# A Genetic Analysis of the Association Between Resistance to *Meloidogyne incognita* and a Necrotic Response to Infection by a Strain of Potato Virus Y in Tobacco

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**ABSTRACT**

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The genetic basis for the association between resistance to the root-knot nematode *Meloidogyne incognita*, and a severe vascular necrosis in response to infection by the $M^SN^R$ strain of potato virus Y (PVY-$M^SN^R$) in tobacco (*Nicotiana tabacum* L.) was investigated. More than 15,000 $F_2$ plants derived from root-knot resistant × root-knot susceptible crosses were inoculated with both pathogens. All plants that did not develop necrosis in response to the virus were root-knot susceptible; ie, recombination between root-knot resistance and the necrotic response to the virus was not detected. Each trait is controlled by a single, dominant gene located on chromosome G of tobacco by monosomic and nullisomic analysis. The virus-induced necrosis may be a pleiotropic response of the gene controlling root-knot resistance. Plants possessing both root-knot and PVY resistance developed little or no necrosis. PVY resistance is conditioned by a recessive gene that appears to be epistatic to the gene conditioning root-knot resistance. A search was made among *Nicotiana* spp. for a new source of root-knot resistance not associated with the necrotic reaction to PVY-$M^SN^R$. *Nicotiana tomentosa*, accession 58, was highly resistant to the nematode and appeared to be immune to the virus.

An association between resistance to the root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood and a severe necrotic reaction to a strain of potato virus Y (PVY) in tobacco (*Nicotiana tabacum* L.) was reported from Virginia by Henderson and Troutman (14). Later, this strain of PVY was reported to be one of three strains that can be classified on the basis of their reaction on flue-cured tobacco cultivars resistant or susceptible to the root-knot nematode (12). The $M^SM^R$ strain (PVY-$M^SM^R$) induces vein-banding and mottling symptoms on both root-knot-resistant and -susceptible cultivars; the $M^SN^R$ strain (PVY-$M^SN^R$) causes necrosis on root-knot-resistant cultivars, but mild symptoms on root-knot susceptible cultivars; and the $N^SN^R$ strain (PVY-$N^SN^R$) induces necrosis on all cultivars.

All tobacco cultivars that have a single gene for resistance to the root-knot nematode develop severe vascular necrosis when infected with PVY-$M^SN^R$, but little is known about the genetic basis for this association. These two reactions could be controlled by two independent genes in tight linkage or by pleiotropic effects of a single gene.

Resistance to the root-knot nematode is controlled by a single dominant factor, or a chromosome block that behaves as a single factor (9). This resistance reportedly originated from T.I. 706 and was transferred into flue-cured tobacco cultivars by E. E. Clayton in the 1930s (6). All modern tobacco cultivars resistant to the root-knot nematode derive resistance from Clayton's original material. An alternative hypothesis states that the source of root-knot resistance was not T.I. 706, but *N. tomentosa* accession 58 (20). The factor controlling root-knot resistance has been reported to be located on tobacco chromosome G (22).

A recessive factor for resistance to the necrotic reaction induced by PVY-$N^SN^R$ has been reported to be located on tobacco chromosome E (13). On the other hand, the mode of inheritance of the necrotic reaction to PVY-$M^SN^R$ has not been investigated, but it has been reported that tobacco plants homozygous for root-knot resistance develop severe necrosis in response to PVY-$M^SN^R$, whereas heterozygous plants develop a mild or attenuated form of necrosis. This suggests that the reaction to PVY may be incompletely dominant (15).

Root-knot resistance in tobacco cultivars involves a necrotic or hypersensitive reaction in the roots that prevents the nematode from establishing a feeding relationship. This reaction is temperature dependent and is not expressed above 32 C soil temperature (10,21). The virus-induced necrosis is also temperature sensitive and is inhibited at the same temperature as root-knot resistance (18). These reports suggest that hypersensitivity to root-knot and PVY-induced necrosis may be pleiotropic responses of a single gene. This is a potential problem in North Carolina because the incidence of PVY-$M^SN^R$ in the state has been increasing in recent years (11) and many popular flue-cured cultivars are root-knot resistant.

