Identification of the Chromosome Carrying the Factor for Resistance to *Meloidogyne incognita* in Tobacco[1]

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Abstract: To identify the chromosome carrying the factor for resistance to *Meloidogyne incognita* in tobacco, crosses were made between resistant tobacco 'NC95' as pollen parent and each of the 12 tobacco monosomics (A-L) representative of the Tomentosae half of the *Nicotiana tabacum* chromosome complement. Of the F_1 seedlings, 927 plants were grown for observation. From these, 223 plants were selected as possible monosomics on the basis of morphological characteristics. These plants were self-pollinated, and the resulting F_2 plants were inoculated with both *M. incognita acrita* and *M. incognita incognita*. Sixteen F_2 populations, derived from the haplo-G monosome, were completely resistant. All of the F_2 populations derived from the other 11 monosomic crosses segregated into a 3:1 (resistant:susceptible) ratio. These results indicate that the factor for resistance to *M. incognita* is located on the G chromosome of *N. tabacum*. This is the first report establishing the *N. tabacum* chromosome that carries the factor for root-knot resistance. The results are consistant with our earlier evidence that *M. incognita* resistance in tobacco is derived from *N. tomentosa*, a species in the section Tomentosae of the subgenus Tabacum, genus *Nicotiana*. The other 12 chromosomes of *N. tabacum* have affinities with *N. sylvestris*, section Alatae, subgenus Petunoides, genus *Nicotiana*. *Key words:* genetics, *Meloidogyne incognita*, resistance, monosomic analysis, *Nicotiana tabacum*.

Root-knot, caused by *Meloidogyne* species, is a major disease of tobacco in much of the world. In 1961 Moore et al. (8) released a *M. incognita*-resistant flue-cured tobacco, 'NC95.' It was proposed that its monogenic dominant resistance was derived from the tobacco breeding line 'RK42,' which had been crossed with an interspecific hybrid, putatively *N. sylvestris* x *N. tomentosiformis* (3,7).

The source of this high degree of resistance is unique. Resistance in 'RK42' was from 'TI 706' and was shown to be polygenic in inheritance and closely linked with small leaf size (7). 'RK42' was crossed with the interspecific hybrid and backcrossed with '402' to produce progeny in which resistance was controlled by a single pair of dominant genes (4).

In 1975 (13) results from inoculations with five *Meloidogyne* isolates suggested that monogenic dominant resistance in 'NC95' was not derived from 'RK42' but from one of the *Nicotiana* species in the interspecific hybrid originally crossed with 'RK42.' It was hypothesized that this species was *N. tomentosa* or a closely related species that had the high degree of resistance to *M. incognita* that is present in *N. tomentosa* (14).

This hypothesis has been supported by further data.

In 1976 (12) 63 *Nicotiana* species were inoculated with *M. i. acrita* and *M. i. incognita*. In 1978 (9) their reaction to *M. arenaria, M. grahami,* and *M. javanica* was reported. *N. tomentosa* was the only species that had the same degree of resistance as 'NC95' to each of these five nematode populations.

In 1977 Stavely et al. (15) compared the Fraction 1 polypeptide composition of 'NC95' tobacco, related species, and hybrids by isoelectric focusing. The results confirm that *N. tomentosa* could have been the interspecific hybrid from which resistance to root-knot nematodes was derived. In 1978 (4,10) resistance in *N. tomentosa* was shown to be controlled by a single dominant factor as in 'NC95.'

This study, portions of which have been previously reported (11), was undertaken to determine which tobacco chromosome carries the monogenic dominant gene for root-knot resistance. A resistance gene located on one of the 12 *N. tabacum* chromosomes having affinities with section Tomentosae of the genus would further indicate that *N. tomentosa,* or a close relative, was the source of resistance. This resistance gene could theoretically be located in the complement

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from either the section Alatae (*N. sylvestris*) or the section Tomentosae of the allotetraploid *N. tabacum* genome.

Cameron (1) described the use of monosomic analysis as a means for locating genes on specific chromosomes. In 1973 Gupton and Burk (6) used the monosomics to locate the recessive factor for resistance to Potato Virus Y on the E chromosome of tobacco. In 1974 Wernsman et al. (17) investigated resistance to black shank, *Phytophtora parasitica* (Dast.) var. *nicotianae* Tucker, with monosomics. They identified chromosomal differences for disease reactions but could locate no major genes for resistance.

