Genetics of Tobacco Resistance to *Globodera tabacum tabacum*

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ABSTRACT

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Genotypes of tobacco (*Nicotiana tabacum*) resistant (R) or susceptible (S) to the nematode *Globodera tabacum tabacum* were crossed. F_1 progeny were selfed and backcrossed to produce additional progeny for evaluation of resistance in greenhouse experiments. Plants without female nematodes visible (×10 magnification) on the root surface 6 wk after inoculation were classified as resistant and those in which one or more females were evident were classified as susceptible. Segregation ratios were 3:1 for F_2 ($F_1 \times F_1$) lines and 1:1 for BC_1 ($F_1 \times S$) lines, indicating that resistance to *G. t. tabacum* is conferred by a single, dominant gene.

The tobacco cyst nematode (*Globodera tabacum tabacum* (Lownsbery & Lownsbery) Stone) is an important pathogen of shade-grown cigar wrapper and field-grown broadleaf cigar tobaccos (*Nicotiana tabacum* L.) in the Connecticut River Valley. The nematode suppresses the growth of shade tobacco directly (10) and increases the incidence and severity of Fusarium wilt of broadleaf tobacco (9). All Connecticut shade and broadleaf tobacco cultivars tested were susceptible to *G. tabacum* (6; J. A. LaMondia and G. S. Taylor, *unpublished*).

G. tabacum solanacearum (Miller & Gray) Stone suppresses the growth and yield of flue-cured tobacco in Virginia (7). *G. t. tabacum* and *G. t. solanacearum* appear to be closely related to each other and to another cyst nematode, *G. tabacum virginiae* (Miller & Gray) Stone, described on horsenettle (*Solanum carolinense* L.) (12). All three subspecies reproduce on tobacco and common horsenettle but can be distinguished morphologically and by host preference (6,13). Two flue-cured tobacco cultivars with resistance to *G. t. solanacearum* (7) were found to be resistant to *G. t. tabacum* (8). Resistance to *G. t. solanacearum* in tobacco was described to be multigenically inherited for Clemson PD-4 (3), for the advanced germ plasm line VA-81 (4), and for BVA 523 and DVA 606, two burley and dark-fired breeding lines (11,14).

Neither Clemson PD-4 nor VA-81 is suitable for production in Connecticut. Both genotypes are being utilized, however, as sources of resistance in our breeding program to incorporate resistance into locally adapted shade and broadleaf tobacco lines. Determination of the number of genes conditioning resistance to *G. t. tabacum* is important, as this may influence both the ability to transfer resistance and the stability of resistance over time.

The objective of this research was to determine the number of genes conditioning resistance to *G. t. tabacum* in crosses between flue-cured and Connecticut types of tobacco.

MATERIALS AND METHODS

VA-81 and PD-4 plants resistant to *G. t. tabacum* were used as controls and as either male or female parents in crosses with susceptible Connecticut shade or broadleaf tobacco lines. F_1 hybrids of Connecticut × flue-cured types were either selfed to produce F_2 progeny or backcrossed to susceptible Connecticut types with desirable horticultural characteristics (BC_1). The Connecticut broadleaf line 86-4 was planted as a susceptible check in all three experiments.

Two resistant flue-cured lines (VA-81 and Clemson PD-4), seven F_1 hybrids between the resistant flue-cured lines and susceptible Connecticut shade and broadleaf types, seven F_2 lines ($F_1 \times F_1$ plants), and four BC_1 lines (F_1 backcrossed to the susceptible Connecticut parent) were each evaluated for resistance to *G. t. tabacum* on two or three occasions from 1989 to 1990. Approximately one-half of the F_1 and BC_1 lines had a flue-cured line as the female parent.

Plant resistance to *G. t. tabacum* was evaluated in greenhouse tests. Appropriate cultivars, lines, or progeny from crosses were directly seeded to two rows (28 cavities) in 14 × 14 row, 196-cavity seedling trays containing 20 cm^3 of Sunshine potting mix per cavity. Plants were thinned to one seedling per cavity after emergence (about 3 wk after seeding) and were inoculated approximately 6 wk after seeding with 5,000 second-stage juveniles per cavity. The *G. t. tabacum* population used in these experiments was a composite collected from shade and broadleaf tobacco types. Seedlings were grown at 18–30 C. Approximately 6 wk after inoculation, the plants were removed from the trays and the numbers of white, developing females of *G. t. tabacum* on the root system were determined at ×10 magnification. Plants without visible females were classified as resistant and those with one or more females visible were considered susceptible. Roots of known susceptible control plants were stained in acid fuchsin (2) to determine the optimum time to examine roots for presence of visible developing females. Data on the frequency of resistance and susceptibility to *G. t. tabacum* were subjected to chi-square analysis. The experiment was repeated three times; results were similar, so the data were combined.

