Genetic Resistance to Tobacco Mosaic Virus, Cyst Nematodes, Root-Knot Nematodes, and Wildfire from *Nicotiana repanda* Incorporated into *N. tabacum*

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ABSTRACT

Gwynn, G. R., Barker, K. R., Reilly, J. J., Komn, D. A., Burk, L. G., and Reed, S. M. 1986. Genetic resistance to tobacco mosaic virus, cyst nematodes, root-knot nematodes, and wildfire from *Nicotiana repanda* incorporated into *N. tabacum*. Plant Disease 70:958-962.

Nicotiana repanda was crossed as the female with *N. tabacum*, using the bridge-cross technique with *N. sylvestris* as the intermediary species. The resulting material was backcrossed to *N. tabacum* cultivars and screened for tobacco mosaic resistance. Pollen infertility was overcome by the use of a pollen fertility restorer, which allowed the test material to be used as a male with the subsequent elimination of *N. repanda* cytoplasm. Breeding material with the local-lesion type of hypersensitive response to tobacco mosaic virus was obtained from *N. repanda*. Segregation was unpredictable and chromosome instability was indicated. Frequency of resistance was increased through subsequent backcrossing. Other breeding material from the same crosses indicated the possibility of obtaining resistance to the pathogens *Meloidogyne incognita*, *Globodera tabacum solanacearum*, and *Pseudomonas syringae* pv. *tabaci*.

Additional key words: interspecific, hybridization, tobacco diseases

Genetic resistance to tobacco mosaic virus (TMV) was incorporated into *Nicotiana tabacum* L. by Holmes (9) in 1938. In North Carolina in 1984, the most costly disease was TMV, accounting for an estimated loss of $16.4 million (11), yet only 4.1% of the acreage was planted to TMV-resistant cultivars. Some growers feel that TMV-resistant cultivars that derive their resistance from *N. glutinosa* (8) are inferior in quality, but other species also carry resistance to TMV (18). One of these is *N. repanda* Will. ex Leh., which is a source of resistance to eight other diseases (3,5,7,12–20), including wildfire, incited by *Pseudomonas syringae* pv. *tabaci* (*P. s.* pv. *tabaci*) Wolf & Foster (1). *N. repanda* does not cross readily with *N. tabacum*, but crossing has been accomplished by the bridge-cross technique (2,14,19), in which *N. sylvestris* Speg. & Comes is used as an intermediary species, or by increasing the ploidy level of *N. repanda* before crossing with *N. tabacum* (10). Stavely et al (17) transferred immunity to *Cercospora nicotiana* Ell. & Ev. and *Meloidogyne javanica* (Treub) Chitwood from *N. repanda* with such techniques, but chromosomes presumably carrying resistance were eliminated and stable immune lines were not obtained.

Schweppenhauser et al (15) reported resistance to *M. javanica* in hybrids between *N. repanda* and *N. longiflora* and between *N. repanda* and *N. palmeri*. Both hybrids were sterile. Male sterility exists in hybrids between *N. repanda* and *N. tabacum*, but the use of a pollen fertility restorer can produce normal anthers (8). Difficulties in successful introgression of *N. repanda* germ plasm into *N. tabacum* has hampered transfer of disease resistance, and patterns of inheritance are sometimes irregular and undependable (14,17).

The primary purpose of this study was to transfer genetic resistance to TMV from *N. repanda* to *N. tabacum*. Segregating breeding material was also evaluated in this study for resistance to root-knot nematode species (*M. incognita* race 3 (Kofoid & White) Chitwood and race 4 (Golden & Slana) and *M. javanica*), cyst nematodes (*Globodera tabacum solanacearum* (Miller & Gray) Stone), and wildfire (incited by *P. s.* pv. *tabaci*).