Studies reported herein were conducted with the following objectives: to determine the mode of inheritance of the necrotic reaction induced by PVY-$M^SN^R$; to determine whether the association between root-knot resistance and the virus-induced necrosis is due to linkage or pleiotropism; to search for segregation between the two reactions in $F_2$ populations derived from root-knot resistant × root-knot susceptible crosses and among haploid and dihaploid lines of tobacco; to locate the chromosome bearing the locus controlling the necrotic reaction induced by the virus and to confirm the location of the root-knot resistant gene on chromosome G by aneuploid analysis; and to search for new sources of root-knot resistance among *Nicotiana* spp. that are not associated with the necrotic reaction to PVY-$M^SN^R$.

## MATERIALS AND METHODS

**Inheritance studies.** Homozygous lines resistant to the root-knot nematode were used as the female or male parent in crosses with root-knot susceptible genotypes. Cultivar NC 744 (root-knot susceptible) was used in some crosses because it has a recessive gene for resistance to PVY-$N^SN^R$. Progenies in the $F_2$ generations were initially inoculated with PVY-$M^SN^R$ (type isolate NC 138), and later inoculated with race 3 of *M. incognita*. PVY tests were conducted at 20–28 C and root-knot tests at 30–35 C aerial temperatures. Seeds were broadcast on the surface of a 2:1 mixture of steam-sterilized sand and sandy-loam soil covered with a thin layer of vermiculite in wooden flats (54 × 37 × 8 cm). Approximately 50 g of slow-release fertilizer (Osmocote®) were added to the soil mixture in each flat. Seedlings were inoculated

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with PVY when the oldest leaves reached 2–4 cm in length. Inoculum was prepared by homogenizing systemically-infected leaves from cultivar Burley 21 tobacco plants in 0.05 M $Na_2HPO_4$-$KH_2PO_4$ buffer, pH 7.2, (1 g tissue: 5 ml buffer). The homogenate was pressed through cheesecloth and 1 g of 22-$\mu$m (600-mesh) carborundum was added to each 100 ml of inoculum. Inoculations were performed with a Thayer and Chandler model E airbrush. Each flat received approximately 15 ml of inoculum propelled by $CO_2$ at 3 kg/$cm^2$ pressure and applied about 10 cm from the leaf surface. Necrotic plants were removed and counted for a period of 7–10 days beginning 1 wk after inoculation. The ratio of necrotic to non-necrotic plants in response to PVY-$M^SN^R$ was computed and a $\chi^2$ goodness-of-fit test was conducted to test the hypothesis of a 3:1 segregation ratio indicative of a single, dominant gene. Plants that did not develop necrosis were moved to a warmer greenhouse and allowed to acclimate for 3–4 days before they were transplanted.

For the root-knot test, plastic containers divided into 12 compartments each holding 100 $cm^3$ of the same soil mixture as above were used. A single plant was placed in each compartment. Nematodes were maintained on tomato plants (*Lycopersicon esculentum* L. 'Homestead'). For inoculation, infected tomato roots were washed and cut in 3–4 cm pieces, agitated for 3 min in 0.5% sodium hypochlorite, and rinsed through nested sieves (74-$\mu$m [200-mesh] on top and 38-$\mu$m [400-mesh] on bottom) to collect eggs. Each plant received approximately 4,000 eggs in 5 ml of water. Eight weeks after inoculation, roots were examined for presence or absence of galling. Plants that did not show evidence of root-knot infection were transplanted into 15-cm-diameter clay pots and reinoculated; first with the virus and 2 wk later with the nematode. This procedure reduced the possibility that plants may have escaped infection. Five $F_2$ plants which appeared non-necrotic and root-knot resistant after the second inoculation were self pollinated to produce the $F_3$ generation. Approximately 200 $F_3$ individuals from each $F_2$ plant were then inoculated with both pathogens.

**Haploids and dihaploids.** Fifty anther-derived (2), 25 maternally derived (3) haploid (plants having only one chromosome of each known pair) lines of cultivar NC 95, and 25 anther-derived dihaploid (having the normal complement of paired chromosomes) lines of cultivar Coker 86 × VY 32 (all root-knot resistant) were inoculated with PVY-$M^SN^R$ in search of lines that would not develop necrosis. The anther-culture procedure was chosen because it has been reported to induce genetic changes among plantlets, even when derived from a highly homozygous source (1,7,8). All entries were seeded as previously described and grown in the greenhouse (28–32 C) in 10-cm-diameter clay pots filled with a steam sterilized mixture of sand, sandy loam, and peat (1:1:1, v/v) to which 1 g of slow-release fertilizer (Osmocote®) was added. Virus inoculum was prepared as above and rubbed on the two oldest leaves with a cotton swab when the plants were about 10 cm tall. Plants were arranged in a completely randomized design and observed for symptom development for 3 wk.

