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The role of SA in the hypersensitive response and systemic acquired resistance induced by elicitor PB90 from *Phytophthora boehmeriae*

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Abstract

We examined the role of salicylic acid (SA) accumulation in the tobacco hypersensitive response (HR) and systemic acquired resistance (SAR) induced by PB90, a 90 kDa protein elicitor that is secreted by *Phytophthora boehmeriae*. The elicitor induced HR of a consistent shape and size on tobacco plants expressing the bacterial gene *nahG*. Salicylate hydroxylase is encodec by *nahG* and inactivates SA by converting it into catechol. The mutant NahG does not accumulate SA. In contrast, infiltration of a wild type tobacco (cv. Xanthi nc) leaves with the elicitor caused the increase of SA levels. The same SA levels were observed in leaves not treated by the elicitor. HR appears to be mediated by a SA-independent signaling pathway. PB90 treatment resulted in enhanced resistance of wild-type plants to infection by black shank fungus, *P. nicotianae*, and TMV, but not in NahG plants. Moreover, the elicitor-induced expression of an SAR marker gene encoding PR-1a was suppressed in NahG plants. These results indicate that SA mediates SAR but not HR in tobacco treated with PB90. During plant-elicitor interactions, HR and SAR may be regulated by distinct signal pathways, or SA may function as an intermediate signal upstream of SAR but downstream of HR, and HR may not be a direct defense mechanism against pathogen infection.

Keywords: PB90; Salicylic acid; Hypersensitive response; Systemic acquired resistance

1. Introduction

Incompatible interactions between plants and pathogens are characterized by the induction of various defense mechanisms [16]. One common feature of many incompatible interactions is the development of a hypersensitive response (HR) in the plant. A HR results from recognition of a specific pathogen-derived elicitor by a corresponding receptor in the host [2,19,21]. HR coincides with numerous metabolic changes in the affected plant cells, such as the strengthening of structural barriers, the synthesis of lytic enzymes, the production of phytoalexins, and the accumulation of pathogenesis-related proteins (PRs). HR is a multifaceted defense mechanism that is active against viruses, fungi, and bacteria [8]. Rapid and localized death of a few plant cells at the site of attempted penetration by the pathogen is the early macroscopic indication of HR. Although this early cell death has been regarded as mainly a means for the plant to inhibit pathogen development, the dying cells may also secrete signals that induce systemic resistance in the whole plant.

Salicylic acid (SA) has been intensively studied due to its role as a signal molecule involved in HR. SA has been shown to play a crucial role in the establishment of both local and systemic defense responses [5,12,22,23,37] and the induction of HR [24,46], although these ideas are not free of controversy. Evidence for the role of SA in HR resulted from the analysis of transgenic plants expressing the bacterial gene *nahG*. This gene encodes the enzyme

Abbreviations: HR, hypersensitive response; SAR, systemic acquired resistance; TMV, tobacco mosaic virus; PR, pathogenesis-related; SA, salicylic acid; PAL, phenylalanine ammonia-lyase; POD, peroxidase.

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salicylate hydroxylase, which inactivates SA by converting it to catechol. NahG transgenic plants are unable to accumulate salicylic acid and are incapable of developing SAR and HR, indicating that salicylic acid accumulation is required for SAR to occur [10,11,15]. However, in other systems, salicylic acid has been shown not to be involved in hypersensitive cell death, and acquired resistance mechanisms have been demonstrated to operate independently of SA [17,20]. These findings are supported by the discovery that several defense responses can be activated without increases in the levels of salicylic acid or the expression of a marker gene for salicylic acid, *PR-1a*. In addition, other plant signal molecules, including jasmonic acid and ethylene, have been determined to function as important defense signals, mediating defense responses during certain incompatible interactions between plants and pathogens [31,36,38,40]. In summary, salicylic acid plays various roles in different interactions between plants and pathogens. Elucidation of the multiple roles of salicylic acid in these interactions will greatly advance our knowledge of the diversity of plant defense mechanisms and aid in the development of genetic strategies for pathogen resistance by crops.

Plant defense responses are initiated by the direct or indirect recognition of microorganism-derived molecules called elicitors, which function as signal molecules in the plant [25,27,28,35,50]. As application of a purified elicitor to plants usually mimics an attack by an avirulent pathogen, elicitors are useful in studying the molecular mechanisms of HR and SAR [35]. PB90, a novel protein elicitor with a molecular mass of 90 kDa, was purified from culture filtrate of the cotton blight agent *Phytophthora boehmeriae*, which is an avirulent pathogen of tobacco, a non-host plant for this pathogen [42]. Infiltration of this elicitor into tobacco triggers HR, elicits increases in the activities of phenylalanine ammonia-lyase (PAL) and peroxidase (POD), and induces systemic acquired resistance to *Phytophthora nicotianae*, *Alternaria alternata*, bacterial pathogens, and TMV [42]. The high levels of hydrogen peroxide induced by the elicitor in tobacco play an important role in HR and systemic acquired resistance [43].