MATERIALS AND METHODS

Tobacco 'NC95' was crossed as pollen parent with the *N. tabacum* 'Red Russian' monosomics designated A through L and with normal 'Red Russian' in the greenhouse in the spring of 1977 (Table 1). The individual 'Red Russian' tobacco monosomics were obtained as cuttings at Raleigh from D. U. Gerstel, Crop Science Department, North Carolina State University, and were established and maintained in the greenhouse at Beltsville, Maryland.

The F_1 progeny were seeded in the greenhouse. They were transplanted to peat pots 4 wk after seeding, and 791 of them were set in the field on 10 June 1977. All 116 monosomic G × 'NC95' and 20 monosomic H × 'NC95' plants were transplanted to the field on 5 July 1977. At flowering, 223 of the F_1 plants were selected as possible monosomics based on morphological and physiological characterics; e.g., plant size, branching, leaf size and shape, presence and size of auricles, calyx and flower morphology, time until maturity, and capsule size, shape, and fullness (2). They were bagged and selfed to produce F_2 seed.

Single-plant progenies from 176 selfed plants were seeded in 16.5-cm clay pots containing the Beltsville soil-silica-sand mixture (14). Four weeks later three seedlings were transplanted into each of 2,500 6.6-cm clay pots. Soil and pots were sterilized before use. Each seedling was inoculated with a suspension of about 750 second-stage larvae 2 wk after transplanting.

Both *Meloidogyne incognita* subspecies, *M. i. acrita* and *M. i. incognita,* were increased on tomato, *Lycopersicon esculentum* Mill. 'Rutgers,' for inoculum. Heavily infected tomato roots were washed and placed

Table 1. 'Red Russian' tobacco monosomics A-L crossed with 'NC95,' their monosomic transmission percentage, the number of plants that were field-grown, selected, and selfed, and the number of F_1 selections and F_2 plants tested for resistance to *Meloidogyne incognita*.

Monosome	Monosomic ovular transmission* (%)	F_1 field plants (no.)			F_2 plants tested (No.)
		Transplanted from greenhouse	Bagged and selfed†	Progenies tested‡	
A	78.7	25	9	8	249
B	32.3	74	19	12	504
C	45.8	50	10	8	302
D	41.2	75	12	9	369
E	81.9	25	6	5	116
F	59.8	75	15	15	986
G	6.4	116	39	36	1,439
H	70.4	56	10	8	246
I	7.7	115	33	17	636
J	6.0	118	38	38	2,398
K	48.3	73	9	6	135
L	18.6	125	23	14	410
	Totals	927	223	176	7,790

*Monosomic Ovular Transmission Percentage observed by Clausen and Cameron (2).

†Plants were selected for bagging as possible monosomics for chromosome A through L according to morphological characteristics indicative of the monosomic condition.

‡Not all bagged and selfed plant progenies were tested.

under constant mist at 28 C. Freshly hatched second-stage larvae (L_2) were collected, counted, and pipetted directly onto the soil surface at the base of the stem (14). This was followed by 10 s of misting. Misting was repeated six times during the next 48 h. Air and soil temperatures were maintained at 25–29 C.

After 4 wk the roots were washed in water and examined for galling and nematode reproduction. The F_2 plants were divided into resistant (clean roots) or susceptible (galled roots) classes (Figs. 1, 2). Each monosomic (A-L) × 'NC95' F_2 generation was rated, and the chi-square values were calculated (Table 2).

RESULTS

Inoculation with *Meloidogyne incognita* caused severe galling on controls and susceptible F_2 plants derived from crosses of the 'Red Russian' monosomics A-L with 'NC95.' Of the F_2 plants, 75.5% were resistant. Chi-square values for goodness-of-fit for a 3:1 ratio of resistant:susceptible F_2 plants are given in Table 2. All observed values fit the 3:1 ratio, except that for monosomic G which was significantly different at the $P = 0.05$ level (16).

Only monosomic G × 'NC95' has F_2 populations that were totally resistant or both resistant and susceptible to *M. incognita* (Table 3). Data from 20 populations fit the expected 3:1 ratio for resistance, and all 643 F_2 plants of 16 populations were resistant. These 16 populations were single-plant progenies from a total of 116 F_1 field plants from the monosomic G × 'NC95' cross, indicating that the monosomic character was transmitted to at least 13.79% of the progeny.