RESULTS AND DISCUSSION

The ratios of transplants resistant or susceptible to *G. t. tabacum* were similar for all three experiments (Table 1). The Connecticut broadleaf tobacco cultivar CT86-4 was a susceptible host of *G. t. tabacum*, with 0–79 developing white

Table 1. Phenotypic ratios of tobacco lines resistant to *Globodera tabacum tabacum* based on the presence or absence of developing females on roots

Tobacco seedlings	Number of resistant/susceptible plants				Test ratio[a]	χ^{2b}	*P* value[c]
	Expt. 1	Expt. 2	Expt. 3	Sum			
Resistant cultivar	40/1	25/0	22/0	87/1	1:0	···	···
Susceptible cultivar	1/38	4/51	1/64	6/153	0:1	···	···
$R \times S = F_1$	105/5	57/3	122/3	284/11	1:0	···	···
$F_1 \times F_1 = F_2$	114/42	48/12	69/22	231/76	3:1	0.01	0.90
$F_1 \times S = BC_1$	28/34	37/37	36/29	101/100	1:1	0.00	0.95

[a] Consistent with a single dominant gene for resistance.
[b] Combined data for three experiments.
[c] Probability of obtaining a greater chi-square value.

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females visible on the root system ($\bar{x}$ = 23.2; *data not shown*). Resistant parental lines and F_1 progeny of homozygous resistant × homozygous susceptible parent lines were overwhelmingly resistant—87 of 88 and 284 of 295 plants classified as resistant, respectively. The segregation of resistant to susceptible phenotypes in the F_2 lines was consistent with a 3:1 ratio, and progeny of F_1 plants backcrossed to homozygous susceptible lines segregated with a 1:1 ratio for resistance and susceptibility. Reciprocal crosses had no effect on resistance/segregation ratios. Segregation ratios of resistant to susceptible phenotypes were consistent with a proposed model for a single dominant major effect gene conferring resistance to *G. t. tabacum*.

Only one of 87 plants of Clemson PD-4 was classified as susceptible in these experiments, with a single developing female present on the root system. This is consistent with the observation that both *G. t. solanacearum* and *G. t. tabacum* invade roots of resistant as well as susceptible plants but fail to produce more than a few cysts (1,8). Fewer than 4% of susceptible plants were classified as resistant. The lack of *G. t. tabacum* reproduction on these plants may be due to escapes or to cross-contamination of tobacco seed within trays.

VA-81, Clemson PD-4, and hybrids of these cultivars and Connecticut tobacco lines appear to possess a single gene for resistance to *G. t. tabacum*. This single dominant gene segregates in a diploid manner in the allotetraploid tobacco genome. VA-81 and PD-4 have previously been reported to possess multigenic resistance to *G. t. solanacearum* (4). It is not inconsistent that the genetics of resistance to *G. t. tabacum* may differ from reports of multigenic resistance to *G. t. solanacearum* for a number of reasons. First, tobacco resistance to these two different nematode subspecies may involve some of the same genes, but more genes may be required for the expression of resistance to *G. t. solanacearum*. Also, the multigenic nature of this resistance to *G. t. solanacearum* was apparently based on the burley and dark-fired breeding lines BVA 523, DVA 606, and hybrids between these lines and flue-cured tobacco types (11,14). VA-81 and PD-4 were apparently not evaluated. It is possible that a single gene for resistance was selected from several in the development of VA-81 and PD-4. Finally, differences in methods may have influenced the interpretation of results. Visually observing roots for developing females, then classifying plants as resistant or susceptible on the basis of presence or absence of females, is quite different from counting numbers of cysts produced per root system (11,14). Differences in total cysts per plant may be due to variation such as that seen in susceptible checks in this experiment. Also, different levels of susceptibility may be conditioned by additional genes.

The results of these experiments do not indicate either intermediate resistance in the F_1 lines or a continuous range of resistance in the F_2 lines inconsistent with the range of females produced on a susceptible cultivar. Both conditions have been cited in determining the multigenic nature of resistance to *G. t. solanacearum* (14). Future research will examine the effects of these same resistant cultivars and progeny lines on the development of *G. t. solanacearum* under the same experimental conditions.

F_3 and BC_2 lines resistant to *G. t. tabacum* have been identified with horticultural characteristics similar to Connecticut shade and broadleaf tobacco types. The fact that resistance to *G. t. tabacum* is inherited as a single dominant gene segregating in a diploid manner has allowed the quick incorporation of resistance into adapted tobacco types. Further development of these lines and selection for tolerance to *G. t. tabacum* (5) will continue.

The long-term effectiveness of a single dominant gene for resistance against *G. t. tabacum* is unknown and remains to be determined. The *G. t. tabacum* population used in these experiments was a composite of shade and broadleaf tobacco types collected at the Valley Laboratory in Windsor, Connecticut. The potential variability of *G. t. tabacum* in the Connecticut River Valley is unknown, but tobacco cyst nematode collections from various locations are currently being increased for future studies. Currently, *G. t. tabacum* is managed by rotation with nonhost crops or by the application of nematicides. The availability of resistant lines as a means of reducing nematode densities by up to 80% (8) will be an important management tool in an integrated nematode management program.

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