

LOCAL AND SYSTEMIC HYDROXYPROLINE-RICH GLYCOPROTEIN ACCUMULATION IN TOBACCO TREATED WITH SALICYLIC ACID AND ACIBENZOLAR-S-METHYL

V. Raggi

*Dipartimento di Scienze Agrarie e Ambientali- Arboricoltura e Protezione delle Piante,
Università degli Studi, Borgo XX Giugno 74, 06121 Perugia, Italy*

SUMMARY

The accumulation of cell wall hydroxyproline-rich glycoproteins (HRGPs) involved in plant defence response was studied, in terms of hydroxyproline accumulation on purified cell walls of leaves of tobacco cvs Havana 425, Xanthi-nc (wild type), and NahG-transformed Xanthi (carrying the gene for salicylate hydroxylase). The leaves (7th and 8th leaves) were analysed after treatment with salicylic acid (SA) and the SA analogue acibenzolar-S-methyl (ASM). Upper untreated leaves (9th leaf) were also analysed. Infiltration of 1 mM SA solution into leaves 8 and 9 of Havana 425 and Xanthi-nc, induced significant HRGP accumulation, which also occurred in the upper untreated leaves, more evident at 4 and 5 days after treatment, whereas in NahG plants no accumulation was observed, both in treated and upper leaves. Spraying 1 mM ASM solutions induced insignificant or modest and transitory HRGP accumulation in sprayed leaves of Havana and Xanthi respectively and a clear accumulation in the upper leaves, at 4, 5 and, generally, at 6 days. In NahG plants, modest accumulation was observed only in the upper leaves at 5 or 5 and 6 days. The pattern of HRGP accumulation induced by SA and ASM in Havana and Xanthi-nc appears consistent with the involvement of HRGPs in SAR. In SA-insensitive NahG plants, the lack (SA treatment) or poor (ASM treatment) HRGP accumulations also hint at their involvement in SAR.

Key words: Havana, NahG, Xanthi-nc, leaf ageing, salicylic acid, acibenzolar-S-methyl

INTRODUCTION

Many defence mechanisms are triggered by pathogenic attacks, which induce a broad spectrum response against the inducers and/or other pathogens, referred to as systemic acquired resistance (SAR). Bel and Gaupels (2004) stated that, despite the long-standing knowledge

of the triggering of long-distance SAR, the translocation pathway and identity of the signal involved have not been determined precisely, as indicated by the recent report of involvement of sieve tubes in signal translocation (Kiefer and Slusarenko, 2003; Durrant and Dong, 2004).

According to Métraux (2001), the importance of salicylic acid (SA) for SAR was demonstrated by Gaffney *et al.* (1993) and others, using different *Arabidopsis* mutants. Although numerous studies have implicated SA as an important component of the plant transduction pathway in pathogen-induced resistance, its function in processes involved in cell death and resistance is still poorly understood (Durrant and Dong, 2004; Bel and Gaupels, 2004). In tobacco carrying the dominant "N" gene, which induces a hypersensitive reaction (HR) to *Tobacco mosaic virus* (TMV) and limits its replication and movement, considerable evidence indicates that SA is an endogenous signal involved in the induction of *PR-1* gene expression and SAR activation (Malamy *et al.*, 1996). NahG plants (tobacco plants transformed with a bacterial salicylate hydroxylase gene *nahG*) are unable to activate SAR (Gaffney *et al.*, 1993) and show increased susceptibility to TMV (Delaney *et al.*, 1994). Many results, particularly those obtained utilising *nahG*-expressing tobacco rootstocks (showing strong reduction of SA levels, Vernooij *et al.*, 1995), support the conclusion that SA does not seem to be the systemic signal, but is probably an intermediate signal between pathogen perception and gene induction (Willits and Ryals, 1998). Based on studies by Sticher *et al.* (1997), using leaf detachment and various grafting experiments (particularly with NahG plants), Métraux (2001) stated that SA is necessary for the induction of SAR, but a signal other than SA may be translocated and required for the onset of induced resistance. Gene activation by these diverse stimuli is mediated via an *as-1*-like element (that is a *cis*-acting sequence) in the *PR-1a* upstream region (Grüner *et al.*, 2003). These results suggest that *PR-1a* gene expression is mediated by the *as-1*-like element and the *PR-1a* promoter is targeted by at least two signal transduction pathways elicited by SA and by a yet unknown signal, produced during the HR (Grüner *et al.*, 2003).

Acibenzolar-S-methyl (ASM), also named benzothiazole derivative, is a potent inducer of both gene in-

duction and inherent disease resistance mechanisms (Görlach *et al.*, 1996). ASM can act as an SA analogue in natural SAR signal pathways, activating essentially the same set of defence genes and inducing resistance against the same spectrum of pathogens, with long lasting effects in monocots and less pronounced effects in dicots (Oostendorp *et al.*, 2001). ASM has the same effect on *PR-1a* gene expression as TMV and SA, based on both the promoter elements involved (*cis*-acting promoter) and the tissue specificity of *PR-1a* expression (Uknes *et al.*, 1993). Pretreating roses with ASM increases resistance against *Diplocarpon rosae* and, after infection, induces increased accumulation of many extracellular proteins (Suo and Leung, 2002). Enhanced resistance in susceptible *Vigna unguiculata*, after ASM-induction followed by infection with *Colletotrichum destructivum*, was associated with a rapid and transient increase (but not in non-inoculated tissues) of the two key enzymes of the phenylpropanoid/flavonoid pathway (inducing early accumulation of kievitone and phaseolin) possibly by potentiating an early defence response, rather than by altering the constitutive resistance of the tissues (Latunde-Dada and Lucas, 2001). ASM systemically protected tomato plants against attacks by *Pseudomonas syringae* pv. *tomato*, and is considered resistance inducer, also because it did not inhibit bacterial growth *in vitro* and the protection it exert was observed after the compound has almost completely disappeared *in planta* (Scarponi *et al.*, 2001). Similar results have been obtained on pepper protected by this compound against *Xanthomonas campestris* pv. *vesicatoria* (Buonaurio *et al.*, 2002).

