European Journal of Plant Pathology **105:** 513–517, 1999.

Short communication

Pseudomonas aeruginosa 7NSK2-induced systemic resistance in tobacco depends on *in planta* salicylic acid accumulation but is not associated with PR1a expression

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Accepted 26 May 1999

Key words: plant growth-promoting rhizobacteria, systemic acquired resistance

Abstract

Root colonization by rhizobacteria can induce a systemic resistance in plants that is phenotypically similar to systemic acquired resistance induced by a localized pathogen infection. We used the tobacco–tobacco mosaic virus model to investigate whether the systemic resistance induced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 is mediated by the systemic acquired resistance signal transduction pathway. Experiments with *nah*G-transformed tobacco revealed that *Pseudomonas aeruginosa* 7NSK2-induced resistance depended on *in planta* salicylic acid accumulation for its expression but not for its induction and is, in this respect, similar to systemic acquired resistance. However, *Pseudomonas aeruginosa* 7NSK2-induced resistance was, unlike systemic acquired resistance, not associated with PR1a expression at the time of challenge with tobacco mosaic virus. This suggests that *Pseudomonas aeruginosa* 7NSK2 treatment would only potentiate defense gene expression in systemic tissue, which would also explain why its level of resistance is lower than in case of systemic acquired resistance. Because we demonstrated that induced resistance by *Pseudomonas aeruginosa* 7NSK2 exclusively depends on the production of salicylic acid by this strain our conclusions might also account for other salicylic acid-producing and resistance-inducing rhizobacteria.

Introduction

Induced systemic resistance (ISR) defines an increased resistance to disease that develops systemically throughout plants after appropriate stimulation (reviewed by Hammerschmidt and Kuć, 1995). Systemic resistance induced by a localized pathogen infection is the most studied form of ISR and is generally termed systemic acquired resistance (SAR). Studies of SAR in *Arabidopsis*, cucumber and tobacco revealed that the secondary plant metabolite salicylic acid (SA) is involved in SAR. SA accumulates to several $\mu g\ g^{-1}$ leaf around localized pathogen infections, is subsequently detected in phloem and in distant plant parts where it precedes the onset of SAR (Malamy et al., 1990; Métraux et al., 1990). In addition, the absence of SAR in transgenic tobacco and *Arabidopsis* that cannot accumulate significant amounts of SA because of expression of the *nah*G gene, that encodes a salicylate hydroxylase, showed that SA is even essential for SAR development (Gaffney et al., 1993; Lawton et al., 1995). Despite the demonstrated SA transport from the site of pathogen infection to systemic tissue (Shulaev et al., 1995; Mölders et al., 1996) two arguments question that the essential role for SA includes long distance signaling for SAR. First, in cucumber, the systemic signal for SAR was generated before significant accumulation of SA at the site of

pathogen infection (Smith-Becker et al., 1998). Second, in tobacco, grafting experiments demonstrated that the systemic signal for SAR was still generated in *nah*G-transformed (NahG) plants that do not allow significant SA accumulation (Vernooij et al., 1994). In addition to the systemic increase in SA levels, SAR development in systemic tissue is also characterized by expression of genes that encode specific pathogenesis-related (PR) proteins (Ward et al., 1991). By consequence the expression of these genes is considered as a molecular marker for SAR. Based on the above-mentioned advances the purely phenotypic definition of SAR was recently broadened with the typical features of the signal transduction pathway involved (Ryals et al., 1996).

Another stimulation that can lead to ISR is root colonization by specific strains of non-pathogenic bacteria (van Loon, 1997). At least some of these strains are able to produce SA in iron-limited environments (Maurhofer et al., 1994; Buysens et al., 1996; Leeman et al., 1996; Press et al., 1997) and in two cases this trait has been related to ISR. Introduction of SA-biosynthetic genes in *Pseudomonas fluorescens* P3 turned this strain into an inducer of systemic resistance (Maurhofer et al., 1998) and ISR was not observed for SA-deficient mutants of the resistance-inducing strain *P. aeruginosa* 7NSK2 (De Meyer and Höfte, 1997). It is, however, unclear whether these SA-producing rhizobacteria induce systemic resistance by activating the SAR-pathway because the amount of SA produced by these strains, about 10–100 ng per plant (Maurhofer et al., 1998; De Meyer et al., 1999), is significantly lower than the amount of SA that accumulates upon pathogen infection.

In this study we verified whether, in tobacco, SA production by *P. aeruginosa* 7NSK2 is essential for ISR to tobacco mosaic virus (TMV) and used the tobacco–TMV model (Ryals et al., 1996) to investigate involvement of the SAR signal transduction pathway in ISR by *P. aeruginosa* 7NSK2.

