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Metabolic responses of tobacco to induction of systemic acquired resistance

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Abstract

Tobacco (*Nicotiana tabacum* L. cv. Xanthi-nc) plants carry the *N* resistance gene against *Tobacco mosaic virus* (TMV) and localize the infection to cells adjacent to the site of viral entry, developing a hypersensitive response in the form of local necrotic lesions. Previous studies have shown changes in metabolic activity, lipid peroxidation and lipid composition in TMV-inoculated Xanthi-nc plants. Here we report the comparative analysis of these changes in wild-type (WT) Xanthi-nc and salicylic acid-deficient phenotype NahG tobacco plants during TMV-induced cell death. TMV-inoculated WT plants develop systemic acquired resistance (SAR), while NahG tobacco plants are not able to establish SAR against a second inoculation by TMV. Heat efflux and ethane emission from uninoculated leaves of NahG plants were found to be significantly lower than from WT tobacco. However, the rates of heat efflux and lipid peroxidation increased up to similar levels in TMV-inoculated WT plants either expressing or not expressing SAR, and in NahG plants. TMV-inoculated leaves of NahG tobacco had a higher ratio of stigmasterol to sitosterol than those of WT plants. Furthermore, ratio of linoleic acid to linolenic acid was also higher in NahG tobacco. Possible reasons why induction of SAR had little or no effect on TMV-induced lipid peroxidation and metabolic activity are discussed.

Keywords: *Nicotiana tabacum*; *Tobacco mosaic virus*; Systemic acquired resistance; Isothermal calorimetry; Membrane lipid composition

1. Introduction

Tobacco mosaic virus (TMV) infection is localized in resistant tobacco plants, such as *Nicotiana tabacum* L. cv. Xanthi-nc that contains the *N* gene for hypersensitive response (HR). A coordinated programmed cell death in virus-inoculated leaves of Xanthi-nc plants is accompanied by the restriction of virus proliferation to small zones around the sites of infection and by the induction of systemic acquired resistance (SAR). The first TMV infection of Xanthi-nc tobacco enhances the localizing response to a second attack by TMV, resulting in the appearance of fewer and smaller lesions on the leaves distal to the primary infection site [1]. SAR depends on the accumulation of salicylic acid (SA), which is produced locally and systemically throughout the plant after TMV inoculation [2]. Xanthi-nc tobacco plants expressing the bacterial *nahG* gene encoding salicylate hydroxylase, an enzyme that converts SA to catechol, contain reduced amounts of SA [3]. Expression of *nahG* gene is sufficient to abolish the SAR response in these transgenic plants. NahG tobacco plants are rendered more susceptible to TMV, characterised by larger necrotic lesions as compared to the wild-type (WT) Xanthi-nc plants, indicating that increased production of SA is critical for resistance to TMV [4].

The rise of endogenous SA in inflorescence of arum lily has been shown to play a regulatory role in thermogenesis during flowering [5]. Similarly, elevated level of SA in TMV-inoculated leaves of WT Xanthi-nc tobacco is associated with thermogenesis and energy expenditure [6]. High level of SA, either externally applied [7] or accumulated upon TMV infection [8]

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has also been observed to induce an increase in lipid peroxidation in tobacco. Furthermore, SA activates expression of the *Arabidopsis thaliana αDOX1* gene, which encodes a 16- and 18-C fatty acid-oxidizing α-dioxygenase [9]. As to lipid composition, the saturation level of fatty acids decreased in wheat plants during frost hardening in correlation with an increase in SA content [10]. The involvement of lipids and lipid compositional dynamics in SAR in plants is also supported by genetic studies in *A. thaliana* suggesting a role for fatty acid desaturase activity [11]. These experiments provide evidence for roles of SA in thermogenesis, lipid peroxidation and composition as well.

The work reported here was initiated to extend the previous studies of our laboratory on TMV-inoculated WT Xanthi-nc tobacco to NahG transgenic plants. As a result, we can estimate the effect of naturally produced SA on the above-mentioned metabolic responses of Xanthi-nc tobacco to TMV. We have employed isothermal calorimetric measurements of heat evolution to determine changes in metabolic activity of TMV-inoculated tobacco leaf tissues. Rate of ethane evolution, a commonly used indicator of lipid peroxidation, was assayed using gas chromatography. Ethane is a volatile scission product released during the breakdown of ω-3 unsaturated hydroperoxy fatty acids [12]. Since membranes play a crucial role in HR of plant cells [13], fatty acid composition of the phospholipid fraction and sterol composition were also determined by gas chromatography. Because lipid composition is known to alter the fluidity of membranes [14,15], it was assessed by measuring steady-state fluorescence polarisation in tobacco protoplasts. Comparative analysis of metabolic responses in WT and NahG transgenic tobacco plants to TMV inoculation may help to further elucidate the mode of action of SA in TMV-induced SAR.

