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Induction of gentisic acid 5-*O*-β-D-xylopyranoside in tomato and cucumber plants infected by different pathogens

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Abstract

Tomato plants infected with the citrus exocortis viroid exhibited strongly elevated levels of a compound identified as 2,5-dihydroxybenzoic acid (gentisic acid, GA) 5-*O*-β-D-xylopyranoside. The compound accumulated early in leaves expressing mild symptoms from both citrus exocortis viroid-infected tomato, and prunus necrotic ringspot virus-infected cucumber plants, and progressively accumulated concomitant with symptom development. The work presented here demonstrates that GA, mainly associated with systemic infections in compatible plant-pathogen interactions [Bellés, J.M., Garro, R., Fayos, J., Navarro, P., Primo, J., Conejero, V., 1999. Gentisic acid as a pathogen-inducible signal, additional to salicylic acid for activation of plant defenses in tomato. Mol. Plant-Microbe Interact. 12, 227–235], is conjugated to xylose. Notably, this result contrasts with those previously found in other plant-pathogen interactions in which phenolics analogues of GA as benzoic or salicylic acids, are conjugated to glucose.

Keywords: *Lycopersicon esculentum*; *Cucumis sativus*; Compatible interactions; Gentisic acid 5-*O*-β-D-xyloside; Phenolics

1. Introduction

Gentisic acid (GA, 2,5-dihydroxybenzoic acid), a biosynthetic derivative of salicylic acid (SA, 2-hydroxybenzoic acid) has been reported to accumulate to high levels in systemic, non-necrotizing infections, as for example in citrus exocortis viroid (CEVd)-infected Rutgers tomato plants, but not in these plants infected with the necrotizing pathogen *Pseudomonas syringae*. Moreover, GA acts as a pathogenic signal additional to SA in activating defence genes in tomato (Bellés et al., 1999). For long time, a defensive role of phenolics in plant pathogen interactions has been assumed. Phenolics induced upon infection of plants by pathogens can act as antimicrobial defense and signals for induced resistance (Métraux and Raskin, 1993; Dixon, 2001). In plants, free forms of phenolic acids are, normally, less abundant than their conjugated counterparts (Harborne, 1980). In general, when induced in plants after pathogen infection, these compounds are conjugated to glucose as is the case of benzoic acid and SA in tobacco mosaic virus-infected tobacco plants (Chong et al., 2001; Malamy et al., 1992). GA is also predominantly found in a conjugated form that produced GA upon hydrolysis with β-glucosidase. Consequently, and based on what happens with SA in tobacco, in which this phenolic is converted into β-*O*-D-glucosyl SA (Enyedi et al., 1992; Malamy et al., 1992), the possibility that the conjugated form of GA could correspond to a GA β-*O*-D-glucoside has been entertained (Bellés et al., 1999). Related to this question, we here present results demonstrating that, unexpectedly, GA is joined to xylose, instead of glucose by a β-*O*-glycosidic linkage between the anomeric form of C-1 on xylose and the

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hydroxyl oxygen atom on C-5 of GA. This occurs, not only in the previously studied interaction, Rutgers tomato and CEVd, but also in other compatible one between cucumber and the non-necrotizing pathogen, prunus necrotic ringspot virus (PNRSV) (Sánchez-Navarro et al., 1998). As far as we know, this is the first report on the accumulation of gentisic acid 5-*O*-β-D-xylopyranoside in plants after pathogen infection.

2. Results and discussion

Studies that point out the importance of phenylpropanoid compounds in plant pathogen defence has been carried out in tobacco (Maher et al., 1994; Shadle et al., 2003; Niggeweg et al., 2004.), *Arabidopsis* (Hagemeier et al., 2001; Hahlbrock et al., 2003; Tan et al., 2004), and tomato plants (Von Roepenack-Lahaye et al., 2003). In our laboratory we are interested in elucidating the structure and, as a long-term goal, the function of phenolics induced during compatible plant-pathogen interactions. Using as our main experimental system the interaction between Rutgers tomato and CEVd, a systematic analysis by TLC of the fractions obtained after column chromatography of the methanolic extracts from control and CEVd-infected plants, was performed. TLC of appropriate fractions from CEVd-infected tissues exhibited a strong fluorescence which was not present in the equivalent fractions from control, non-infected plants. HPLC preparative analysis and UV detection allowed the purification of the compound responsible for the fluorescence. Its initial characterization was achieved by HPLC/ESI-MS in negative mode (60 mV of cone voltage) which produced a good ion fragmentation of the analyte. Fig. 1(a) shows a total ion chromatogram for this analysis presenting a main peak (retention time 6.96 min), whose mass spectrum yielded the following ions: [*m*/*z* (%)]: 285 (15), 153 (15), 152 (41), 109 (17), 108 (100) (Fig. 1(c)). Negative electrospray ionization at mild conditions of low voltage (20 mV) yielded only a molecular ion of $[M-H]^-$ $m/z = 285$ in negative ESI-MS, corresponding to a M_r of 286 (Fig. 1(b)). This was confirmed by HRMS which gave the molecular ion exact mass as 286.072537 (286.068868 calculated for $C_{12}H_{14}O_8$). The *m*/*z* ion 153 is compatible with, and typical of, a dihydroxybenzoic acid, while 108 *m*/*z* ion correspond to a fragment of the latter that has lost the carboxyl group. The chromatographic behaviour of the compound, both in the open column as well as in the HPLC, indicated that it possessed some polarity which could be conferred by a sugar. Moreover, the difference between the molecular weight of the compound and that of the dihydroxybenzoic acid coincided with the molecular weight of a pentose. In plants, hydroxybenzoic acids normally occur as glucosides, although glucose esters are also present (Klick and Herrmann, 1988; Edwards, 1994). To more precisely elucidate the structure of the dihydroxybenzoic acid and also the nature (α or β)