MATERIALS AND METHODS

Crosses were made between *N. repanda* as the female and *N. sylvestris* as the male (Fig. 1). This hybrid was doubled using a colchicine treatment (6) in the first generation followed with pollination by *N. sylvestris*. The sesquidiploid resulting from this cross was pollinated by four flue-cured tobacco (*N. tabacum*) cultivars: North Carolina (NC) 95, NC 2326, Coker (C) 139, and South Carolina (SC) 58. Twenty-five plants from each of these families were grown to about 15 cm high in the greenhouse. A detached leaf from each was inoculated with an aqueous suspension of TMV-infested green tissue at Oxford, NC (6). An attempt was made to grow resistant plants from each of these families to flowering and use them as females in backcrosses with their respective flue-cured parent. From several hundred pollinations, a limited amount of seed was obtained on two plants in the NC 2326 family, one plant in the SC 58 family, and six plants in the C 139 family. After the seeds germinated, one plant from the NC 2326 family and 49 from the Coker 139 family were recovered and grown to flowering. These plants were pollinated by a pollen fertility restorer labeled R 22 (8). After the fertility restorer was used, seed was obtained from one plant in the NC 2326 family (labeled M 1) and from 28 plants in the C 139 family (labeled M 2-M 29).

Subselections within the M lines were made and tested further for disease resistance. Several of the M lines with mosaic resistance were studied further and crossed with flue-cured cultivars; most of this report deals with the lines M 1 and M 11 and their descendants because of their increased fertility and resistance. A selection made within M 11, designated M 11-3, was crossed a second time with the pollen fertility restorer and a TMV-resistant selection coded 9071 developed from it. Another selection that produced many TMV-resistant offspring from M 11 was 0005. Sublines were developed from each of these and crossed once or twice with various flue-cured cultivars. Selfed progenies were developed from the flue-cured backcrosses after mosaic screening.

Subselections within the M 1 line were tested under greenhouse conditions for

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reactions to *Meloidogyne* spp. The subselections were also crossed with various susceptible flue-cured cultivars to produce segregating materials, which were tested for their reactions to the cyst nematode (*G. tabacum solanacearum*) and *P. s.* pv. *tabaci*.

Disease-screening procedures. *TMV.* TMV screening involved rubbing green leaves from greenhouse- or field-grown plants with an aqueous solution of TMV and 600-mesh Carborundum. In some greenhouse tests, five to 10 inoculated leaves were detached and their petioles kept in water during the completely randomized test (6). Various numbers of plants were inoculated in greenhouse tests, but 22 plants per plot were used in field tests. All TMV resistance classifications were based on the presence or absence of necrotic spots.

Meloidogyne. Testing for *Meloidogyne* was conducted in the greenhouse on the 15 subselections from the M 1 line. Individual plants about 15 cm high were transplanted into 15-cm-diameter clay pots and inoculated with a soil drench of NaOCl-extracted eggs (4), 10,000 eggs per plant for *M. javanica* and *M. incognita* race 3 and 4,800 eggs per plant for *M. incognita* race 4 because of a shortage of eggs for this race. Eggs were extracted from the roots after 2 mo, and plant reaction was expressed as number of eggs per gram of roots. Four replicates were used in a randomized complete block design.

G. tabacum solanacearum. Tests were conducted in both the greenhouse and the field. Greenhouse seedlings were transplanted into 15-cm-diameter clay pots filled with a 50:50 mixture of commercial sand and a sandy loam soil containing cyst nematodes. Plants set in this mixture were grown in a greenhouse at 26–29 C in a randomized complete block design with three replicates (four plants per replicate) for 87 days, then the roots were washed and evaluated using a scale of 0–5 based on the percentage of roots containing cysts (0 = no cysts, 1 = 1–10%, 2 = 11–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100%). Field tests were conducted in a cyst-nematode nursery at Blackstone, VA, in 1983 and 1984. Four replicates of 12 plants each were used in a randomized complete block design. Cyst-nematode counts were taken at transplanting and again at the end of normal harvest (about 110 days later). Results were expressed as the difference between these two counts, with a buildup of nematodes over the season indicating susceptibility and a decline indicating resistance.

P. s. pv *tabaci.* A strain of *P. s.* pv. *tabaci* was obtained from H. A. Skoog, USDA, Beltsville, MD, and maintained on beef peptone-dextrose broth. A 1:10 dilution of the stock culture (O.D. = 15 at 540 nm) was used to inoculate leaves in the greenhouse by rubbing with a gauze pad (6). Plants were preshaded for 1 day, inoculated, misted, and shaded again for 1 day. Four days after inoculation, the plants were rated on a scale of 0–5, where 0 = lesions, 1 = <10%, 2 = 10–24%, 3 = 25–50%, 4 = 51–75%, and 5 = 76–100% of leaf area infected. Four replicates of a randomized complete block design were used, with each plant constituting a replicate.