The critical signals involved in the SAR induced by PB90 and the potential involvement of salicylic acid in the HR and systemic acquired resistance triggered by PB90 are of great interest, which will contribute to understand the molecular mechanisms for PB90 recognition of plant and to manipulate defense signaling to improve disease resistance. To address these issues, we first analyzed the kinetics of salicylic acid accumulation in tobacco leaves infiltrated with the elicitor. Next, the HR and SAR induced by PB90 in wild-type or NahG tobacco plants were compared. Our results suggest that SA mediates SAR but not HR in tobacco plants treated with PB90.

2. Materials and methods

2.1. Plant material and pathogen cultures

Nicotiana tabacum cv. Xanthi nc harboring the N gene, which confers resistance against TMV [47], and NahG transgenic plants harboring *nahG* gene, which encodes a bacterial salicylate hydroxylase, were cultivated at 25 °C in a greenhouse under a 12 h light/12 h dark cycle. Plants were watered on alternate days and once a week were supplied with half-strength Hoagland's nutrient solution (2 mM KNO_3, 5 mM $Ca(NO_3)_2$, 1 mM KH_2PO_4, 1 mM $MgSO_4$, and trace elements, pH 7.0). All elicitor treatments and pathogen inoculations were performed at 10 weeks after seeding as described previously [42]. *Phytophthora nicotianae* isolate YC02010 cultures were grown on LBA at 25 °C in darkness.

2.2. Plant treatments and inoculations

The elicitor PB90 was purified from culture medium of *Phytophthora boehmeriae* JX-9 as described [42]. The elicitor was diluted with buffer (0.1 M Tris·HCl +0.1 M EDTA, pH 7.5) and infiltrated using a blunt-ended syringe into the mesophyll of tobacco leaves, covering areas of 1.5–3 cm^2. Stem inoculations of tobacco with *P. nicotianae* isolate YC02010 were performed by applying mycelia disks to stems at sites 20 mm from the axils of treated leaves.

Tobacco mosaic virus (TMV) infection assays were performed by challenging plants with the common strain of TMV 24 h after treatment with PB90. Inoculation was performed by rubbing the leaves with an aqueous suspension of highly purified virus at described [5], after which the plants were incubated at 25 °C.

2.3. Extraction and analysis of SA

Extraction and purification of total SA from leaf tissues were performed as described previously [48]. Analysis of total SA was performed using a direct competitive enzyme-linked immunosorbent assay (ELISA) according to Wang et al. [41]. SA concentrations are expressed in nmol per gram fresh weight.

2.4. RNA extraction and gel blot hybridization analysis

Tobacco plants were treated with PB90 at a concentration of 10 nmol L^{-1} by foliar infiltration, and treated leaves were harvested for extraction of total RNA 5 days after the treatment. Total RNA was extracted from 1 g of plant material using the guanidine isothiocyanate method with modifications [4]. Homogenization was performed by pulverizing the samples in liquid nitrogen and suspending the powder in 6 ml of a guanidine isothiocyanate solution containing 25 mM sodium citrate, 0.5% sodium N-lauroylsarcosine, and 0.1 mol L^{-1}

β-mercaptoethanol. The aqueous solution was centrifuged at 14,000×*g* at 4 °C for 5 min. The supernatant was added to 1/10 volume of 2 mol L^{-1} sodium acetate (pH 4.0), and the mixture was subjected to phenol-chloroform-isoamyl alcohol (250:49:1 [v/v]) extraction. After centrifugation at 4 °C for 15 min, the total RNA in the aqueous phase was precipitated with 1 volume of isopropanol. The pellet was washed with 70% ethanol, dried under vacuum, resuspended in 40 μ//l of sterile water, and stored at -70 °C for further analysis.

2.5. Northern blot analysis

The total RNAs extracted as described above were separated on denaturing 1% agarose gels containing formaldehyde, with each lane loaded with approximately 15 μg of total RNA. After migration, the RNA gels were stained with ethidium bromide and the RNAs were transferred onto a nylon membrane (Hybond N+, Amersham, Buckinghamshire, England). Northern hybridization was performed according to the DIG DNA labeling and detection kit (Roche, Germany) following the manufacturer's instructions, using a tobacco *PR-1a* cDNA as a probe.

