Long-Term Induction of Defense Gene Expression in Potato by *Pseudomonas* sp. LBUM223 and *Streptomyces scabies*

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ABSTRACT

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Streptomyces scabies is a causal agent of common scab of potato, which generates necrotic tuber lesions. We have previously demonstrated that inoculation of potato plants with phenazine-1-carboxylic acid (PCA)-producing *Pseudomonas* sp. LBUM223 could significantly reduce common scab symptoms. In the present study, we investigated whether LBUM223 or an isogenic *phzC*– mutant not producing PCA could elicit an induced systemic resistance response in potato. The expression of eight defense-related genes (salicylic acid [SA]-related *ChtA*, *PR-1b*, *PR-2*, and *PR-5*; and jasmonic acid and ethylene-related *LOX*, *PIN2*, *PAL-2*, and *ERF3*) was quantified using newly developed TaqMan reverse-transcription quantitative polymerase chain reaction assays in 5- and 10-week-old potted potato plants. Although only wild-type LBUM223 was capable of significantly reducing common scab symptoms, the presence of both LBUM223 and its PCA-deficient mutant were equally able to upregulate the expression of *LOX* and *PR-5*. The presence of *S. scabies* overexpressed all SA-related genes. This indicates that (i) upregulation of potato defense-related genes by LBUM223 is unlikely to contribute to common scab's control and (ii) LBUM223's capacity to produce PCA is not involved in this upregulation. These results suggest that a direct interaction occurring between *S. scabies* and PCA-producing LBUM223 is more likely involved in controlling common scab development.

Common scab of potato (*Solanum tuberosum* L.) is a bacterial disease causing necrotic lesions and is frequently caused by *Streptomyces scabies* (22). *S. scabies* attacks actively growing tubers by producing thaxtomins, which are phytotoxins that inhibit the biosynthesis of cellulose (32), resulting in cell death.

Previously, we showed that inoculating potato plants with phenazine-1-carboxylic acid (PCA)-producing *Pseudomonas* sp. LBUM223 could significantly reduce potato common scab symptoms by twofold when compared with plants inoculated with *S. scabies* alone or in combination with an isogenic mutant of LBUM223 (*phzC*–) incapable of producing PCA. Surprisingly, pathogen populations did not decrease in the rhizosphere or the geocaulosphere surrounding potato plants in the presence of LBUM223. However, in the geocaulosphere, the presence of the wild-type LBUM223 but not its mutant significantly repressed the expression of *txtA* (2), involved in thaxtomin production in *S. scabies*, suggesting a reduction in thaxtomin A production. A decrease in thaxtomin A production may, at least in part, account for the fewer symptoms observed when potato plants were treated with LBUM223 (38). However, the implication of PCA production at other levels, such as triggering a systemic defense response, could not be ruled out.

Fluorescent *Pseudomonas* spp. have repeatedly been shown to be useful biocontrol agents, in part due to their capacity to produce various antimicrobial compounds, including phenazines (7), but also due to their capacity to induce plant defense responses (4). This enhanced state of defense that occurs systemically in a plant has been termed induced systemic resistance (ISR). In this state, a plant subsequently subjected to a pathogen attack is better protected and will produce more defense proteins due to a priming phenomenon, which occurs following exposure to the ISR-inducer (8). *Pseudomonas*-mediated ISR seems to rely on the detection of a variety of molecules by the host plant, including several bacterial components such as flagella and lipopolysaccharides, but also secreted compounds such as siderophores and antibiotics (4). The production of the antibacterial diacetylphloroglucinol by several fluorescent *Pseudomonas* spp. has been shown to be an important element in inducing systemic resistance in *Arabidopsis thaliana* (44). A type of phenazine, pyocyanin, produced by *Pseudomonas aeruginosa* 7NSK2, has been shown to elicit an ISR response against *Botrytis cinerea* in tomato (3) and also against *Magnaporthe grisea* in rice (11). At least one strain explicitly described as being a PCA producer, *P. chlororaphis* sp. O6, has been shown to elicit ISR (35); however, to our knowledge, the production of PCA has never been shown to directly induce a systemic response in plants.

The perception of a pathogen by a plant has also been shown to stimulate plant defenses. Similarly to ISR, a plant that has been exposed to a pathogen and survived the infection will be better protected against a subsequent infection by the same or another pathogen. Such a response is known as systemic acquired resistance (SAR) (13). To our knowledge, *S. scabies*' ability to induce SAR in potato plants has never been studied. However, some reports suggest that *S. scabies*, or the thaxtomin A it produces, are capable of inducing defense responses in *A. thaliana* (5,14,15,23).