Results and discussion

In three independent experiments root colonization by *P. aeruginosa* 7NSK2 significantly reduced lesion diameters of TMV infection in Samsun NN tobacco (Table 1). Because 7NSK2 could not be detected on challenged leaves by standard detection techniques (as described by De Meyer and Höfte, 1997) this effect is mediated by ISR. The resistance level induced by 7NSK2 was generally lower than the SAR after TMV treatment of three lower leaves (Table 1). This is consistent with other results in tobacco (Maurhofer et al., 1994; Press et al., 1997) where rhizobacterial ISR was also less performant than SAR. ISR to TMV was also observed after root-inoculation with *P. aeruginosa* KMPCH, a pyoverdin and pyochelin-deficient mutant of 7NSK2 (Table 1), indicating that pyoverdin and pyochelin are not essential for ISR. Because ISR was no longer observed for MPFM1-569, that is additionally deficient in SA production (Table 1) it can be concluded that, like in bean (De Meyer and Höfte, 1997), SA is essential for ISR to TMV by 7NSK2 and that SA is the only determinant for ISR by KMPCH.

In NahG tobacco, root colonization by *P. aeruginosa* 7NSK2 or its mutants did not result in ISR to TMV (Table 1). In this respect, ISR by 7NSK2 is similar to SAR induced by TMV, which is also not expressed in NahG tobacco (Gaffney et al., 1993). The restoration of ISR by 7NSK2 and KMPCH in Samsun NN scions grafted on NahG rootstocks (Table 1) demonstrates that 7NSK2 and KMPCH can activate ISR in a Xanthi nc background. Therefore the absence of ISR in Xanthi nc NahG tobacco must be due to the dependency of ISR by 7NSK2 on *in planta* accumulation of SA. The restoration of ISR by 7NSK2 and KMPCH in Samsun NN scions grafted on NahG rootstocks also demonstrates that *in planta* accumulation of SA is only essential for the expression of ISR in leaves and not for its induction in roots. The same conclusion was reached for SAR because TMV inoculation on leaves of a NahG rootstock induced resistance in a wild-type scion (Table 1; Vernooij et al., 1994). It thus appears that the SA-dependent induction of systemic resistance by 7NSK2 is mediated by the SAR-pathway. At first sight the dependency of ISR by 7NSK2 and KMPCH on bacterial SA seems contradictory to the observation of ISR by these strains in Samsun scions grafted on NahG rootstocks because, in the NahG rootstock, the bacterial SA could be converted to catechol. In this respect it should be noted that in NahG tobacco not all SA is converted to catechol and that amounts up to 100 ng SA g^{-1} fresh weight can still be present (Friedrich et al., 1995). Therefore it is possible that the SA produced by 7NSK2 (10–100 ng SA per plant) is not metabolized in NahG tobacco and either by itself functions as a signal for SAR or triggers an alternative long distance signal for SAR.

Table 1. Effect of root treatment with *P. aeruginosa* strains derived from 7NSK2 and prior inoculation with TMV on induction of systemic resistance to subsequent TMV infection in *Nicotiana tabacum* cv. Samsun NN and cv. Xanthi nc NahG

Treatment	Mean lesion diameter (mm)[a]								
	Samsun			NahG[b]			NahG rootstock/Samsun scion[c]		
	exp 1	exp 2	exp 3	exp 1	exp 2	exp 3	exp 1	exp 2	exp 3
Control	3.9 b	5.3 a	3.8 a	5.1 a	5.0 a	5.6 a	3.4 a	3.4 a	4.4 a
TMV[d]	1.6 d	3.1 d	1.9 b	ND[e]	ND	ND	2.4 b	ND	ND
7NSK2[f]	2.4 c	4.6 b	2.2 b	4.7 a	4.7 a	5.5 a	2.6 b	2.9 b	3.6 b
KMPCH	2.1 c	3.8 c	2.0 b	4.6 a	5.0 a	5.6 a	2.4 b	2.9 b	3.4 b
MPFM1-569	4.5 a	5.3 a	3.7 a	4.8 a	4.7 a	5.8 a	3.3 a	3.3 a	4.4 a
LSD[g]	0.5	0.6	0.4	0.6	0.5	0.4	0.5	0.4	0.6

[a]Mean lesion diameter of 10 randomly chosen lesions per leaf determined 5 (NahG) or 7 (Samsun) days after challenge-inoculation on one upper leaf. Plants were challenged 7 weeks after transfer of seedlings to potting soil.

[b]NahG-10, Gaffney et al. (1993).

[c]Grafting was performed 4.5 weeks after seedling transfer.

[d]Standard induction of SAR by TMV inoculation of three lower leaves that resulted in 50–100 lesion per leaf was performed one week before challenge.

[e]ND: not determined.

[f]*P. aeruginosa* strains were grown as previously described (De Meyer and Höfte, 1997) and applied as a combined root and soil treatment at transfer of 4 week-old seedlings to compost potting soil (Klassmann Deilman, Geeste, Germany). Seedling roots were dipped in bacterial suspension with 10^9 CFU ml^{-1} and transferred to soil with 5×10^7 CFU g^{-1}. In addition 10 ml of a 10^7 CFU ml^{-1} bacterial suspension were applied to each plant 4 and 6 weeks after seedling transfer. Phenotype of the bacterial strains: 7NSK2: wild type, Pvd$^+$, Pch$^+$, SA$^+$; KMPCH: Pvd$^-$, Pch$^-$, SA$^+$; MPFM1-569: Pvd$^-$, Pch$^-$, SA$^-$ with Pvd: pyoverdin, Pch: pyochelin and SA: salicylic acid (De Meyer and Höfte, 1997).

