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A genetically controlled polyacrylic acid induced resistance in *Nicotiana* species

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Summary

Several *Nicotiana* species and cultivars, with and without the N gene, were screened for the effect of polyacrylic acid (PAA) and salicylic acid (SA) on b-protein production and induced resistance. Whilst SA was effective in producing either b-proteins and resistance to a challenge viral infection in all the cultivars tested, the effectiveness of PAA appeared cultivar dependent. Only the cultivars Xanthi-nc (NN), Xanthi (nn) and hybrids between Xanthi-nc (NN) and other *N. tabacum* cultivars responded to PAA. This is the first time that the genetic determinant for the PAA response has been shown to be sexually transmitted as a dominant character, and also that it is distinct from the N gene.

Nicotiana sp.; TMV; TNV; b-proteins; polyacrylic acid; salicylic acid; induced resistance

Introduction

Tobacco varieties carrying the N gene from *Nicotiana glutinosa* respond to infection with tobacco mosaic virus (TMV) by the formation of necrotic local lesions and the subsequent localization of the virus around its point of entry [1]. The occurrence of the hypersensitive reaction in tobacco in response to TMV infection not only localizes the virus, but also induces resistance throughout the plant to a secondary infection called induced resistance [2]. Such induced resistance appears to be non-specific since it is active against other viruses, fungi, bacteria and even insects [3–6].

Various chemicals such as polyacrylic acid (PAA) [7–9] and salicylic acid (SA) [10]

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can also induce this aspecific resistance in tobacco plants. The expression of either TMV- or chemically-induced resistance is always associated with the synthesis of new soluble host-coded proteins (b- or PR-proteins) [7,9–13]. Among the chemical compounds known to induce resistance in *Nicotiana* species, one, PAA, is thought to be cultivar specific since it induces a very high level of resistance to TMV and *Pseudomonas syringae* in Xanthi-nc (NN) but not in Samsun NN [9,14].

Whilst it is well established that the hypersensitive reaction to TMV in *Nicotiana* species is controlled by the presence of the dominant gene N, the genetic basis of induced resistance is not known. The use of a selective inducer of resistance such as PAA could therefore represent an interesting tool for research into the genetic and molecular mechanisms involved in induced resistance.

In the present study we have compared the effect of PAA with that of SA, a cultivar aspecific inducer of resistance, on different tobacco varieties with or without the N gene and on their reciprocal hybrids. Using this system we hoped to gain insight into the relationship between expression of the N gene, the genes coding for the b-proteins and those for induced resistance.

Materials and Methods

Plant materials and viruses

The following plants were used: *Nicotiana glutinosa* (NN), *N. tabacum* cv Amersfort, Cridlo Corentino, Izmir, Judy's Pride Burley, Samsun NN, Xanthi-nc (NN), Xanthi (nn) and the reciprocal hybrids between the cultivars cited above and *N. tabacum* Xanthi-nc (NN). Plants were grown in a greenhouse in 15-cm pots containing a peat/soil/gravel mixture until they were 6 to 7 weeks old and then transferred to a constant environment room (20°C, 16 h day, 16 000 lux, 70% r.h.) one week prior to experiments.

The common strains of tobacco mosaic virus (TMV) and tobacco necrosis virus (TNV) were used to elicit a hypersensitive reaction. Plants were inoculated with these viruses by rubbing the surface of leaves dusted with carborundum with either a suspension of purified TMV, or the sap of leaves of *N. debneyi* infected with TNV. Water inoculated leaves served as controls.

Chemical treatments

Half-leaves of plants were injected with a 1 mM solution of PAA (Aldrich, mol. wt. = 2000) or SA (Sigma). The solutions were adjusted to pH 6.5 with KOH prior to injection. Control opposite half-leaves were injected with distilled water.

Protein extraction and electrophoresis

Tissue samples from control and chemically-treated half-leaves were harvested 7 days after injection. Protein extraction and electrophoresis were carried out as described by Gianinazzi et al. [15].

Determination of induced resistance

Seven days after injection of SA, PAA or water, leaves were inoculated with either TMV or TNV as specified for each experiment. Resistance was measured as percentage of reduction in local lesion numbers and/or size on treated leaves as compared to controls 7 days after inoculation. At least 10 half leaves were used for each test and 30 lesions randomly taken for each treatment, were measured using a stereoscopic microscope equipped with an ocular micrometer at a magnification of 10 ×.

Results

Effects of PAA and SA on several Nicotiana species and cultivars

Among the *Nicotiana* species and cultivars carrying the N gene, only *N. tabacum* cv 'Xanthi-nc' (NN) showed an induced resistance to a subsequent TMV-inoculation 7 days following the injection of leaves with PAA (Table 1). Cultivars lacking the N gene respond to TMV by formation of typical mosaic symptoms; the hypersensitive reaction was therefore elicited in these cultivars by a challenge inoculation with TNV. A resistance to TNV infection after PAA treatment was only observed in Xanthi (nn) (Table 2).