**Aneuploid analysis.** Monosomes (plants lacking one of a pair of chromosomes) and nullisomes (lacking a specific pair of chromosomes) of cultivar Red Russian were used for locating genes on specific chromosomes. Red Russian plants are susceptible to the root-knot nematode and develop vein banding in response to PVY-$M^SN^R$.

Cultivar Red Russian Nullisomic E plants were used in a series of crosses because the recessive factor for resistance to the necrotic reaction induced by PVY-$N^SN^R$ is reported to be located on chromosome E (13). These crosses were made to determine whether the counterpart to this recessive allele, ie, the dominant allele conditioning necrosis to PVY-$N^SN^R$, is the same allele involved in the necrotic reaction to PVY-$M^SN^R$ and thereby to discern whether these two reactions are genetically the same and independent of the root-knot resistance gene, and whether the gene controlling root-knot resistance is located on chromosome E.

Nullisomic E plants were used as the female parent and fertilized with pollen from cultivar NC 95 (resistant to root-knot and develops necrosis when inoculated with PVY-$M^SN^R$) and cultivar NC 107 (susceptible to root-knot and resistant to PVY-$N^SN^R$). $F_1$ progeny monosomic for chromosome E were used as the male parent and crossed to cultivar NC 2326 (root-knot susceptible and

TABLE 1. Response of *Nicotiana* spp. inoculated with *Meloidogyne incognita* and strain $M^SN^R$ of potato virus Y (PVY) under different aerial temperatures

| | Average disease severity | | | | | |
|---|---|---|---|---|---|---|
| | *M. incognita*[a] | | | | PVY[b] | |
| (*Nicotiana* spp. entry) | 33–35 C | Plants tested (no.) | 28–32 C | Plants tested (no.) | 28–32 C | Plants tested (no.) |
| *N. otophora* accession | | | | | | |
| Summer Bloomer | 4.4 | 14 | 3.7 | 22 | 1.5 | 15 |
| Cochabamba I | 4.9 | 32 | 3.0 | 36 | 1.5 | 32 |
| Cochabamba II | 4.4 | 7 | 2.4 | 24 | 1.5 | 9 |
| Jujuy | 4.2 | 4 | 2.2 | 6 | 1.5 | 4 |
| LaQuinta | 4.4 | 5 | 2.4 | 5 | 1.5 | 5 |
| *N. glutinosa* | 4.2 | 18 | 3.1 | 7 | 2.0 | 16 |
| *N. setchelli* | 2.8 | 6 | 1.7 | 4 | 1.8 | 14 |
| *N. sylvestris* | 4.7 | 12 | 3.3 | 9 | 2.0 | 8 |
| *N. plumbaginifolia* (43-A) | 1.9 | 9 | 1.0 | 7 | 2.0 | 8 |
| *N. repanda* | 1.0 | 7 | 0.3 | 7 | 2.0 | 6 |
| *N. longiflora* var. *breviflora* (30-A) | 1.0 | 5 | 0.6 | 5 | 2.0 | 6 |
| *N. tomentosiformis* | 4.3 | 7 | 2.3 | 18 | 0.0 | 15 |
| *N. tomentosa* (58-A) | 4.2 | 10 | 1.9 | 16 | 0.3 | 16 |
| *N. tomentosa* (58) | 0.0 | 22 | 0.0 | 9 | 0.0 | 16 |
| *N. tabacum* | | | | | | |
| NC 95 | 0.0 | 15 | 0.0 | 4 | 5.6 | 15 |
| Hicks | 5.0 | 12 | 3.0 | 15 | 2.0 | 14 |
| NC 2326 | 5.0 | 15 | 2.8 | 16 | 2.0 | 7 |
| VY 32 | 5.0 | 12 | 3.0 | 12 | 1.2 | 12 |
| *N. sylvestris* × *tomentosiformis* (4N) | 4.3 | 6 | 3.0 | 7 | 2.0 | 6 |
| LSD $P = 0.05$ | 1.8 | | 0.8 | | 0.7 | |
| $P = 0.01$ | 2.9 | | 1.2 | | 1.0 | |

[a] Root-knot disease severity rating (expressed as percentage of total root system galled): 0 = No galling, 1 = 1–10%, 2 = 11–30%, 3 = 31–70%, 4 = 71–90%, and 5 = 100%.