Hormonal signals are involved in translating a local response at the site of recognition or infection to the entire plant, and triggering the upregulation in the expression of specific defense genes. Traditionally, jasmonic acid (JA) and ethylene (ET) have been considered to be the signals involved in ISR, while salicylic acid (SA) mediates the SAR response. Although these seem to be the backbone of systemic resistance in several plant systems,

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additional key molecules that can also modulate plant responses, including cytokinins, gibberellins, and auxins, are constantly being discovered (9,29). There also seem to be various levels of crosstalk between the different hormonal pathways (30,39). To date, most signaling studies have been performed using the model plant *A. thaliana*. It is likely that a given signaling response may vary between plant species and will also depend on the microorganism encountered (18). For example, a study has shown that SA and JA are both activated in response to pathogen-associated molecular patterns extracted from the late blight pathogen *Phytophthora infestans* (20). This information renders the prediction of the exact mechanisms of resistance involved in potato in response to the presence of nonstudied microorganisms highly difficult. To our knowledge, there are no reports investigating the transcriptional activity of a variety of defense-related genes in potato plants in response to *Pseudomonas* spp. or *S. scabies* and, therefore, there is no information on the signaling mechanisms that may be utilized by potato in such a system. Of the few systemic resistance studies that have been conducted using potato, either ISR or SAR, most have focused on fungal or oomycete pathogens, the majority on *P. infestans* (12,17,42,43) and *Verticillium dahliae* (10).

The aim of this study was to determine whether *Pseudomonas* sp. LBUM223, through its production of PCA or other mechanisms, is able to induce a long-term systemic resistance response in potato, and determine, if such a response exists, whether it can contribute to the biocontrol of common scab. In parallel, we also examined whether *S. scabies* was able to induce the overexpression of defense-related genes. In order to detect a systemic defense response, RNA transcripts were isolated from the leaves of 5- and 10-week-old potato plants; treated with all combinations of LBUM223, its PCA- mutant, or *S. scabies*; and analyzed by newly designed reverse-transcription quantitative polymerase chain reaction (RT-qPCR) assays. Eight commonly used marker genes for which sequences were available for potato were targeted: *PR-1b*, *PR-2*, *PR-5*, and a class II chitinase (*ChtA*), which are generally associated with an SA response; lipoxygenase (*LOX*), phenylalanine ammonia-lyase (*PAL-2*), and proteinase inhibitor II (*PIN2*), which are generally associated with a JA response; and, finally, ET response factor 3 (*ERF3*), which is associated with an ET response.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Thaxtomin-A-producing *S. scabies* LBUM848 was isolated from a common scab lesion on a potato tuber harvested in Bloomfield, NB, Canada (37). *Pseudomonas* sp. LBUM223 was previously isolated from the rhizosphere of strawberry plants in Bouctouche, NB, Canada (28). An isogenic mutant of LBUM223, LBUM223 *phzC*–, incapable of producing PCA, was generated and validated as previously described (36). All strains were routinely grown in tryptic soy broth (BD, Franklin Lakes, NJ) with shaking at 25°C.

Soil and seed preparation. The soil used (sandy loam, pH 6.7) was collected from the Agriculture and Agri-Food Canada S.H.J. Michaud Research Farm (Bouctouche, NB, Canada) and sieved (4 mm). As a potting soil mix, three parts of soil were mixed with one part of sand and autoclaved twice during two consecutive days at 121°C for 1 h prior to use. Seed tubers ('Kennebec') were kept at room temperature until multiple sprouts had started to appear on each tuber, then cut in ≈30-cm^3 pieces bearing at least one sprout. Tuber pieces were left to heal for 3 days at 15°C before planting.

***S. scabies* and *Pseudomonas* sp. inocula.** The *S. scabies* inoculum was prepared in soil as previously described (2). A negative control was also prepared using the autoclaved soil without adding *S. scabies*. Standard microbiological plating on tryptic soy agar was used to estimate *S. scabies* concentration (CFU per gram of soil). For *Pseudomonas* sp. LBUM223 treatments (wild type and isogenic mutant *phzC*–), liquid inocula were prepared by growing each respective strain at 25°C for 3 days with shaking at 250 rpm. Populations were estimated using spectrophotometer readings (λ= 600 nm) and a standard curve.

