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The genetic and molecular basis of b-proteins in the genus Nicotiana

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Abstract

Screening for the pathogenesis-related (b) protein patterns of 11 *Nicotiana* species and 30 *N. tabacum* varieties has revealed both inter- and intraspecific variability and 7 different b-proteins (b_0, b_1, $b_{1'}$, $b_{1''}$, b_2, b_3 and b_4) have been clearly defined. Their genetic determinants are sexually transmitted independently of the *N* gene conferring resistance to TMV, and a monogenic inheritance has been demonstrated for one of them ($b_{1'}$). Grafting experiments have revealed the existence of a species-aspecific 'mobile compound' responsible for the expression of the b-protein genes, the production of which is probably under the control of the *N* gene. Among the 5 intraspecific and 6 interspecific hybrids studied, one of them, the *N. glutinosa* × *N. debneyi* together with its amphidiploid, synthesizes b-protein ($b_{1''}$) in a constitutive way and possesses a high level of resistance to necrosis-inducing viruses. The amphidiploid is able to transfer these two properties to other *Nicotianae* not only by crossing but also by grafting; it therefore appears to permanently synthesize the 'mobile compounds'. Furthermore, the hypersensitive reaction to TMV in these hybrids is only completely broken down at 35 °C, whereas this normally occurs at 30 °C in plants with the *N* gene.

Additional keywords: interspecific and intraspecific hybrids, grafting experiments, *N* gene, resistance, temperature effects.

Introduction

In their early work, Gianinazzi et al. (1970) and Van Loon and Van Kammen (1970) reported that the pathogenesis-related (b) proteins synthesized during the hypersensitive reaction to virus infection by the three *Nicotiana* species they studied were different, revealing the existence of an interspecific variability among the b-protein families. White (1979) and Ahl (1979) have recently shown that the b-protein patterns in *N. tabacum* 'White Burley' and 'Judy's Pride Burley' are different from the one in *N. tabacum* 'Xanthi-nc' (Gianinazzi et al., 1970): a new band, $b_{1'}$, is present in the former and has an Rf between that of the bands b_1 and b_2 of 'Xanthi-nc' tobacco. Thus, an intraspecific variability of b-protein patterns also exists. In a more detailed study, Ahl et al. (1982) clearly demonstrated this inter- and intraspecific variability and pointed out the interest of using b-proteins as genetic markers.

Inter- and intraspecific variability of b-protein patterns

Up to now, 7 different b-proteins have been clearly defined according to their relative migration in 10% polyacrylamide gels: b_0 (Rf = 0.88; mol. wt = 15 700), b_1 (Rf =

Table 1. b-Protein patterns of different inter- and intraspecific *Nicotiana* hybrids and of their parents, as revealed by analysis in 10% polyacrylamide gels.