ASM treatment induces resistance against the same spectrum of pathogens and the same biochemical changes as used to define SAR in tobacco (e.g. a co-ordinate induction of the same set of mRNAs that are induced by TMV and SA), but does not induce the accumulation of either free or total SA. Consequently it is unlikely that it acts through SA (Görlach *et al.*, 1996). Accordingly, ASM treatment induces PR-1 mRNA accumulation in NahG plants with essentially the same time course as wild-type Xanthi-nc and induces resistance to *Peronospora tabacina* in a dose-dependent manner, independent of SA and the expression of the *nahG* gene (Uknes *et al.*, 1993).

Hydroxyproline-rich glycoproteins (HRGPs) are abundant structural proteins in the plant cell wall. This generic term includes molecules rich in hydroxyproline/proline: extensin, arabinogalactan-proteins, proline/hydroxyproline-rich proteins and solanaceous lectin (Sommer-Knudsen *et al.*, 1998). These proteins are known to be involved in plant defence, both in dicots (Esquerré-Tugayé *et al.*, 1979; Mazau and Esquerré-Tugayé, 1986) and in monocots (Kang and Buchenauer, 2000; 2003; Shailasree *et al.*, 2004). The involvement of HRGPs in plant defence is likely because: (i) they accumulate early and massively in the cell wall together with

the relative transcripts (Templeton *et al.*, 1990) and in tissues immediately adjacent to the inoculation site in incompatible combinations (Benhamou *et al.*, 1990); (ii) their accumulation is highly localized at sites where bacterial and fungal growth is arrested (O'Connell *et al.*, 1990); (iii) artificial induction of HRGP increases resistance, whereas inhibition decreases it (Toppan *et al.*, 1982).

HRGP accumulation and cross-linking processes in response to pathogen attacks has been noted (Bradley *et al.*, 1992; Brisson *et al.*, 1994; Brady and Fry, 1997; Shailasree *et al.*, 2004) and the HRGP gene-encoding sequence has been studied (García-Muniz *et al.*, 1998). HRGP mRNA accumulation has been induced by application of elicitors isolated from fungi and accumulation of the transcripts has also been induced by exogenous SA administration to cultured parsley cells (Thulke and Conrath, 1998), but the relationships between SA or ASM level and HRGP accumulation are still largely unknown.

The aim of the present work was to understand the effect of SA and ASM treatment on HRGP accumulation in SA-accumulating plants (Havana 425 and Xanthi-nc) and SA non-accumulating plants (NahG-transformed Xanthi, carrying the gene for salicylate hydroxylase), both in directly treated leaves and in untreated leaves immediately above.

MATERIALS AND METHODS

The experiments were carried out by infiltrating salicylic acid or spraying ASM on the basal leaves (7th and 8th true leaves from the bottom) of tobacco Havana 425, Xanthi-nc, NahG-transformed Xanthi and then determining HRGP levels in treated leaves, in those immediately above and in respective controls, by analyzing hydroxyproline content in purified cell walls. On tobacco Havana 425 the potential for inducible HRGP accumulation of younger compared to older leaves was also tested.

Host materials, growth conditions and treatments.

Seeds of *Nicotiana tabacum* L., Havana 425, Xanthi-nc (wild type) and NahG-transformed Xanthi (carrying the bacterial gene for salicylate hydroxylase) were sown in sterilized standard potting compost soil (sand and peat, 50/50) and the plants grown in a greenhouse at 20±4°C, 65-75% R.H., 14 h natural light conditions, supplemented with artificial light (400 Watt, Philips HLRG) and at 14-16°C, 80-90% R.H., during 10 h of darkness. A batch of uniform plants, at the three leaf stage, were transferred to a growth chamber under the following conditions: 23±1°C, 70% R.H., 10 h illumination at 240 µmol m⁻² sec⁻¹ photosynthetic photon flux density (PPFD), produced by daylight and by fluorescent lamps (Powerstar HQI-T 400W/D Osram daylight lamp, and

58W/33 fluorescent lamp Philips TLD); 20±1°C and 80-90% R.H., during the 10 h of darkness; 20±1°C, 60-65% R.H. and 120 µmol PPF for 2+2 h transitions (dark to light and vice versa).

After 4 days acclimatisation in the growth chamber plants: (i) were infiltrated under the lower epidermis of the basal leaves (7th and 8th leaves), with a hypodermic syringe with 1 mM salicylic acid solution (the maximum concentration showing no phytotoxicity), in 50 mM potassium phosphate buffer (pH 7), until almost all the tissues appeared water-soaked. Control plants were infiltrated with the buffer (sham treated plants); (ii) were accurately sprayed with a nebulizer on the basal leaves (7th and 8th leaves), with 1 mM acibenzolar-S-methyl (ASM), ex benzo(1, 2, 3)thiadiazole-7-carbothionic acid S-methyl ester (also known as benzothiadiazole derivative, BTH) water solution. The controls plants were sprayed with water.