[b] PVY disease severity rating: 0 = no symptoms; 1 = vein clearing, mild mottling; 2 = vein banding, severe mottling or mosaic; 3 = localized necrosis, restricted to inoculated leaf; 4 = mild, systemic necrosis; 5 = severe, systemic necrosis; and 6 = plant death.

develops vein banding when inoculated with PVY-$M^SN^R$). Offspring populations were inoculated with the virus and the nematode.

Monosomic G plants of cultivar Red Russian were crossed as the female parent with NC 95 to verify whether the gene controlling root-knot resistance is located on chromosome G, and to determine if the gene conditioning the necrotic response to PVY-$M^SN^R$ might also be located on that chromosome. Monosomic and disomic $F_1$ plants were identified, crossed as the male parent to root-knot susceptible tobacco cultivar Hicks females, and self-pollinated. Progenies from all crosses were inoculated with the virus and the nematode.

**Inoculation of *Nicotiana*.** *Nicotiana* spp. plants were inoculated with PVY-$M^SN^R$ and with the root-knot nematode in search of a new source of root-knot resistance that does not develop necrosis when infected with this strain of PVY (Table 1). When the two oldest leaves expanded to 4–5 cm, plants were transplanted into 10-cm-diameter clay pots containing a steam-sterilized 1:1 mixture of sand and sandy loam soil for the root-knot test, and a mixture of sand, sandy loam soil, and peat (1:1:1, v/v) for the PVY test. One-half of the plants were grown at 33–35 C and the other half at 28–32 C, and inoculated with root-knot nematodes as previously described. Roots were washed 8 wk after inoculation and rated for galling by using the following index (expressed as percentage of total root system galled): 0 = no galling; 1 = 1–10%; 2 = 11–30%; 3 = 31–70%; 4 = 71–90%; and 5 = 100%. Virus inoculation was accomplished as described for haploid and dihaploid plants. Initially, plants were evaluated for virus symptoms at both temperatures listed above, but the cooler temperature (28–32) gave better results and further tests were conducted only in this temperature range. Plants were observed for PVY symptoms for 8 wk. Symptomless entries were assayed for the presence of virus by preparing inoculum from their leaves and using it to inoculate susceptible cultivar Burley 21 plants.

A completely randomized design was used in every case with varying numbers of replications. Entry means were weighted by the number of observations in the analysis of variance by using a general linear model procedure.

## RESULTS

Populations of $F_2$ plants derived from root-knot resistant × root-knot susceptible crosses segregated in close agreement to a 3 necrotic:1 non-necrotic ratio upon inoculation with PVY-$M^SN^R$ (Table 4). A chi-square value of 2.28 was obtained with a probability value for goodness-of-fit of 0.25–0.10, which indicated that the necrotic reaction to the virus is conditioned by a single, dominant gene. All $F_2$ plants that did not become necrotic in response to the virus were root-knot susceptible. Segregation between root-knot resistance and the necrotic response to PVY-$M^SN^R$ was not detected. Approximately thirty plants that only developed mild mosaic-type symptoms when infected with PVY-$M^SN^R$ appeared to be root-knot resistant. These plants were reinoculated with the virus and most became necrotic; those that did not developed galls after a second inoculation with nematodes. Five plants continued to be non-necrotic and root-knot resistant even after the second inoculation. Consequently, they were allowed to self-pollinate to produce the $F_3$ generation. About 200 $F_3$ individuals from each $F_2$ plant were inoculated with both pathogens. A high proportion of the $F_3$ population was expected to be root-knot resistant and non-necrotic if the parental $F_2$s showed the trait. However, segregation occurred for both root-knot resistance and the virus-induced necrosis and a high proportion of necrotic plants was found. Therefore, the $F_2$ plants were heterozygous for both traits. It has been reported that plants heterozygous for root-knot resistance show an attenuated form of necrosis compared to homozygous lines (15).