2.6. Determination of transcript levels of pathogenesis-related genes

Semi-quantitative RT-PCR was performed for the *PR-1a*, *PR-1b*, and *PR-1c* genes. Two micrograms of total RNA were digested with DNase I (TaKaRa Biotech. Co. Ltd, Dalian, China), and then reverse-transcribed for 1 h at 42 °C using 200 units of M-MLV (Promega, USA) in a solution containing a 1× concentration of the corresponding buffer, 10 mM dithiothreitol, 0.4 mM of each dNTP, and 0.5 μg μL^{-1} oligo(dT) primer (TaKaRa Biotech. Co. Ltd). The cDNA was used in PCR with 1 unit of Taq polymerase (TaKaRa Biotech. Co. Ltd), a 1× concentration of the corresponding buffer, 0.2 mM of each dNTP, and 10 μM of the *EF-1α*, *PR-1a*, *PR-1b*, and *PR-1c* primers. The *PR-1* primers were designed based on published sequences [30] and deposited in the National Center for Biotechnology Information database with the accession numbers D90196 (*PR-1a*), X05453 (*PR-1b*), and X05454 (*PR-1c*). Primer sequences are as follows: *PR-1a*, *PR-1b*, and *PR-1c* forward primer, 5′-ATGCCCATAACAGCTCG-3′; *PR-1a* reverse primer, 5′-GAGGATCATAGTTGCAAGAG-3′; *PR-1b* reverse primer, 5′-GTATGGACTTTGGCCATGAC-3′; and *PR-1c* reverse primer, 5′-GGATCATAGTTGCAAGAGAC-3′. Amplification of a constitutively expressed gene, the translation elongation factor gene *EF1α*, served as an internal control in RT assays, using specific primers (5′-AGACCACCAAGTACTACTGCAC-3′; 5′-CCACCAATCTTGTACACATCC-3′) synthesized based on a highly conserved region of the *EF1α* cDNA sequence (GenBank accession number AF120093) to produce a 495-bp sequence. The thermal cycle conditions used were 5 min of initial denaturation followed by a variable number of cycles (25 for EF1α and 30 for *PR-1a*, *PR-1b*, and *PR-1c*) of denaturation at 94 °C for 30 s, annealing at 52 °C (for the *PR-1a* gene) or 56 °C (for the *PR-1b* and *PR-1c* genes) for 30 s, and extension at 72 °C for 30 s, followed by a final extension at 72 °C for 7 min. The intensities of the PCR fragments were analyzed and quantified using Gel Doc 2000 and Quantity One version 4.2.1 (Bio-Rad, Milan, Italy).

3. Results

3.1. Kinetics of salicylic acid accumulation in tobacco plants treated with the PB90 elicitor

Leaves of wild-type tobacco (cv. Xanthi nc) were infiltrated with 10 nM PB90, and the total SA content was determined in these leaves and the non-elicitor-treated leaves just above and below the infiltrated leaves. Three independent experiments were performed with similar results. In wild-type plants, SA accumulated in a distinctly bisphasic manner within 28 h (Fig. 1). An increase was observed by 1 h post-treatment, with peaks at 4 and 12 h. In the first upper and lower non-elicitor-treated leaves, SA accumulated in a monophasic manner within 28 h, with a peak at 4 h. The level of SA in the above three leaf types remained elevated at 28 h post-infiltration. Infiltration of buffer did not induce accumulation of SA in the wild type, and infiltration of elicitor or buffer did not trigger accumulation of SA in NahG plants (unpublished data). These data clearly show that PB90 can trigger SA accumulation in tobacco plants.

3.2. The role of SA in HR triggered by PB90 treatment

Forty-eight hours after infiltration of leaves of wild-type and NahG tobacco with PB90 at doses ranging from 1–100 nM, visible necroses appeared on the leaves (Fig. 2). The size and color of the necroses were similar in wild-type and NahG plants. These results strongly indicate that SA is

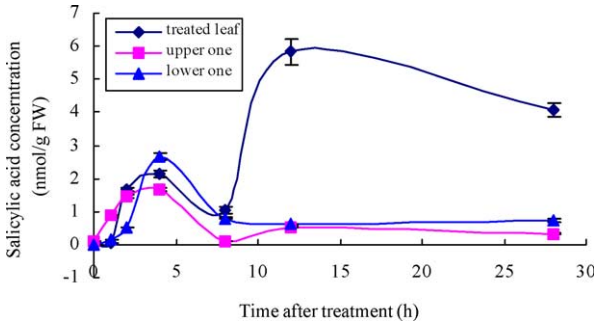

Fig. 1. Kinetics of salicylic acid (SA) in tobacco (cv. Xanthi nc) leaves infiltrated with PB90. Curves represent the means±SD (bars) from 3 independent replicate tests.

Fig. 2. Hypersensitive response on tobacco leaves induced by PB90. 1, 2 and 3 indicated treated with 100, 10 and 1 nmol L^{-1}, respectively. 4: CK. Photograph was taken 24 h post-inoculation.

not involved in the hypersensitive cell death signal pathway that is triggered by PB90 in tobacco.

3.3. The role of SA in systemic acquired resistance induced by PB90

We have demonstrated that in tobacco, PB90 can induce SAR against TMV, bacteria, and fungi [42]. In *Nicotiana tabacum* cv. Xanthi nc transgenic plants harboring the N gene, which confers resistance against TMV [47], the defense response to TMV infection results in necrotic lesions. SA and SAR activators enhance this resistance and reduce the size of the lesions [13,14,44]. In order to elucidate the role of SA in this process, PB90 was infiltrated into the middle leaves of wild-type and NahG plants. Twenty-four hours post-treatment, TMV was applied to the elicitor-treated leaves and to the first and second leaves above and below the elicitor-treated leaves to test for SAR. Five days after TMV inoculation, significantly fewer lesions were observed on the leaves of treated plants than on NahG leaves and buffer-treated wild-type leaves (Table 1 and Fig. 3). At 7 days after TMV inoculation, wild-type plants exhibited smaller lesions than did NahG transgenic plants. In wild-type plants, the average number of lesions was approximately 70% that on NahG plants and approximately 50–60% that on buffer-treated wild-type plants. However, in NahG plants, elicitor treatment did not result in a reduction in the average lesion size. These results indicate that induction of N-gene-mediated resistance by PB90 is dependent on the SA signaling pathway.