Sampling. Samples were taken from the two treated leaves and the untreated leaves immediately above, and consisted of 3 replicates each from 2 plants. Controls were taken from correspondent leaves of basal sham treated plants. Samples were taken on days 3, 4, 5 and 6 after treatment. Using the same sampling pattern, possible effects on leaf water content were previously evaluated by determining the dry weight (constant weight at 85°C) and then the fresh weight /dry weight (f.w./d.w.) ratio, in two independent trials for each treatment.

In the experiment designed for the evaluation of leaf

age on the inducibility of HRGP accumulation, treatment with SA or ASM was carried out on basal and upper leaves of tobacco “Havana 425”. Older and younger treated leaves and respective controls were sampled on days 4 and 5 after treatment.

Preparation of purified cell walls and HRGP hydrolysis. Interveinal leaf tissues (5 g f.w.) were homogenized in 50 ml ice-cold 500 mM potassium phosphate buffer pH 7, by 2 min grinding at 6000 rev min⁻¹ (Omnimixer Ultra-Turrax T25, Kunkel GmbH & Co. IKA Labortechnik, Germany). The insoluble material was collected by filtration on Miracloth, washed with 100 ml cold buffer and 180 ml cold distilled water, resuspended in 50 ml cold chloroform:methanol (1:1, v/v) and again homogenized for 3 min. The solid material was collected by filtration and washed with 50 ml cold chloroform:methanol (1:1, v/v) and 100 ml acetone, then dried at room temperature (Young and Sequeira, 1986; Minardi, 1990; Ye *et al.*, 1992; Raggi, 1998, 2000; Shailasree *et al.*, 2004) and briefly stored at -20°C.

The HRGPs were hydrolyzed in HCl (about 200 mg dry purified cell walls per 30 ml 6N HCl) at 110°C under vacuum for 18 h, in nitrogen flushed Vendura tubes (Schott Glass Werke Germany). The hydrolysate was filtered with Whatman GF/C, dried in Rotavapor (Büchi, Flawil, Schweiz), solubilized in 6 ml distilled H₂O, filtered with syringe filters and briefly stored at -20°C (modified from Mazau and Esquerré-Tugayé, 1986; Shailasree *et al.*, 2004).

Table 1. SA- and ASM-induced hydroxyproline accumulation (µmol g⁻¹ f.w.) in tobacco “Havana 425”, compared to respective sham treated controls, and differences between induced accumulation in older and younger leaves (age effect), at 4 and 5 days after treatments. Data are the means of 3 independent samples. Asterisks indicate significant differences: *** significant at P≤0.001; ** at P≤0.01; * at P≤0.05.

Salicylic acid

Days	Older leaves			Younger leaves			Older minus younger
	Treated	Controls	Accumulation	Treated	Controls	Accumulation	
4	0.294±0.011	0.218±0.002	0.076**	0.379±0.018	0.202±0.007	0.177***	-0.101**
5	0.310±0.007	0.237±0.005	0.073**	0.419±0.005	0.226±0.004	0.193***	-0.120***

Acibenzolar-S-methyl

Days	Older leaves			Younger leaves			Older minus younger
	Treated	Controls	Accumulation	Treated	Controls	Accumulation	
4	0.321±0.010	0.277±0.005	0.044*	0.440±0.009	0.268±0.004	0.172***	-0.128***
5	0.334±0.005	0.288±0.008	0.046**	0.450±0.012	0.282±0.007	0.168***	-0.122**

Hydroxyproline-rich glycoprotein determination by hydroxyproline (Hyp) analysis. Since almost all the hydroxyproline of purified cell walls comes from wall glycoproteins (Ye *et al.*, 1992), the HRGPs were determined by analysing Hyp in the purified cell wall hydrolysate. Hyp was determined by eluting the amino acid on ion exchange resins (aminex A4, 600x10 mm diameter column) at 40°C, with lithium buffer (pH 2.8 ± 0.02), staining with ninhydrin at 100°C and colour reading in continuous flow at 440 nm (Raggi, 1998, 2000, and modified from Shailasree *et al.*, 2004), using an aminoacid analyser (Aminolyzer Optica, Milano, Italy) coupled to a spectrophotometric detector SPD-10 AV (Shimadzu, Analytical Instrument Division, Milano, Italy).

Statistical analysis. The data were submitted to variance analysis. Standard errors and significant differences (Student's t-test), for treated versus sham treated leaves (controls), are reported in the figures and in Table 1. Differences between pools of data were also analysed by Student's t-test.

RESULTS

Fresh weight/dry weight ratio. SA and ASM treatments did not appreciably alter the f.w./d.w. ratio of treated and upper leaves, compared to the respective controls (sham-treated plants) of the 3 tobacco varieties. The differences were in fact quite modest (± 5%) and not significant (data not shown).