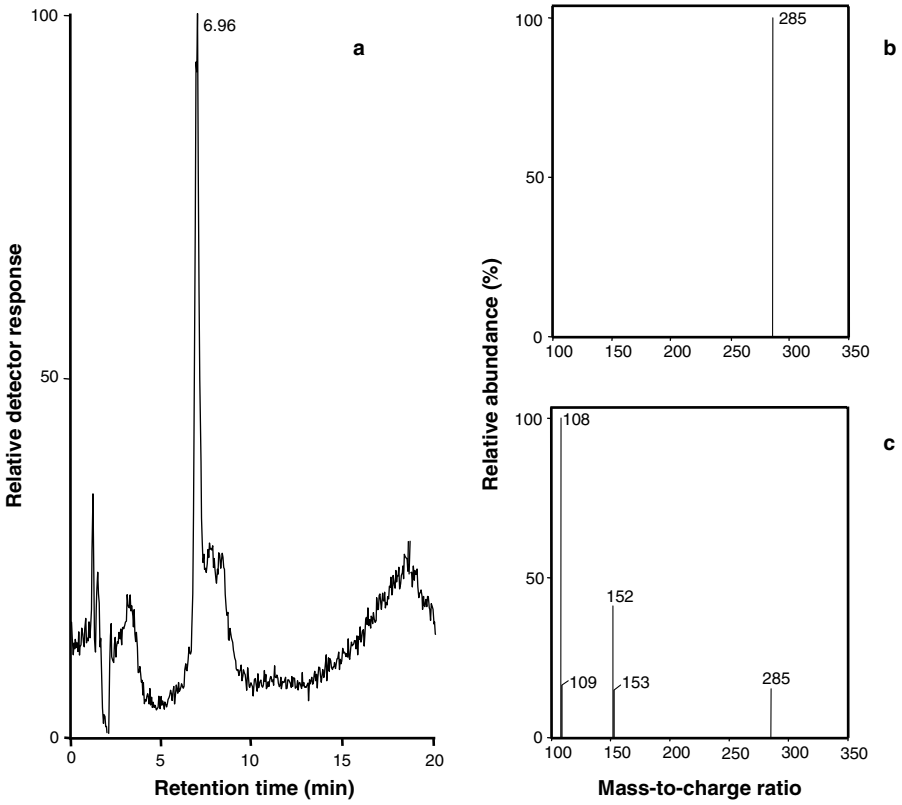

Fig. 1. Total ion current HPLC/ESI-MS chromatogram in negative ion mode of GA 5-*O*-β-D-xyloside (a). Mass spectra of GA 5-*O*-β-D-xyloside (peak of retention time 6.96 min) in electrospray ionization negative ion mode at a cone voltage of 20 V (b) or 60 V (c).

of the bond between the acid and the sugar, enzymatic hydrolysis of the purified compound with β-glucosidase or esterase was performed. β-Glucosidase but not esterase, specifically released the dihydroxybenzoic acid. Its retention time, data on mass spectra obtained by electrospray ionization in negative mode at 20 V ($m/z = 153$; $m/z = 108$), and UV-absorption characteristics ($\lambda_{max} = 327$ nm) were consistent with those of authentic GA. On the other hand, no cleavage of the *O*-glycosidic bond was detected after esterase digestion, indicating that the link between GA and the sugar was through a β-glucosidic bond (Table 1). The sugar joined to GA was identified by hydrolysis of the compound with HCl and analysis of the products in a pulsed electrochemical detector. As shown in Fig. 2(b), hydrolysis of the compound produced a new peak (retention time 7.8 min) in the chromatogram that was absent in the non-hydrolysed sample (Fig. 2(a)). Identification of the sugar after hydrolysis was achieved by spiking with the pentoses xylose, arabinose, or ribose. Only authentic xylose coeluted with the sugar product of the hydrolysis (Fig. 2(c)). These results contrast with what occurred for the analogues of GA, benzoic acid and SA in other plant biotic or abiotic interactions, in which these phenolics were conjugated to glucose (Bowling et al., 1994; Coquoz et al., 1998; Chong et al., 2001; Enyedi et al., 1992; Hammond-Kosack et al., 1996; Malamy et al., 1992). Further confirmation of the proposed identity of the compound was assessed by ^{1}H and ^{13}C NMR (Table 2). The ^{1}H NMR signals for the sugar moiety were similar to those previously described for β-D-xylose (Hounsell, 1995). The proton-proton nuclear Overhauser effects (NOESY) showed a correlation between the sugar $H_{1'}$ (δ 4.96) and the GA protons H_6 (δ 7.54) and H_4 (δ 7.25) but not with H_3 (δ 6.93). These date demonstrated that the sugar is joined to GA through hydroxyl oxygen atom on C-5 and not through C-2 one. Based on the above results, we propose glycoside to be GA 5-*O*-β-D-xylopyranoside (GAX) (Fig. 3). Key ions in the ESI mass spectrum appear at m/z 152 ($C_7O_4H_4$), and 108 ($C_7O_4H_4$–CO_2). Fig. 4(a) shows a time course for