DISCUSSION

The F_2 progenies from self-pollinated monosomic F_1 plants consist of disomic, monosomic, and nullisomic individuals. The occurrence of viable nullisomics in tobacco is rare or nonexistant, depending upon which chromosome is involved (5,17). Male gametes with 23 chromosomes usually abort or are noncompetitive with those that have 24 chromosomes (17). Consequently, in this study the viable selfed progeny from F_1 monosomic crosses were assumed to consist of disomic and monosomic individuals, with their proportions varying for each of the 24 chromosomes in the genome (2,17). This variability has been measured by Clausen

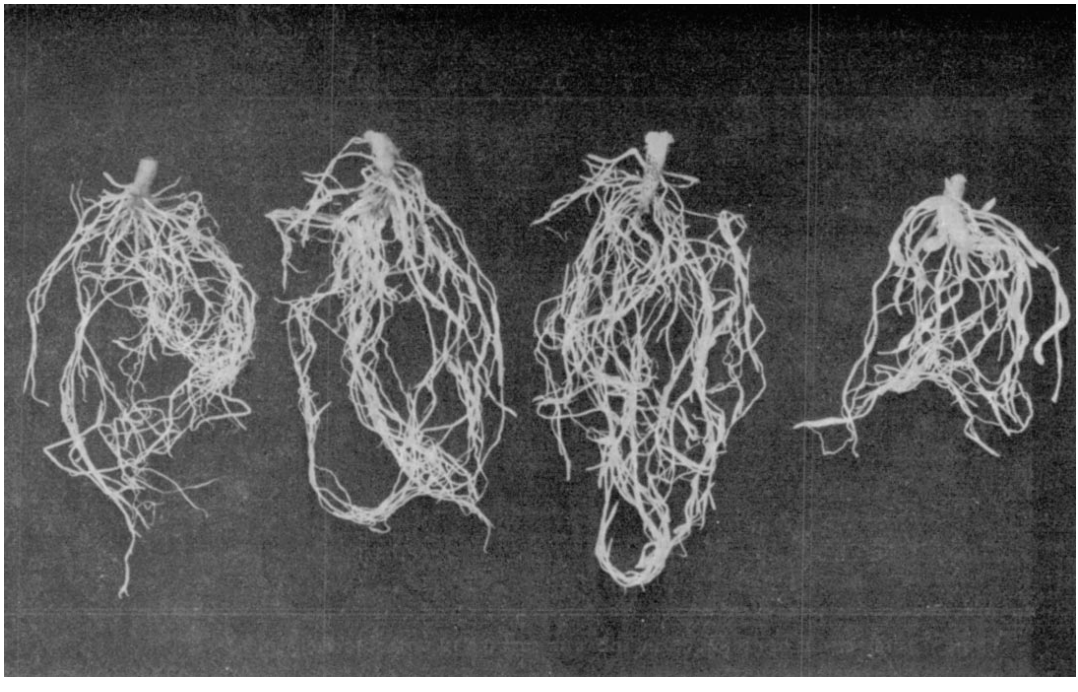

Fig. 1. Root systems of F_2 plants derived from 'Red Russian' monosomic A crossed with 'NC95' tobacco 4 wk after inoculation with *Meloidogyne incognita acrita,* demonstrating the 3:1 (resistant:susceptible) ratio.