2. Materials and methods

2.1. Plant material and inoculations

Tobacco plants (*N. tabacum* cv. Xanthi-nc and *N. tabacum* cv. Xanthi-nc/NahG transgenic) were grown in greenhouse (18–23 °C, 160 mE m^{-2} s^{-1} for 8 h d^{-1} supplemental light, 60% RH). Seeds of transgenic line NahG-10 were kindly provided by NOVARTIS, Agricultural Biotechnology Research, Research Triangle Park, NC, USA. *Tobacco mosaic virus* (TMV) U1 strain was propagated in *N. tabacum* cv. Samsun tobacco plants. Leaves of TMV-infected Samsun tobacco plants showing typical mosaic symptoms were ground in mortar (1 g in 10 ml water) and the homogenate was used for inoculation without an abrasive. Control plants were mock-inoculated with water only. The third and fourth true leaves of tobacco plants were mock-inoculated or inoculated with TMV at the age of 8 weeks. A subsequent TMV challenge was applied on the upper leaves (fifth and sixth leaf positions above the hypocotyl) 14 days after inoculation of the lower leaves. Mock-inoculated plants were used as controls. Our treatments are summarised in Table 1. All measurements were performed in the upper fifth and sixth true leaves of 10-week-old WT and NahG plants.

Table 1
Plant inoculations[a]

Designation	Inoculations	
	On the lower leaves	On the upper leaves
TMV−−	Mock	Mock
TMV−+	Mock	TMV
TMV++	TMV	TMV

[a] The third and fourth leaves of tobacco plants were mock-inoculated or inoculated with TMV at the age of 8 weeks. The fifth and sixth leaves were inoculated 2 weeks later.

2.2. Calorimetric measurements

Leaf segments (5 mm × 35 mm) were cut from the interveinal leaf area. Two leaf segments from different tobacco plants were placed into a sample ampoule of a Thermal Activity Monitor LKB-2277 (Thermometric, Järfälla, Sweden), tightly sealed and allowed to equilibrate for 20 min before analysis. Heat production was recorded continuously for 20 min at 25 °C in five replicates. Maximum emission rate of heat in mW was calculated per g dry weight basis.

2.3. Ethane assay

Lipid peroxidation was detected through the analysis of thermally produced ethane. Leaf samples (about 400 mg) were placed into a 16-ml flask and sealed under nitrogen atmosphere. *In situ* decomposition of ω-3 unsaturated hydroperoxy fatty acids into ethane was accelerated by a brief heat treatment (80 s) of the samples in a microwave oven [16]. After cooling to room temperature, 1 ml of the gas phase was withdrawn from the flasks and injected into a gas chromatograph (Hewlett Packard 5890 Series II equipped with a 30-m GS-Alumina column) for ethane determination. The gas chromatograph was operated at injector temperature of 120 °C, detector temperature of 150 °C and oven temperature of 100 °C. The carrier gas used was helium (350 kPa).

2.4. Lipid analysis

Total lipid was extracted from tobacco leaf tissue with chloroform:methanol (1:2, v/v) [17]. After phase separation against 0.1 M KCl [18], the organic phase was dried under nitrogen at 38 °C. The samples were resuspended in chloroform, fractionated by silicic acid column chromatography, and methyl esthers of fatty acids were prepared as described by Żur et al. [19]. Phospholipids were analysed by gas chromatography for fatty acid composition using GS-Alumina (30 m × 0.542 mm, J&W Scientific) capillary column and operated isothermally at 200 °C [19]. An internal standard of heptadecanoic acid (C17:0) was added to extracts. Helium was used as a carrier gas (350 kPa).

Sterols were acetylated by using acetic anhydride and pyridine (2:1, v/v) for 24 h at room temperature and separated using DB-1701 (15 m × 0.254 mm, J&W Scientific) column at 270 °C [19]. Cholestan was used as an internal standard. The carrier gas was helium with a head pressure of 350 kPa.