Table 1. Frequency of plants showing resistance to tobacco mosaic virus (TMV) in F_2 and F_3 populations of crosses between *Nicotiana repanda* breeding lines and flue-cured cultivars and cytological results on certain lines

Entry no.	Pedigree	Resistant to TMV: No. resistant/ total tested	Resistant to TMV: Frequency	Cytology of parent plant
3223	F_2 C 319 × (NC 2326 × 0005-3MR)	2/22	0.09	···
3223-1	F_3	0/24	0.00	24II
3223-2	F_3	10/23	0.43	···
4210	F_2 NC 2326 × 3223-2	1/88	0.01	···
4211	F_2 × 3223-4	1/44	0.02	···
3780	F_2 C 319 × (NC 2326 × 0005-3MR)	11/77	0.14	···
3780-1	F_3	8/15	0.53	···
3780-2	F_3	5/21	0.24	23II + 2I
3780-3	F_3	7/24	0.29	23II + 2I
3780-4	F_3	3/8	0.38	···
3780-5	F_3	13/31	0.42	···
3780-6	F_3	5/16	0.31	···
3780-7	F_3	2/24	0.08	···
3780-8	F_3	3/23	0.13	···
3780-9	F_3	2/24	0.08	···
3780-10	F_3	5/23	0.22	···
4226	F_2 C 319 × 3780-1	1/88	0.01	···
3792	F_2 C 319 × 9071-1MR	9/57	0.16	···
3792-1	F_3	7/21	0.33	···
3792-2	F_3	9/20	0.45	23II + 2I
3792-3	F_3	4/21	0.19	···
3792-4	F_3	0/24	0.00	···
3792-5	F_3	10/28	0.36	···
3792-6	F_3	3/23	0.13	···
3792-7	F_3	16/45	0.36	23II + 2I
3792-8	F_3	5/28	0.18	23II + 2I
4232	F_2 C 319 × 3792-3	2/88	0.02	···
3767	F_2 NC 82 × (NC 2326 × 0005-1MR)	5/44	0.11	···
3767-2	F_3	13/32	0.41	···
3767-3	F_3	11/24	0.46	23II + 2I
3767-4	F_3	4/16	0.25	23II + 2I
3767-5	F_3	8/28	0.29	···
3790	F_2 NC 82 × 9071-2-2MR	10/88	0.11	···
3790-1	F_3	2/17	0.12	Mixture
3790-2	F_3	14/35	0.40	24II
3790-3	F_3	9/34	0.26	···
3790-4	F_3	8/22	0.36	···
3790-5	F_3	9/21	0.43	···
3790-6	F_3	3/14	0.21	···
3790-7	F_3	11/39	0.28	···
4638	F_2 NC 82 × 3790-1	4/66	0.06	···
4639	F_2 NC 82 × 3790-2	0/66	0.00	···
4640	F_2 NC 82 × 3790-3	1/66	0.02	···
4641	F_2 NC 82 × 3790-4	1/66	0.02	···
4642	F_2 NC 82 × 3790-5	0/66	0.00	···

RESULTS AND DISCUSSION

TMV. Results of detached leaf tests for TMV reactions showed seven of the 29 M lines with the necrotic spotting indicating resistance: M 2, M 6, M 11, M 12, M 24, and M 29. Results of TMV tests on backcross and selfed material developed from 0005 and 9071 selections crossed with flue-cured cultivars are given in Table 1. Resistant lines 0005-1MR, 0005-3MR, and 9071-1MR served as the source of TMV resistance and were either used directly in crosses with susceptible flue-cured cultivars, as in the case of entry 3792, or in three-way crosses, as in the case of entries 3223, 3780, and 3767. Further backcrossing to flue-cured cultivars and selfing occurred, as indicated in Table 1. Entries in Table 1 that were in the F_2 generation of inbreeding came from individual TMV-resistant F_1 plants, and entries that were in the F_3 generation of inbreeding came from individual, TMV-resistant plants selected in the F_2.

