Selection of Tobacco Lines with a High Degree of Resistance to Tobacco Etch Virus

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ABSTRACT

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Resistance of tobacco introduction 1406 to tobacco etch virus, as defined by a lack of visible symptoms, is controlled by a single recessive gene that must be homozygous to give resistance. However, studies with plants of the resistant phenotype indicated that modifying factors may be acting to determine levels of virus concentration reached in the resistant, symptomless plants. Selection and selfing of resistant plants that supported very low levels of virus multiplication showed that this character was heritable.

Tobacco introduction (TI) 1406 has been used as the source of resistance in a burley tobacco breeding program to control the diseases caused by two potyviruses, tobacco etch virus (TEV) and tobacco vein-mottling virus (TVMV) (1). These diseases can significantly reduce yields and alter the chemical composition of leaf tissue of infected plants (6,7). All burley varieties are susceptible to these viruses and develop easily observed systemic symptoms (8,9). Because control of the aphid vectors of the viruses and elimination of overwintering weed hosts have thus far been impractical (2), introducing resistance to TEV and TVMV into the standard burley varieties has been emphasized as the major control measure. The backcross method has been used in improving burley tobacco varieties with the resistance from TI 1406 (1).

Initial breeding work, in which progeny were characterized as resistant on the basis of lack of symptom expression, indicated that the resistance of TI 1406 to TEV was inherited in a recessive manner (1). The purpose of the following study was to investigate further the inheritance of TI 1406 resistance to TEV and to determine the degree of resistance to virus multiplication in the donor parent (TI 1406) and in resistant selections of the breeding program.

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MATERIALS AND METHODS

Six- to eight-week-old tobacco plants were mechanically inoculated with a previously described isolate (M) of TEV (4). In keeping with the previous study (1), plants were classified as susceptible or resistant to the virus based on whether symptoms developed after inoculation with TEV. Individual plants were assayed for virus content 3–5 wk after inoculation. Ten or 20 1-cm leaf disks were taken from leaves above the ones inoculated, ground in 1 or 2 ml of water, respectively, and assayed on half-leaves of *Chenopodium amaranticolor* using a Latin square design.

Because of the large differences in virus content between resistant and susceptible plants, it was necessary to assay the sap extracts at different dilutions. Extracts of resistant plants were assayed without any further dilution. Extracts of susceptible plants were diluted 30- or 50-fold before assay. To delineate differences in virus content, a virus concentration index was developed by multiplying the number of lesions per half-leaf by the dilution factor. Although it was recognized that this did not necessarily reflect the exact magnitude of the differences, it did give an indication of their magnitude; the average number of lesions produced per half-leaf could be calculated by dividing the index by the dilution factor.

Virus titers in extracts from resistant individuals (resistance derived from TI 1406) from successive backcross populations, in which standard burley cultivars Ky 10 or Ky 16 served as the recurrent parent, were compared. In addition, variation in virus content among plants of the resistant phenotype of F_2 generations was determined. Some of these F_2 plants were selfed, and the seed was collected and sown. Resulting F_3 individuals were inoculated and assayed for virus content. All experiments were done under screened greenhouse conditions.

RESULTS

The resistance of TI 1406 to TEV, as characterized by a lack of visible symptoms, was confirmed to be recessive to susceptibility. Distribution of plants in F_2 generations of crosses between TI 1406 plants and burley plants Ky 10 or Ky 16 fit the three susceptible to one resistant ratio expected on the basis of monogenic inheritance (Table 1). Two types of observation suggested, however, that the inheritance of resistance was more complex than that suggested by the 3:1 ratio. First, there were at least two levels of symptom expression within the susceptible phenotype; about 75% of the F_2 plants rated as susceptible exhibited symptoms that were distinctly less severe than those exhibited by the susceptible parent, whereas the remaining 25% exhibited symptoms that were at least as severe as those exhibited by the susceptible parent.

Second, when resistant F_3 generation hybrids and resistant individuals from F_3 backcrosses, in which the susceptible Ky 10 served as the recurrent parent, were assayed, there was no consistency in the patterns of virus content. For example, some F_3BC_2 individuals contained fivefold to 50-fold less virus than F_3BC_1 and F_3BC_3 plants in the same backcross series. Because the resistant plant selected in an F_2 generation to continue the backcross program was not necessarily the same plant selfed to produce the above F_3 populations, we hypothesized that segregation (with respect to the level of virus multiplication a given plant can support) was occurring among the resistant plants of an F_2 population. Two F_2 populations of TI 1406 × Ky 16 and TI

Table 1. Distribution of plants in the F_2 generation categorized as resistant or susceptible to tobacco etch virus (TEV) on the basis of symptom production

Population	Number of plants		Total	χ^2 probability
	TEV symptoms	Symptomless		3:1 ratio
F_2 (TI 1406 × Ky 10)	96	28	124	0.70–0.50
F_2 (TI 1406 × Ky 16)	101	23	124	0.20–0.10

1406 × Ky 10 were thus chosen for detailed studies.

In the F_2 population from TI 1406 × Ky 16, four plants were of the resistant and 24 were of the susceptible phenotype. Among the resistant plants, there was up to a fivefold difference in the virus titers (Table 2). Selection and selfing of plant B (Table 2), which supported very low levels of virus multiplication, produced F_3 progenies in which virus multiplication was curtailed (Table 3). Selfing of plant D (Table 2), in which relatively high levels of virus multiplication occurred, resulted in F_3 progenies that also supported relatively high levels of virus multiplication (Table 3).