Table 2
Inspection of virus-induced visible necrotic symptoms 48 h after TMV inoculation[a]

	Lesion size (mm)		Lesion number (cm^{-2})		Necrotisation (% of leaf area)	
	WT	NahG	WT	NahG	WT	NahG
TMV−+	1.08 ± 0.31	1.54 ± 0.41	2.98 ± 1.55	2.50 ± 1.38	2.95	4.95
TMV++	0.66 ± 0.18	1.58 ± 0.47	3.07 ± 1.63	2.57 ± 1.25	1.11	5.47

[a] Number and size of TMV-induced lesions were inspected visually on the upper leaves of 10-week-old tobacco plants. WT, wild-type Xanthi-nc plants. For inoculations see Table 1. The mean lesion density was used to calculate the necrotic lesion area. Values are means ± S.D. based on three independent assays. Lesions of three inoculated plants were counted for each experiment.

2.5. Protoplast isolation

Interveinal leaf segments (1 g) were finely chopped with a razor blade and incubated in 10 ml of 600 mM mannitol, 5 mM $CaCl_2$, 1% cellulase Onozuka RS, 1% driselase and 0.075% pectolyase Y-23 (pH 5.6). Leaf segments were agitated by gentle shaking at 25 °C in the dark for 3 h. The digest was filtered through 155 and 75 μm nylon mesh and centrifuged (65 × *g*, 5 min). The protoplasts were washed three times (600 mM mannitol and 5 mM $CaCl_2$, pH 5.7), centrifuged (65 × *g*, 5 min) and resuspended (10^4 cells/ml).

2.6. Membrane fluidity

Membrane fluidity was characterised by measuring fluorescence polarisation value of 1,6-diphenyl-1,3,5-hexatriene (DPH) in the protoplast membranes [20]. Protoplast suspensions were incubated in 65 μM DPH for 1 h at 35 °C. DPH was diluted from a 4.3 mM stock solution in acetonitrile. Fluorescence polarisation measurements were performed with a Perkin-Elmer LS50B spectrofluorimeter equipped with rotating polarisers in both the excitation and emission beams and a thermostatic chamber. Excitation and emission wavelengths were 360 and 430 nm, respectively. All measurements were done at 22 °C. The degree of polarisation (P) was calculated using the following equation: $P = (I_{\parallel} - I_{\perp}) \times (I_{\parallel} + I_{\perp})^{-1}$ in which $I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensities measured with emission analyser parallel and perpendicular orientations, respectively.

2.7. Statistical analysis

At least three independent experiments were conducted in each case. Measurements were carried out in five repetitions. Statistical analysis was performed using Student's t-test. Differences were considered to be significant at $P < 0.05$.

3. Results

3.1. Development of TMV-induced lesions

Upper leaves of WT and NahG tobacco plants were challenged with TMV 2 weeks after the first inoculation on lower leaves. Virus-induced necrotic lesions appeared on the upper leaves 40–48 h postinoculation. Necrotic symptoms were assessed by visual inspection of TMV-infected leaves (Table 2). Since TMV inoculation of the lower leaves of WT plants induced SAR against a subsequent TMV infection in remote leaves, significantly (at $P < 0.05$) smaller necrotic lesions developed on the upper leaves of WT plants. As a result, total necrotic area decreased by 70% in WT plants expressing SAR compared to that in uninduced plants. NahG plants, incapable of mounting an SAR response, showed impaired resistance to TMV and produced significantly larger necrotic lesions (the ratio of necrotic area was increased by 70%), as compared to WT. A previous TMV infection on the lower leaves did not affect significantly the lesion size in upper leaves of NahG tobacco. Expression of *nahG* gene or induction of SAR in WT plants did not affect significantly the lesion density.

3.2. Leaf heat production

The rate of exothermic heat flow was significantly lower in leaves of control, uninoculated NahG plants than in WT tobacco (Fig. 1). No changes were observed in heat emission 24 h after TMV inoculation in either of the tobacco lines (data not shown). However, 48 h postinoculation when TMV-induced necrotic symptoms had already developed, heat efflux was apparently induced in the virus-inoculated leaves (Fig. 1A). Rates of heat flow from the TMV-inoculated leaves of NahG