[g]Treatments consisted of at least 8 plants and statistical analysis was performed per experiment by analysis of variance with $P = 0.05$. Treatment means were compared with Fisher's least significant difference (LSD) test ($P = 0.05$). Means followed by the same letter are not statistically different.

The induction of the SAR signal transduction pathway by 7NSK2 correlates with the induction of phenylalanine ammonia-lyase activity in roots and increased SA levels in leaves of *P. aeruginosa* KMPCH-treated bean plants (De Meyer et al., 1999) but seems not consistent with the induction of systemic resistance by the SA-producing rhizobacteria *S. marcescens* 90-166 and *P. fluorescens* WCS417 in, respectively, NahG tobacco (Press et al., 1997) and *Arabidopsis* (Pieterse et al., 1996). It should be noted, however, that in these plants ISR by *S. marcescens* 90-166 and *P. fluorescens* WCS417 does not solely depend on bacterial SA production (Press et al., 1997; van Wees et al., 1997) and thus could involve other bacterial components. Because these additional bacterial components might induce resistance by a signaling pathway different from the SAR pathway (Pieterse et al., 1998) it is not unlikely that the alternative signaling pathway might compensate for the loss of SAR expression in NahG plants. This compensation is unlikely to occur for 7NSK2 because, in tobacco, ISR by this strain exclusively depends on SA production.

Prior to challenge with TMV, expression of PR1a, the most abundant SAR marker in tobacco (Ward et al., 1991), was observed in upper leaves from plants induced by a prior TMV inoculation on 3 lower leaves, but not in leaves from *P. aeruginosa* 7NSK2- or KMPCH-treated plants (Figure 1). This observation could be explained in two ways. Firstly, the difference could relate to timing of both induction treatments because expression of molecular marker genes is particularly related to the onset of SAR (Ryals et al., 1996) but can afterwards decrease to undetectable levels in systemically resistant tissue (Pieterse et al., 1996). Therefore, the absence of PR1a expression in *P. aeruginosa* 7NSK2-treated plants could indicate that resistance had already been induced at an earlier stage in the experiment. To investigate this possibility, we

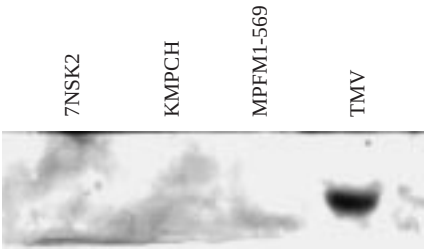

Figure 1. Effect of TMV inoculation and *P. aeruginosa* root colonization on PR1a mRNA accumulation in *Nicotiana tabacum* cv. Samsun NN. *P. aeruginosa* was applied as a combined root and soil treatment at seedling transfer and by an additional application 4 and 6 weeks after seedling transfer. Phenotype of the bacterial strains: 7NSK2: wild type, Pvd^+, Pch^+, SA^+; KMPCH: Pvd^-, Pch^-, SA^+; MPFM1-569: Pvd^-, Pch^-, SA^- with Pvd: pyoverdin, Pch: pyochelin and SA: salicylic acid. TMV was inoculated on 3 lower leaves of untreated plants 6 weeks after seedling transfer. Leaf samples were collected from unchallenged upper leaves 7 weeks after seedling transfer. Per treatment 30 µg of total RNA was analyzed on Northern blots via hybridization with a dioxigenin-labeled PR1a probe (Payne et al., 1988) using standard techniques. The experiment was performed twice with similar results.

analysed expression of PR1a in upper unchallenged leaves of 7NSK2-treated plants at earlier time points than as shown in Figure 1 i.e. 2, 4 and 6 days before the normal TMV-challenge. At none of these time points, PR1a expression could be detected (data not shown). In addition, PR1a expression was also not detected in leaves of 7NSK2-treated plants 3, 4, 5 and 6 weeks after seedling transfer (data not shown). These results show that the absence of PR1a in 7NSK2-treated plants is most likely not caused by differences in timing of SAR-induction. The second explanation for absence of PR1a expression in 7NSK2-treated plants is that systemic resistance by 7NSK2 is not comparable to the full pathogen-induced SAR that activates defense genes in unchallenged tissue but only has a potentiating effect on the expression of defense genes upon pathogen infection (Mur et al., 1996). This is consistent with the essential role for SA in expression of ISR by 7NSK2 (see above) and would also explain why ISR by 7NSK2 is less performant than SAR (Table 1).

Acknowledgements

This work was supported by a grant from the Fund for Scientific Research-Flanders (FWO, Belgium) and by a specialization fellowship of the Flemish Institute for the stimulation of Scientific-Technological Research in Industry (IWT, Belgium) that was given to Geert De Meyer and Kris Audenaert. We thank Dr. J. Ryals and Dr. M. Hunt from Novartis Crop Protection (Triangle Park, NC) for providing the PR-1a cDNA clone and NahG-10 tobacco seeds and I. Mollet and K. Capieau for help with plant experiments.

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