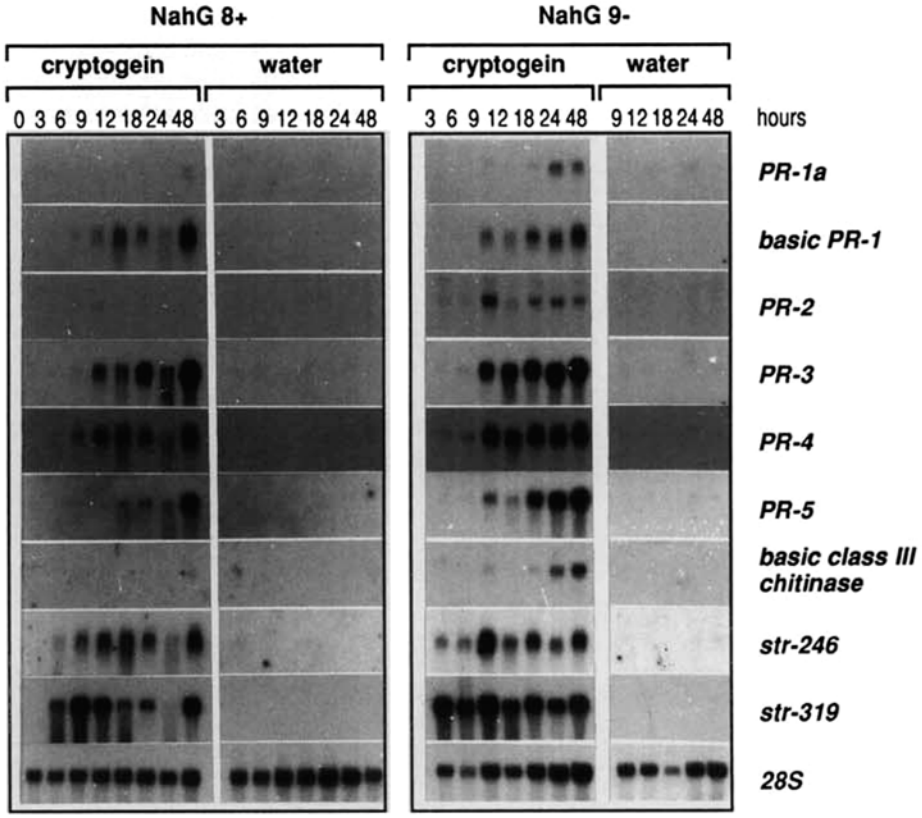

Fig. 4. Effect of salicylic acid depletion on the accumulation of cryptogein-inducible tobacco mRNAs. Young plants of tobacco lines NahG 8+ and NahG 9– were treated with 0.1 nmol cryptogein or water prior to RNA extraction from three whole plants at time points of 0, 3, 6, 9, 12, 18, 24, and 48 h after the onset of the treatments. Electrophoresis was performed with 6 µg total RNA per lane. Labeled probes were derived from cDNAs encoding tobacco PR-1a, basic PR-1, PR-2, PR-3, PR-4, PR-5, and basic class III chitinase (Friedrich et al. 1991; Payne et al. 1988a,b, 1989, 1990; Ward et al. 1991a,b), as well as tobacco str-246 (Gough et al. 1995) and str-319 (Marco et al. 1990). Gel loading was controlled by hybridization of the filters with a probe of the *P. parasitica* 28*S* rDNA.

After exogenous application of SA (360 nmol/plant), the highest induction and the strongest suppression of mRNA accumulation in NahG 9– and NahG 8+ tobacco, respectively, were observed for the *PR-1a*, *PR-2*, *PR-4*, *PR-5* and *basic class III chitinase* genes. Transcript accumulation of *PR-3* and *str-246*, as well as a weak induction of *str-319* appeared not to be affected by the activity of salicylate hydroxylase in NahG 8+ plants (Fig. 5).

Although not affected in necrosis, NahG tobacco plants show enhanced susceptibility during the interaction with elicitin producing *Phytophthora* spp.

Pathogenicity of *Phytophthora* spp. on tobacco is negatively correlated with elicitin production. To determine if SA is required for the resistance responses of tobacco during the interaction with the fungus, NahG 8+ and NahG 9– plants were inoculated with *P. cryptogea*, *P. capsici*, and three isolates of *P. parasitica*.