Hydroxyproline content. In Havana leaves, Hyp was significantly higher, both in infiltrated and in immediate upper leaves, compared to the respective controls, on days 4, 5 and 6 after infiltration (Fig. 1A, 1st and 2nd trial). Induced accumulations were significantly lower in SA-treated basal leaves than in the upper untreated ones, in absolute value (a.v.): -0.034*, -0.039**, -0.014* $\mu\text{mol g}^{-1}$ f.w. of Hyp at 4, 5 and 6 days respectively (-0.030**, -0.044*** and -0.008* in a.v.) in the 1st trial; -0.079*, -0.082**, -0.033*, respectively (-0.058**, -0.080***, -0.025* $\mu\text{mol g}^{-1}$ f.w. of Hyp, in a.v.) in the 2nd trial. While ASM-sprayed Havana leaves showed no significant changes, compared to controls, highly significant Hyp accumulations were documented in the upper leaves on days 4, 5 and 6 after treatment in the first trial, days 4 and 5 in the second (Fig. 1B). The differences were: -0.131*, -0.179*, -0.112***, at days 4, 5 and 6, respectively (-0.094*, -0.158**, and -0.126***, in a.v.) in the 1st trial; -0.111*, -0.182*, -0.079** $\mu\text{mol g}^{-1}$ f.w. at days 4 and 5 respectively (-0.090*, -0.136 ns, -0.047*, in a.v.) in the parallel trial.

Significant Hyp accumulations were also observed in SA-infiltrated and especially in the upper leaves of to-

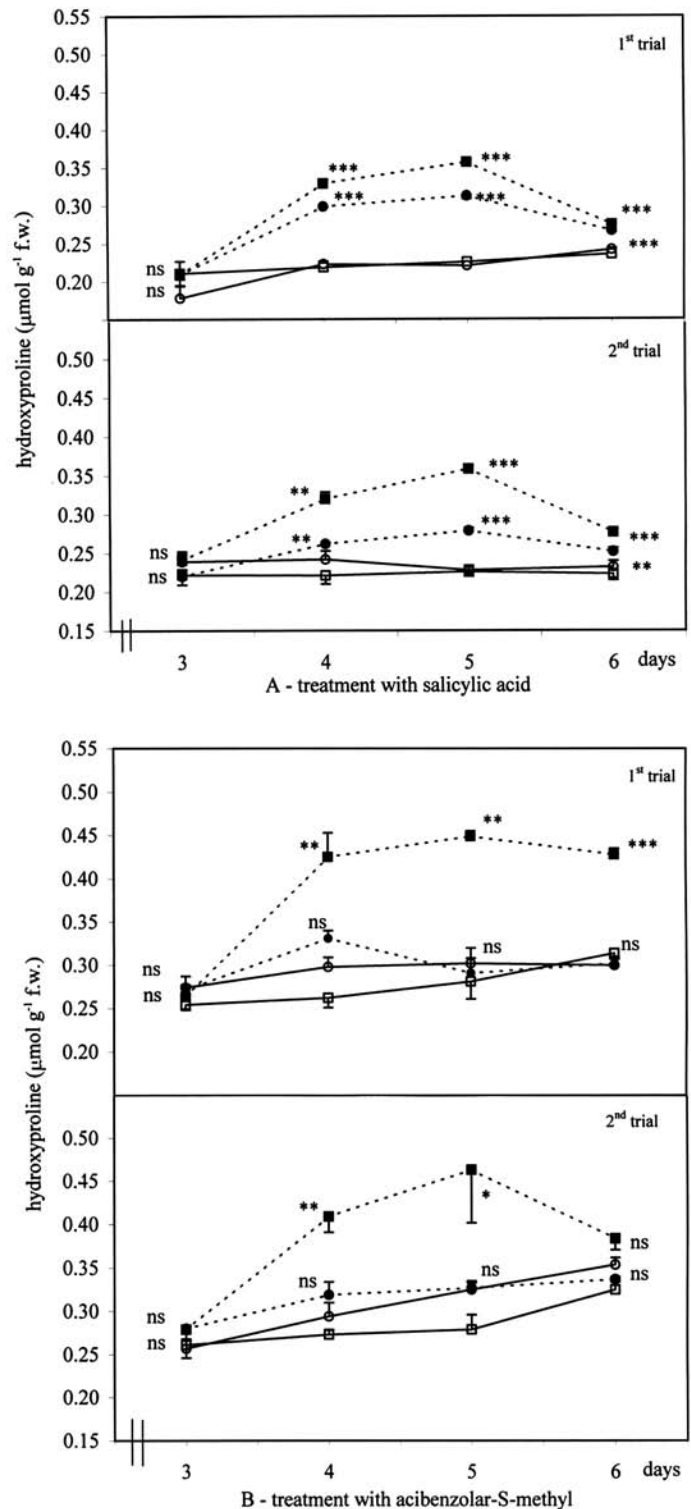


Fig. 1. Hydroxyproline content ($\mu\text{mol g}^{-1}$ f.w.) in tobacco "Havana 425" basal treated with salicylic acid (A) or acibenzolar-S-methyl (B), 3-6 days after treatment: treated leaves (—●—) and relative controls (---○---), upper leaf (---■---) and relative controls (---□---), in two independent trials (1st and 2nd trial). Each data is the means of 3 independent sample of two plants each at days 3, 4, 5, and 6 after treatment. Asterisks indicate significant differences between treated or upper leaves versus their respective sham treated controls: *** significant at $P \leq 0.001$; ** at $P \leq 0.01$; * at $P \leq 0.05$; ns, not significant. Vertical bars represent \pm SE.

bacco Xanthi (Fig. 2A). The differences were significant if calculated on the pool of days 4 and 5 (-0.047 and -0.052): -0.0495 ** $\mu\text{mol g}^{-1}$ f.w. average (-0.036*, -0.046* for days 4 and 5 in a.v.). ASM spray induced modest Hyp accumulations (significant only on day 4) in Xanthi treated leaves, while highly significant accumulation in the upper leaves was documented on days 4, 5 and 6 after treatment (Fig. 2B), with the following differences: -0.100**, -0.132**, -0.098* $\mu\text{mol g}^{-1}$ f.w. for days 4, 5 and 6 after treatment, respectively (-0.081**, -0.138*** and -0.084**, in a.v.).