Table 1
Fragment ions of GA 5-*O*-β-D-xyloside obtained by electrospray ionization in negative ion mode at a cone voltage of 20 V, before and after its hydrolysis with β-glucosidase or esterase

Treatment	*m/z*	Fragment ion	UV spectral data λ_{max} (nm)
No hydrolysis	285	M − H	315
β-Glucosidase	153	Gentisic acid–H	327
	108	Gentisic acid–CO_2H	
Esterase	285	M − H	315

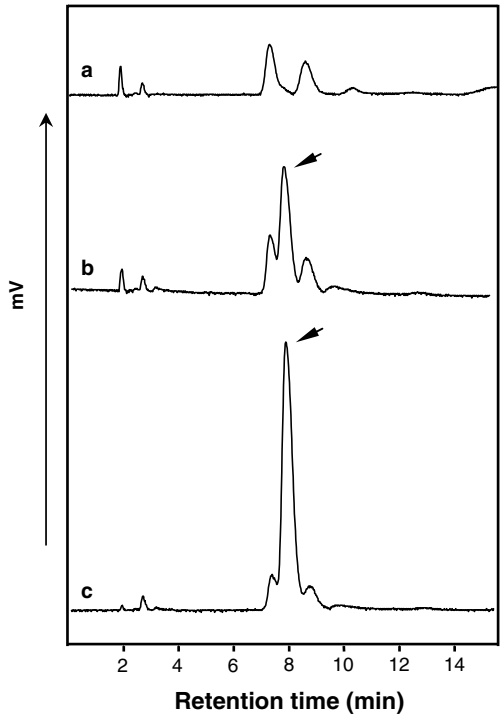

Fig. 2. HPLC profiles of GA 5-*O*-β-D-xyloside before (a), after its hydrolysis (b), and after its hydrolysis coinjected with authentic xylose (c). Arrow indicates xylose peak. Sugars from the hydrolysed mixture were detected in a Waters 464 pulsed electrochemical detector.

Table 2
^{1}H and ^{13}C NMR spectral data of gentisic acid 5-*O*-β-D-xyloside

Site	δ_H	Multiplicity[a]	J_H (Hz)	δ_C
Gentisic acid				
1				114.47
2				156.28
3	6.93	(*d*)	9.0	118.33
4	7.25	(*dd*)	9.0, 3.0	125.92
5				149.37
6	7.54	(*d*)	3.0	118.43
7				172.65
β-Xylose				
1′	4.96	(*brd*)	7.8	102.30
2′	3.50–3.56[b]	(*m*)	–	73.22
3′	3.50–3.56	(*m*)	–	75.87
4′	3.65–3.76	(*m*)	–	69.44
5′ax	3.44[b]	(*dd*)	11.3, 10.5	65.62
5′eq	3.99	(*dd*)	11.3, 5.4	

[a] *d*, doublet; *dd*, doublet of doublet; *brd*, broad doublet; *m*, multiplet.
[b] Data assigned by 2D-COSY correlation.

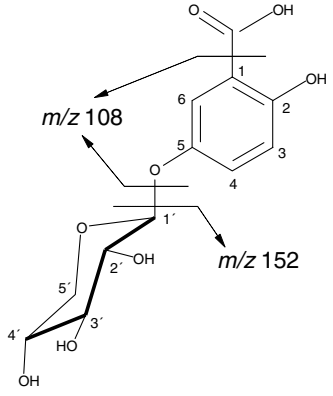

Fig. 3. Structure of GA 5-*O*-β-D-xyloside and scheme of fragmentation of the compound obtained by electrospray ionization in negative ion mode at a cone voltage of 60 V.