NahG 9– plants, as well as untransformed tobacco were completely resistant to infection with *P. cryptogea* isolate 52 (Table 2). The interaction with this cryptogein-producing fungus was characterized by the formation of necrosis on the plant leaves (Fig. 6). Although necrosis was not affected by the activity of salicylate hydroxylase after inoculation, invasion occurred on NahG 8+ and NahG 10+ plants (Fig. 6; Table 2).

P. capsici (isolate 147) and the isolates 26 and 310 of *P. parasitica* produce acidic elicitins. Inoculation of NahG 9- tobacco with these nonpathogenic species and isolates resulted in limited infection of the plants, when compared to the extent of invasion after inoculation with the pathogenic *P. parasitica* isolate 183 (Fig. 7), which does not produce an elicitin. In contrast, application of mycelia of the non-pathogenic species and isolates onto NahG 8+ tobacco led to a two- to threefold higher extent of invasion (Fig. 7). On NahG 8+ tobacco, infection by *P. parasitica* isolate 310 reached similar levels as those observed for the highly virulent isolate 183. After inoculations with *P. capsici* or *P. parasitica*, visible necrosis did not occur.

DISCUSSION

Elicitins are well-characterized proteins that have been shown to induce SAR in tobacco. Elicitin-induced SAR is effective against different pathogens (Bonnet et al. 1996) and is preceded by induction of the same set of *SAR* genes (Keller et al. 1996) as those induced after preinfection with TMV or treatment with SA and INA (Ward et al. 1991b). However, in contrast to INA, elicitin-induced SAR is mediated by SA, as *nahG* expressing tobacco were not able to acquire SAR to *P. parasitica* var. *nicotianae* infection. These results indicate that elicitins trigger SAR upstream of SA accumulation.

Acidic elicitins like capsicein induce SAR in tobacco without the appearance of necrotic symptoms on the leaves. In contrast, treatment of the plants with basic elicitins like cryptogein leads to SAR, which is accompanied by the formation of intensive necrosis. This necrotizing activity of cryptogein was reported to be located in a domain involving a lysine residue at position 13 of the protein. An exchange of this lysine into valine, as found in acidic elicitins, decreases the protein activity in necrosis induction (O'Donohue et al. 1995). The formation of this hypersensitive-like necrosis after cryptogein application is preceded by active defense mechanisms like the biosynthesis of ethylene (Milat et al. 1991), the transcriptional activation of defense-related genes (Keller et al. 1996) and the local accumulation of sesquiterpenoid phytoalexins (H. Keller, P. Czernik, M. Ponchet, P. Ricci and Y. Marco, unpublished results). While NahG tobacco plants were not able to acquire elicitin-induced SAR, they were not altered in their capacity to establish cryptogein-induced necrosis. Recently, it was shown that TMV-infected NahG tobacco plants do not show increases in SA accumulation in systemic tissues, but still accumulate small amounts in cell layers immediately surrounding TMV-induced lesions. However, this accumulation is highly reduced and represents only 0.5% of the amount measured in TMV-inoculated nontransformed Xanthi.nc leaves (Friedrich et al. 1995). As shape and size of cryptogein-induced necrosis was neither altered nor reduced in NahG plants, this plant response appears to be mediated by a signaling pathway which does not involve SA. This contrasts to findings in TMV infected NahG tobacco carrying the resitance gene N, where spreading of the virus in absence of SA accumulation is accompanied by continually expanding necrotic lesions (Delaney et al. 1994). Thus, it appears that necrosis after cryptogein application may be an associated response that is not mechanistically linked to SAR.

The requirement for SA in elicitin-induced SAR, but not in necrosis, is reflected in NahG 8+ plants on the molecular level by the suppression or the unaffected expression, respectively, of different elicitin-inducible genes. Expression of the genes encoding PR-1a, PR-2, PR-5, and basic class III chitinase appears to be mediated by SA, as their mRNA accumulated abundantly in response to exogenous SA application and as the breakdown of SAR in NahG tobacco correlated with the suppression of an accumulation of these gene transcripts. Furthermore, the induction of *PR-1a* mRNA accumulation in systemic tissues at the site of challenge inoculation with *P. parasitica* var. *nicotianae* was suppressed in NahG plants (data not shown). These findings are in agreement with previous results, demonstrating suppressed mRNA accumulation of *PR-1a* and *PR-2* in biologically induced NahG tobacco (Friedrich et al. 1995; Vernooij et al. 1994) and of *PR-1a* in NahG Arabidopsis (Delaney et al. 1994; Lawton et al. 1995).