TABLE 2. Segregation ratios of necrotic and non-necrotic $F_2$ tobacco plants derived from the cross 9844 (root-knot resistant; PVY susceptible) × NC 744 (root-knot susceptible; resistant to strain $N^SN^R$ of PVY) after inoculation with strain $M^SN^R$ of PVY

| Test | Number of plants | | Ratio | 3:1 | | 9:7 | |
|---|---|---|---|---|---|---|---|
| | Necrotic | Non-necrotic | | $\chi^2$ | $P$ | $\chi^2$ | $P$ |
| 1 | 524 | 415 | 1.26:1 | 183.89 | <0.005 | 0.07 | 0.75–0.90 |
| 2 | 492 | 367 | 1.34:1 | 143.34 | <0.005 | 0.38 | 0.50–0.75 |
| 3 | 64 | 41 | 1.56:1 | 11.50 | <0.005 | 0.97 | 0.25–0.50 |
| Total | 1,080 | 823 | 1.31:1 | 252.96 | <0.005 | 0.09 | 0.75–0.90 |

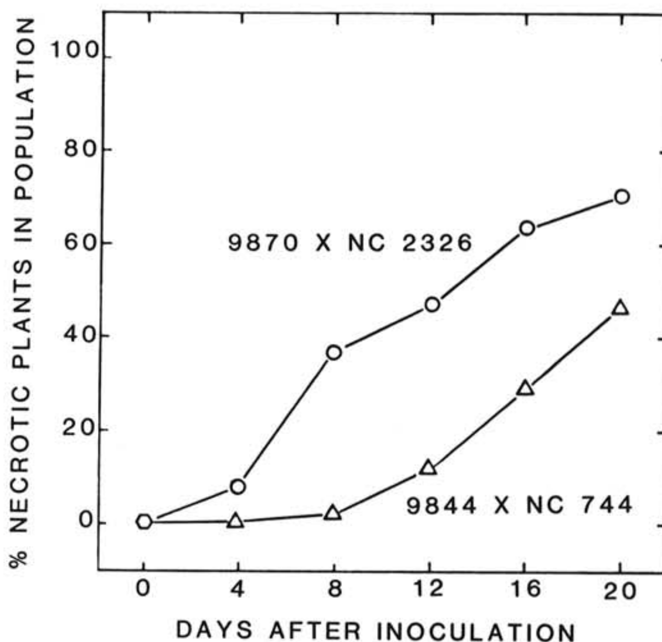


**Fig. 1.** Plant necrosis over time in two $F_2$ populations of tobacco plants inoculated with strain $M^SN^R$ of potato virus Y expressed as the percent of necrotic plants in the total population. Both populations are segregating for root-knot nematode resistance. Population II is also segregating for PVY resistance.

TABLE 3. Segregation pattern of aneuploid crosses designed to determine chromosomal location of genes conditioning resistance to the root-knot nematode and potato virus Y in tobacco

| Cross | Generation | PVY test | | Root-knot test | |
|---|---|---|---|---|---|
| | | Necrotic | Non-necrotic | Resistant | Susceptible |
| Nullisomic E × NC 95 | $F_1$ | 2,735 | 0 | 308 | 0 |
| Nullisomic E × NC 107 | $F_1$ | 0 | 2,585 | 0 | 296 |
| NC 2326 × Monosomic E (Nullisomic E × NC 95) | $F_1$ | 395 | 380 | 130 | 124 |
| NC 2326 × Monosomic E (Nullisomic E × NC 107) | $F_1$ | 0 | 941 | 0 | 206 |
| Monosomic G × NC 95 | $F_1$ | 1,798 | 0 | 266 | 0 |
| Hicks × Disomic G (Monosomic G × NC 95) | $F_1$ | 267 | 249 | 132 | 118 |
| Disomic G × Disomic G | $F_2$ | 188 | 106 | 239 | 87 |
| Hicks × Monosomic G (Monosomic G × NC 95) | $F_1$ | 498 | 0 | 235 | 0 |
| Monosomic G × Monosomic G | $F_2$ | 212 | 0 | 252 | 0 |

Populations of $F_2$ plants having cultivar NC 744 as one of the parents developed necrosis at a rate significantly slower than that of the other $F_2$ populations (Fig. 1). Cultivar NC 744 is root-knot susceptible, but resistant to PVY-$N^SN^R$. When cultivar NC744 was crossed with a root-knot-resistant line, the $F_2$ segregating ratios deviated from the 3:1 pattern observed with other populations (Table 2). It appears that the recessive gene for PVY resistance in cultivar NC 744 is epistatic to the dominant gene controlling the necrotic reaction as indicated by close agreement with the expected 9:7 segregation ratio of necrotic to non-necrotic plants. Although this genetic mechanism for PVY resistance slows the onset and consequent development of the necrotic reaction, it does not eliminate it completely since some of the plants that appeared non-necrotic when data were collected became faintly necrotic some weeks later. The gene for resistance to PVY-$N^SN^R$ is located on chromosome E (13) and originated from VY 32, which in turn derived its resistance from cultivar Virgin A mutant (16).