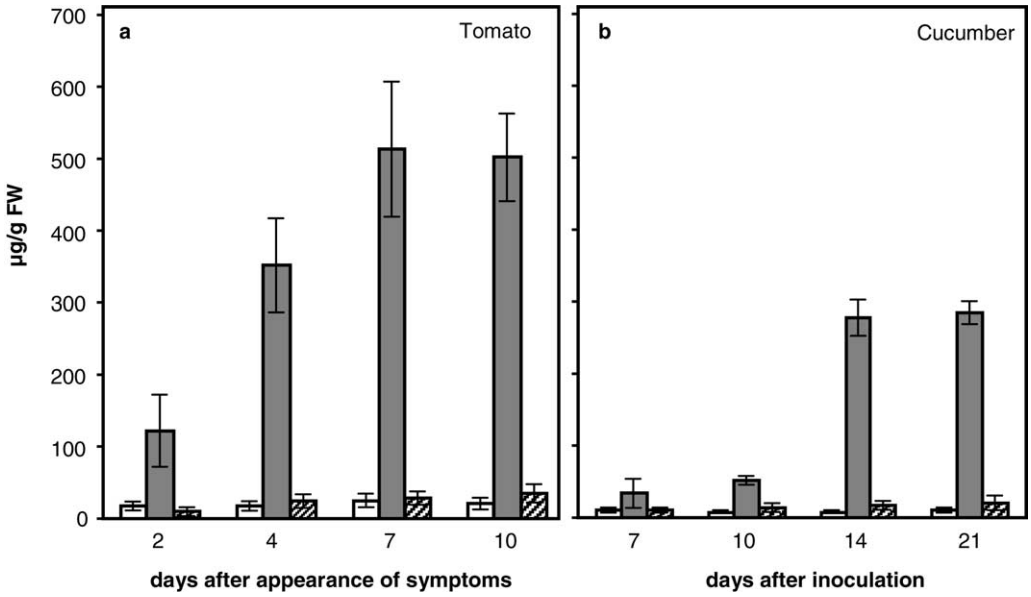

Fig. 4. Time courses for the accumulation of GA 5-*O*-β-D-xyloside and free GA in CEVd-infected Rutgers tomato (a), and PNRSV-infected cucumber plants (b). Open bars, GA 5-*O*-β-D-xyloside in control plants; dark bars, GA 5-*O*-β-D-xyloside in CEVd- or PNRSV-infected plants; cross-hatched bars, free GA in CEVd- or PNRSV-infected plants. Results are the means ± SE of three replicates.

GAX accumulation in leaves from CEVd-infected plants during the period of symptoms development. Visual symptoms, consisting of downward curling and rugosity of the leaves, develop 2–3 weeks after inoculation of cotyledons. A significant increase of GAX commenced early after appearance of symptoms, and was associated with mild symptoms in the leaves and progressively increased up to 50-fold relative to mock-inoculated plants, coinciding with the stronger symptoms of the disease. To establish whether the accumulation of this novel glycoside was restricted to CEVd-infected tomato or it was a more general phenomenon also occurring in other plant pathogen interactions, GAX was also measured in PNRSV-infected cucumber plants. Fig. 4(b) shows a time course for its accumulation in cucumber following inoculation with PNRSV over the period of symptom development. At day 4 following cotyledon inoculations, when the first leaves commenced to present curling and malformations, a significant increment in the level of GAX was already evident. As the disease progressed and symptoms became progressively more severe, GAX accumulation also increased reaching about 60% the level (approximately 300 µg/g FW) observed in CEVd-infected tomato (approximately 500 µg/g FW). The pattern of evolution of free GA after pathogen infection was similar to that of GAX, but free GA represented less than 10% of GAX (Fig. 4(a) and (b)). These results are in accordance with what has been previously found in tomato (Bellés et al., 1999). The GAX found in CEVd-infected tomato and PNRSV-infected cucumber was not present in tomato leaves inoculated with *Pseudomonas syringae* producing a hypersensitive-like reaction in the leaves (data not shown). This is consistent with previous results (Bellés et al., 1999) showing that GA was not induced in tomato leaves infected with this pathogen. Conjugation of phenolics with sugars through glycosyltransferases may modulate the endogenous level of free phenolics protecting plants from phenolic toxicity (Jones and Vogt, 2001). On the other hand, assuming phenolic glycosides to be by themselves an inactive storage form of phenolics, as it is the case of SA 2-*O*-β-D-glucoside (Henning et al., 1993), conjugation may also regulate the pool of active free form in those physiological situations in which elevated levels of phenolics are formed. Thus, the question arises about the enzyme that conjugates GA to xylose. Although plant glycosyltranferases usually transfer glucose (Keegstra and Raikhel, 2001; Lim and Bowles, 2004), enzymes that specifically recognize UDP-xylose as glycosyl donor have been reported (Martin et al., 1999; Rose et al., 1996). Also, 4-methylguaiacol and vanillyl alcohol, phenolic wood components, were converted by the fungus *Coriolus versicolor* to their β-xylosides, indicating that UDP-glycosyltransferase is involved in the xylosylation of lignin model compounds (Kondo et al., 1993). It has been reported that leaves from tomato plants infected with citrus exocortis viroid (CEVd) exhibited elevated levels of a conjugated form GA (Bellés et al., 1999). Based on the fact that the major part of conjugated GA was hydrolyzed by almond β-glucosidase, it was hypothesised (Bellés et al., 1999) that this conjugated form could be a β-*O*-D-glycosyl GA but the nature of the sugar moiety was not investigated. β-Glucosidase specifically hydrolyses β-glucosidic bonds, but its specificity for the sugar, however, is much lower. The work presented here demonstrates that in CEVd-infected tomato leaves, GA is conjugated to xylose contrary to what happens in *Cotoneaster orbicularis* (El-Mousallamy et al., 2000), and buckwheat (Schulz et al., 1993), in which GA is joined to glucose by a β-1,2-and a β-1,5-glycosidic linkage, respectively. On the other hand,