On NahG plants, no Hyp accumulation was noted in SA-infiltrated and upper leaves, compared to respective controls (Fig. 3A, 1st and 2th trial). ASM spraying induced no Hyp accumulation in treated leaves and a modest one in the upper leaves at day 5 (Fig. 3B, 1st and 2th trial), with the following differences: -0.067** (-0.160***, in a.v.) at day 5, 1st trial; -0.086** and -0.043* (-0.153***, -0.119***, in a.v.) at days 5 and 6 after spraying respectively, 2nd trial.

Effect of leaf ageing on the potential for inducible HRGP accumulation. SA or ASM treatment induced lower Hyp accumulation in older than in younger Havana leaves (basal and upper treated versus corresponding sham-treated leaves, Table 1) at days 4 and 5, clearly showing a negative age effect.

DISCUSSION

The unchanged f.w./d.w. ratio, in both SA- and ASM-treated and upper leaves of the 3 varieties, indicates that no essential changes occurred in water relations of these leaves and justify basing Hyp and corresponding HRGP content on f.w.

HRGP contents of sham treated Havana leaves were close to that documented in a previous study on the same sham treated tobacco cultivar (Raggi, 1998; 2000), indicating reliability of both the analysis and the plant response. A higher HRGP content in younger than in older control leaves of NahG plants, particularly in sham ASM-treated ones, not observed in Xanthi, might be attributed to changes in HRGP content during leaf growth.

In agreement with the modest accumulation of HRGP transcripts induced by exogenous SA administration to cultured parsley cells (Thulke and Conrath, 1998), the accumulation of HRGPs in SA-treated Havana (Fig. 1A) and Xanthi (Fig. 2A) were moderate. Our results, particularly those for upper leaves, taken together show the similarity between HRGP accumulation trends induced by SA and ASM in both Havana and Xanthi (see also Fig. 1B and 2B) in agreement with the results of Lawton *et al.* (1996), indicating that these compounds activate the same signal transduction pathways downstream of SA.

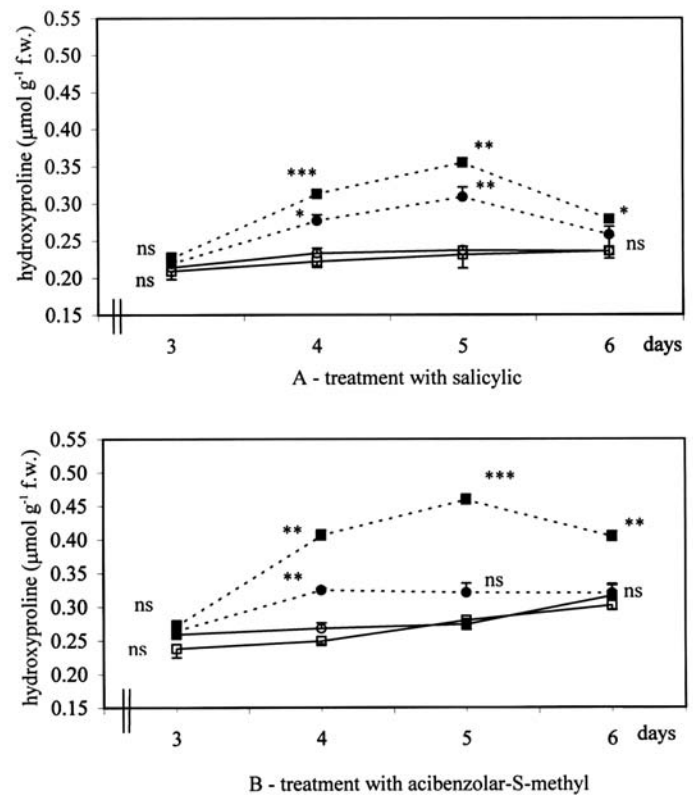


Fig. 2. Hydroxyproline content in tobacco Xanthi basal treated with salicylic acid (A) or acibenzolar-S-methyl (B): treated leaves (---●---) and relative controls (---○---), upper leaf (---■---) and relative controls (---□---). For further details see Figure 1.

The lack of significant HRGP accumulation after SA infiltration, both in treated and upper leaves (Fig. 3A, 1st and 2nd trial), was expected in NahG plants, since these plants carry the bacterial salicylate hydroxylase gene (*nahG* gene) and are insensitive to SA (Gaffney *et al.*, 1993). HRGP accumulation induced by ASM in the upper leaves of these plants (Fig. 3B, 1st and 2nd trial) appears consistent with induction of: 1) the same signal transduction pathways downstream of SA in NahG as in wild-type Xanthi plants (Lawton *et al.*, 1996; Görlach *et al.*, 1996); 2) PR-1 mRNA accumulation with essentially the same time course in NahG as in the wild-type Xanthi plants; 3) resistance to *Peronospora tabacina* in these plants (Uknes *et al.*, 1993). The much less evident and more transitory HRGP accumulation induced by ASM in NahG plants (Fig. 3B), than in Havana and Xanthi (compare with Figs. 1B and 2B), also agree with the suggestion of Molina *et al.* (1998) that ASM-protection is transient and repeated stimulations are required for resistance activation, and this is probably because ASM-treatment can compensate only partially for an impaired signal transduction pathway in that plant.