To confirm the role of SA triggered by PB90 in induced resistance to fungi, tobacco plants were treated with PB90, and 24 h later *P. nicotianae* was applied. The stem invasion length was significantly shorter in PB90-treated wild-type plants than it was in PB90-treated NahG plants and buffer-pretreated wild-type plants ($P=0.01$) (Fig. 4). In contrast, there was no significant difference in lesion diameter between elicitor-pretreated NahG plants, untreated NahG plants, and buffer-treated wild-type plants. These results suggest that SA accumulation is necessary for PB90-induced resistance of tobacco plants to *P. nicotianae*.

3.4. SA mediates the accumulation of pathogenesis-related transcripts induced by PB90

Several genes for acid PR proteins are coordinately expressed in tobacco leaves during the induction and maintenance of SAR, and are also expressed during SAR induced by elicitor treatment or infection with incompatible pathogens [10]. The expression of *PR-1a*, which encodes one of these PR proteins, is used as a molecular marker for the SA-dependent SAR response [32]. To investigate whether SA is involved in the SAR induced by PB90, we monitored *PR-1a* expression in elicitor-treated NahG transgenic plants and wild-type plants. Following elicitor treatment, the *PR-1a* gene was activated in leaves of wild-type plants but not in NahG transgenic plants (Figs. 5 and 6). These results confirm that the SAR response induced by PB90 is effectively blocked in NahG plants, suggesting that the SAR induced in tobacco by treatment with the elicitor is dependent on SA accumulation.

Following infiltration of leaves of wild-type tobacco plants with 10 nM PB90, accumulation of the *PR-1a*, *PR-1b*, and *PR-1c* transcripts began as early as 1 h after elicitation, and transcript levels continued to increase for at least 48 h (Fig. 6). PB90 did not induce transcription of the *PR-1a* gene in NahG tobacco plants, but expression of the *PR-1b*

Table 1
Acquired resistance to TMV on tobacco plants expressing the *nahG* gene and wild-type tobacco (cv. Xanthi nc) by PB90

Leaf position	Xanthi nc		NahG	
	Lesion number (mean ± SD)	Lesion diameter (mm, mean ± SD)	Lesion number (mean ± SD)	Lesion diameter (mm, mean ± SD)
Control	157.7 ± 7.9 A	2.8 ± 0.34 A	166.7 ± 7.3 A	4.7 ± 0.40 A
Second upper leaf	51.9 ± 6.9 B	1.4 ± 0.33 B	155.8 ± 5.4 A	4.4 ± 0.75 A
First upper leaf	45.6 ± 3.6 B	1.1 ± 0.11 B	156.3 ± 9.4 A	4.6 ± 0.43 A
Treated leaf	45.7 ± 3.1 B	1.0 ± 0.13 B	150.9 ± 8.0 A	4.5 ± 0.32 A
First lower leaf	51.0 ± 4.1 B	1.2 ± 0.14 B	150.9 ± 10.3 A	4.6 ± 0.39 A
Second lower leaf	49.6 ± 6.0 B	1.3 ± 0.12 B	149.6 ± 8.4 A	4.5 ± 0.52 A

The mean lesion numbers followed by the same letter are not significantly different at 0.01 level according to Duncan's new multiple range test. Lesion number was measured 5 days following the TMV inoculation. Lesion diameter was detected at 7 days after TMV inoculation. Each experiment was performed with 7 plants and 5 leaves of each were inoculated with TMV. Lesion diameter is shown as the means ± SD of a single experiment. The experiment was repeated three times with similar results.

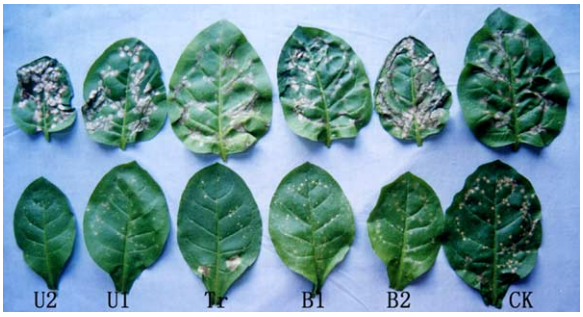

Fig. 3. AR against TMV induced by PB90. Wild-type and NahG transgenic plants were pretreated with 10 nmol L^{-1} by foliar infiltrating 1 day prior to TMV inoculation on treated and the 1st and 2nd upper of lower leaves of the elicitor-treated leaves on the same plants. The bottom row leaves (U2-B2) from wild-type plant and top row leaves (U2-B2) from tobacco plant expressing the *nahG* gene. U2, second upper leaf; U1, first upper leaf; Tr, treated leaf; B1, first lower leaf; B2, second leaf. Photograph was taken 7 days post-inoculation.

and *PR-1c* genes increased, similar to the behavior of these genes in PB90-treated wild-type tobacco plants (Fig. 6). A constitutively expressed gene encoding the translation elongation factor *EF1α* served as an internal standard in these assays. These data show that PB90-induced resistance of non-host tobacco against pathogens may involve hormone(s) other than SA.