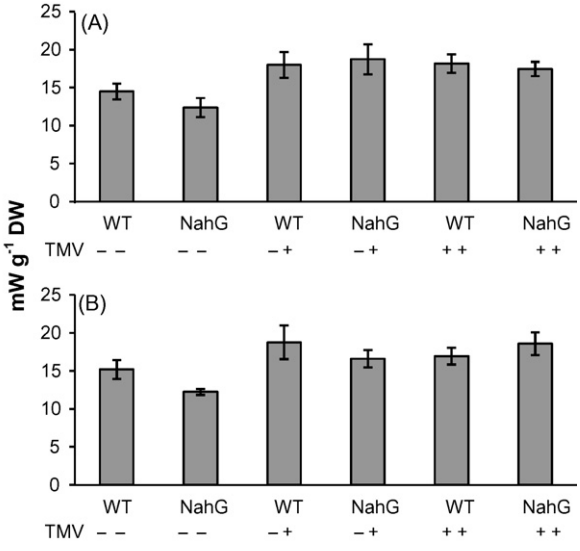

Fig. 1. Metabolic heat rates in the inoculated upper leaves of 10-week-old wild-type Xanthi-nc and transgenic NahG tobacco plants 48 h (A) and 72 h (B) after inoculation. Means of five replicates ± S.D. are shown. WT, wild-type Xanthi-nc plants. For symbols see Table 1.

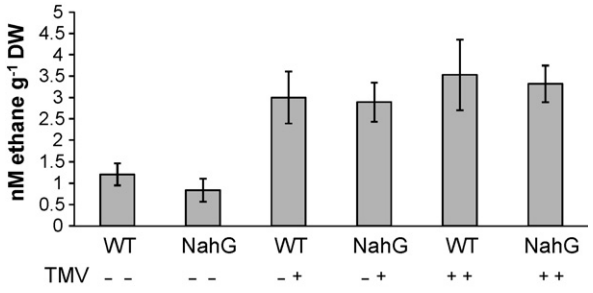

Fig. 2. Thermally produced ethane emitted from the upper leaves of 10-week-old wild-type Xanthi-nc and transgenic NahG tobacco plants 48 h after TMV inoculation. Means of five replicates ± S.D. are shown. WT, wild-type Xanthi-nc plants. For symbols see Table 1.

plants and WT plants expressing or not expressing SAR were not significantly different, in spite of the large differences in visible necrotic leaf damage. Three days after inoculation the heat emission was comparable to that detected at 2 days after inoculation (Fig. 1B).

3.3. Lipid peroxidation

TMV inoculation induced the peroxidation of membrane lipids as demonstrated by the emission of thermally produced ethane, derived from the decomposition of the 16-hydroperoxide of linolenic acid. The rates of ethane release from tobacco leaves were congruent with the results obtained with calorimetry. The level of thermally produced ethane was lower by 30% in uninoculated leaves of control NahG plants than in WT (Fig. 2). Ethane emission was significantly elevated in tobacco leaves inoculated with TMV. However, the severity of TMV-induced necrotic symptoms did not correlate with ethane emission 48 h after inoculation (Fig. 2). Lipid peroxidation was similar in TMV-inoculated leaves of NahG as well as of uninduced and induced (SAR-expressing) WT tobacco plants.

3.4. Lipid composition

The proportions of fatty acids in the phospholipid fractions prepared from the leaves of WT and transformed NahG tobacco plants are shown in Table 3. Linolenic acid accounted for about 57% of the total pool in leaves of WT tobacco. We observed a remarkable decrease in linolenic acid in the transgenic NahG plants. Furthermore, the proportion of linoleic acid was slightly elevated in leaves of NahG tobacco plants that resulted in a decrease in the ratio of linolenic to linoleic acid as compared to WT plants. TMV inoculation itself did not change significantly the fatty acid composition in either of the tobacco lines.

The major sterol accumulated within tobacco leaves was sitosterol, followed by stigmasterol, campesterol and cholesterol. SA-deficient NahG plants contained slightly higher levels of campesterol than WT (Table 4). Virus infection did not affect the sterol composition in leaves of WT plants. However, ratio of stigmasterol to sitosterol increased in NahG plants by 37% as a result of TMV inoculation.

Table 3
Fatty acid composition (in mol%) of phospholipids isolated from inoculated upper leaves of 10-week-old tobacco plants 3 days after mock inoculation or TMV inoculation[a]

Fatty acid	WT C	WT TMV	NahG C	NahG TMV
16:0	16.10 ± 1.10	16.30 ± 1.00	17.00 ± 1.10	16.00 ± 0.90
16:1	2.70 ± 0.30	2.60 ± 0.20	3.70 ± 0.40	2.90 ± 0.40
18:0	3.90 ± 0.20	4.30 ± 0.30	4.50 ± 0.30	4.70 ± 0.50
18:1	1.30 ± 0.20	1.50 ± 0.20	1.80 ± 0.20	1.50 ± 0.30
18:2	18.80 ± 1.10	19.00 ± 1.20	20.20 ± 1.60	21.20 ± 1.30
18:3	57.20 ± 2.20	56.30 ± 1.90	52.80 ± 2.00	53.70 ± 2.00
18:3/18:2	3.12 ± 0.19	2.94 ± 0.09	2.63 ± 0.29	2.52 ± 0.12
U/S	3.95 ± 0.21	3.85 ± 0.28	3.66 ± 0.26	3.77 ± 0.25
DBI	2.14 ± 0.10	2.12 ± 0.07	2.04 ± 0.04	2.05 ± 0.05