The lower (Havana and Xanthi) or absent (NahG plant) HRGP accumulation in ASM-treated leaves compared with the upper ones, may be mainly attributed to

a lower potential of the older leaves for HRGP accumulation, as documented by the experiment on the effect to leaf ageing (comparison between basal and upper treated leaves versus respective sham treated control leaves, Table 1).

Comparison of the present data on the upper untreated leaves of SA or ASM treated Havana, with the corresponding data on the same cultivar basal infected with *Tobacco mosaic virus* (TMV; Raggi, 1998) or with a

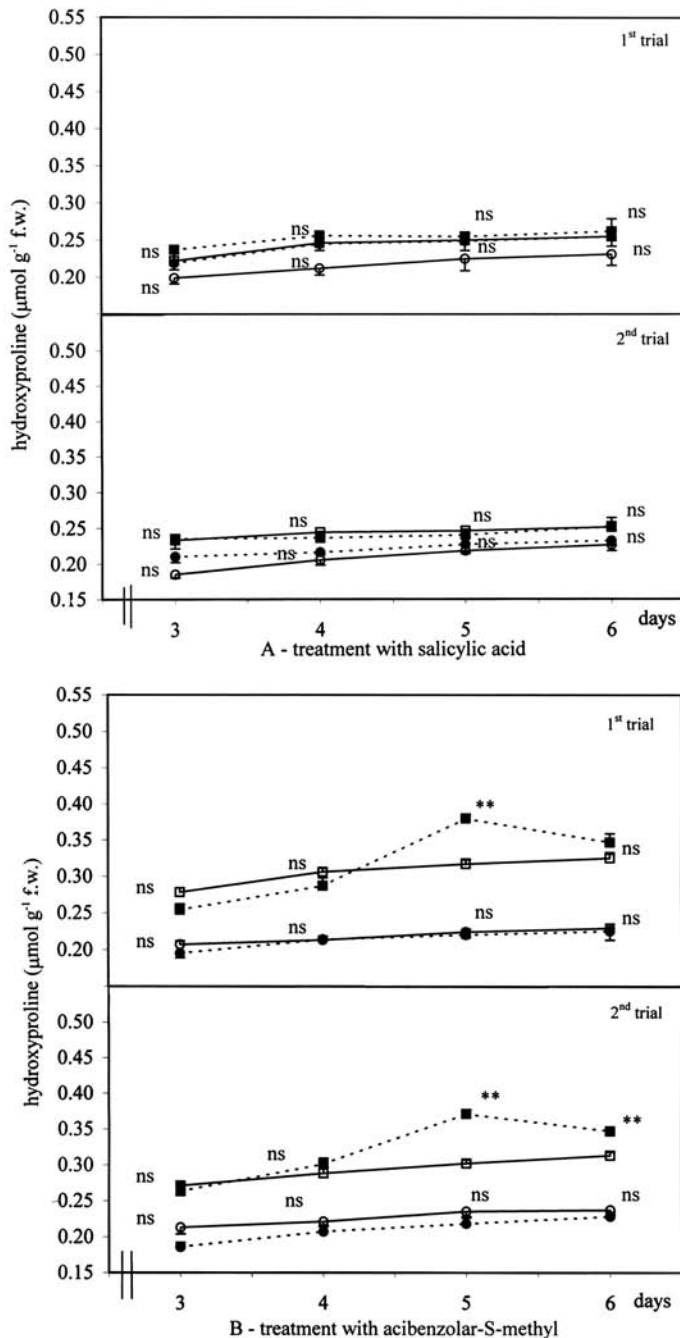


Fig. 3. Hydroxyproline content in tobacco NahG basal treated with salicylic acid (A) or acibenzolar-S-methyl (B): treated leaves (---●---) and relative controls (---○---), upper leaf (---■---) and relative controls (---□---). For further details see Figure 1.

necrotic strain of *Potato virus Y* (PVY^N; Raggi, 2000), shows earlier, more transitory and lower HRGP accumulation induced by SA and ASM compared to TMV and PVY^N. Earlier and shorter elicitation of the host reaction might depend on the most rapid spread and shorter lifetime of SA or ASM molecules, compared to the viruses (TMV spread and necrotization last one week or more, Malamy *et al.*, 1990); while the lower accumulation might depend on weaker and shorter induction. Regarding this aspect, our results are also in agreement with the lower SA accumulation induced in tobacco by the elicitor of *Phytophthora megasperma* (Dorey *et al.*, 1997) compared to that induced by TMV infection (Malamy *et al.*, 1990). The data obtained on virus-infected Havana, showing lower HRGP accumulation in TMV- or PVY^N-infected older leaves, than in uninfected upper leaves (Raggi, 1998; 2000), also indicate a lower potential for HRGP accumulation in older compared to younger leaves.