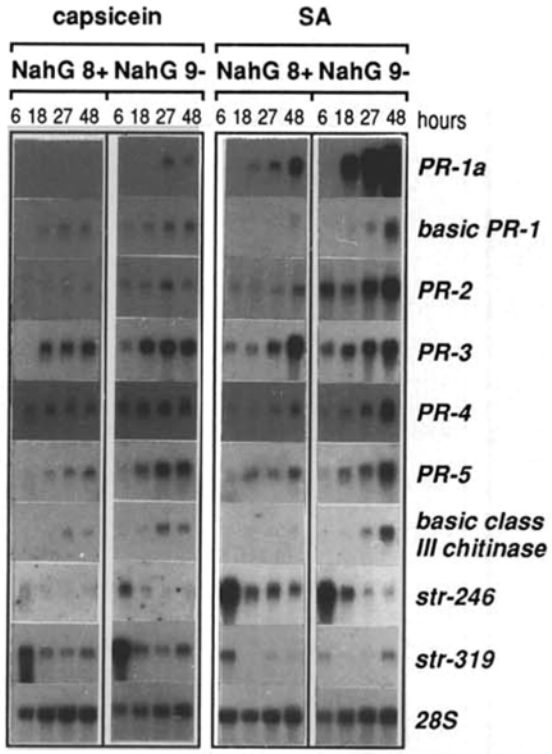

Fig. 5. Induction of mRNAs in response to treatment with capsicein and salicylic acid in NahG tobacco. Accumulation of *SAR* and *str* transcripts was detemined as described for Figure 4. Three plants for each treatment were collected at 6, 18, 27, and 48 h after application of either 2 nmol capsicein or 360 nmol SA.

Table 2. Leaf necrosis and stem invasion 7 days after inoculation of tobacco with *Phytophthora cryptogea* (isolate 52)

Tobacco line	Leaf necrosis[a] (%)	Invasion[b] (mm)
Xanthi.nc	20.0 ± 6.6	5.0 ± 1.0
NahG 9–	39.8 ± 3.3	2.7 ± 2.3
NahG 8+	21.7 ± 1.4	12.0 ± 2.0
NahG 10+	30.9 ± 7.8	12.0 ± 8.5

[a] Extent of necrosis of total leaf area, as calculated from the nine upper leaves of a plant; means and standard deviations of measurements of three plants.

[b] Extent of stem invasion by mycelia of the fungus after application of mycelium plugs to the decapitated stem; means and standard deviations of measurements on three individual plants.

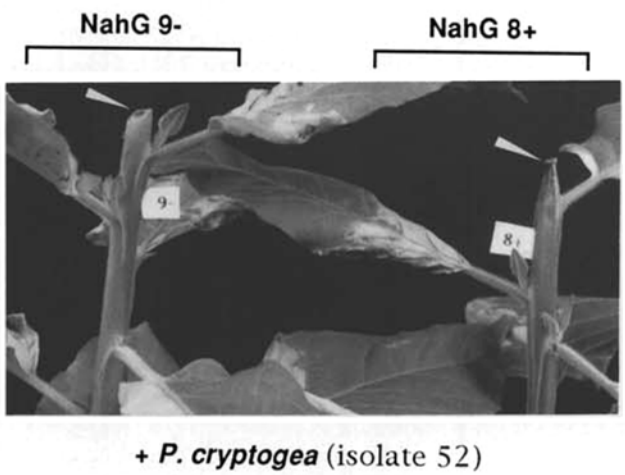

Fig. 6. During interaction, *nahG*-expressing plants are not not altered in necrosis, but show enhanced susceptibility to *Phytophthora cryptogea*. Tobacco plants were decapitated and mycelia of *P. cryptogea* (gerbera isolate 52) were applied to the cut surface. A shrunken and rotted stem along 1 to 2 cm basal of the inoculation site were the symptoms of infection on NahG 8+ plants. The photograph was taken 7 days after inoculation.