4. Discussion

We demonstrated previously that PB90, a novel protein eli citor from *P. boehmeriae*, can trigger HR and SAR in tobacco [42] and that H_2O_2 is involved in the HR and SAR induced by this elicitor [43]. In this study we elucidate the role of SA in HR and SAR induced by PB90.

Several lines of evidence suggest that SA has various roles in signaling host cell death in different incompatible interaction systems. SA has been shown to play a crucial role in mediating cell death, as determined by the analysis of

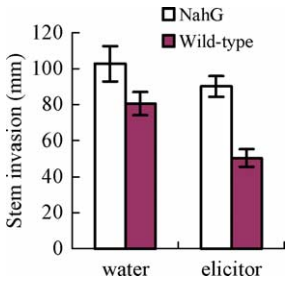

Fig. 4. AR against *Phytophthora nicotianae* by PB90. Wild-type and NahG transgenic plants were pretreated with 10 nmol L^{-1} by foliar infiltrating 1 day prior to *P. nicotianae* inoculation on the stem at site 20 mm away from the axil of treated leaf. Lesions were measured 6 days following the *P. nicotianae* inoculation. Each experiment was performed with 6 plants. Values are shown as the means ± SD of a single experiment. The experiment was repeated three times with similar results.

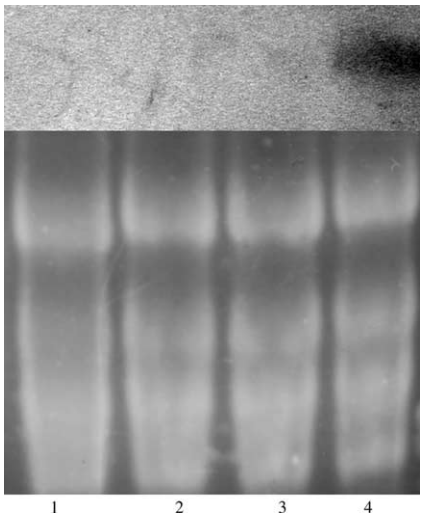

Fig. 5. Induction of SAR marker gene *PR-1a* mRNA accumulation after treatment with PB90. Wild-type and NahG transgenic tobacco plants were treated with 10 nmol L^{-1} or water in leaves by foliar infiltrating. Treated leaves of wild-type plants and NahG plants were collected 5 days after treatment and used for Northern blot analysis following RNA extraction. Uniform loading was standardized by staining with ethidium bromide. 1and 2 indicated NahG tobacco leaves treated with water and PB90, respectively. 3 and 4 indicated wild-type tobacco leaves treated with water and PB90, respectively. Three replicates of determination gave the same results.

Arabidopsis mutants. Several *Arabidopsis* mutants, including those in the lesion-simulating disease resistance response (*lsd*) [7] and in a suppressor of salicylate insensitivity of npr1–5 (*ssi1*) [33], accumulate high levels of SA and spontaneously develop lesions in the absence of pathogen attack. When SA accumulation is prevented in these mutants by expression of the bacterial gene *nahG*, the spontaneous lesion phenotype is suppressed [7,33]. However, the phenotype can be restored by treating the *nahG*-expressing mutants with SA [7]. Evidence for the critical role of SA in the induction of cell death has also come from studies of plant-pathogen interactions, including the *Pseudomonas syringae* pv *glycinea* and soybean suspension cell culture system [7] and the TMV and tobacco interaction

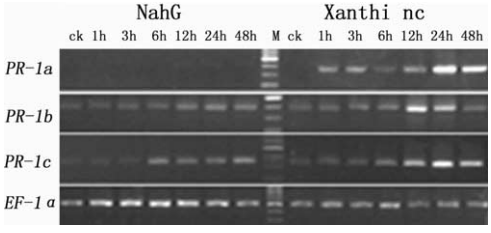

Fig. 6. Kinetics of *PR* genes expression in wild-type and NahG tobacco plants treated with PB90 by semi-quantitative RT-PCR. Expression of the EF-1α used as a quantitative standard. DNA 100 ladders (M) indicate that *PR-1a* (360 bp), *PR-1b* (389 bp), *PR-1c* (358 bp) and *EF-1α* (495 bp) were produced. The same results were obtained in 3 replicates.