If mosiac resistance were conditioned by a single dominant gene, as it is in *N. glutinosa*, and if that gene had been introgressed into an *N. tabacum* chromosome, one would expect an F_2 population of a homozygous-resistant selection crossed on a susceptible flue-cured cultivar to segregate 75% resistant/25% susceptible. F_3 progenies of resistant individual F_2 plants (F_3 lines)

would be either 100% resistant or segregate 75% resistant/25% susceptible under the same conditions. Results showed that the frequencies of TMV-resistant plants were lower than expected under these conditions. The frequency of resistant plants increased in the advanced selections (i.e., F_3 lines) in most cases, e.g., lines 3780-1, 3792-2, and 3767-3. The deviation from expected ratios indicates that the genetic factors for TMV resistance may not yet be introgressed into the *N. tabacum* genome.

A preliminary cytological evaluation of some of the TMV-resistant plants was performed to determine if chromosome constitution varied (Table 1). Seven of the nine plants studied formed 23 bivalents and two univalents at metaphase I. The progeny of these lines segregated for TMV resistance, which is consistent with the assumption that one of the univalents may be an *N. repanda* chromosome and the other an *N. tabacum* chromosome. These plants are tentatively considered heterozygous alien-substitution lines. One of the resistant plants (3790-2) formed 24 bivalents, whereas the final plant studied (3790-1) formed predominantly 24 bivalents but also had cells with 23 bivalents plus 2 univalents, 22 bivalents, a trivalent plus a univalent and 22 bivalents plus a quadrivalent. Both of these plants segregated for TMV resistance after selfing. The chromosome configurations observed in these two plants indicate that some integration of genetic material from *N. repanda* into an *N. tabacum* chromosome may have occurred; however, this cannot be confirmed until additional research is conducted with resistant and susceptible progeny of these lines. These studies are under way and will be reported later.

Meloidogyne. The reactions of line M 1 and its 15 subselections to *M. javanica* and *M. incognita* races 3 and 4 in greenhouse tests is shown in Table 2. Included in the test were R 22 (the pollen fertility restorer), NC 95 (resistant to *M. incognita* races 3 and 4), and the susceptible Hicks. The parent line, M 1, and all subselections had egg levels that were not significantly different from resistant NC 95 when exposed to *M. incognita* race 3. Subselections M 1-5, M 1-12, and M 1-13 had the lowest values. There was little indication of resistance to *M. incognita* race 4 in this group and no indication of significant resistance to *M. javanica*. Even though some entries had lower egg counts of *M. javanica* than others, the differences among lines were not significant.

Cyst nematode and wildfire. F_3 lines resulting from crosses of various susceptible flue-cured cultivars crossed with sublines from the line M 1 were tested in the greenhouse by inoculation with *G. tabacum solanacearum* and *P. s.* pv. *tabaci* (Table 3). When compared