However, surrounding TMV lesions *nahG*-expressing tobacco accumulates substantial amounts of *SAR* gene mRNA, which suggests either compartmentalization of SA or an independent signal transduction pathway leading to *SAR* gene expression (Friedrich et al. 1995). Compartmentalization of SA around lesions might explain a higly reduced, but still detectable accumulation of *PR-1a, PR-2, PR-5,* and *basic class III chitinase* mRNAs in NahG tobacco after elicitin application, but is unlikely the reason for elicitin-induced, high level mRNA induction of *basic PR-1, PR-3, str-246,* and *str-319* in NahG plants. *PR-3* and *str-246* are highly inducible by exogenous application of SA, regardless of whether or not the plant expresses the *nahG* gene. If expression of those genes is mediated by SA, then it occurrs either rapidly before degradation of SA, or else a low threshold of nondegraded SA is sufficient to trigger the full response in gene expression. On the other hand, *basic PR-1* and *str-319* responded only weakly to exogenously applied SA, suggesting an SA independent signal transduction pathway leading to expression of these genes. In this context, it was shown that *basic PR-1* of tobacco is specifically activated by ethylene (Eyal et al. 1993; Sessa et al. 1995). Ethylene production also was shown to be one of the early responses of tobacco leaves to cryptogein treatment (Milat et al. 1991) and thus might be responsible for *basic PR-1* and *str-319* gene expression. Taken together, these observations make it seem likely that cryptogein triggers a dual signal transduction pathway in tobacco. One involves SA, leading to specific *SAR* gene expression (reflected by *PR-1a, PR-2, PR-5* and basic class III chitinase) and finally to SAR. A further, SA-independent signal transduction pathway leads to mRNA accumulation of *basic PR-1, str-319* and, probably, *PR-3* and *str-246.*

NahG tobacco and Arabidopsis were reported not only to fail in establishing SAR, but also to show enhanced susceptibility against viral, fungal, and bacterial pathogens, which extends even to host/pathogen combinations normally resulting in genetic resistance (Delaney et al. 1994). Elicitins are supposed to act as avirulence factors in the *Phytophthora*/tobacco interaction, and to be determinants in host specificity of *P. parasitica* (Bonnet et al. 1994; Ricci et al. 1989, 1992). This results in resistance of tobacco to *P. cryptogea* and *P. capsici,* as well as in resistance to elicitin producing *P. parasitica* isolates. Infection of tobacco with *P. cryptogea* usually results in delocalized, systemic HR on the leaves, while the fungus growth is restricted to the site of application (Bonnet 1985). While no changes in the necrosis-inducing capacity of the fungus were observed on NahG tobacco, *P. cryptogea* showed an enhanced infection on plants lacking SA accumulation. However, degradation of SA in NahG plants was not sufficient for converting an incompatible *Phytophthora*/tobacco non-host interaction into a fully susceptible one. This contrasts to findings in Arabidopsis. NahG plants containing the single dominant resistance gene *Rpt2* are no longer able to recognize the bacterial avirulence gene *avrRpt2* and react to infection with *Pseudomonas syringae* pv. *tomato* harboring this gene with complete susceptibility (Delaney et al. 1994). In this case, the activity of salicylate hydroxylase suppresses the action of the host resistance gene. In tobacco, the situation appears to be not as clear as in Arabidopsis. In TMV-infected NahG plants the virus develops rapidly and enters even the vascular system. However, NahG plants do not show systemic movement of the virus in the phloem, as observed in genetically susceptible plants (Delaney et al. 1994). The lack of SA accumulation in tobacco is apparently not sufficient to completely suppress genetic resistance. Similarly, during the *Phytophthora*/tobacco interaction, SA modulates resistance of the plant to elicitin-producing *Phytophthora* species and isolates, but is apparently not the only determining factor for the plant in restricting fungal invasion.

Taken together, our results demonstrate that SA is an essential intermediate in the establishment and maintenance of elicitin-induced SAR and an important factor for disease resistance to *Phytophthora*, but SA seems not to be the general mediator of HR. Elicitins with different necrotizing potentials and NahG tobacco, in combination, will be valuable resources for a further dissection and characterization of the signal transduction pathway leading to SAR.

MATERIALS AND METHODS

Plant materials and fungal cultures.

All experiments were performed with nontransgenic tobacco (Xanthi.nc) or transgenic plants expressing (NahG 8+ and NahG 10+) or not expressing (NahG 9–) the *nahG* gene (Gaffney et al. 1993). Plants were grown in a growth chamber at 24°C with a 16-h light period. All treatments and inoculations were performed at 3 or 6 weeks after seeding, as indicated in the Results section. Mycelia of *P. cryptogea* (isolate 52), *P. capsici* (isolate 147), *P. parasitica* (isolates 26 and 310), and *P. parasitica* var. *nicotianae* (isolates 183 and 329) were from the *Phytophthora* collection of INRA, Antibes (Lacourt et al. 1994; Ricci et al. 1989). They were cultivated on V8 agar at 24°C in the dark and transferred to fresh medium every week. Zoospore production was performed as described elsewhere (Keller et al. 1996).