Growth chamber experiments. The experimental set-up consisted of six replicates of each of the following treatments: (i) no inoculum (control), (ii) LBUM223, (iii) LBUM223 *phzC*–, (iv) *S. scabies* (no antagonist), (v) *S. scabies* + LBUM223, and (vi) *S. scabies* + LBUM223 *phzC*–. The entire experiment was repeated three times. All bacterial inoculations were done at planting. For pots requiring *S. scabies*, the *S. scabies* soil inoculum was blended with the potting soil mix to obtain ≈5 × 10^7 CFU/g of soil, and a total of 1 kg of soil was added per 6-in. pot. For pots not requiring *S. scabies*, the same amount of negative control soil inoculum was used. For antagonist treatments, seed tubers were first dipped in a bacterial culture of either LBUM223 or LBUM223 *phzC*–; then, the rest of the culture was subsequently added to the pot around the tuber to obtain a final concentration of 4 × 10^7 CFU/g of soil. Negative controls received the same volume of water. Tuber pieces (one per pot) were planted at a depth of ≈5 cm. Pots were placed in the growth chamber (Conviron CMP5000 series; Controlled Environments, Winnipeg, MB, Canada) using a completely randomized design and grown following a previously described light and temperature regime (2). For each replication of the experiment, destructive harvesting of three replicates per treatment occurred at 5 and 10 weeks after planting. For each plant, all leaves were rapidly collected, immediately frozen in liquid nitrogen and stored at –80°C.

Extraction of plant RNA. Leaf samples were ground in liquid nitrogen using RNAse-free mortars and pestles. RNA was extracted from the homogenized leaf samples using the RNeasy Plant Mini kit (Qiagen, Mississauga, Canada). Manufacturer instructions were followed using the provided RLT buffer and a final elution with 2 × 50 µl of elution buffer. The optional on-column DNase (Qiagen) was performed. An additional DNAse treatment was required (TURBO DNA-free; Ambion, Applied Biosystems, Foster City, CA) following the manufacturer's instructions. RNA was quantified (NanoDrop Technologies, Wilmington, DE) and assessed for quality using an Experion system (Bio-Rad, Mississauga, Canada). All samples obtained an RNA quality indicator value >7, indicating that the RNA was of good quality and could be used for expression analyses.

Primers and probes. Primers and TaqMan probes (Table 1) were newly designed using the Primer Express 3.0 software (Applied Biosystems) and purchased from Integrated DNA Technologies (Coralville, IA) and Applied Biosystems, respectively. The TaqMan probes were each constructed using FAM at the 5′ end and minor groove binding (MGB) at the 3′ end. To ensure specificity, all sequences were analyzed using the BLASTn algorithm (National Center for Biotechnology Information) and all primer–probe combinations were also validated on potato DNA and cDNA. The resulting amplicons were all 60 to 69 bp and qPCR reaction efficiencies were 93 to 100%.

Relative RT-qPCR analysis with normalization using two reference genes. RNA samples were diluted to 30 ng/µl prior to RT. Synthesis of 100 µl of cDNA was accomplished using oligo-dT and the TaqMan Reverse Transcription Reagents Kit (Applied Biosystems). The resulting cDNA was diluted 1:2. Eight genes were targeted: *ChtA*, *PR-1*, *PR-2*, *PR-5*, *ERF3*, *LOX*, *PAL-2*, and *PIN2*. Interplate calibrators, consisting of identical samples of potato DNA, were used to ensure consistency. Reactions (20 µl) were prepared using the iTaq reagent (10 µl of iTaq Supermix [Bio-Rad], primers and probe [200 nM], and 4.8 µl of cDNA). qPCRs were performed in triplicate and included no-template controls using an ABI 7500 Real-Time PCR System (Applied Biosystems). Because several potential reference genes had previously been discovered for potato (27), we were able to select

and validate two references genes that were deemed stable in our system using the geNorm function of the qbase^{PLUS} software (Biogazelle, Zwijnaarde, Belgium): *EF1-α* and *CyP*. Target gene cycle thresholds (Cts) were normalized by subtracting the geometric mean of the Cts from both reference genes using the qbase^{PLUS} software. The resulting data were then divided by the arithmetic mean of all control plants for a given time and experiment in order to obtain a relative fold change for each individual unit (plant).

Statistical analyses. Three-factor analyses of variance (time, type of *Pseudomonas* treatment, and presence or absence of *S. scabies*) were performed with subsequent Tukey-Kramer adjustment to determine significance ($P < 0.05$). Mixed models were used to include a random factor, which removes variation between different replications of experiments and allowed the analysis of all experiments together ($n = 9$ for each treatment at each time). The interaction between factors was verified and, if an interaction occurred, multiple comparisons were made using these results and the analysis did not utilize the effect of each individual factor. The absence of an interaction with the time factor confirmed that the trend observed was the same at all time points. The absence of an interaction between both treatment factors (*Pseudomonas* spp. and *S. scabies*) indicates that the presence of one treatment does not modify the effect that the other has on gene expression. The data required log transformations to achieve suitable homogeneity and normality. Each harvested pot represented an independent experimental unit. The SAS Statistical Analysis Software (v. 9.2; SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Infection by *S. scabies* upregulates the expression of *ChtA*, *PR-1b*, *PR-2*, and *PR-5* and downregulates the expression of *ERF3*. The presence of the pathogen, regardless of whether or not *Pseudomonas* sp. LBUM223 or its *phzC*– mutant was also inoculated, significantly upregulated the expression of *ChtA* (Fig. 1A), *PR-1b* (Fig. 1B), *PR-2* (Fig. 1C), and *PR-5* (Fig. 1D), generally by two to three times, at both 5 and 10 weeks ($P < 0.0001$ for all genes). The expression profile of *PR-5* was the only one varying in time ($P = 0.004$), with the expression at week 10 being generally higher. In addition to inducing the expression of these genes, the application of *S. scabies* also slightly downregulated the expression of *ERF3* (Fig. 2A). An interaction occurred between time and the presence of *S. scabies* ($P < 0.0001$), indicating that the effect of *S. scabies* was not the same at all times. Multiple comparisons showed a reduced expression to ≈60% of that of the control treatment, only at week 5. At week 10, the presence of the pathogen had no effect. The expression of *LOX* (Fig. 2B), *PAL-2* (Fig. 2C), and *PIN2* (Fig. 2D) did not vary significantly in response to the presence of *S. scabies*.