system [24]. Recently, harpin from *Xanthomonas oryzae* pv. *oryzae* and $HrpN_{Ea}$ were reported to induce hypersensitive cell death in wild-type but not NahG tobacco plants [29,45]. However, other data have shown that SA is not involved in hypersensitive cell death. For example, tobacco and *Arabidopsis* plants expressing the *nahG* gene and therefore not accumulating SA exhibit hypersensitive cell death when infected with an incompatible pathogen [24,39], and cryptogein is able to trigger HR in NahG tobacco plants [20]. The present study demonstrated that the PB90 elicitor of *P. boehmeriae* triggers SA accumulation in tobacco plants and causes HR in both wild-type and NahG tobacco plants, suggesting that the HR induced in tobacco by PB90 is independent of SA accumulation, similar to that induced by cryptogein [20].

Treatment of plants with various elicitors derived from *Phytophthora* activates different signaling transduction pathways, leading to biologically induced resistance responses that differ in their requirements for SA accumulation. Treatment with cryptogein or a 32-kDa glycoprotein elicitor of *Phytophthora megasperma* does not activate *PR-1a* gene expression in NahG tobacco plants, although these treatments activate the expression of both acidic and basic PR genes in wild-type tobacco, suggesting that these elicitors activate both SA-dependent and SA-independent signaling pathways [3,5,6,12,20]. PB90 treatment induces both local and systemic accumulation of SA (Fig. 1) and *PR-1a* gene expression in wild-type tobacco plants (Figs. 5 and 6), two phenomena that are major indicators of SAR in tobacco [32,34]. No resistance against TMV or *P. nicotianae* was induced by PB90 treatment of NahG transgenic tobacco plants (Figs. 3 and 4), indicating that the PB90 signaling pathway is SA-dependent and that SA functions as an intermediate signal upstream of SAR. Moreover, PB90 can induce expression of the basic PR gene *PR-1b* (Fig. 6), which is specifically activated by jasmonic acid [26]. Therefore, the results show that in tobacco the elicitor likely triggers dual signal transduction pathways that include both SA-dependent and SA-independent routes.

Does HR confer systemic acquired resistance during incompatible interactions between plants and pathogens? Specific pathogen recognition mechanisms, governed by resistance gene products that interact with matching avirulence gene products from the pathogen, usually lead to HR at the site of pathogen invasion, isolating the pathogen and preventing it from invading the rest of the plant. Cells that die during HR have also been suggested to release mobile signals that condition adjacent cells to become responsive to pathogens and that activate systemic acquired resistance throughout the plant. Alvarez et al. observed in Arabidopsis that hypersensitive cell death is not restricted to the macroscopic HR lesions at the inoculation site, but also occurs at a distance in 'micro-HR' lesions that are not visible to the naked eye, suggesting that this HR in Arabidopsis depends on secondary oxidative bursts in distant tissues and that the secondary oxidative bursts generate a signal capable of inducing SAR and LAR [1]. However, several reports have suggested that HR and defense gene expression result from distinct pathways. For example, the *dnd*1 mutation, which lacks the HR phenotype, leads to activation of generalized defense mechanisms, so that in an R gene background, elicitation of the resistance mechanisms may occur earlier in the infection cycle than in wild-type plants [49]. The caspase-specific peptide inhibitors Ac-YVAD-CMK (which is irreversible and more specific for caspase-1) and Ac-YVAD-CHO (which is reversible and more specific for caspase-3) abolish expression of the bacteria-induced tobacco HR marker genes *HSR203J* and *HIN1* as well as hypersensitive cell death, but do not significantly affect the expression of defense genes including *PR-1a* and *PR-2* [9]. Treatment of bean plants with *Pseudomonas syringae* strains harboring mutations in the *hrp* genes does not elicit an HR, but induces defense gene expression [18]. In the Pto/avrPto interaction system, Zhou et al. used the yeast two-hybrid system to find that Pti1 is involved in the HR of tomato induced by *P. syringae*, and that the Pti4/5/5/6 proteins are involved in a separate pathway governing defense gene expression [51]. These observations are consistent with the notion that the signal perceived by an R gene product is transduced *via* two separate pathways, an HR pathway and a defense gene activation pathway, although cross-talk may exist between the two pathways. In this study, we observed that PB90 treatment induces HR in NahG transgenic tobacco as well as wild-type tobacco, but does not elicit SAR against TMV and *P. nicotianae* or the expression of *PR-1a*, a SAR marker gene. These results suggest that these SAR and HR effects may be regulated by distinct pathways that are coordinately activated through the same signal, PB90. It is also possible that the same pathway mediates SAR and HR, with SA functioning downstream of HR, but upstream of SAR, and that cells dying due to the HR may release signals that condition adjacent cells to become responsive to the elicitor and activate SA accumulation and SAR throughout the plant. Our results show that the HR cell death induced by PB90 may act as a signaling system rather than a direct defense mechanism.

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References

[1] Alvarez ME, Pennell RI, Meijer PJ, Ishikawa A, Dixon RA, Lamb C. Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell 1998;92:773–84.