[a] Means ± S.D. of five replicates are shown. WT, wild-type Xanthi-nc; C, mock-inoculated control; TMV, TMV-inoculated; 18:3/18:2, the ratio of linolenic to linoleic acid; U/S, the ratio of unsaturated and saturated fatty acids; DBI, double bond index = Σ(mol% fatty acid × number of double bonds)/100.

Table 4
Sterol composition (in mol%) of inoculated upper leaves of 10-week-old tobacco plants 3 days after mock inoculation or TMV inoculation[a]

Sterol	WT C	WT TMV	NahG C	NahG TMV
Cholesterol	4.70 ± 0.75	5.03 ± 0.59	4.15 ± 0.37	4.02 ± 0.78
Campesterol	18.46 ± 0.35	17.30 ± 0.44	20.78 ± 0.49	19.59 ± 0.70
Stigmasterol	23.86 ± 0.35	24.69 ± 2.37	22.21 ± 1.11	27.93 ± 1.04
β-Sitosterol	52.98 ± 0.56	52.98 ± 2.76	52.86 ± 1.91	48.46 ± 0.94

[a] Means ± S.D. of five replicates are shown. WT, wild-type Xanthi-nc; C, mock-inoculated control; TMV, TMV-inoculated.

The above-mentioned changes in fatty acid and sterol composition, however, did not alter the fluidity of membranes in protoplasts prepared from these tobacco leaves (Table 5).

4. Discussion

Previous reports have shown that TMV inoculation of WT Xanthi-nc tobacco leaves results in local and systemic increases in SA and is associated with changes in heat production, lipid peroxidation and composition. The aim of this study was to characterise the role of SA in these metabolic responses with the help of a SA-deficient NahG transgenic line of Xanthi-nc tobacco.

The degree of unsaturation of fatty acids has been shown not to be affected by the induction of SAR in Xanthi-nc plants [21]. In NahG transgenic plants we observed a lower proportion of

Table 5
Fluorescence polarisation (P_{DPH}) in protoplasts prepared from uninoculated upper leaves of 10-week-old tobacco plants[a]

	P_{DPH}
WT C	0.295 ± 0.025
NahG C	0.304 ± 0.046
WT SAR	0.307 ± 0.027

[a] Means ± S.D. of five replicates are shown. WT, wild-type Xanthi-nc; C, control, the lower leaves were mock-inoculated at the age of 8 weeks; SAR, plants expressing SAR, the lower leaves were inoculated with TMV at the age of 8 weeks.

C18:3 fatty acid (by 8%) and a lower ratio of C18:3 to C18:2 fatty acid (by 16%) as compared to WT tobacco. It is noteworthy that when the content of trienoic fatty acids was reduced by 15–20% in the antisense *fad7* transgenic line of Xanthi-nc tobacco, the area of TMV-induced necrotic lesions became about two times larger than that in WT plants [22]. Likewise, molar ratio of linolenic acid within the fatty acids of the phospholipid fraction has been found to decrease approximately by 20% in TMV-inoculated Xanthi-nc leaves [23]. However, Ruzicska et al. [23] have applied a temperature shift from 33 to 25 °C to develop confluent necrosis on the virus-inoculated leaves. In our case, TMV inoculation caused much less severe necrotic symptoms and concomitantly did not induce significant changes in fatty acid composition.

An elevated ratio of stigmasterol to sitosterol was detected in TMV-inoculated leaves of NahG plants. Since aging of tobacco leaves has also been found to be accompanied by an increase in the ratio of stigmasterol to sitosterol [24,25], our result suggests that TMV inoculation induces senescence in NahG plants. Changes in lipid composition can affect membrane fluidity and permeability, therefore we compared the fluidity in protoplasts prepared from leaves of untreated WT and NahG plants, as well as of WT plants that had established SAR. Fluorescence polarisation measurements showed that these plants maintained their appropriate membrane fluidity. Similarly, induction of SAR in WT tobacco plants did not induce significant changes in fluorescence polarisation in the upper uninfected leaves [21].