The presence of LBUM223 or its isogenic mutant upregulate the expression of *PR-5* and *LOX*. In addition to responding to the presence of *S. scabies*, *PR-5* expression (Fig. 1D) was also upregulated equally by the presence of LBUM223 or its *phzC*– mutant ($P < 0.0001$). This upregulation was approximately two- to threefold at both weeks studied, when comparing *Pseudomonas* spp.-containing treatments with their respective controls (containing or not containing *S. scabies*). Because no interaction occurred between the factors analyzed, the increase in expression induced by the application of LBUM223 or its mutant is independent from the expression induced by the application of the pathogen, meaning that neither has an influence on the other. Both LBUM223 and its isogenic mutant also overexpressed *LOX* at all time points (Fig. 2B) ($P < 0.005$), increasing it two- to threefold when compared with control treatments. The expression of all other genes did not vary significantly in response to LBUM223 or its isogenic mutant.

The expression of *PAL-2* and *PIN2* was not affected by either treatment with LBUM223 or infection with *S. scabies*. The expression of *PAL-2* (Fig. 2C) and *PIN2* (Fig. 2D) remained unchanged regardless of treatment or time point.

DISCUSSION

To our knowledge, this is the first report demonstrating a systemic long-term (up to 10 weeks postinoculation) upregulation in the expression of several defense-related genes in full-grown potato plants exposed to multiple microorganisms.

The presence of *Pseudomonas* sp. LBUM223 or its isogenic *phzC*– mutant equally overexpressed *LOX* and *PR-5* in potato, regardless of the presence or absence of *S. scabies*. PCA production was not required in order for LBUM223 to have an effect on the expression of these two genes, suggesting that another bacterial determinant triggered the signal. Also, the fact that both LBUM223 and its non-PCA-producing mutant induced the same response in potato, whereas only PCA-producing

TABLE 1. Quantitative polymerase chain reaction primers and probes used in this study and corresponding GenBank accession number of potato sequences used for design

Gene	Name used	Accession number	Primers (5′–3′)	Probe (5′–3′)
Chitinase class II	*ChtA*	U49970.1	stChtA-F: TTCTGGATGACAGCACAGGATAA stChtA-R: GGCGTCCATTGCCCAAT	stChtA-Pr: AGCCATCATGCCACAAC
Pathogenesis-related protein 1b	*PR-1b*	AY050221	stPR1b-F: GGCATCCCGAGCACAAAAT stPR1bR: CTGCACCGGAATGAATCAAGT	stPR1b-Pr: ATGCCAATTCAAGAACTG
Endo-1,3-β-D-glucanase	*PR-2*	U01902.1	stPR2-F: GTGAAGCTGGTTTGGGAAATG stPR2-R: TTGCCAATCAACGTCATGTCTAC	stPR2-Pr: CATTAAGGTCTCAACGTC
Putative thaumatin-like protein	*PR-5*	AY737317.1[a]	stPR5-F: GGAGGCAGACGACTCGACTT stPR5-R: CCATGGTTGTTCCTGGATTCA	stPR5-Pr: CCAAACTTGGAACATTAA
Ethylene response factor 3	*ERF3*	EF091875.1	stERF3-F: GTGTTGACGTGAAACCAACCAT stERF3-R: CCGGTGGAGGAAAGTTAAGGT	stERF3-Pr: CCGGTCTAAATCTG
Lipoxygenase; *lox1-St-2*	*LOX*	Y18548.1	stLOX-F: CAGATCAGGCCCCGTTAATG stLOX-R: CCTGTAAGTCCACCTTCACTTGTTG	stLOX-Pr: TCCGTATACGTTGCTTTT
Phenylalanine ammonia-lyase 2	*PAL-2*	X63104	stPAL2-F: GGTCACTGCCTCGGGTGAT stPAL2-R: CCTGCCAGTGAGCAAACCA	stPAL2-Pr: TTGTACCTTTGTCCTACATTG
Proteinase inhibitor II	*PIN2*	X04118.1	stPIN2-F: ATGAGCCCAAGGCAAATATGTAC stPIN2-R: GCCAATCCAGAAGATGGACAA	stPIN2-Pr: CTGCAATGTGACCCTAG
Elongation factor 1 α	*EF1-α*	AB061263.1	stEF1α-F: AGGTTCTAGTTCTCTGCCTTGTATGTC stEF1α-R: CGCCACCGCCTATCAAGTAC	stEF1α-Pr: AGTACTCATTCTCAGAACTG
Cyclophilin	*CyP*	AF126551.1	stCyP-F: AGCACGTCGTGTTTGGACAA stCyP-R: AACAGCCTCGGCCTTCTTAAT	stCyP-Pr: TTGTTGAAGGCTTGGATGT