In conclusion, SA infiltration induced significant HRGP accumulation in treated leaves and even more in the upper untreated leaves of Havana and Xanthi, while no accumulation was observed in NahG plants (in SA-treated or upper leaves). ASM spraying induced transitory and poor accumulation of these glycoproteins in treated leaves and a highly significant accumulation in the untreated upper (younger) leaves of cv Havana and Xanthi, as well as a modest and transitory accumulation in younger leaves of NahG plants. The pattern of HRGP accumulation induced by SA or ASM treatment appears consistent with the involvement of HRGPs in host defence responses, both in “Havana 425” and “Xanthi-nc” as well as in “NahG transformed tobacco” (i.e. no accumulation, no resistance). Ageing seems to reduce the potential for HRGP accumulation induced by both SA and ASM treatments.

ACKNOWLEDGEMENTS

I am grateful to Dr. M. Orfei for his accurate and skillful technical support, to Syngenta for NahG-transformed plants and to NOVARTIS for benzothiadiazole derivative acibenzolar-S-methyl.

REFERENCES

- Bel A.J.E., van Gaupels F., 2004. Pathogen-induced resistance and alarm signals in the phloem. *Molecular Plant Pathology* 5: 495-504.
- Benhamou N., Mazau D., Esquerré-Tugayé M.T., Asselin A., 1990. Immunological localization of hydroxyproline-rich glycoproteins in necrotic tissue of *Nicotiana tabacum* L. cv. Xanthi-nc infected by tobacco mosaic virus. *Physiological and Molecular Plant Pathology* 36: 129-145.

- Bradley D. J., Kjellbom P., Lamb C. J., 1992. Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response. *Cell* **70**: 21-30.
- Brady J. D., Fry S. C., 1997. Formation of di-isodityrosine and loss of isodityrosine in the cell walls of tomato cell-suspension cultures treated with fungal elicitors or H₂O₂. *Plant Physiology* **115**: 87-92.
- Brisson L. F., Tenhaken R., Lamb C., 1994. Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. *Plant Cell* **6**: 1703-1712.
- Buonaurio R., Scarponi L., Ferrara M., Sidoti P., Bertona A., 2002. Induction of systemic acquired resistance in pepper plant by acibenzolar-S-methyl against bacterial spot disease. *European Journal of Plant Pathology* **108**: 41-49.
- Delaney T.P., Ukness S., Vernooij B., Friederich L., Weymann K., Negrotto D., Gaffney T., Gut-Rella M., Kessmann H., Ward E., Ryals J., 1994. A central role of salicylic acid in plant disease resistance. *Science* **266**: 1247-1250.
- Dorey S., Billieul F., Pierrei M.A., Saindrenan P., Fritig B., Kauffmann S., 1997. Spatial and temporal induction of cell death, defence genes, and accumulation of salicylic acid in tobacco leaves reacting hypersensitively to a fungal glycoprotein elicitor. *Molecular Plant-Microbe Interaction* **10**: 646-655.
- Durrant W.E., Dong X., 2004. Systemic acquired resistance. *Annual Review of Phytopathology* **42**: 185-209.
- Esquerré-Tugayé M.T., Lafitte C., Mazau D., Toppan A., Touzé A., 1979. Cell surfaces in plant-microorganism interaction. II. Evidence for accumulation of hydroxyproline-rich glycoproteins in cell wall of diseased plants as a defence mechanism. *Plant Physiology* **64**: 320-326.
- Gaffney T., Friederich L., Vernooij B., Negrotto D., Nye G., Ukness S., Ward E., Kessmann H., Ryals J., 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. *Science* **261**: 754-756.
- García-Muniz N., Martínez-Izquierdo J.A., Puigdomènech P., 1998. Induction of mRNA accumulation corresponding to a gene encoding a cell wall hydroxyproline-rich glycoprotein by fungal elicitors. *Plant Molecular Biology* **38**: 623-632.
- Görlach J., Volrath S., Knauf-Beiter G., Hengy G., Beckhove U., Kogel K.H., Oostendorp M., Staub T., Ward E., Kessmann H., Ryals J., 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *The Plant Cell* **8**: 629-643.
- Grüner R., Strompent G., Pfitzner A.J.P., Pfitzner U.M., 2003. Salicylic acid and the hypersensitive response initiate distinct signal transduction pathways in tobacco that converge on the *as-1-like* element of the *PR-1a* promoter. *European Journal of Biochemistry* **270**: 4876-4886.
- Latunde-Dada A.O., Lucas J.A., 2001. The plant defence activator acibenzolar-S-methyl primes cowpea (*Vigna unguiculata* (L.) Walp.) seedlings for rapid induction of resistance. *Physiological and Molecular Plant Pathology* **58**: 199-208.
- Lawton K.A., Friedrich L., Hunt M., Weymann K., Delaney T., Kessmann H., Staub T., Ryals J., 1996. Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. *The Plant Journal* **10**: 71-82.
- Kang Z., Buchenauer H., 2000. Ultrastructural and immunocytochemical investigation of pathogen development and host responses in resistant and susceptible wheat spikes infected by *Fusarium culmorum*. *Physiological and Molecular Plant Pathology* **57**: 255-268.
- Kang Z., Buchenauer H., 2003. Immunochemical localization of cell wall-bound thionins and hydroxyproline-rich glycoproteins in *Fusarium culmorum*-infected wheat spikes. *Journal of Phytopathology* **151**: 120-129.
- Kiefer W., Slusarenko A., 2003. The pattern of acquired resistance induction within the *Arabidopsis* rosette in relation to the pattern of translocation. *Plant Physiology* **132**: 840-847.
- Malamy J., Carr J.P., Klessig D.F., Raskin I., 1990. Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. *Science* **250**: 1002-1004.
- Malamy J., Sánchez-Casas P., Hennig J., Guo A., Klessig D.F., 1996. Dissection of the salicylic acid signalling pathway in tobacco. *Molecular Plant-Microbe Interaction* **9**, 474-482.
- Mazau D., Esquerré-Tugayé M.T., 1986. Hydroxyproline-rich glycoproteins accumulation in the cell walls of plants infected by various pathogen. *Physiological and Molecular Plant Pathology* **29**: 147-157.
- Métraux J.P., 2001. Systemic acquired resistance and salicylic acid: current state of knowledge. *European Journal of Plant Pathology* **107**: 13-18.
- Minardi P., 1990. Riconoscimento cellulare pianta-batteri nel sistema eterologo tabacco-Pseudomonadi. Ph.D. Thesis, Università degli Studi di Bologna, Italy.
- Molina A., Hunt M.D., Ryals J.A., 1998. Impaired fungicide activity in plants blocked in disease resistance signal transduction. *Plant Cell* **10**: 1903-1914.
- O'Connell R.J., Brown I.R., Mansfield J.W., Bailey J.A., Mazau D., Runeau D., Esquerré-Tugayé M.T., 1990. Immunocytochemical localization of hydroxyproline-rich glycoproteins accumulating in melon and bean at sites of resistance to bacteria and fungi. *Molecular Plant-Microbe Interaction* **3**: 33-40.
- Oostendorp M., Kunz W., Dietrich B., Staub T., 2001. Induced disease resistance in plants by chemicals. *European Journal of Plant Pathology* **107**: 19-28.
- Raggi V., 1998. Hydroxyproline-rich glycoprotein accumulation in TMV-infected tobacco showing systemic acquired resistance to powdery mildew. *Journal of Phytopathology* **146**: 321-325.
- Raggi V., 2000. Hydroxyproline-rich glycoprotein accumulation in tobacco leaves protected against *Erysiphe cichoracearum* by potato virus Y infection. *Plant Pathology* **49**: 179-186.
- Scarponi L., Buonaurio R., Martinetti L., 2001. Persistence and translocation of a benzothiadiazole derivative in tomato plants in relation to systemic acquired resistance against *Pseudomonas syringae* pv. *tomato*. *Pest Management Science* **57**: 262-268.
- Shailasree S., Ramachandra Kini K., Deepak S., Kumudini B. S., Shekar Shetty H., 2004. Accumulation of hydroxypro-