Lipid peroxidation and heat production have been known to accompany the hypersensitive response of tobacco plants to TMV infection. In order to further elucidate the role of SA in lipid peroxidation and thermogenicity, we analysed ethane and heat emission in TMV-inoculated leaves of WT tobacco plants expressing SAR and of transgenic NahG tobacco plants that are impaired in the SAR response. This system is useful for examination of SA-mediated and SA-independent responses of tobacco to TMV inoculation.

It has been demonstrated earlier that the severity of hypersensitive symptoms in TMV-inoculated tobacco leaves is closely correlated with the level of lipid peroxidation [8]. In contrast, we found that lipid peroxidation is comparable in TMV-inoculated leaves of WT and NahG tobacco plants as demonstrated by the emission of ethane. Furthermore, ethane release was similar in WT plants after the primary and secondary inoculation with TMV, although we expected lipid peroxidation, like HR symptoms, to decrease in tobacco plants after establishment of SAR. These results support previous observations suggesting that SA potentiates the oxidative burst and increases cell death program in combination with reactive oxygen species [26,27]. Accordingly, activation of SAR by TMV inoculation induced single-cell microscopic lesions that appeared on both uninfected leaf areas and distal leaves of TMV-inoculated WT tobacco plants [28,29]. Our results indicate that ethane emission may arise from TMV-induced single-cell lesions in addition to that from visible necrotic spots. This hypothesis is supported by evidence that SA and its biologically active analogues induced a dose-dependent increase of lipid peroxidation in tobacco [7]. Furthermore, level of ethane released from uninfected leaves of NahG plants was lower by 30% than that in WT tobacco. Consistent with this result, catechol has been found to be a potent inhibitor of lipoxygenase activity in mammalian cells [30]. Increased lipoxygenase activity is characteristic of TMV-inoculated leaves of hypersensitively reacting tobacco plants [23] and lipoxygenase-mediated lipid peroxidation has been found to be responsible for about 95% of the total lipid peroxidation in TMV-infected Xanthi-nc tobacco leaves [8]. Therefore, we need to consider that SA-dependent and SA-independent processes equally contribute to lipid peroxidation in TMV-inoculated tobacco leaves at the time when hypersensitive lesions appear.

An enhanced heat production has previously been observed in TMV-infected leaves of WT Xanthi-nc tobacco plants during the formation of TMV-induced necrotic lesions [6,31]. In TMV-infected tobacco, the development of necrotic lesions is known to be accompanied by an increase in oxygen uptake [32–34] and an oxidative burst [35,36]. We have shown previously that the oxidative burst is correlated with the number and size of TMV-induced necrotic lesions in our TMV-tobacco system [37–39]. A calorimetric study has previously demonstrated that oxygen uptake and superoxide burst are in strong correlation with heat flow rate in *Chlorella vulgaris* alga inoculated by a mycoplasma, *Acholeplasma laidlawii* [40].

In the present study the previous work of our laboratory [6] was extended to include WT Xanthi-nc tobacco plants exhibiting SAR and SA-deficient NahG plants. Heat production reflects the general metabolic activity of tobacco leaf tissue, therefore our results obtained by calorimetry consist of both SA-dependent and SA-independent heat effluxes. The amount of heat emitted by uninoculated leaves of control NahG plants was significantly lower than that of WT tobacco. SA itself has been demonstrated to induce heat efflux [5,41]. SA treatment of tobacco plants has resulted in higher oxygen consumption and heat production through stimulation of the alternative respiratory pathway [42,43] and by the disruption of the proton gradient [44]. Incubation of tobacco cells with 20 μM SA has increased the rate of heat evolution by 60% compared to uninduced controls [42]. It is worth noting that the highest levels of free SA detected in tobacco around TMV-induced necrotic lesions were about 50 μM [45]. Interestingly, most effects of SA depletion in NahG plants were observed in control, uninfected plants, whereas little effect was found after TMV inoculation. This is curious with respect to the fact that SA levels are very low in uninfected plants and only rise after infection [2]. This might indicate that relatively small alterations in basal SA level trigger a significant shift in metabolic activities. Alternatively, the generation of catechol could be responsible for the inhibition of heat production as it was found in case of lipoxygenase activity in mammalian cells [30]; however, this needs further investigations.