[a] Position 1123 to 1795 of reverse complement.

LBUM223 has been previously shown to control common scab in potato, suggests that the enhanced expression of *LOX* and *PR-5* observed in this study is most likely not significantly involved in the reduction of common scab symptoms but could be implicated in other aspects of plant metabolism. The only upregulated gene from the JA/ET pathway in this study was *LOX*. It encodes the enzyme lipoxygenase, which catalyzes the addition of molecular oxygen to specific polyunsaturated fatty acids in order to produce hydroperoxy fatty acids in plant. These can then enter several pathways, one of which leads to the production of JA via octadecanoid precursors in a defense response and another that may produce oxylipids implicated in vegetative growth, among others (31). Because *Pseudomonas* sp. LBUM223 and its non-PCA-producing mutant have previously been shown capable of promoting potato plant growth by significantly increasing total plant weight (2), the upregulation of *LOX* expression described in this study might perhaps be linked to the growth promotion observed (to be determined). *PR-5*, an SA-responsive gene, was also upregulated by *Pseudomonas* sp. LBUM223 and its isogenic *phzC*– mutant. The acidic *PR-5* gene studied here is not well characterized in function compared with its basic counterpart (1) and, therefore, could be implicated, along with *LOX*, in other plant functions not related to defense. It is interesting to note that the expression of *PR-5* is significantly higher in 10-week-old plants than 5-week-old plants for all treatments, which shows that the overexpression of defense genes can be sustained or even increased over a period of several weeks following inoculation with *Pseudomonas* sp. LBUM223 (wild type and *phzC*– mutant).