the xylosides of gentisic and caffeic acids have been recently found to be native components in *Erythrina indica* (Nassar et al., 2003), and *Rubus* species (Hussein et al., 2003), respectively, not associated with plant responses to biotic or abiotic stresses. Flavonoid xylosides involved in plant responses to abiotic stress (Lancaster et al., 2000) or pathogens (Elliger and Halloin, 1994) have been reported. The induction of GAX in CEVd-infected tomato and PNRSV-infected cucumber is similar to that observed for the compound 3′-*O*-*β*-D-ribofuranosyl adenosine which accumulates in tomato and *Arabidopsis* upon infection with virulent pathogens (Bednarek et al., 2004). The accumulation to high levels of this xyloside in compatible interactions which involve very different pathogens (viroid and virus) and plants (tomato and cucumber), in addition to its novelty, constitute an interesting observation that in turn give rises to new important questions as what might be the significance of the accumulation of GA joined to xylose rather than glucose as in the case of SA and the possible role of the presently described xyloside in plant defence. In recent years, the implication of other glycosides in plant defence has started to be investigated. In this respect, engineered alfalfa plants accumulating resveratrol glucoside showed increased resistance to *Phoma medicaginis* (Hipskind and Paiva, 2000), and enhancing the levels of coumarin glucoside (scopolin) or tyrosol glucoside has been correlated with the induction of defence responses in tobacco (Baumert et al., 2001) and potato (Landtag et al., 2002).

3. Experimental

3.1. Plant material and inoculations

Cultivation and inoculation procedures of tomato (*Lycopersicon esculentum* L. cv. Rutgers) plants were as previously described (Bellés et al., 1991). *Cucumis sativus* L. cv. Wisconsin SMR-58 plants were grown from seeds in 10-cm-diameter pots containing a mixture of peat and vermiculite (1:1) at 22/18 °C (day/night) and relative humidity from 50% to 70%. Plants were irrigated with Hoagland solution. Infection of cucumber plants with PNRSV (isolate NCM1; Aparicio et al., 1999) was performed with a viral extract obtained from leaves of PNRSV-infected cucumber plants homogenized in 30 mM sodium phosphate buffer (pH 8) and supplemented with 0.1% 2-mercaptoethanol (3 ml of buffer/g of fresh tissue). Seven days after sowing, cotyledons were dusted with carborundum, and buffer (mock-inoculated control) or viral extract was applied by gently rubbing the upper face of the two cotyledons. The cotyledons were washed with distilled water 5 min later.

3.2. Extraction and preliminary fractionation

Frozen control or CEVd-infected tomato leaves showing strong symptoms of the disease (100 g of each) were homogenized with a Heidolph homogenizer (Diax 900) in methanol (500 ml). The extracts were maintained at 40 °C with constant agitation in magnetic stirrer hotplates during 2 hours, then sonicated for 15 min, and centrifuged at 14,000*g* for 15 min to remove cellular debris. The supernatant (approximately 400 ml) was dried in vacuo. The residue (2 g) was dissolved in 20 ml of methanol, and submitted to column chromatography silicagel using a stepwise gradient of 25 ml each from dichloromethane to methanol as described by González et al. (2003). Aliquots were pooled in six fractions according to their similarity by TLC and dried under vacuum.

3.3. Purification and MS analysis of GA 5-O-β-D-xyloside

Fraction 4 (98 mg) was used as source to purify the compound by HPLC (Waters 600E) under isocratic conditions using a mixture of methanol and 1% acetic acid (30:70) at a flow rate of 15 ml/min. Samples (100 μl) were injected into a SymmetryPrep C18 7 μm (19 × 150 mm) column and the unknown compound was photometrically detected (280 nm) with a Waters 460 tunable absorbance detector. Several runs were performed and the total amount of collected compound was approximately 4 mg. Identification of the glycoside was done by HPLC in combination with electrospray ionization (ESI) mass spectrometry (Willoughby et al., 2002). Samples (10 μg) from purified compound were injected into a reverse-phase X-Terra MS C18 5 μm (3.9 × 150 mm) column. A 20-min linear gradient of 1% (v/v) acetic acid in water to 100% methanol at a flow rate of 1 ml/min (Yalpani et al., 1992) was applied with a Waters 1525 HPLC binary pump. A post-column split delivered approximately 25% of the flow to a Waters ZMD mass spectrometer and the rest to a Waters 996 photodiode array detector (maxplot between 240 and 400 nm). The source parameters of the mass spectrometer for ESI in negative mode were the following: capillary voltage 2500 V, cone voltage 20 or 60 V, extractor 5 V, RF Lens 0.5 V, source block temperature 100 °C and desolvation gas temperature 300 °C. The desolvation and cone gas used was nitrogen at a flow of 300 l and 50 l per min, respectively. Other mass spectrometer conditions were: low mass resolution 13.5, high mass resolution 13.5, ion energy 0.5, multiplier 650. ESI data acquisition was in the conditions of a full scan range from mass-to-charge ratio (*m*/*z*) 100 to 700 at 1 s per scan. HRMS have been performed with a VG Auto Spec Fisons spectrometer.