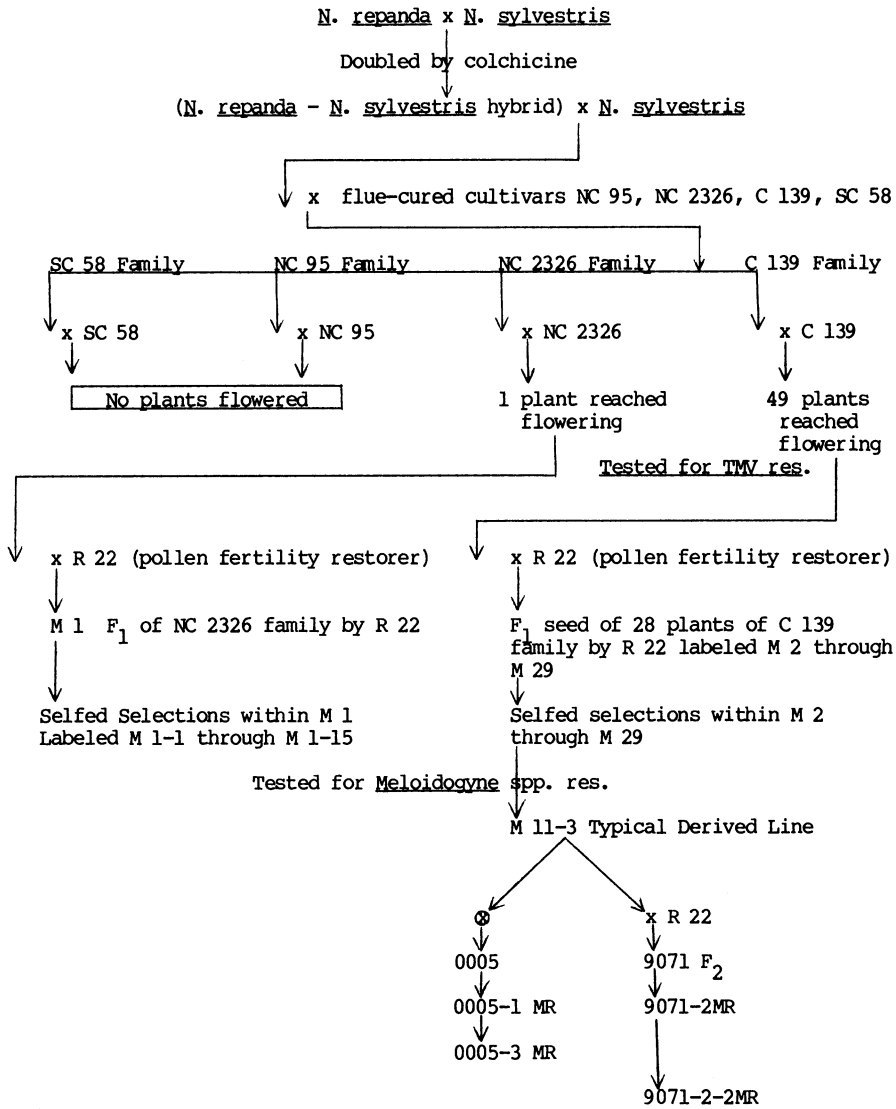

Fig. 1. Summary of crosses and lines tested.

Table 2. Reactions of M 1 lines and subselections derived from M 1 to *Meloidogyne javanica, M. incognita* race 3, and *M. incognita* race 4

Line	No. eggs per gram of roots (in 1,000s)		
	M. javanica	*M. incognita* race 3	*M. incognita* race 4
M 1	36.0 bcde[z]	5.10 fghi	12.7 bcdef
M 1-1	56.7 bcd	10.80 cdefghi	26.7 a
M 1-2	58.7 bcd	...	...
M 1-3	25.3 cde	4.70 ghi	11.7 bcdef
M 1-4	...	7.80 defghi	...
M 1-5	53.7 bcde	0.30 i	11.2 bcdef
M 1-6	58.6 bcd	2.60 hi	14.9 bcdef
M 1-7	28.0 cde	6.30 efghi	9.8 cdef
M 1-8	28.6 cde	8.80 defghi	12.5 bcdef
M 1-9	31.2 cde	13.40 bcdefghi	12.3 bcdef
M 1-10	...	...	...
M 1-11	44.5 bcde	4.00 ghi	13.4 bcdef
M 1-12	35.3 bcde	0.10 i	16.2 abcdef
M 1-13	53.3 bcde	0.80 i	12.9 bcdef
M 1-14	21.9 de	2.40 hi	7.0 f
M 1-15	38.2 bcde	4.70 ghi	15.5 abcdef
R 22 (fertility restorer)	51.3 bcde	21.30 abcde	9.3 def
NC 95	37.6 bcde	0.05 i	9.6 def
Hicks	73.1 ab	31.30 ab	13.7 bcdef

[z] Means within the same column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 3. Reactions of breeding lines from crosses between flue-cured cultivars and M lines to *Globodera solanacearum* and *Pseudomonas syringae* pv. *tabaci* in greenhouse tests

Entry	Pedigree	Cyst index[x]	Wildfire index[y]
9017-2	C 319 × M 1-4	0.25 fgh[z]	5.00 a
9020-1	C 411 × M 1-4	0.13 gh	1.25 cde
9020-2	C 411 × M 1-4	0.13 gh	4.50 a
9020-3	C 411 × M 1-4	0.00 h	0.00 e
9021-1	NC 2336 × M 1-1	0.13 gh	0.50 de
9021-2	NC 2336 × M 1-1	0.13 gh	4.25 ab
9021-3	NC 2336 × M 1-1	0.63 cdefg	5.00 a
9021-4	NC 2336 × M 1-1	1.13 abc	4.50 a
9023-1	NC 2326 × M 1-4	0.13 gh	3.50 abc
9023-2	NC 2326 × M 1-4	0.13 gh	4.00 ab
9025-1	Va 115 × M 1-1	0.88 abcde	···
9025-2	Va 115 × M 1-1	1.00 abcd	4.25 ab
9026-1	Va 115 × M 1-4	0.88 abcde	2.00 bcde
9026-2	Va 115 × M 1-4	0.75 bcdef	5.00 a
9026-3	Va 115 × M 1-4	1.25 ab	2.75 abcd
9028-1	C 298 × M 1-4	0.50 defgh	4.25 ab
9029-1	C 298 × M 1-1	0.38 efgh	1.50 cde
9029-2	C 298 × M 1-1	0.00 h	4.25 ab
9030-1	C 319 × M 1-2	0.13 gh	3.25 abc
9032-1	3150 × M 1-4	0.00 h	4.50 a
9032-2	3150 × M 1-4	0.00 h	4.25 ab
C 319	···	0.88 abcde	3.50 abc
Va 81	···	0.00 h	0.50 de