Parents, hybrids and amphidiploids	Virus inducing HR[1]	b-proteins[2]						
		b_0	b_1	$b_{1'}$	$b_{1''}$	b_2	b_3	b_4
Parents								
N. debneyi	TNV	−	−	−	+	−	−	−
N. glutinosa	TMV, TNV	−	−	−	+	−	−	−
N. sylvestris	TMVa[4], TNV	+	+	−	−	−	+	−
N. tabacum 'Burley 49'	TMV, TNV	−	+	−	−	+	+	−
N. tabacum 'Judy's Pride Burley'	TNV	−	+	+	−	+	+	−
N. tabacum 'Samsun NN'	TMV, TNV	−	+	−	−	+	+	+
N. tabacum 'Samsun nn'	TNV	−	+	−	−	+	+	+
N. tabacum 'Xanthi-nc'	TMV, TNV	−	+	−	−	+	+	+
N. tomentosiformis	TNV	−	−	−	−	+	−	−
Interspecific hybrids								
N. glutinosa × *N. debneyi*	–[3]	−	−	−	+	−	−	−
N. sylvestris × *N. tomentosiformis*	TMVa[4], TNV	+	+	−	−	+	+	−
N. tabacum 'Judy's Pride Burley' × *N. glutinosa*	TMV, TNV	−	+	+	+	+	+	−
N. tabacum 'Judy's Pride Burley' × *N. sylvestris*	TMVa[4], TNV	+	+	+	−	+	+	−
N. tabacum 'Samsun NN' × *N. glutinosa*	TMV, TNV	−	+	−	+	+	+	+
N. tabacum 'Samsun NN' × *N. sylvestris*	TMV, TNV	+	+	−	−	+	+	+
Intraspecific hybrids								
N. tabacum 'Burley 49' × *N. tabacum* 'Judy's Pride Burley'	TMV, TNV	−	+	+	−	+	+	−
N. tabacum 'Samsun NN' × *N. tabacum* 'Judy's Pride Burley'	TMV, TNV	−	+	+	−	+	+	+
N. tabacum 'Samsun nn' × *N. tabacum* 'Burley 49'	TMV, TNV	−	+	−	−	+	+	+
N. tabacum 'Samsun nn' × *N. tabacum* 'Judy's Pride Burley'	TNV	−	+	+	−	+	+	+
N. tabacum 'Xanthi-nc' × *N. tabacum* 'Judy's Pride Burley'	TMV, TNV	−	+	+	−	+	+	+
Amphidiploids								
N. glutinosa × *N. debneyi*	–[3]	−	−	−	+	−	−	−
N. sylvestris × *N. tomentosiformis*	TMVa[4], TNV	+	+	−	−	+	+	−

[1] HR = hypersensitive reaction.
[2] + = presence; − = absence.
[3] No virus was used, because this combination produces b-proteins as a constitutive component.
[4] TMVa = TMV aucuba strain.

0.83; ml. wt = 15 700), $b_{1'}$ (Rf = 0.79; ml. wt = 15 700), $b_{1''}$ (Rf = 0.76; mol. wt = 13 800), b_2 (Rf = 0.66; mol. wt = 15 700), b_3 (Rf = 0.56; mol. wt = 15 700) and b_4 (Rf = 0.53; two polypeptides with mol. wts of 23 000 and 29 000) (Gianinazzi et al., 1980; Antoniw et al., 1980; Ahl et al., 1982; Carr et al., 1982). Of these 7 b-proteins, *N. repanda* has only b_1; *N. glutinosa, N. debneyi* and *N. suaveolens* have only $b_{1''}$; *N. tomentosiformis, N. occidentalis* and *N. rustica* have only b_2; *N. excelsior* and *N. maritima* have both $b_{1''}$ and b_2 whilst *N. sylvestris* has b_0, b_1 and b_3. Varieties of *N. tabacum* can be divided into 4 groups: 1) (made up of 10 varieties) plants with b_1, b_2, b_3 and b_4 (e.g. 'Xanthi-nc'); 2) (made up of 10 varieties) plants with b_1, b_2 and b_3 (e.g. 'Burley 49'); 3) (made up of 8 varieties) plants with b_1, $b_{1'}$, b_2 and b_3 (e.g. 'Judy's Pride Burley'); 4) (made up of 2 varieties) plants with b_1, $b_{1'}$, $b_{1''}$, b_2, b_3 and b_4 (e.g. 'Paraguay P48' (Ahl, 1983).

The homology of b-proteins coming from different species or cultivars is becoming more and more evident; this justifies giving them the same denomination of b-proteins (Gianinazzi et al., 1970; Ahl et al., 1982; Gianinazzi, 1983) or PR-proteins (Antoniw et al., 1980).

Inheritance of the b-protein genetic determinants

Reciprocal crosses between *Nicotiana* species or between tobacco varieties always give hybrids containing the exact sum of the b-proteins of their different parents (Table 1), showing that the b-protein determinants are sexually transmitted (Gianinazzi et al., 1980; Ahl et al., 1982). A monogenic inheritance, independent of that of the *N* gene (the gene conferring hypersensitivity to TMV), has been demonstrated for one of them ($b_{1'}$) (Ahl et al., 1982) and J.P. Carr (personal communication) has recently shown that each b-protein in *N. tabacum* 'Xanthi-nc' possesses its own mRNA. It therefore seems plausible that each b-protein is coded by its own gene and that the b-proteins are not the product of the *N* gene. However, since the latter is necessary for significant production of b-protein after TMV infection, it is possible that the *N* gene may play a regulative role in the expression of the b-protein genes.