3.4. GA 5-O-β-D-xyloside and free GA measurements

Leaf material (0.5 g) from CEVd- and PNRSV-infected leaves and the corresponding controls were ground in mortar using liquid nitrogen and then, homogenized in 1.5 ml of methanol. The extracts were vortexed vigorously, sonicated for 15 min, and centrifuged at 14,000*g* for 15 min in 2-ml Eppendorf tubes to remove cellular debris. The supernatant (1.5 ml) was dried under nitrogen at 40 °C,

and the residue dissolved in 200 μl of methanol. Samples were filtered through a 0.45 μm Spartan 13/0.45RC filters (Schleicher and Schuell), and then 40 μl assayed on an HPLC system (Waters 600E) on a reverse-phase Symmetry 5 μm C18 (4.6 × 150 mm) column at 30 °C. A 20-min linear gradient of 1 % (v/v) acetic acid to 100% methanol at a flow rate of 1 ml/min was applied and glycoside was detected with a Waters 470 scanning fluorescence detector (excitation wavelength 313 nm; emission wavelength 405 nm) and quantified with the Waters Millenium^{32} software, using authentic standards. Free GA was extracted and measured according to Naranjo et al. (2003). Data were corrected for losses in the extraction procedure, and recovery of GA 5-*O*-β-D-xyloside and free GA, based on SA recovery, ranged between 50% and 80%.

3.5. Enzymatic digestions

Hydrolysis with β-glucosidase (14.3 U/mg, Fluka) or esterase (41 U/mg, Sigma) was performed adding 10 units enzyme to 100 μg of GA 5-*O*-β-D-xyloside dissolved in 200 μl of 50 mM sodium acetate (pH 4.5), for β-glucosidase or 50 mM Tris–HCl (pH 8), for esterase, and incubating the mixtures for 2 h at 37 °C. The reaction was stopped by adding 1 ml methanol and centrifuged at 12,000*g* for 10 min to remove enzymes. The supernatants were dried in a flow of nitrogen and dissolved in 100 μl of methanol for HPLC/MS analysis for hydrolysis products as above.

3.6. Sugar analysis

Hundred microgram of GA 5-*O*-β-D-xyloside were hydrolysed at 80 °C for 1 h in 500 μl 0.1 M HCl. After hydrolysis, 20 μl samples were injected into a RCX-10 anion exchange 7 μm (4.6 × 250 mm) column (Hamilton). Compounds were isocratically eluted at room temperature with 30 mM NaOH at a flow rate of 1 ml/min with a Waters 515 HPLC binary pump. Xylose was detected in a Waters 464 pulsed electrochemical detector with a range of 10 μA and the following settings (E1 = −80 mV, T1 = 0.26 s, E2 = 570 mV, T2 = 0.26 s, E3 = −730 mV, T3 = 0.36 s).

3.7. NMR analysis

1D (^{1}H), (^{13}C) and 2D (NOESY), (COSY) NMR spectra was recorded at 298 K on a Bruker spectrometer at a frequency of 300 MHz in D_2O solvent; chemical shifts are reported in δ (parts per million) values and couplings constants in Hz, using TMS as internal standard.

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References

Aparicio, F., Myrta, A., Di Terlizzi, B., Pallás, V., 1999. Molecular variability among fifteen isolates of prunus necrotic ringspot virus (PNRSV) from six different *Prunus* species. Phytopathology 89, 991–999.

Baumert, A., Mock, H.-P., Schmidt, J., Herbers, K., Sonnewald, U., Strack, D., 2001. Patterns of phenylpropanoids in non-inoculated and potato virus Y-inoculated leaves of transgenic tobacco plants expressing yeast-derived invertase. Phytochemistry 56, 535–541.

Bednarek, P., Winter, J., Hamberger, B., Oldham, N.J., Schneider, B., Tan, J., Hahlbrock, K., 2004. Induction of 3′-*O*-β-D-ribofuranosyl adenosine during compatible, but not during incompatible, interactions of *Arabidopsis thaliana* or *Lycopersicon esculentum* with *Pseudomonas syringae* pathovar tomato. Planta 218, 668–672.