[x]Scale based on percentage of roots containing cysts: 0 = no cysts, 1 = 1–10%, 2 = 11–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100%.
[y]Scale based on percentage of leaf area infected: 0 = no lesions, 1 = <10%, 2 = 10–24%, 3 = 25–50%, 4 = 51–75%, and 5 = 76–100%.
[z]Means within columns followed by the same letter are not significantly different ($P = 0.05$) according to Waller-Duncan K-ratio 100.

Table 4. Difference between initial (beginning of season) and final (postharvest) numbers of *Globodera solanacearum* per 500 cm^3 of soil on certain breeding lines and checks in field nurseries

		1983		1984	
Entry no.	Pedigree	Generation	Difference (cysts/500 cm^3)	Generation	Difference (cysts/500 cm^3)
3144	C 411 × M 1	F_2	−103[y] f[z]	F_4	−284 cd
3144C	Composite			F_5	−164 bcd
3151	NC 2326 × M 1	F_4	101 bcdef	F_4	−120 abcd
3154	NC 2326 × M 1	F_4	39 def	F_5	−319 d
3163	NC 2326 × M 1	F_4	−43 ef	F_4	−186 bcd
K 326	Susc. check		183 abcde	C 319 (susc. check)	59 ab
				Repanda (res. check)	−288 cd

[y]Negative values occur when initial counts exceed final counts.
[z]Means with the same letter within columns are not significantly different ($P = 0.05$) according to the Waller-Duncan K-ratio 100.

with the resistant Virginia (Va) 81 and susceptible C 319, some of the lines had high levels of resistance to both cyst nematode and wildfire: 9020-1, 9020-3, 9021-1, and 9029-1. It has been speculated (D. A. Komn, *unpublished*) that a close linkage exists between wildfire resistance and cyst-nematode resistance, but this was not true for nine of the 21 lines tested, because they were resistant to the nematodes but were susceptible to wildfire. No line was encountered that was resistant to wildfire but susceptible to cyst nematodes.

Several breeding lines from crosses of line M 1 with two flue-cured cultivars were tested in a cyst-nematode field nursery in 1983 and 1984. The difference between initial (beginning of season) and final numbers (harvest) are shown in Table 4. The populations declined on line 3144 during both growing seasons, which contrasted with the results obtained on both susceptible flue-cured check Northrup King (K) 326 in 1983 and susceptible C 319 in 1984. It was not different from the resistant species *N. repanda* in 1984. A composite of plants selected in 1983 from 3144 and tested in 1984 continued to show resistance. The three lines with NC 2326 as a parent did not differ significantly from the check in 1983. Selection within line 3154 in 1983 of plants with more vigorous growth and greater height resulted in a F_5 composite that in 1984 was not significantly different from the resistant *N. repanda* check. This may indicate a lack of genetic stability in the F_4 generation.

Although we failed to obtain a TMV-resistant breeding line that consistently transmitted resistance, indications are that the frequency of transmission and ease of manipulation are increasing with additional crossing with flue-cured cultivars. The reaction to TMV inoculation was the local-lesion hypersensitive type, but we observed that necrosis appeared to become systemic more rapidly than with *N. glutinosa* resistance. Until the gene(s) for TMV resistance is incorporated into *N. tabacum* germ plasm, it will be difficult to study the mode of inheritance or any relationship to the dominant gene for resistance from *N. glutinosa*. It may be possible to obtain resistance from *N. repanda* to *M. incognita* race 3, *G. tabacum solanacearum* and *P. s.* pv. *tabaci*.

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