Regulation of the expression of b-protein genes

The above hypothesis is strengthened by results from grafting experiments in which b-proteins can be induced in tobacco lacking the *N* gene by grafting it onto a TMV-inoculated hypersensitive root-stock (Gianinazzi, 1982; Table 2). The most logical explanation for this is that a compound, whose production may be under the control of the *N* gene, is induced by the TMV infection in the root-stock and moves into the scion where it allows the expression of the gene(s) coding for b-proteins. It is not known if this 'mobile compound' is the same as the one responsible for inducing systemic acquired resistance (Ross, 1961), which has up to now always been accompanied by the appearance of b-proteins, but the grafting experiments shown in Table 2 clearly demonstrate that it is likewise not species specific. It may be that this 'mobile compound' is involved in converting the mRNAs coding for b-proteins (Carr et al., 1982) from their latent untranslatable state to a translatable one.

Another interesting fact which contributes towards understanding how b-proteins are synthesized in vivo is the observation that the interspecific hybrid *N. glutinosa* ×

Table 2. b-Protein induction in different scions after grafting on TMV- or TNV-inoculated *Nicotiana* root-stocks or on the amphidiploid *N. glutinosa* × *N. debneyi*.

Plants		Virus infecting root-stock	Root-stock reaction[4]	b-proteins[5]											
				root-stock						scion					
Root-stock (N)[1]	*Scion* (N)														
'Burley 49'	'Judy's Pride Burley' × 'Burley 49'	TMV	HR	b_1	–	–	b_2	b_3	–	b_1	$b_{1'}$	–	b_2	b_3	–
'Judy's Pride Burley' × 'Burley' 49'	'Burley 49'	TMV	HR	b_1	$b_{1'}$	–	b_2	b_3	–	b_1	–	–	b_2	b_3	–
'Samsun NN'	'Samsun NN' × 'Judy's Pride Burley'	TMV	HR	b_1	–	–	b_2	b_3	b_4	b_1	$b_{1'}$	–	b_2	b_3	b_4
'Samsun NN' × 'Judy's Pride Burley'	'Samsun NN'	TMV	HR	b_1	$b_{1'}$	–	b_2	b_3	b_4	b_1	–	–	b_2	b_3	b_4
N. glutinosa × *N. debneyi*	'Xanthi-nc'	–[3]	–	–	–	$b_{1''}$	–	–	–	b_1	–	–	b_2	b_3	b_4
Root-stock (N)	*Scion* (n)[2]														
'Samsun NN'	'Judy's Pride Burley'	TMV	HR	b_1	–	–	b_2	b_3	b_4	b_1	$b_{1'}$	–	b_2	b_3	–
N. glutinosa × *N. debneyi*	'Paraguay P48'	–[3]	–	–	–	$b_{1''}$	–	–	–	b_1	$b_{1'}$	–	b_2	b_3	b_4
Root-stock (n)	*Scion* (N)														
'Judy's Pride Burley'	'Samsun NN'	TMV	S	–	–	–	–	–	–	b_1	–	–	b_2	b_3	b_4
'Judy's Pride Burley'	'Samsun NN'	TNV	HR	b_1	$b_{1'}$	–	b_2	b_3	–	b_1	–	–	b_2	b_3	b_4
Root-stock (n)	*Scion* (n)														
'Judy's Pride Burley'	'Samsun nn'	TNV	HR	b_1	$b_{1'}$	–	b_2	b_3	–	b_1	–	–	b_2	b_3	b_4

[1] Plants carrying the *N* gene for hypersensitivity to TMV.
[2] Plants carrying the n allele of the *N* gene.
[3] No virus was used because this amphidiploid produces b-proteins as a constitutive component.
[4] HR = hypersensitive reaction; S = sensitive reaction.
[5] – = absence.

