## Phylogenetic aspects of pathogenesis-related (b) proteins

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Biochemical analysis of phylogenetic relationships in plant genera and species has recently been developed as a way for studying evolution in plants. The use of b-proteins for such analyses has several advantages: stable and reproducible protein patterns, a specific variability inherited by simple Mendelian laws, a readily available molecular and genetic approach. Up to now such investigations have only been extensively undertaken in *Nicotiana* species: seven different b-proteins have been recognized in about 15 species belonging to the three main subgenera (1).

Similar genetic analyses have also been made using leaf peroxydase banding patterns (2), and chloroplast-DNA restriction patterns (3). Results from the three methods of analysis show some convergence and broadly confirm the previous classification for species in the genus *Nicotiana* established by Goodspeed (4). The Australian group (subgenus *suaveolentes)* seems to be characterized by the proteins $b_{1}$·· and $b_2$ (belonging to the same serological group (5)) and occurrence of $b_2$ in *N. tomentosiformis* and *N. rustica* supports the maternal filiation suggested by the work on chloroplast-DNA patterns. The hypothetical origin of tobacco, *(N. tabacum = N. sylvestris* × *N. tomentosiformis* amphidiploïd) suggested by many workers (cf.1) is also supported by the results of the b-protein analyses; three of the main b-proteins ($b_1$, $b_2$, and $b_3$) found in tobacco, probably come from the two hypothetical parents: $b_2$ from *N. tomentosiformis*, $b_1$ and $b_3$ from the ancestral *N. sylvestris.*

Although available results and data are still incomplete, it appears from these studies that b-proteins could be usefully employed in phylogenetic investigations.

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(2) Sheen, S.J., 1970. Peroxydases in the genus *Nicotiana.* Theor. Appl. Gen. 40: 18-25.
(3) Kung, S.D., Zhu, Y.S. & Shen, G.F., 1982. *Nicotiana* chloroplast genome III. Chloroplast DNA evolution. Theor. Appl. Gen. 61: 73-79.
(4) Goodspeed, T.H., 1954. The genus *Nicotiana*. Chronica Botanica Comp., Waltham, Mass., USA, 536 pp.
(5) Ahl, P., 1983. Aspects génétiques et moléculaires de la résistance (RH) chez les *Nicotiana.* PhD Thesis, University of Geneva, Switzerland.

## A new potential for enhancing resistance to tobacco mosaic virus in Nicotiana species

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Crosses between *Nicotiana glutinosa* and *N. debneyi* produce hybrids which are highly resistant to tobacco mosaic virus (TMV) and to tobacco necrosis virus (TNV). Healthy plants of these hybrids synthesize a protein ($b_{1}$··) which is not present in the healthy parents, but whose appearance can be induced in the parents by infection with

necrosis-inducing viruses (hypersensitive reaction (HR)). Both reciprocal hybrids develop very small local lesions at 20 °C after TMV or TNV inoculation. As compared to 7-day-old local lesions on *N. glutinosa,* resistance, expressed as percent decrease in lesion size, was 93% and 94% for TMV, and 54% and 58% for TNV (1) and complete inhibition of the HR only occurs at 35 °C (2), as compared to 30 °C for other *Nicotianae.*

An amphidiploïd of the *N. glutinosa* × *N. debneyi* hybrid was obtained by culture in vitro. It looks and behaves like the hybrids, particularly for b-protein production and resistance to TMV (93%) and TNV (86%), but is fully fertile.

Grafts onto a rootstock of this amphidiploïd of *N. tabacum* 'Xanthi-nc' (hypersensitive to TMV) or 'Paraguay P48' (sensitive to TMV) induces the appearance in these tobacco cultivars of their own b-proteins ($b_1$, $b_2$, $b_3$, $b_4$, and $b_1$, $b_1'$, $b_2$, $b_3$, $b_4$, respectively) and enhances the level of TMV resistance of 'Xanthi-nc' by 58%. No such phenomena are observed in control grafts between both tobacco cultivars, nor in the reciprocal grafts using *N. tabacum* as a rootstock and the amphidiploïd as a scion. Crosses between the amphidiploïd and *N. tabacum* 'Samsun nn' or 'Judy's Pride Burley' produce plants which synthesize the b-proteins from both of their parents as constitutive components ($b_1$, $b_1''$, $b_2$, $b_3$, $b_4$, and $b_1$, $b_1'$, $b_1''$, $b_2$, $b_3$, respectively) and which also show a high level of TMV resistance (84% and 79%, respectively). By backcrossing the amphidiploïd with *N. debneyi*, plants are produced which show the same behaviour as the amphidiploïd regarding b-protein production and TMV (94%) and TNV (71%) resistance. This only occurs if the amphidiploïd is used as the female parent; in reciprocal backcrosses, plants in the progeny do not have the capacity to produce a high level of constitutive b-proteins together with resistance to TMV, either due to loss or non-expression of the *N* gene, even though after TNV infection a typical HR occurs.

These two types of experiments show that the capacity to synthesize b-proteins as constitutive components and the high level of resistance against TMV and TNV can be transferred to other *Nicotianae.* In the hybrids and in the amphidiploïd, a non-species-specific compound must be present which enables b-proteins to be synthesized constitutively. This moves in the plant from base to top and could be the same as the 'mobile compound' produced in *N. tabacum* during the hypersensitive reaction and thought to be the mediator of systemic acquired resistance (2, 3). This new potential for enhancement of resistance to TMV could be interesting for tobacco breeders.

(1) Ahl, P. & Gianinazzi, S., 1982. b-Protein as a constitutive component in highly (TMV) resistant interspecific hybrids of *Nicotiana glutinosa* × *Nicotiana debneyi.* Pl. Sci. Lett. 26: 173-181.

(2) Gianinazzi, S. & Ahl, P., 1983. The genetic and molecular basis of b-proteins in the genus *Nicotiana.* Neth. J. Pl. Path. 89: 275-281.

(3) Ahl, P., 1983. Aspects génétiques et moléculaires de la résistance (RH) chez les *Nicotiana*, PhD Thesis, University of Geneva, Switzerland.