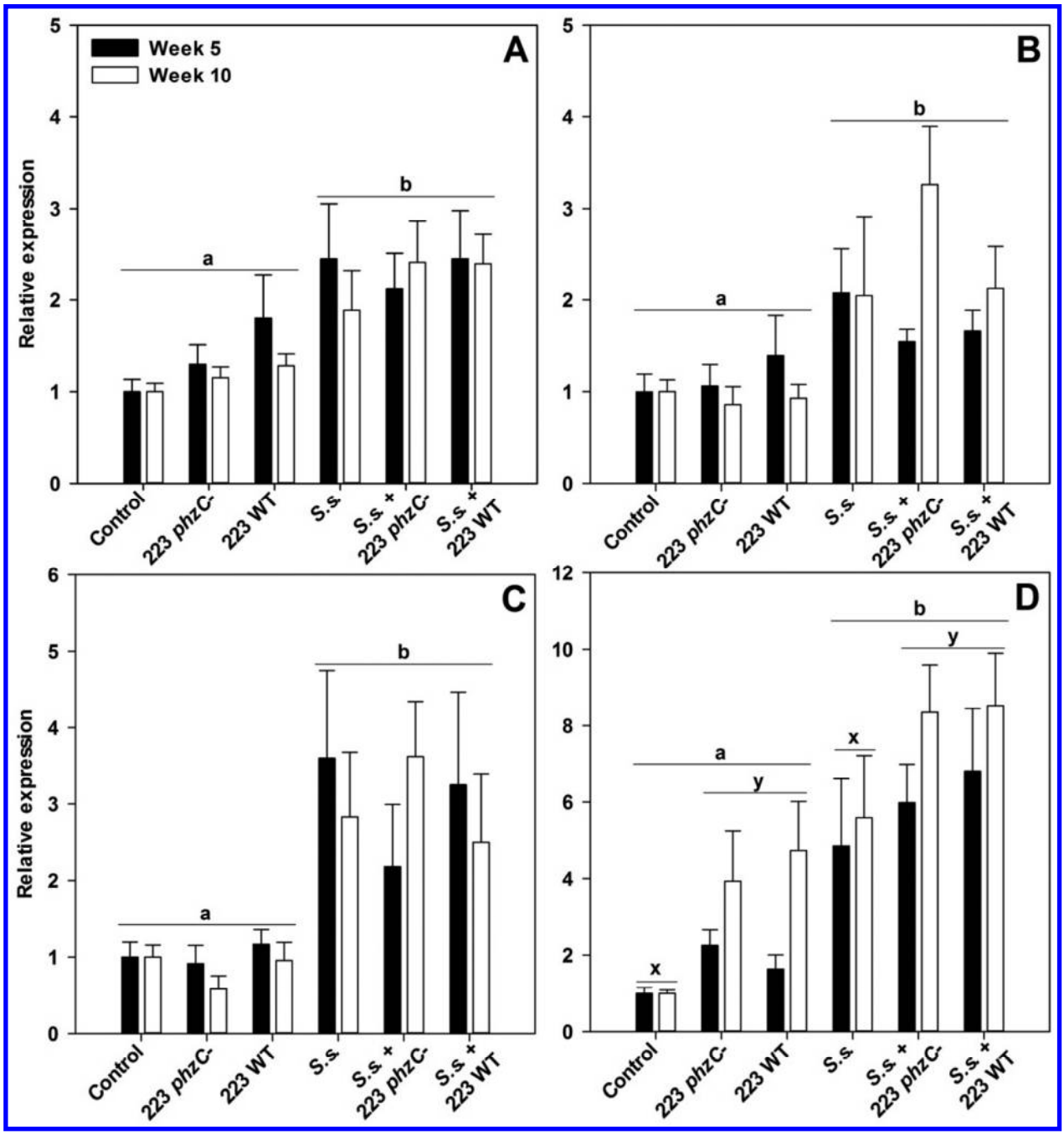

Fig. 1. Expression of salicylic acid-related genes **A,** *ChtA*; **B,** *PR-1b*; **C,** *PR-2*; and **D,** *PR-5* in potato leaves at 5 and 10 weeks. For all panels, values are presented as mean ± standard error of the mean (n = 9). Each panel has its respective Y-axis scale. Significant differences are presented using different letters: a and b for the *Streptomyces scabies* factor and x and y for the *Pseudomonas* factor. Because no interaction occurred between time and both treatment factors, horizontal lines were used to group all time points related to treatments where *S. scabies* (*S.s.*) was applied or not (a and b) or *Pseudomonas* LBUM223 (wild type [WT] or *phzC*–) was applied or not (x and y).

Among the eight genes studied, only *PR-5* was upregulated by both *S. scabies* and *Pseudomonas* sp. LBUM223 (wild type and *phzC*– mutant). However, statistical analyses showed no interaction between both treatment factors, meaning that the modulation of gene expression was additive and independent. In other words, the presence of LBUM223 or its isogenic mutant did not alter the effect *S. scabies* had on the potato plant and vice versa. Therefore, the higher expression of *PR-5* observed when LBUM223 (wild type and *phzC*– mutant) is inoculated with *S. scabies*, compared with the pathogen alone, seems to be a combination of the induction of expression by LBUM223 and that of *S. scabies*. Our results suggest that LBUM223 does not prime the plant for enhanced protection against *S. scabies*, because priming usually involves the potentiation of a broad spectrum of defense genes related to the same signaling pathway (8), which is not the case. Also, ISR inducers do not typically induce the expression of defense-related genes on their own (41), as seen here, but, rather, prepare the plant for an enhanced expression once it is exposed to a pathogen. It is possible that the conditions under which the experiment was conducted, which aimed at simulating field trials during which the seed tuber is immediately exposed to the pathogen once planted, did not allow sufficient contact with LBUM223 prior to the pathogen challenge to efficiently prime the plant. However, disease control was nevertheless observed when