[2] Atkinson MM. Molecular mechanisms of pathogen recognition by plants. Adv Plant Pathol 1993;10:35–64.

[3] Baillieul F, de Ruffray P, Kauffmann S. Molecular cloning and biological activity of alpha-, beta-, and gamma-megaspermin, three elicitins secreted by *Phytophthora megasperma* H20. Plant Physiol 2003;131:155–66.

[4] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156–9.

[5] Cordelier S, de Ruffray P, Fritig B, Kauffmann S. Biological and molecular comparison between localized and systemic acquired resistance induced in tobacco by a *Phytophthora megasperma* glycoprotein elicitin. Plant Mol Biol 2003;51:109–18.

[6] Costet L, Cordelier S, Dorey S, Baillieul F, Fritig B, Kauffmann S. Relationship between localized acquired resistance (LAR) and the hypersensitive response (HR): HR is necessary for LAR to occur and salicylic acid is not sufficient to trigger LAR. Mol Plant-Microbe Interact 1999;8:655–62.

[7] Dangl JL, Dietrich RA, Richberg MH. Death don't have no mercy: cell death programs in plant-microbe interactions. Plant Cell 1996;8: 1793–807.

[8] Dangl JL, Jones JDG. Plant pathogens and integrated defence responses to infection. Nature 2001;411:826–33.

[9] del Pozo O, Eric L. Caspases and programmed cell death in the hypersensitive response of plants to pathogens. Curr Biol 1998;8: 1129–32.

[10] Delaney TP, Ukness S, Vernooij B, Friedrich L, Weymann K, Negrotto N, et al. A central role of salicylic acid in plant disease resistance. Science 1994;266:1247–50.

[11] Dong HS, Delaney TP, Bauer DW, Beer SV. Harpin induces disease resistance in Arabidopsis through the systemic acquired resistance pathway mediated by salicylic acid and the NIM1 gene. Plant J 1999; 20:207–15.

[12] Dorey S, Baillieul F, Pierrel MA, Saindrenan P, Fritig B, Kauffmann S. Spatial and temporal induction of cell death, defense genes, and accumulation of salicylic acid in tobacco leaves reacting hypersensitively to a fungal glycoprotein elicitor. Mol Plant-Microbe Interact 1997;5:646–55.

[13] Durner J, Shah J, Klessig DF. Salicylic acid and disease resistance in plants. Trends Plant Sci 1997;2:266–74.

[14] Friedrich L, Lawton K, Ruess W, Masner P, Specker N, Gut-Rella M, et al. A benzothiadiazole derivative induces systemic acquired resistance in tobacco. Plant J 1996;10:61–70.

[15] Gaffney T, Friedrich M, Vernooij B, Negrotto D, Nye G, Uknes S, et al. Requirement of salicylic acid in systemic acquired resistance. Science 1993;261:754–6.

[16] Hammond-Kosack KE, Jones JDG. Resistance gene-dependent plant defense responses. Plant Cell 1996;8:1773–91.

[17] Huckelhoven R, Fodor J, Preis C, Kogel KH. Hypersensitive cell death and papilla formation in barley attacked by the powdery mildew fungus are associated with hydrogen peroxide but not with salicylic acid accumulation. Plant Physiol 1999;119:1251–60.

[18] Jakobek JL, Lindgren PB. Generalized induction of defense response in bean is not correlated with the induction of the hypersensitive reaction. Plant Cell 1993;5:49–56.

[19] Jones AM, Dangl JL. Logjam at the Styx: programmed cell death in plants. Trends Plant Sci 1996;1:114–9.

[20] Keller H, Bonnet P, Galiana E, Pruvot L, Friedrich L, Ryals J, Ricci P. Salicylic acid mediates elicitin-induced systemic acquired resistance, but not necrosis in tobacco. Mol Plant-Microbe Interact 1996;9: 696–703.

[21] Keen NT. Gene-for-gene complementarity in plant–pathogen interactions. Ann Rev Genet 1990;24:447–63.

[22] Malamy JM, Klessig DF. Salicylic acid and its conjugates during the resistance response to tobacco mosaic virus infection. Plant Cell 1992; 4:359–66.

[23] Martinez C, Baccou JC, Bresson E, Baissac Y, Daniel JF, Jalloul A, et al. Salicylic acid mediated by the oxidative burst is a key molecule in local and systemic responses of cotton challenged by an avirulent race of *Xanthomonas capestris* pv *malvacearum*. Plant Physiol 2000; 122:757–66.

[24] Mur LAJ, Bi YM, Darby RM, Firek S, Draper J. Compromising early salicylic acid accumulation delays the hypersensitive response and increase viral dispersal during lesion establishment in TMV-infected tobacco. Plant J 1997;12:1113–26.

[25] Mysore KS, Ryu CM. Nonhost resistance: how much do we know? Trends Plant Sci 2004;9:97–104.

[26] Niki T, Mitsuhara I, Seo S, Ohtsubo N, Ohashi Y. Antagonistic effect of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco leaves. Plant Cell Physiol 1998;39:500–7.