- line-rich glycoproteins in pearl millet seedlings in response to *Sclerospora graminicola* infection. *Plant Science* **167**: 1227-1234.
- Sommer-Knudsen J., Bacic A., Clarke A.E., 1998. Hydroxyproline-rich plant glycoprotein. *Phytochemistry* **47**: 483-497.
- Sticher L., Mauch-Mani B., Métraux J.P., 1997. Systemic acquired resistance. *Annual Review of Phytopathology* **35**: 235-270.
- Suo Y., Leung D.W.M., 2002. BTH-induced accumulation of extracellular proteins and blackspot disease in rose. *Biologia Plantarum* **45**: 273-279.
- Templeton M.D., Dixon R.A., Lamb C.J., Lawton M.A., 1990. Hydroxyproline-rich glycoprotein transcripts exhibit different spatial patterns of accumulation in compatible and incompatible interactions between *Phaseolus vulgaris* and *Colletotrichum lindemuthianum*. *Plant Physiology* **94**: 1265-1269.
- Thulke O.U., Conrath U., 1998. Salicylic acid has a dual role in the activation of defence-related genes in parsley. *Plant Journal* **14**: 35-42.
- Toppan A., Roby D., Esquerré-Tugayé M.T., 1982. Cell surfaces in plant-microorganism interactions. III. In vivo effect of ethylene on hydroxyproline-rich glycoprotein accumulation in the cell wall of diseased plants. *Plant Physiology* **70**: 82-86.
- Uknes S., Dincher S., Friedrich L., Negrotto D., Williams S., Thompson-Taylor H., Potter S., Ward E., Ryals J., 1993. Regulation of pathogenesis-related protein-1a gene expression in tobacco. *The Plant Cell* **5**: 159-169.
- Vernooij B., Friedrich L., Ahl-Goy P., Staub T., Kessmann H., Ryals J., 1995. 2,6-Dichloroisonicotinic acid-induced resistance to pathogens without the accumulation of salicylic acid. *Molecular Plant-Microbe Interaction* **8**: 228-234.
- Willits M.G., Ryals J.A., 1998. Determining the relationship between salicylic acid levels and systemic acquired resistance induction in tobacco. *Molecular Plant-Microbe Interaction* **11**: 795-800.
- Ye X.S., Jarlfors U., Tuzun S., Pan S.Q., Kuc' J., 1992. Biochemical changes in cell walls and cellular responses of tobacco leaves related to systemic resistance to blue mould (*Peronospora tabacina*) induced by tobacco mosaic virus. *Canadian Journal of Botany* **70**: 49-57.
- Young D.H., Sequeira L., 1986. Binding of *Pseudomonas solanacearum* fimbriae to tobacco leaf cell walls and its inhibition by bacterial extracellular polysaccharides. *Physiological and Molecular Plant Pathology* **28**: 393-402.

Received February 20, 2007

Accepted May 18, 2007