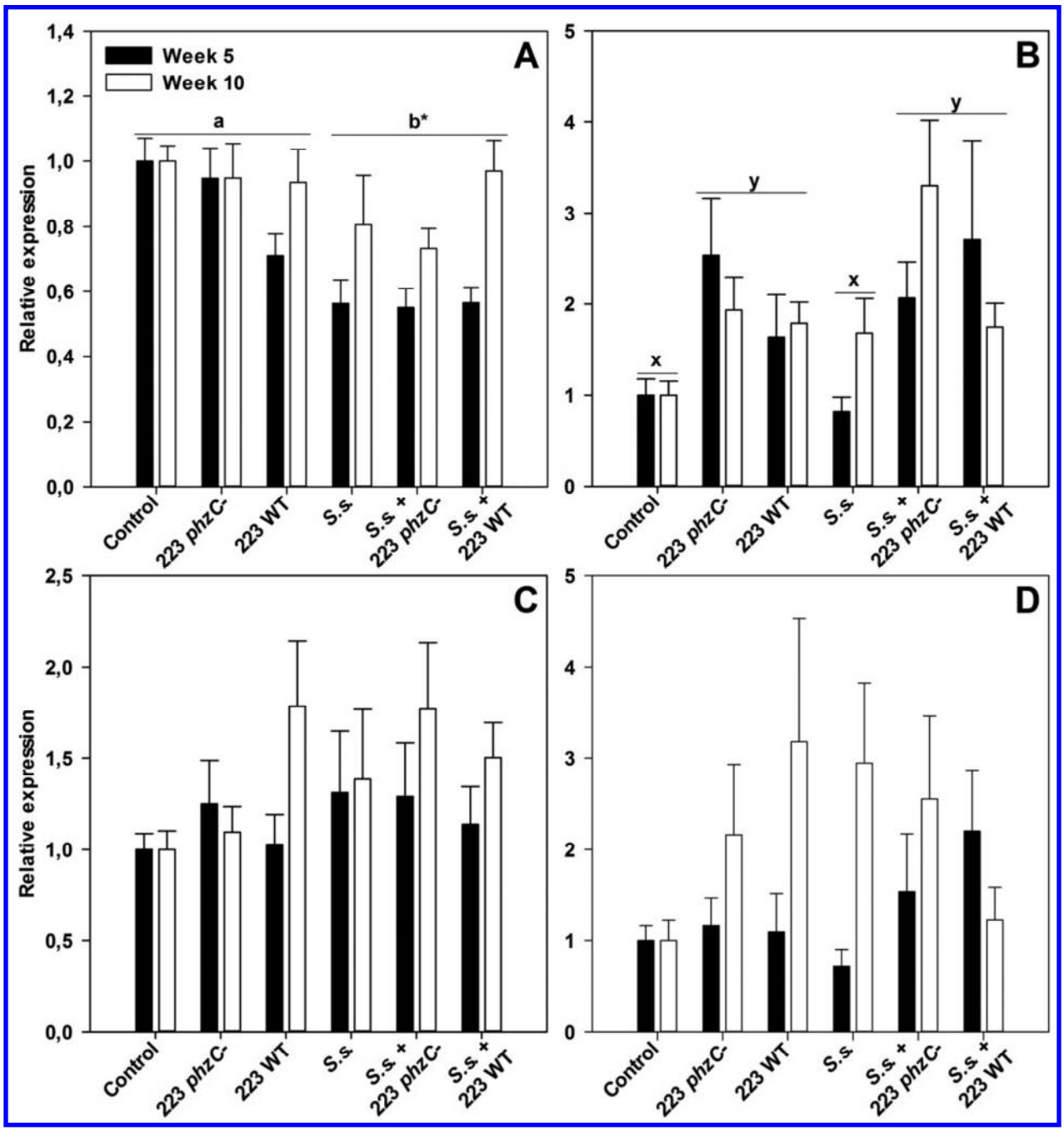

Fig. 2. Expression of ethylene and jasmonic acid-related genes **A,** *ERF3*; **B,** *LOX*; **C,** *PAL-2*; and **D,** *PIN2* in potato leaves at 5 and 10 weeks. For all panels, values are presented as mean ± standard error of the mean ($n = 9$). Each panel has its respective Y-axis scale. Significant differences are presented using different letters: a and b for the *Streptomyces scabies* factor and x and y for the *Pseudomonas* factor. There was an interaction between the time and treatment with *S. scabies* factors for *ERF3* (A). Horizontal lines were used to group treatments where *S. scabies* (*S.s.*) was applied or not, and letters above these lines represent a significant difference when *S. scabies* was applied at week 5 only (an asterisk [*] was used to indicate that the significant difference was only observed at week 5 and not week 10). Because no interaction occurred between time and both treatment factors for *LOX* (B), horizontal lines were used to group all time points related to treatments where *Pseudomonas* LBUM223 (wild type [WT] or *phzC*–) was applied or not (x and y).

LBUM223 was inoculated in this manner (2). Therefore, the implication of LBUM223-mediated ISR in the protection of potato against common scab is unlikely. This corroborates with one other study on defense-related protein activity in potato infected by *S. scabies* and inoculated with a *Pseudomonas* sp. biocontrol treatment (34). Four-week-old potted potato plants were used and *S. scabies* was inoculated for a short period of 24 to 96 h, in the presence or absence of the biocontrol treatment, which consisted of several strains of *Pseudomonas* with or without vermicompost. The activity of two enzymes was characterized in leaves and on the tuber surface: PAL and peroxidase (POX). No significant differences were apparent, suggesting that, for the enzymes under study, ISR did not play a preponderant role in that system as well. However, no SA-related proteins were verified.