Bellés, J.M., Carbonell, J., Conejero, V., 1991. Polyamines in plants infected by citrus exocortis viroid or treated with silver ions and ethephon. Plant Physiol. 96, 1053–1059.

Bellés, J.M., Garro, R., Fayos, J., Navarro, P., Primo, J., Conejero, V., 1999. Gentisic acid as a pathogen-inducible signal, additional to salicylic acid for activation of plant defenses in tomato. Mol. Plant-Microbe Interact. 12, 227–235.

Bowling, S.A., Guo, A., Cao, H., Gordon, S., Klessig, D.F., Dong, X., 1994. A mutation in Arabidopsis that lead to constitutive expression of systemic acquired resistance. Plant Cell 6, 1845–1857.

Chong, J., Pierrel, M.-A., Atanassova, R., Werck-Reichhart, D., Fritig, B., Saindrenan, P., 2001. Free and conjugated benzoic acid in tobacco plants and cell cultures. Induced accumulation upon elicitation of defense responses and role as salicylic acid precursors. Plant Physiol. 125, 318–328.

Coquoz, J.L., Buchala, A., Métraux, J.P., 1998. The biosynthesis of salicylic acid in potato plants. Plant Physiol. 117, 1095–1101.

Dixon, R.A., 2001. Natural products and plant disease resistance. Nature 411, 843–847.

Edwards, R., 1994. Conjugation and metabolism of salicylic acid in tobacco. J. Plant Physiol. 143, 609–614.

Elliger, C.A., Halloin, J.M., 1994. Phenolics induced in *Beta vulgaris* by *Rhizoctonia solani* infection. Phytochemistry 37, 691–693.

El-Mousallamy, A.M.D., Hussein, S.A.M., Merfort, I., Nawwar, M.A.M., 2000. Unusual phenolic glycosides from *Cotoneaster orbicularis*. Phytochemistry 53, 699–704.

Enyedi, A.J., Yalpani, N., Silverman, P., Raskin, I., 1992. Localization, conjugation and function of salicylic acid in tobacco during the hypersensitive reaction to tobacco mosaic virus. Proc. Natl. Acad. Sci. USA 89, 2480–2484.

González, M.C., Lull, C., Moya, P., Ayala, I., Primo, J., Primo, E., 2003. Insecticidal activity of penitrems, including penitrem G, a new member of the family isolated from *Penicillium crustosum*. J. Agric. Food Chem. 51, 2156–2160.

Hagemeier, J., Schneider, B., Oldham, N.J., Hahlbrock, K., 2001. Accumulation of soluble and wall-bound indolic metabolites in *Arabidopsis thaliana* leaves infected with virulent or avirulent *Pseudomonas syringae* pathovar tomato strains. Proc. Natl. Acad. Sci. USA 98, 753–758.

Hahlbrock, K., Bednarek, P., Ciolkowski, I., Hamberger, B., Heise, A., Liedgens, H., Logemann, E., Nurnberger, T., Schmelzer, E., Somssich, I.E., Tan, J., 2003. Non-self recognition, transcriptional reprogramming, and secondary metabolite accumulation during plant/pathogen interactions. Proc. Natl. Acad. Sci. USA 100, 14569–14576.

Hammond-Kosack, K.E., Silverman, P., Silverman, P., Raskin, I., Jones, J.D.G., 1996. Race-specific elicitors of *Cladosporium fulvum* induce changes in cell morphology and the synthesis of ethylene and salicylic acid in tomato plants carrying the corresponding *Cf* disease resistance gene. Plant Physiol. 110, 1381–1394.

Harborne, J.B., 1980. Plant phenolics. In: Bell, E.A., Charlwood, B.V. (Eds.), Secondary Plant Products, New Series, Vol. 8. Springer, New York, pp. 329–402.

Henning, J., Malamy, J., Grynkiewicz, G., Indulski, J., Klessig, D.F., 1993. Interconversion of the salicylic acid signal and its glucoside in tobacco. Plant J. 4, 593–600.

Hipskind, J.D., Paiva, N.L., 2000. Constitutive accumulation of a resveratrol-glucoside in transgenic alfalfa increases resistance to *Phoma medicaginis*. Mol. Plant-Microbe Interact. 13, 551–562.

Hounsell, E.F., 1995. ^{1}H NMR in the structural and conformational analysis of oligosaccharides and glycoconjugates. Prog. Nucl. Mag. Res. Spectrosc. 27, 445–474.

Hussein, S.A.M., Ayoub, N.A., Nawwar, M.A.M., 2003. Caffeoyl sugar esters and an ellagitannin from *Rubus sanctus*. Phytochemistry 63, 905–911.

Jones, P., Vogt, T., 2001. Glycosyltransferses in secondary plant metabolism: tranquilizers and stimulant controllers. Planta 213, 164–174.

Keegstra, K., Raikhel, N., 2001. Plant glycosyltransferases. Curr. Opin. Plant Biol. 4, 219–224.