In the F_2 population from TI 1406 × Ky 10, six plants were of the resistant and 22 were of the susceptible phenotype. There was up to a sixfold difference in virus concentration among individuals from the resistant phenotype (Table 2). Selection and selfing of resistant plant A (Table 2), which supported very low levels of virus multiplication, produced F_3 progenies in which virus multiplication was severely curtailed (Table 4). Selfing of resistant plants B and E (Table 2), which supported intermediate and relatively high levels of virus multiplication, respectively, resulted in F_3 progenies that generally supported relatively high levels of multiplication (Table 4). There was considerable variation in virus concentration among the F_3 progeny of plants B and E, which suggests that genetic segregation was occurring in these populations.

Although some of these resistant F_2 and F_3 plants supported relatively high levels of virus multiplication, virus concentration in them was 30 times lower than the concentrations found in the susceptible Ky 16 or Ky 10 parents.

Table 2. Concentration of tobacco etch virus (TEV) in plants from the resistant (symptomless) phenotype of F_2 populations of TI 1406 × Ky 16 and TI 1406 × Ky 10

Plant	Virus concentration index[a]
F_2 (TI 1406 × Ky 16)	
A	45
B	32
C	94
D	154
Controls[b]	
F_2 (TI 1406 × Ky 16)[c]	2,530
Ky 16	2,075
F_2 (TI 1406 × Ky 10)	
A	31
B	59
C	103
D	116
E	164
F	177
Controls[b]	
F_2 (TI 1406 × Ky 10)[c]	4,230
Ky 10	3,960

[a] Average number of lesions × dilution factor; extracts of resistant plants were assayed without further dilution and those of controls were diluted 30-fold. Assays were made 4 wk after inoculation with TEV by inoculating extracts of uninoculated leaves of each plant onto eight half-leaves of *Chenopodium amaranticolor*.

[b] Plants with typical TEV symptoms.

[c] Plants of the susceptible phenotype from the F_2 population assayed.

Table 3. Assay of tobacco etch virus (TEV) in resistant F_3 individuals resulting from the selection and selfing of resistant F_2 (TI 1406 × Ky 16) plants B and D[a]

F_3 plant	Lesions per half-leaf[b]
B1	0
B2	2
B3	22
B4	1
B5	1
B6	<1
B7	0
B8	0
D1	311
D2	181
D3	263
D4	155
D5	186
D6	195
D7	280
D8	221

[a] See Table 2. Plants B and D supported low and relatively high levels, respectively, of virus multiplication.

[b] Average number of lesions on four half-leaves of *Chenopodium amaranticolor*. Uninoculated leaves of F_3 plants were assayed 5 wk after inoculation with TEV.

Table 4. Assay of tobacco etch virus (TEV) in resistant F_3 individuals resulting from the selection and selfing of resistant F_2 (TI 1406 × Ky 10) plants A, B, and E[a]

F_3 plant	Lesions per half-leaf[b]
A1	0
A2	0
A3	<1
A4	5
A5	2
A6	8
A7	<1
A8	0
B1	0
B2	42
B3	67
B4	338
B5	117
B6	<1
B7	130
B8	234
E1	334
E2	<1
E3	30
E4	37
E5	344
E6	146
E7	15
E8	218

[a] See Table 2. Plants A, B, and E supported low, intermediate, and relatively high levels, respectively, of virus multiplication.

[b] Average number of lesions on four half-leaves of *Chenopodium amaranticolor*. Uninoculated leaves of F_3 plants were assayed 5 wk after inoculation with TEV.

DISCUSSION

The resistance of TI 1406 to TEV, as defined by a lack of visible symptoms, appears to be controlled by a single recessive gene that must be homozygous to give resistance (1). A similar finding was made in studies of resistance in TI 1406 to potato virus Y (3) and TVMV (5); based on distribution of symptomless and symptom-producing progeny, it was concluded that resistance is controlled by a single locus.

That different levels of TEV symptom expression occur within the susceptible phenotype of F_2 generations of crosses between TI 1406 plants and susceptible burley plants is similar to the findings of Legg et al (5) on the inheritance of the resistance in TI 1406 to TVMV. They explained these different levels of symptom expression with a completely additive type of gene action. Using this genetic model, a heterozygous plant would not be as severely affected as one with the homozygous susceptible genotype.

Although the lack of symptom production on TI 1406 infected with TEV appeared to be a simply inherited characteristic, studies with plants of the resistant phenotype indicated that modifying factors may be acting in determining levels of virus concentration reached in the symptomless plants. Virus titers in any plant of the susceptible phenotype were at least 30 times higher than those in any plant of the resistant phenotype; yet there was considerable variation in virus concentration among plants of the resistant phenotype.

Such considerations as ability to serve as a source for secondary spread of virus, potential synergistic effects resulting from infection by other pathogens, and—particularly for tobacco—effects of virus infection on chemical constituents suggest that selection of plants that support the lowest amount of virus multiplication would be most desirable in a breeding program. Selection of this type is possible by using quantitative assays, such as local lesion or enzyme-linked immunosorbent assay. Our results showed that severe curtailment of virus multiplication within some resistant plants was heritable. Selection of plants solely on the basis of visual symptoms fails to differentiate these differences in virus multiplication among individuals of the resistant phenotype.

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