Independently of the presence of LBUM223 or its non-PCA-producing mutant, the application of *S. scabies* upregulated the expression of all SA-related genes targeted in this study (*ChtA*, *PR-1b*, *PR-2*, and *PR-5*), suggesting that the recognition of this pathogen by potato plants triggers the SA-signaling pathway (to be determined), and could be considered an SAR response. To our knowledge, this is the first report on the ability of *S. scabies* to induce an SAR response in potato plants. The exact signaling mechanisms by which these upregulations occur remain to be determined. The function of PR-1 is not completely understood (40) and, therefore, its upregulation in the presence of *S. scabies* cannot be directly linked to a mechanism that may help the plant resist common scab development. However, recent studies have presented the hypothesis that PR-1 may be involved in protease-mediated programmed cell death (24), in order to limit infection to a particular area of the plant. PR-1 has been vastly used as a marker for the induction of the SA-signaling pathway and exists in both a basic and acidic form, which may differ substantially in their biological activities (40). The potato *PR-1b* targeted in this study encodes a basic protein, and has previously been shown to be upregulated systemically following infection of potato by *P. infestans* (21). The PR-2 family is composed of β-1,3-glucanases (40), which in potato are also upregulated systemically by *P. infestans* (33). When paired with chitinases, which cleave poly-β-1,4-N-acetylglucosamine, these enzymes are very efficient in preventing fungal growth by degrading essential components of fungal cell walls (25). The class II chitinase studied here has been shown to be systemically upregulated in potato plants infected by *P. infestans* or treated with SA (6). The upregulation of *ChtA* in this case most likely does not target *S. scabies* because it also produces chitinases (45). *S. scabies* was also able to repress *ERF3* expression in 5-week-old plants. This trend was not sustained in 10-week-old plants. ERFs are transcriptional factors that bind to unique DNA motifs. Plants possess several ERFs which, in some cases, can bind the promoter regions of several pathogenesis-related and JA- or ET-induced genes (19). It is possible that *S. scabies* could limit ERF activity and reduce the number of defense proteins produced by the plant, although this remains to be demonstrated.

Based on the results obtained, it seems that the response triggered by *S. scabies* in potato is a general one, not leading to the production of defense proteins that could specifically repress common scab development. However, it could potentially contribute to controlling other diseases if pathogens are sensitive to the enhanced expression and production of the SA-related defense proteins under study. Although few studies on the defense responses induced by *S. scabies* in potato exist, focusing mostly on responses in individual tubers and not entire plant systems (16), there have been studies performed in *A. thaliana* plant systems. Interestingly, the inoculation of *A. thaliana* seedlings with *S. scabies*, after preinoculation with a nonpathogenic endophytic *Streptomyces* isolate, also induced an SA-related SAR response (23). Some reports on the effect of thaxtomin A, the phytotoxin responsible for scab symptoms, have also been accomplished in *A. thaliana*. In seedlings, RT-qPCR analyses showed that SA-related *PR-1*, *PR-5*, and chitinase were all upregulated in response to the application of thaxtomin A (5), which corroborates with our results. However, three JA-responsive genes were also upregulated—*vegetative storage protein* (*VSP) 1*, *VSP2*, and *CHI-B* (5)—whereas in our study, *S. scabies* did not alter JA-related gene expression. Other studies have found JA-responsive genes to be upregulated with the exogenous application of thaxtomin A to *A. thaliana* cells. It induced an influx of calcium and the expression of *PAL-1* but not *PR-1* in one case (15), and induced the expression of many defense-related genes in a microarray analysis, including *PAL2*, *POX*, a thaumatin family PR protein, and a *PIN*-related protein (14). These results differ from those we have obtained, suggesting that perhaps thaxtomin, or other bacterial determinants of *S. scabies*, induce different responses in different plant systems, as seen in a study which demonstrated that thaxtomin A did not induce a similar cellular response in *A. thaliana* and tobacco, another solanaceous species (26). Thaxtomin A or *S. scabies* may also have a different effect in potted plants compared with cell suspensions, detached tissues, or in vitro plantlets. For example, inoculation of detached potato tubers with *S. scabies* did not seem to generate a significant amount of unique expressed sequence tags, none of which related to defense responses (16), whereas we observed over-expression of many SA-related genes in full-grown plants.

In this study, we have demonstrated that *S. scabies* and *Pseudomonas* sp. LBUM223 (wild type and *phzC*– mutant) are independently able to induce an upregulation of defense-related gene expression profiles for a period of at least 10 weeks following contact with potato plants under soil conditions that mimic natural field conditions. However, results showed that the expression profiles were not consistent with that of a biocontrol agent capable of inducing a systemic resistance leading to disease control. Inoculation with LBUM223 or its *phzC*– mutant induced identical gene expression profiles in potato, while only PCA-producing LBUM223 was able to significantly reduce common scab symptoms (2). These results suggest that PCA is not an ISR elicitor in this system. The biocontrol mechanism by which LBUM223 controls common scab of potato does not seem to rely on plant-mediated defenses but, rather, on a direct interaction occurring between *S. scabies* and *Pseudomonas* sp. LBUM223.

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