Klick, S., Herrmann, K., 1988. Glucosides and glucose esters of hydroxybenzoic acids in plants. Phytochemistry 27, 2177–2180.

Kondo, R., Yamagami, H., Sakai, K., 1993. Xylosylation of phenolic hydroxyl groups of the monomeric lignin model compounds 4-methylguaiacol and vanillyl alcohol by *Coriolus versicolor*. Appl. Environ. Microbiol. 59, 438–441.

Lancaster, J.E., Reay, P.F., Norris, J., Butler, R.C., 2000. Induction of flavonoids and phenolic acids in apple by UV-B and temperature. J. Hort. Sci. Biotechnol. 75, 142–148.

Landtag, J., Baumert, A., Degenkolb, T., Schmidt, J., Wray, V., Scheel, D., Strack, D., Rosahl, S., 2002. Accumulation of tyrosol glucoside in transgenic potato plants expressing a parsley tyrosine decarboxylase. Phytochemistry 60, 683–689.

Lim, E.K., Bowles, D.J., 2004. A class of plant glycosyltransferases involved in cellular homeostasis. EMBO J. 23, 2915–2922.

Maher, E.A., Bate, N.J., Ni, W., Elkind, Y., Dixon, R.A., Lamb, C.J., 1994. Increased disease susceptibility of transgenic tobacco plants with suppressed levels of preformed phenylpropanoid products. Proc. Natl. Aca. Sci. USA 91, 7802–7806.

Malamy, J., Henning, J., Klessig, D.F., 1992. Temperature-dependent induction of salicylic acid and its conjugates during the resistance response to tobacco mosaic virus infection. Plant Cell 4, 359–366.

Martin, R.C., Mok, M.C., Mok, D.W., 1999. A gene encoding the cytokinin enzyme zeatin *O*-xylosyltransferase of *Phaseolus vulgaris*. Plant Physiol. 120, 553–558.

Métraux, J.P., Raskin, I., 1993. Role of phenolics in plant disease resistance. In: Chet, I. (Ed.), Biotechnology in Plant Disease Control. Wiley-Liss, New York, pp. 191–209.

Naranjo, M.A., Romero, C., Bellés, J.M., Montesinos, C., Vicente, O., Serrano, R., 2003. Lithium treatment induces a hypersensitive-like response in tobacco. Planta 217, 417–424.

Nassar, M.I., Gaara, A.H., Marzouk, M.S., El-Din, E., El-Khrisy, A.M., 2003. A new gentisic acid glycoside and C-glycosylflavones from *Erythrina indica* with the antioxidant activity evaluation. Bull. Fac. Pharm. Cairo Univ. 41, 207–209.

Niggeweg, R., Michael, A.J., Martin, C., 2004. Engineering plants with increased levels of the antioxidant chlorogenic acid. Nat. Biotechnol. 22, 746–754.

Rose, A., Glassgen, W.E., Hopp, W., Seitz, H.U., 1996. Purification and characterization of glycosyltransferases involved in anthocyanin biosynthesis in cell-suspension cultures of *Daucus carota* L. Planta 198, 397–403.

Sánchez-Navarro, J.A., Aparicio, F., Rowhani, A., Pallás, V., 1998. Comparative analysis of ELISA, nonradioactive molecular hybridization and PCR for the detection of prunus necrotic ringspot virus in herbaceous and *Prunus* hosts. Plant Pathol. 47, 780–786.

Schulz, M., Schnabl, H., Manthe, B., Schweihofen, B., Casser, I., 1993. Uptake and detoxification of salicylic acid by *Vicia faba* and *Fagopyrum esculentum*. Phytochemistry 33, 291–294.

Shadle, G.L., Wesley, S.V., Korth, K.L., Chen, F., Lamb, C., Dixon, R.A., 2003. Phenylpropanoid compounds and disease resistance in transgenic tobacco with altered expression of L-phenylalanine ammonia-lyase. Phytochemistry 64, 153–161.

Tan, J., Bednarek, P., Liu, J., Schneider, B., Svatos, A., Hahlbrock, K., 2004. Universally occurring phenylpropanoid and species-specific indolic metabolites in infected and uninfected *Arabidopsis thaliana* roots and leaves. Phytochemistry 65, 691–699.

Von Roepenack-Lahaye, E., Newman, M.A., Schornack, S., Kim, E., Hammond-Kosack, K.M., Lahaye, T., Jones, J.D.G., Daniels, M.J., Dow, J.M., 2003. Coumaroyladrenaline, a novel plant metabolite implicated in tomato defense against pathogens. J. Biol. Chem. 278, 43373–43383.

Willoughby, R., Sheehan, E., Mitrovich, S., 2002. A Global View of LC/MS, second ed. Global View Publishing, Pittsburgh, PA.

Yalpani, N., Schulz, M., Davis, M.P., Balke, N.E., 1992. Partial purification and properties of an inducible uridine 5′-diphosphate-glucose:salicylic acid glucosyltranferase from oat roots. Plant Physiol. 100, 457–463.