All twenty-five root-knot-resistant dihaploids derived from the cross cultivar Coker 86 × VY 32 developed severe systemic necrosis 17 days after inoculation with PVY-$M^SN^R$. There was no variation among plants. All fifty anther-derived, and 25 maternally derived, haploid lines of cultivar NC 95 developed severe necrosis when inoculated with the virus. The rate of development of the necrotic reaction in these haploids was somewhat slower and less severe than in the diploid cultivar NC 95 control plants, which were severely necrotic 7 days after inoculation and died by the 2nd wk. Haploids required at least 10 days to become necrotic and did not die until the 3rd or 4th wk. All 2,735 $F_1$ plants from the Nullisome E × NC 95 cross developed necrosis when inoculated with PVY-$M^SN^R$ (Table 3). Three hundred and eight $F_1$ plants of the same cross inoculated with root-knot nematodes were resistant. None of the 2,585 $F_1$ plants from the cross Nullisome E × NC 107 became necrotic in response to the virus, and all plants inoculated with the nematode were susceptible. $F_1$ plants monosomic for chromosome E from NC 95 or NC 107 were used as the male parent in crosses with NC 2326. The critical feature of this cross was the choice of the $F_1$ as the pollen parent which produces two kinds of gametes: one with a copy of chromosome E and one lacking it. Pollen with the full complement of chromosomes out-competes any non-aborted chromosome-deficient pollen which is transmitted at a rate of less than 1%. Therefore, two possible outcomes from the cross with monosomic E from cultivar NC 95 were expected: 1) Ninety-nine plus percent of the individuals would be root-knot resistant and necrotic if the dominant factor controlling viral necrosis and the dominant factor controlling root-knot resistance are both located on chromosome E (linked). 2) One-half of the individuals would be root-knot resistant and the other half would be root-knot susceptible indicating that the factor controlling the necrosis induced by PVY-$N^SN^R$ and the gene controlling root-knot resistance are not the same chromosome. Progeny from the cross NC 2326 × Monosome E (Nullisome E × NC 95) segregated in close agreement to a 1:1 ratio for both root-knot resistance and the virus-induced necrosis. Therefore, the factor conditioning the necrotic reaction to PVY-$M^SN^R$ is not located on chromosome E as is the gene for resistance to necrosis induced by the $N^SN^R$ strain. Consequently, the dominant allele that conditions necrosis in response to the $N^SN^R$ strain is genetically different and independent of the dominant allele for susceptibility to necrosis induced by the $M^SN^R$ strain; ie, these two reactions are controlled by genes at separate loci. Were these two reactions identical, all progeny from the cross NC 2326 × Monosome E would have been necrotic and root-knot resistant. These results lend support to the hypothesis of pleiotropism, but the possibility that the root-knot resistance gene is linked to a second gene which controls the necrotic reaction to PVY-$M^SN^R$ cannot be totally excluded.

Further evidence that the necroses induced by the two strains of PVY are different comes from the fact that when plants have the dominant gene for root-knot resistance plus the recessive gene for resistance to PVY located on chromosome E (conditioning resistance to the necrosis induced by strain $N^SN^R$), these plants eventually develop necrosis. Nevertheless, this recessive gene does retard the viral reaction as stated above and as reported earlier (4). Progeny from the cross NC2326 × Monosome E (Nullisome E × NC 107) were all non-necrotic and root-knot susceptible. These plants developed very mild vein-banding symptoms and were thus quite tolerant of the virus. Results of this cross corroborate the location of the gene for resistance to PVY-$N^SN^R$ on chromosome E.

Results from the crosses involving Monosome G are also shown in Table 3. The monosomic maternal gamete is transmitted at a rate of about 6% (5). All $F_1$ individuals were expected to be root-knot resistant. Monosomic $F_1$ plants (6%) could be distinguished phenotypically by their decreased vigor and morphological traits from the disomic $F_1$s. However, verification of the chromosomal condition was also done by test-crossing the two types of plants (Table 3). $F_1$ plants considered to be disomic were crossed as the male parent with the root-knot susceptible cultivar Hicks. If disomic, their progenies were expected to segregate equally into two types: one-half root-knot resistant and one-half root-knot susceptible. In the second test-cross, $F_1$ plants believed to be monosomic were also crossed as the male parent with Hicks. If these were monosomic plants, 99+% of the progeny were expected to be root-knot resistant, since transmission of the monosomic condition through the pollen is less than 1%.