[27] Nurnberger T, Brunner F. Innate immunity in plants and animals: emerging parallels between the recognition of general elicitors and pathogen-associated molecular patterns. Curr Opin Plant Biol 2002;5: 318–24.

[28] Nurnberger T, Scheel D. Signal transmission in the plant immune response. Trends Plant Sci 2001;6:372–9.

[29] Peng JL, Dong HS, Dong HP, Delaney TP, Bonasera JM, Beer SV. Harpin-elicited hypersensitive cell death and pathogen resistance require the NDR1 and EDS1 genes. Physiol Mol Plant Pathol 2003;62: 317–26.

[30] Pfitzner UM, Goodman HM. Isolation and characterization of cDNA clones encoding pathogensis-related proteins from tobacco mosaic virus infected tobacco plants. Nucleic Acids Res 1987;15:4449–65.

[31] Pieterse CMJ, Van Loon LC. Salicylic acid-independent plant defence pathways. Trends Plant Sci 1999;4:52–8.

[32] Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY, Hunt MD. Systemic acquired resistance. Plant Cell 1996;8:1809–19.

[33] Shah J, Kachroo P, Klessig DF. The Arabidopsis ssi mutation restores PR gene expression in npr1 plants and renders defensin gene expression SA dependent. Plant Cell 1999;11:191–206.

[34] Sticher L, Mauch-Mani B, Metraux JP. Systemic acquired resistance. Annu Rev Phytopathol 1997;35:235–70.

[35] Thordal-Christensen H. Fresh insights into processes of nonhost resistance. Curr Opin Plant Biol 2003;6:351–7.

[36] Turner JG, Ellis C, Devoto A. The jasmonate signal pathway. Plant Cell 2002;14:S153–S64.

[37] Uknes S, Winter AM, Delaney T, Vernooij B, Morse A, Friedrich L, et al. Biological induction of systemic acquired resistance in Arabidopsis. Mol Plant-Microbe Interact 1993;6:692–8.

[38] Verberne MC, Hoekstra J, Bol JF, Linthorst HJM. Signaling of systemic acquired resistance in tobacco depends on ethylene perception. Plant J 2003;35:27–32.

[39] Vernooij B, Friedrich L, Morse A, Reist R, Kolditz-Jawhar R, Ward E, Uknes S, Kessmann H, Ryals J. Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. Plant Cell 1994;6:959–65.

[40] Wang KLC, Li H, Ecker JR. Ethylene biosynthesis and signaling networks. Plant Cell 2002;14:S131–S51.

[41] Wang SC, Li GJ, Xia K, Xu LL, Chen FY, Zhou X. Preparation and application of monoclonal antibodies specific for salicylic acid. Acta Botanica Sinica 2001;43:1207–10.

[42] Wang YC, Hu DW, Zhang ZG, Ma ZC, Zheng XB, Li DB. Purification and immunocytolocalization of a novel Phytophthora boehmeriae protein inducing the hypersensitive response and systemic acquired resistance in tobacco and Chinese cabbage. Physiol Mol Plant Pathol 2003;63:223–32.

[43] Wang YC, Zhang ZG, Li J, An CC, Chen ZL, Zheng XB. H_2O_2 is involved in hypersensitive response and systemic acquired resistance induced by the 90 kDa protein elicitor of *Phytophthora boehmeriae*. J Plant Physiol Mol Biol 2003;29:185–91.
[44] Ward E, Uknes S, Williams S, Dincher S, Wiederhold D, Alexander D, et al. Coordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell 1991;3:1085–94.
[45] Wen WG. Study on elicitors of hypersensitive response from *Xanthomonas oryzae*. PhD thesis, Nanjing Agricultural University, 2001.
[46] Weymann K, Hunt M, Uknes S, Neuenschwander U, Lawton K, Steiner HY, Ryals J. Suppression and restoration of lesion formation in Arabidopsis lsd mutants. Plant Cell 1995;7:2013–22.
[47] Whitham S, Dinesh-Kumar SP, Choi D, Hehl R, Corr C, Baker B. The product of the tobacco mosaic virus resistance gene N: similarity to Toll and the interleukin-1 receptor. Cell 1994;78:1101–15.
[48] Yalpani N, Silverman P, Wilson TMA, Kleier DA, Raskin I. Salicylic acid is a systemic signal and an inducer of pathogensis-related proteins in virus-infected tobacco. Plant Cell 1991;3:809–18.
[49] Yu IC, Parker J, Bent AF. Gene-for-gene disease resistance without the hypersensitive response in Arabidopsis dnd1 mutant. Proc Natl Acad Sci USA 1998;95:1819–7824.
[50] Yu LM. Elicitins from Phytophthora and basic resistance in tobacco. Proc Natl Acad Sci USA 1995;92:4088–94.
[51] Zhou J, Tang X, Frederick R, Martin G. Pathogens recognition and signal transduction by the Pto kinase. J Plant Res 1998;111:353–6.