Contrary to the cultivar specificity of PAA, SA induced resistance in all the *Nicotiana* species and cultivars tested (Tables 1 and 2). In each case where resistance occurred after injection of SA, it was expressed by a reduction in both the number and size of local lesions formed after the challenge viral infection.

The production of b-proteins was studied 7 days after chemical injection. The synthesis of b-proteins occurred in the injected leaves only when resistance to TMV or

TABLE 1

Effect of PAA and SA treatments on b-protein production and resistance to TMV in *Nicotiana* carrying the N gene

Plants	Treatment	b-protein production before virus inoculation	Local lesions			
			Number	Reduction (%)	Size (mm)	Reduction (%)
N. tabacum						
Xanthi-nc (NN)	H_2O	-	247		1.96	
	PAA	+	14**	94.3	0.53**	73
	SA	+	75**	70	0.67**	65.8
Samsun (NN)	H_2O	-	197		1.47	
	PAA	-	197	-	1.75	-
	SA	+	43**	78	0.43**	70.7
N. glutinosa	H_2O	-	177		1.25	
	PAA	-	180	-	1.3	-
	SA	+	54*	69.5	0.47**	62.4

Significant difference (Student's *t*-test) between control and treated leaves at $P<0.05$ (*) or $P<0.01$ (**).

TABLE 2

Effect of PAA and SA treatments on b-protein production and resistance to TNV in several *N. tabacum* cultivars without the N gene

Plants	Treatment	b-protein production before virus inoculation	Local lesions			
			Number	Reduction (%)	Size (mm)	Reduction (%)
Xanthi (nn)	H_2O	-	542		2.07	
	PAA	+	50**	90.8	0.92*	55.6
	SA	+	nd	-	nd	-
Amersfort	H_2O	-	59		2.7	
	PAA	-	60	-	2.77	-
	SA	+	12**	79.7	0.8**	70.3
Cridlo	H_2O	-	18		1.92	
Corentino	PAA	-	15	16.7	1.72	10.4
	SA	+	3**	83.3	0.58**	69.8
Izmir	H_2O	-	31		2.61	
	PAA	-	36	-	2.4	0.9
	SA	+	7**	77.4	0.97**	62.8
Judy's Pride	H_2O	-	32		2.03	
Burley	PAA	-	24	25.0	1.79	11.8
	SA	+	13**	59.3	0.93*	54.2

Significant difference (Student's *t*-test) between control and treated leaves at $P<0.05$ (*) or $P<0.01$ (**). nd = not determined.

TNV was induced either by PAA or SA treatment (Tables 1 and 2). More detailed analysis (Table 3) showed that SA treatment induced the same b-proteins as the viral infection and that their patterns varied intra- and interspecifically. *N. tabacum* cv 'Xanthi-nc' (NN), 'Samsun' (NN) and 'Xanthi' (nn) produced 4 new proteins with R_f values of 0.83, 0.66, 0.56 and 0.53 and called b_1, b_2, b_3 and b_4 respectively; b_1, b_2, b_3 and an additional protein $b_{1'}$ with a R_f of 0.79 was detected in cultivars Amersfort, Cridlo Corentino, Izmir and Judy's Pride Burley. *N. glutinosa* synthetised only the $b_{1'}$ protein with a R_f of 0.76. PAA only induced the production of the proteins in Xanthi-nc (NN) and Xanthi (nn); b_1, b_2 and b_3, characteristic of these varieties, were present but not b_4, as has been seen in viral infection [7,9].

Inheritance of PAA-induced resistance

Effectiveness of PAA treatment did not depend on the virus used but instead was dependent on the cultivar of tobacco. Its action appeared to be independent of the N gene since it was effective in Xanthi cultivars both with and without this gene. Since the hypersensitive reaction to TMV is controlled by the dominant N gene, all the hybrids with Xanthi-nc (NN) reacted to TMV infection by the formation of typical local lesions. Table 4 shows the effect of PAA injection on resistance to TMV and production of b-proteins in such hybrids. After PAA injection, all the hybrids studied whatever the direction of the cross, showed a resistance to the challenge TMV infection expressed by a reduction in number and size of local lesions formed. This resistance

TABLE 3

b-protein patterns in *Nicotiana* species with or without the N gene as revealed by analysis in 10% polyacrylamide gels following a hypersensitive reaction or treatment with SA or PAA

Plants	Treatment	b-proteins					
		b_1	$b_{1'}$	$b_{1''}$	b_2	b_3	$b_{4'}$
N. glutinosa	TMV	-	-	+	-	-	-
	SA	-	-	+	-	-	-
	PAA	-	-	-	-	-	-
N. tabacum							
Xanthi-nc (NN)	TMV	+	-	-	+	+	+
	SA	+	-	-	+	+	(+)
	PAA	+	-	-	+	+	-
Samsun (NN)	TMV	+	-	-	+	+	+
	SA	+	-	-	+	+	(+)
	PAA	-	-	-	-	-	-
Xanthi (nn)	TNV	+	-	-	+	+	+
	SA	+	-	-	+	+	(+)
	PAA	+	-	-	+	+	-
Amersfort	TNV	+	+	-	+	+	-
	SA	+	+	-	+	(+)	-
	PAA	-	-	-	-	-	-
Cridlo Corentino	TNV	+	+	-	+	+	-
	SA	+	+	-	+	(+)	-
	PAA	-	-	-	-	-	-
Izmir	TNV	+	+	-	+	+	-
	SA	+	+	-	+	+	-
	PAA	-	-	-	-	-	-
Judy's Pride Burley	TNV	+	+	-	+	+	-
	SA	+	+	-	(+)	(+)	-
	PAA	-	-	-	-	-	-