$F_1$ progenies of Monosome G × NC 95 were all root-knot resistant and developed necrosis when inoculated with PVY. Test crosses correctly accomplished the identification of Monosomic G and Disomic G $F_1$ plants. Offsprings from Hicks × $F_1$ Disomic G (Red Russian Monosome G × NC 95) segregated in close agreement to a 1:1 ratio for both root-knot resistance and the necrotic reaction to the virus. $F_1$ progenies from the cross Hicks × $F_1$ Monosomic G (Red Russian Monosome G × NC95) produced all root-knot resistant and necrotic plants since transmission of the monosome through the pollen is virtually zero. Monosomic G and Disomic G $F_1$ plants were self-pollinated to produce the $F_2$ generations. The $F_2$ population derived from Disomic G segregated close to a 3:1 ratio for both root-knot resistance and virus-induced necrosis, but the $F_2$ population derived from Monosomic G plants did not segregate, which indicated that the factor controlling root-knot resistance is located on chromosome *G*.

Reactions of *Nicotiana* spp. to the root-knot nematode and to PVY-$M^SN^R$ are shown in Table 1. *Nicotiana tomentosa* (Ruiz and Pavon), accession 58, was as resistant to the nematode as NC 95 (index = 0), but these plants did not become necrotic when inoculated with the virus. Moreover, *N. tomentosa* was immune to PVY-$M^SN^R$. *Nicotiana repanda* (Willdenow ex Lehmann) and *N. longiflora* (Cavanilles) var. *breviflora* were also quite resistant to root-knot nematodes (index = 1), but these species showed severe mottling, vein banding, and stunting in response to the virus. All other species were highly susceptible to both pathogens.

TABLE 4. Segregation ratios of necrotic to non-necrotic $F_2$ tobacco plants derived from root-knot resistant and × root-knot susceptible (and vice versa) crosses after inoculation with strain $M^SN^R$ of potato virus Y

| $F_2$ populations from crossing | Number of plants | | Ratio | $\chi^{2}$[a] | *P* |
|---|---|---|---|---|---|
| | Necrotic | Non-necrotic | | | |
| 9942 × NC 89 | 1,338 | 543 | 2.46:1 | 15.11 | <0.005 |
| 9870 × NC 2326 | 2,898 | 1,020 | 2.84:1 | 2.17 | 0.25–0.10 |
| 9914 × Coker 86 | 4,227 | 1,377 | 3.06:1 | 0.55 | 0.50–0.25 |
| 9942 × Coker 86 | 1,350 | 445 | 3.13:1 | 0.59 | 0.50–0.25 |
| Total | 9,813 | 3,371 | 2.98:1 | 2.28 | 0.25–0.10 |

[a] Chi-square test performed to test the hypothesis of a 3:1 segregation ratio of necrotic to non-necrotic $F_2$ plants.

## DISCUSSION

More than 15,000 $F_2$ plants from root-knot-resistant × root-knot-susceptible cultivar crosses were inoculated with PVY-$M^SN^R$ and the root-knot nematode, but none were found to be root-knot resistant and non-necrotic in response to the virus. Thus, separation of these two reactions was not achieved. A few $F_2$ plants initially appeared to be root-knot resistant with only mild virus

symptoms, but most of these plants developed galls upon reinoculation with the nematode. The rest of the plants remained free of root-knot infection even after reinoculation with both nematode and virus. These plants were self-pollinated to produce the $F_3$ generation. Segregation for root-knot resistance and viral necrosis was observed among $F_3$ plants. Evidently, viral necrosis had been masked in the $F_2$ plants which were heterozygous for root-knot resistance. Plants homozygous for root-knot resistance reportedly develop more severe necrosis than do heterozygous plants (15). Also, mature plants under warm temperatures show a reduced degree of virus-induced necrosis even if completely homozygous for root-knot resistance (18). The $F_2$ plants were grown at aerial temperatures of 32–35 C because these temperatures favor development of galls, but the same temperatures inhibit development of the necrotic reaction in response to the virus (18). In addition, $F_2$ plants were fairly mature by the time they were reinoculated. Interestingly, NC 95 haploids also behave like heterozygous plants in that they develop necrosis at a slower rate than their diploid counterparts. This may represent a gene-dosage phenomenon.

On the other hand, the gene conditioning root-knot resistance is fully expressed in the heterozygote, and $F_1$ plants from crosses of root-knot-resistant × root-knot-susceptible parents are completely resistant. Conceivably, the necrotic reaction involved in resistance to the root-knot nematode could also be attenuated in the heterozygote, but the reaction may still be sufficient to prevent the nematode from feeding given its sedentary behavior.