The elevated level of heat efflux detected in the TMV-inoculated NahG leaves indicates that heat production can be independent of SA. Although we detected an increase of heat emission in tobacco after TMV inoculation as compared to control noninoculated plants, no remarkable differences in heat flow rate were observed among TMV-inoculated leaves of NahG, uninduced and induced (SAR-expressing) WT tobacco plants. In other words, as with lipid peroxidation, levels of metabolic

heat production were similar in TMV-inoculated tobacco leaves producing severe or mild symptoms in association with their own endogenous SA levels. We assume that the low rate of heat flow caused by a shortage of SA in NahG tobacco can be compensated for by a strong oxidative burst. In contrast, heat efflux augmented by elevated SA levels in WT plants may be compensated for by a down-regulated oxidative burst. This hypothesis is supported by previous studies from this laboratory showing that the higher the rate of TMV-induced tissue necrotisation, the higher the level of oxidative burst in tobacco leaves [37–39]. Recent data obtained from oilseed rape suggest that enhanced defence responses induced by 24-epibrassinolide against an incompatible bacterium are accompanied by elevated heat emission from the infected tissues, as compared to the untreated plants [46]. Furthermore, the extent of natural herbicide-resistance of wild oat biotypes was tightly correlated with the rate of heat production upon exposure to herbicide [47]. These results strongly indicate that increases of heat production are often characteristic of challenged plants possessing elevated levels of resistance to necrotising biotic or abiotic agents and an activation of metabolic pathways is required for defence responses.

In conclusion, a substantial body of evidence indicates that SA can induce lipid peroxidation and heat efflux. Therefore, the visual inspection of TMV-induced necrotic symptoms can yield misleading conclusions about the benefit of SAR in tobacco. We assume that the biological cost of mild tissue damage in TMV-inoculated tobacco leaves expressing SAR may be comparable to that of severe tissue damage in NahG tobacco impaired in its ability to mount an SAR response. Energy requirements of resistance responses can impose costly detrimental effects on plants resulting in early senescence or retarded growth [48,49].