N. debneyi (Ahl and Gianinazzi, 1982) and its amphidiploid (Table 1) produce b-proteins as constitutive components of healthy plants and at the same time show a high intrinsic resistance to TMV without any inducing agents (Ahl et al., 1983). This situation seems to be an exception, since all the other intra- and interspecific healthy hybrids investigated so far (Table 1) do not contain b-proteins as constitutive components and do not show such a high inherent resistance to virus infection.

The fact that b-proteins are present as a constitutive component in the *N. glutinosa* × *N. debneyi* hybrids suggests that their cells also inherently produce the 'mobile compound' thought to be responsible for b-protein synthesis. Indirect proof for this comes from grafting experiments (Table 2) which show that the ability to synthesize constitutive b-proteins can be induced in other *Nicotianae* by the amphidiploid together with resistance to TMV.

b-Proteins, N gene, temperature and resistance to TMV

It is known that in *Nicotiana* species the hypersensitive reaction to TMV due to the *N* gene breaks down at around 30 °C (Martin, 1966). However, in the hybrids and in the amphidiploid *N. glutinosa* × *N. debneyi,* localization of the virus can only be completely inhibited at 35 °C as compared to 32 °C in *N. tabacum* 'Xanthi-nc' (Table 3). Furthermore, virus multiplication and spread are slower in the hybrids than in the parent carrying the *N* gene *(N. glutinosa)* or in *N. tabacum* 'Xanthi-nc'. For example, when the hybrids are transferred to 32 °C for 2 days before returning them to 20 °C, there is only an increase in lesion size whereas a similar temperature shift is sufficient to give a complete generalization of TMV in *N. glutinosa* (Ahl and Gianinazzi, 1982). This relative insensitivity to temperature of the hybrids *N. glutinosa* × *N. debneyi* is even more striking since they are heterozygous for the *N* gene, a situation in which plants usually have a lower temperature limit for resistance to TMV than homozygous *NN* plants. (Jockusch, 1966; Table 3).

Table 3. Effect of temperature on the resistance to TMV of plants with or without the *N* gene.

Temperature (°C)	*N. glutinosa* × *N. debneyi* *(Nn)*	*N. tabacum* 'Xanthi-nc' *(NN)*	*N. tabacum* 'Xanthi-nc' × *N. tabacum* 'Xanthi' *(Nn)*	*N. tabacum* 'Xanthi' *(nn)*
20	HR[1]	HR	HR	S
27	HR	HR	HR + S	S
32	HR + S[2]	S	S	S
35	S	S	S	S
40	S	S	S	S

[1] HR = hypersensitive reaction.
[2] S = systemic infection.

The amount of constitutive b-protein decreases with increasing temperature, but its synthesis can only be totally suppressed at 40 °C. Weakening of the expression of the *N* gene by increasing temperature is accompanied therefore by a reduction in the amount of b-protein synthesized and when the hypersensitive reaction is fully in-

hibited (35 °C), only a small amount of b-protein can be detected. The presence of the *N* gene does not seem to be the only way of obtaining a high level of b-protein production in *Nicotiana* since transfer of TMV-infected sensitive tobacco ('Samsun nn') from 25 °C to 11 °C also results in a hypersensitive-like reaction (Gianinazzi et al., 1977; Gianinazzi and Schneider, 1979) with subsequent production of a high amount of b-proteins (Gianinazzi, 1978). This indicates that under certain conditions, plants lacking the *N* gene can also synthesize considerable amounts of b-proteins after TMV infection, in a way similar to *N* gene-carrying plants.

Conclusion

Knowledge concerning the genetic determinants coding for the b-proteins has been considerably increased by recent genetic investigations. In particular, these have clearly demonstrated that b-protein genes are different from the *N* gene. Furthermore they have given some insight into how the synthesis of b-proteins must occur; a 'mobile compound' which is not species-specific appears to be involved in the process of b-protein synthesis, and the ability to produce this compound permanently, as in the amphidiploid *N. glutinosa* × *N. debneyi*, can be sexually transmitted (Ahl et al., 1983). As the *N* gene strengthens the expression of the b-protein genes, it is possible that production of the 'mobile compound' is directly under the control of the *N* gene. These observations clearly illustrate the potential interest of using b-proteins as biochemical markers in studies of gene expression in relation to plant resistance.

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