Anther culture is known to produce genetic changes. Variability may be due to a higher than normal rate of mutation in tissue culture, residual heterozygosity in the parental anther source, and/or nuclear differentiation and DNA amplification in the pollen vegetative nuclei from which haploid plants arise (1,7,8). In these studies, haploid and dihaploid plants behaved similarly to the diploid parents and the technique did not induce separation between root-knot resistance and the necrotic reaction to PVY-$M^SN^R$.

The fact that disruption of the association between root-knot resistance and the necrotic reaction to PVY-$M^SN^R$ was not found in these experiments does not demonstrate that the two reactions are not coded by two separate and tightly linked genes. It is possible for these two reactions to be controlled by functional loci. For example, the loci may be in close proximity because they have similar functions. However, based on the following lines of evidence, it is highly probable that the two reactions are pleiotropic effects of the same gene: 1) Both root-knot resistance and the necrotic response to the virus are inherited in the same fashion and both are controlled by single, dominant genes. 2) A large number of $F_2$ plants were examined without disassociation of the two reactions. The $F_2$ populations used in this study were derived from homozygous breeding lines as the root-knot-resistant parent. Therefore, in developing these lines, there had been ample opportunity for crossing-over between the two putative loci, but this did not occur. Furthermore, it is believed that all root-knot-resistant tobacco cultivars available today originated from the same source of resistance. Extensive crosses have been performed in the development of these tobacco cultivars, but segregation between root-knot resistance and the necrotic reaction to PVY-$M^SN^R$ has never been detected. In addition, this single gene for root-knot resistance is present in many genetic backgrounds and expression of the two reactions is not greatly modified by other loci. It must be pointed out, however, that among root-knot-resistant cultivars, NC 95 develops the strongest necrotic reaction in response to the virus. 3) Both root-knot resistance and the virus-induced necrosis are temperature-sensitive, necrotic reactions and are inhibited by the same temperatures (18). 4) Both reactions are cell-specific; ie, expressed in all cells of a root-knot resistant genotype and the virus-induced necrosis is not translocatable to root-knot-susceptible cells (18). Pleiotropism is well documented in *Drosophila*, in sickle-cell anemia in humans (23), and in the susceptibility to *Helminthosporium victoriae* and resistance to *Puccinia coronata* in cultivar Victoria oats (17). Interestingly, the latter case also involves hypersensitivity or necrosis in response to both pathogens.

In these experiments, we confirmed that the root-knot resistance locus is located on chromosome G and established that the locus involved in the necrotic reaction to PVY-$M^SN^R$ is also located on this chromosome, but we could not distinguish between linkage and pleiotropism from these data. Whether these two reactions were truly conditioned by one or two separate genes becomes primarily an academic question because it appears that it will be very difficult to separate the two reactions by conventional breeding techniques. Meanwhile, a practical solution to the problem is desirable. Some progress may be made by selection (as indicated by results shown in Fig. 1) among plants with resistance to both root knot and PVY. Necrosis develops slower in these plants and this phenomenon is independent of virus concentration at the time of inoculation. Plants of NC 95 inoculated with a dilution series of the virus ranging from 10 to $10^{-5}$ developed necrosis at the same time, indicating that the onset of necrosis and its rate of development are independent of virus titer at the time of inoculation. It is possible that less damage will occur under field conditions if cultivars have combined resistance to both pathogens, but this remains to be tested. Our results in this aspect concur with earlier reports (4).

The other alternative for control would be to transfer the root-knot resistance found in *Nicotiana tomentosa* 58 into *N. tabacum*. This accession of *N. tomentosa* was reported earlier (20,21) to be root-knot resistant and we have confirmed those results even though we used a different race of the nematode. Furthermore, *N. tomentosa* does not become necrotic when inoculated with PVY-$M^SN^R$ and it actually appears to be immune to the virus. Sievert (19) found similar results although he did not indicate which strain of the virus he used in his studies. It has been speculated that *N. tomentosa* is the source of root-knot resistance found in NC 95 and other resistant cultivars. Since *N. tomentosa* does not develop necrosis when inoculated with the virus, it is either not the original source of root-knot resistance, or if it is, a segment of *N. tomentosa* chromosome (versus a single gene) was transferred into *N. tabacum* and a mutation has since occurred within this linkage block.

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