+, -, (+) = presence, absence or trace of b-protein.

was always accompanied by the production of all the b-proteins from both parents. Interestingly b_4, which was not induced by PAA in Xanthi-nc (NN) (Table 3), was never present in the hybrids treated with this chemical.

Discussion

In the present study we report that resistance induced by PAA depends on the *Nicotiana* species and cultivar used. In addition, resistance induced by either PAA or SA is always associated with production of b-proteins. These b-proteins show the same intra- and interspecific variability as when they are induced by viral infection [16].

It has previously been demonstrated that injection of PAA into leaves of Xanthi-nc (NN) leads to the appearance of resistance to viral infection and of proteins b_1, b_2 and b_3 [7,9]. The hypothesis that effectiveness of PAA against pathogens could be cultivar

TABLE 4

Effect of PAA on b-protein production and resistance to TMV in several reciprocal hybrids between Xanthi-nc (NN) and other tobacco cultivars with or without the N gene

Hybrids	Treatment	b-protein production					Local lesions			
		b_1	$b_{1'}$	b_2	b_3	b_4	Number	Reduction (%)	Size (mm)	Reduction (%)
Xanthi-nc × Amersfort	H_2O	–	–	–	–	–	450		2.1	
	PAA	+	+	+	(+)	–	67	85**	0.83	60.5**
Xanthi-nc × Cridlo Corentino	H_2O	–	–	–	–	–	347		2.16	
	PAA	+	+	+	(+)	–	73	78.9**	0.75	65.3**
Xanthi-nc × Izmir	H_2O	–	–	–	–	–	346		2.08	
	PAA	+	+	+	(+)	–	30	91.3**	0.6	71.2**
Xanthi-nc × Judy's Pride Burley	H_2O	–	–	–	–	–	301		2.28	
	PAA	+	+	+	(+)	–	41	86**	0.6	73.7**
Xanthi-nc × Samsun NN	H_2O	–	–	–	–	–	91		2.52	
	PAA	+	–	+	(+)	–	4	95.6**	0.24	90.5**
Amersfort × Xanthi-nc	H_2O	–	–	–	–	–	164		1.32	
	PAA	+	+	+	(+)	–	33	79.8**	0.48	63.6**
Cridlo Corentino × Xanthi-nc	H_2O	–	–	–	–	–	57		2.83	
	PAA	+	+	+	(+)	–	2	96.5**	0.65	77**
Izmir × Xanthi-nc	H_2O	–	–	–	–	–	144		1.37	
	PAA	+	+	+	(+)	–	35	75.7**	0.5	63.5**
Judy's Pride Burley × Xanthi-nc	H_2O	–	–	–	–	–	66		1.86	
	PAA	+	+	+	(+)	–	1	98.5**	0.96	48.4*
Samsun NN × Xanthi-nc	H_2O	–	–	–	–	–	360		2.01	
	PAA	+	–	+	(+)	–	28	94.6**	0.25	87.6**

Significant difference (Student's *t*-test) between control and treated leaves at $P<0.05$ (*) or $P<0.01$ (**). +, –, (+) = presence, absence or trace of b-protein.

specific was first put forward by Antoniw and White in the case of viruses [9] and reinforced recently by Ahl et al. [14] using a bacterial infection in Xanthi-nc (NN) and Samsun (NN). Our results confirm that resistance induced in tobacco by PAA is cultivar dependent; no other varieties tested in the present study show a resistance after PAA injection, whilst they all respond to SA treatment. Moreover, we have demonstrated that this PAA-induced resistance does not depend on the N gene which controls the hypersensitive reaction to TMV. Firstly, other *Nicotiana* species like *N. tabacum* cv Samsun (NN) and *N. glutinosa* which have the N gene do not respond to PAA and, secondly, the cultivar Xanthi (nn), without the N gene shows a PAA-induced resistance.

Furthermore, by crossing *N. tabacum* cv 'Xanthi-nc' (NN) with several varieties of tobacco which do not respond to PAA, we have shown in all the hybrids tested that PAA can induce resistance and the production of all the b-proteins from both parents. Therefore the genetic determinant for PAA response can be sexually transmitted through either male or female gametes and behaves as a dominant character, distinct from the N gene. Its expression is always accompanied by the production of b-proteins. This genetic determinant for PAA response may have been inherited from *N. tomentosiformis* since this species responds to PAA treatment [16] and is thought to be, with *N. sylvestris*, an ancestor of present-day tobacco [17].

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