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References

[1] A.F. Ross, Virology 14 (1961) 340–358.
[2] J. Malamy, J.P. Carr, D.F. Klessig, I. Raskin, Science 250 (1990) 1002–1004.
[3] T. Gaffney, L. Friedrich, B. Vernooij, D. Negrotto, G. Nye, S. Uknes, E. Ward, H. Kessmann, J. Ryals, Science 261 (1993) 754–756.
[4] T.P. Delaney, S. Uknes, B. Vernooij, L. Friedrich, K. Weymann, D. Negrotto, T. Gaffney, M. Gut-Rella, H. Kessmann, E. Ward, J. Ryals, Science 266 (1994) 1247–1250.
[5] I. Raskin, I.M. Turner, W.R. Melander, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 2214–2218.
[6] A.L. Ádám, F. Dubert, A. Gilly, B. Barna, A. Skoczowski, Acta Phytopathol. Entomol. Hung. 28 (1993) 195–200.
[7] M.D. Anderson, Z.X. Chen, D.F. Klessig, Phytochemistry 47 (1998) 555–566.
[8] J.L. Montillet, J.P. Agnel, M. Ponchet, F. Vailleau, D. Roby, C. Triantaphylidès, Plant Physiol. Biochem. 40 (2002) 633–639.
[9] I.P. de León, A. Sanz, M. Hamberg, C. Castresana, Plant J. 29 (2002) 61–72.
[10] T. Janda, G. Szalai, K. Lesko, R. Yordanova, S. Apostol, L.P. Popova, Phytochemistry 68 (2007) 1674–1682.
[11] A. Nandi, R. Welti, J. Shah, Plant Cell 16 (2004) 465–477.
[12] E.E. Dumelin, A.L. Tappel, Lipids 12 (1977) 894–900.
[13] T. Yaeno, O. Matsuda, K. Iba, Plant J. 40 (2004) 931–941.
[14] M.-A. Hartmann, Trends Plant Sci. 3 (1998) 170–175.
[15] M. Kates, E.L. Pugh, G. Ferrante, Biomembranes 12 (1984) 379–395.
[16] N. Degousée, C. Triantaphylidès, S. Starek, G. Iacazio, D. Martini, C. Bladier, R. Voisine, J.L. Montillet, Anal. Biochem. 224 (1995) 524–531.
[17] E.G. Bligh, W.J. Dyer, Can. J. Biochem. Physiol. 37 (1959) 911–917.
[18] J. Flosh, M. Lees, G.H. Sloaney-Stanley, J. Biol. Chem. 226 (1957) 497–509.
[19] I. Żur, A. Skoczowski, E. Niemczyk, F. Dubert, Acta Physiol. Plant. 24 (2002) 3–10.
[20] M. Shinitzky, Y. Barenholz, Biochim. Biophys. Acta 515 (1978) 367–394.
[21] A. Ádám, B. Barna, T. Farkas, Z. Király, Plant Sci. 66 (1990) 173–179.
[22] Y. Im, M.S. Kim, K.Y. Yang, Y. Kim, K. Back, B.H. Cho, Can. J. Bot. 82 (2004) 297–303.
[23] P. Ruzicska, Z. Gombos, G. Farkas, Virology 128 (1983) 60–64.
[24] C. Grunwald, Plant Physiol. 61 (1978) 76–79.
[25] B. Barna, B. Györgyi, Physiol. Mol. Plant Pathol. 40 (1992) 247–257.
[26] M.E. Alvarez, Plant Mol. Biol. 44 (2000) 429–442.
[27] K. Shirasu, P. Schulze-Lefert, Plant Mol. Biol. 44 (2000) 371–385.
[28] M.E. Alvarez, R.I. Pennell, P.J. Meijer, A. Ishikawa, R.A. Dixon, C. Lamb, Cell 92 (1998) 773–784.
[29] E. Balázs, I. Sziráki, Z. Király, Physiol. Plant Pathol. 11 (1977) 29–37.
[30] J. Simpson, R. Forrester, M.J. Tisdale, D.C. Billington, D.L. Rathbone, Bioorg. Med. Chem. Lett. 13 (2003) 2435–2439.
[31] L. Chaerle, W. Van Caeneghem, E. Messens, H. Lambers, M. Van Montagu, D. Van Der Straeten, Nat. Biotechnol. 17 (1999) 813–816.
[32] M. Weintraub, W.G. Kemp, H.W. Ragetli, Can. J. Microbiol. 6 (1960) 407–415.
[33] R. Mittler, V. Shulaev, M. Seskar, E. Lam, Plant Cell 8 (1996) 1991–2001.
[34] S. Chivasa, J.P. Carr, Plant Cell 10 (1998) 1489–1498.
[35] A.C. Allan, R. Fluhr, Plant Cell 9 (1997) 1559–1572.
[36] N. Doke, Y. Ohashi, Physiol. Mol. Plant Pathol. 32 (1988) 163–175.
[37] J. Fodor, É. Hideg, A. Kecskés, Z. Király, Plant Cell Physiol. 42 (2001) 775–779.
[38] Z. Király, B. Barna, A. Kecskés, J. Fodor, Free Radic. Res. 36 (2002) 981–991.
[39] Y.M. Hafez, J. Fodor, Z. Király, Acta Phytopathol. Entomol. Hung. 39 (2004) 347–360.
[40] N.L. Loseva, L.K. Gordon, F.V. Minibayeva, A.J. Alyabyev, V.M. Chernov, O.A. Chernova, I.N. Andreyeva, G.G. Rachimova, V.I. Tribunskih, R.I. Estrina, J.B. Gogolev, R.B. Kemp, Thermochim. Acta 390 (2002) 39–46.
[41] I. Raskin, A. Ehman, W.R. Melander, B.J.D. Meeuse, Science 237 (1987) 1601–1602.
[42] Y. Kapulnik, N. Yalpani, I. Raskin, Plant Physiol. 100 (1992) 1921–1926.
[43] D. Van Der Straeten, L. Chaerle, G. Sharkov, H. Lambers, M. Van Montagu, Planta 196 (1995) 412–419.
[44] L.K. Gordon, F.V. Minibayeva, D.F. Rakhmatullina, A.J. Alyabyev, T.I. Ogorodnikova, N.L. Loseva, Y.N. Valitova, Thermochim. Acta 422 (2004) 101–104.
[45] A.J. Enyedi, N. Yalpani, P. Silverman, I. Raskin, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 2480–2484.
[46] A. Janeczko, I. Tóbiás, A. Skoczowski, F. Dubert, G. Gullner, B. Barna, Thermochim. Acta 458 (2007) 88–91.
[47] A. Stokłosa, A. Janeczko, A. Skoczowski, J. Kieć, Thermochim. Acta 441 (2006) 203–206.
[48] K. Morris, S.A.H. Mackerness, T. Page, C.F. John, A.M. Murphy, J.P. Carr, V. Buchanan-Wollaston, Plant J. 23 (2000) 677–685.
[49] S. Yoshida, M. Ito, I. Nishida, A. Watanabe, Plant J. 29 (2002) 427–437.