Plant treatments and inoculations.

Purification of elicitins was performed as described (LeBerre et al. 1994). Cryptogein, capsicein, SA, or water was

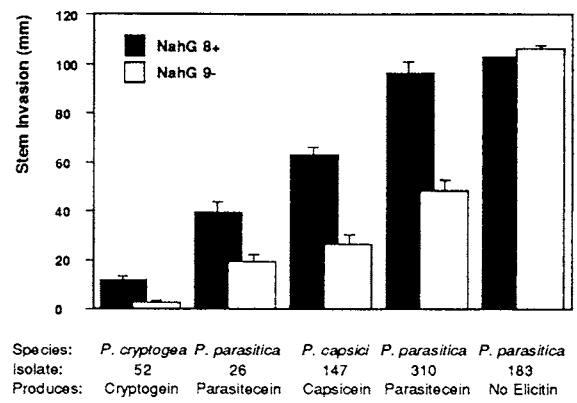

Fig. 7. Salicylic acid modulates resistance of tobacco to different elicitin-producing *Phytophthora* species and isolates. Plants were inoculated with mycelium plugs of *P. cryptogea* (isolate 52), *P. parasitica* (carnation isolate 26), *P. capsici* (pepper isolate 147), and *P. parasitica* (tobacco isolates) showing low (isolate 310) or high (isolate 183) virulence on tobacco. With the exception of isolate 183, all other isolates secrete elicitins (Lacourt et al. 1994; Ricci et al. 1989). Invasion of NahG 8+ and NahG 9– plants was determined 7 days after inoculation. Stem invasions (in mm) by the different species and isolates of *Phytophthora* are indicated as means with standard deviations of measurements on three plants per inoculation.

applied as 5-µl solutions to cut surfaces of decapitated, 3-week-old plants. Root inoculations with isolate 329 of *P. parasitica* var. *nicotianae* were performed by pipetting 1 ml of zoospore suspensions (adjusted to 20,000 spores/ml) directly into the soil. For treatment of 6-week-old plants, decapitation was performed above the third highest fully expanded leaf and 20-µl solutions containing elicitin, INA, or water were applied to the freshly cuts. All concentrations are indicated in the Results section. Untreated plants were inoculated by placing mycelium plugs onto the cut stem. Challenge inoculations of tobacco with *P. parasitica* var. *nicotianae* isolate 183 were performed by the application of mycelia to the petiole of the second leaf basal of the site of pretreatment. The extent of leaf necrosis was determined according to Bonnet (1988).

RNA extraction and gel blot hybridization analysis.

Total RNA was extracted either from 3-week-old tobacco plants (three plants per treatment and per time point) or from leaf tissue of *P. parasitica* var. *nicotianae*-inoculated, 6-week-old plants as described by Logemann et al. (1987), except that an additional $CHCl_3$ washing step was added after phenol/$CHCl_3$ extraction of guanidine hydrochloride treatment. Total RNA was separated on 1.2% agarose gels containing 6.3% formaldehyde, blotted to nylon membranes (Hybond N^+, Amersham) and crosslinked by alkali fixation with 0.05 N NaOH. The probes, described in detail in Keller et al. (1996), were labeled to high specific activity using the Multiprime Labeling Kit (Amersham, Buckinghamshire, England) with 25 µCi [α-^{32}P]-dCTP according to the supplier's instructions. Hybridizations were performed at 42°C for 15 h in 50% formamide, 5 × Denhardt's reagent, 5× SSC (1× is 0.15 M NaCl plus 0.015 M sodium citrate), 50 mM sodium phosphate (pH 6.5), 0.1% sodium dodecyl sulfate (SDS), and 10% dextran sulfate after addition of 100 µg/ml denatured salmon sperm DNA. Prior to autoradiography the filters were washed with 0.2× SSC, 0.1% SDS at 65°C. Gel loading was controlled by hybridization of the filters with a 257-bp fragment, corresponding to the extreme 3′ region of the *P. parasitica* 28S rDNA, which cross-hybridizes to tobacco rRNA (stringency of washing: 0.2× SSC, 0.1% SDS, 50°C).

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