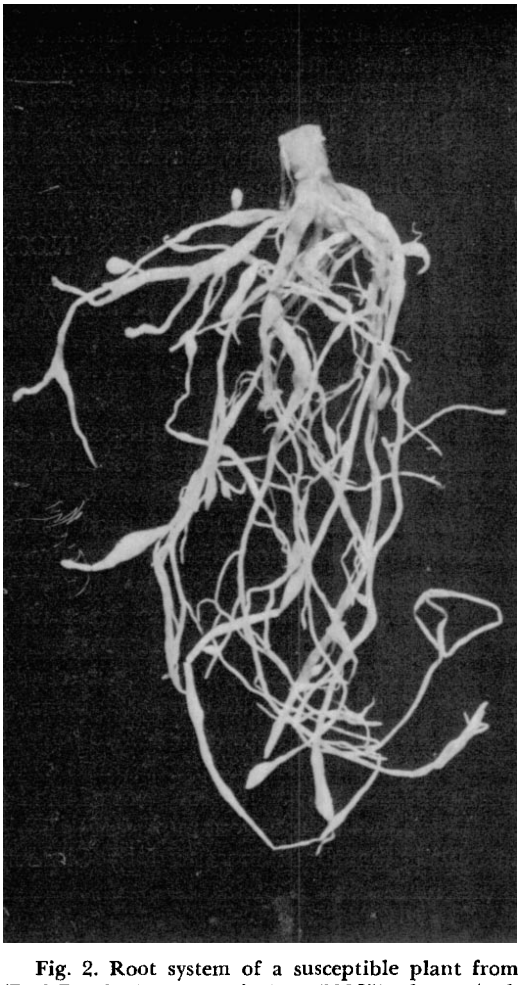

Fig. 2. Root system of a susceptible plant from 'Red Russian' monosomic A × 'NC95' tobacco 4 wk after inoculation with *M. i. acrita*.

and Cameron (2) and is referred to as the ovular monosomic transmission percentage (Table 1).

Crosses of the resistant variety 'NC95,' with a dominant gene for root-knot resistance (RR), to susceptible 'Red Russian' female, monosomic and recessive (r—) for a chromosome with the locus affecting disease resistance, produced resistant disomic (Rr) and resistant monosomic (R—) F_1 progenies (Table 4). Selfing the F_1 monosomic produced homozygous resistant disomics (RR), resistant monosomics (R—), and occasionally nullisomics (——). The all-resistant populations in the F_2 of monosomic G × 'NC95' were thus RR and R—. The absence of even a single susceptible plant in these 16 populations suggested that there were no nullisomics.

The progeny of plants monosomic for chromosomes other than the one carrying the locus influencing resistance segregated into a normal 3:1 resistant:susceptible ratio.

In order to maximize the chances of successfully locating the locus for resistance and to make this study logistically practical, three assumptions were made: 1) the locus most likely resided in the Tomentosae rather than the Alatae, *N. sylvestris,* subgenome, thus chromosomes A-L were the focus of this investigation; 2) field growth of F_1 plants facilitated selection for the slight differences in certain characteristics associated with each particular monosomic; and 3) the number of field plants grown, selected, and bagged, and from which F_2 progenies were tested, was based on the ovular monosomic transmission percentages given by Clausen and Cameron (2). Monosomics G and J, having the lowest transmission rates, had the largest number of F_1 plants from which to select.

In this study, 11 of the 12 monosomic F_2 progenies contained both resistant and susceptible individuals segregating in normal Mendelian fashion (Table 2). The chi-square value 110.60 for the 1,439 tested plants of monosomic G × 'NC95' was significant at the $P = 0.05$ level, indicating a divergence from the expected 3:1 ratio.

Test results of all the progenies from the 36 selections from the monosomic G × 'NC95' cross are included in Table 3. All progenies from 16 selections were resistant. This is reflected in the chi-square value of 110.60. These 16 populations provided strong evidence that resistance to *M. incognita* is located on chromosome G, and that at least 16 of the 116 F_1 plants grown in the field were monosomic for chromosome G. In this experiment the ovular monosomic transmission percentage was at least 13.8; it might have been higher if all of the 116 F_1 progenies had been selfed and tested.

Twenty populations of monosomic G × 'NC95' F_1 selections included 187 susceptible and 609 resistant plants, with a chi-square value of 0.9648. These data fit the 3:1 (resistant:susceptible) ratio expected for progenies from plants that did not possess the chromosome for resistance in the monosomic condition. These progenies were from selfed plants that were not monosomics. It

Table 2. Numbers of resistant and susceptible plants and chi-square values for goodness-of-fit to 3:1 ratios of F_2 populations from the crosses of 'Red Russian' monosomics A through L with *Meloidogyne incognita* resistant 'NC95' tobacco.

Monosome	Resistant*		Susceptible*		Total	χ^2 (3:1)	Actual ratio
	Observed	Expected	Observed	Expected			
A	191	186.75	58	62.25	249	0.3869	3.29:1
B	393	378.00	111	126.00	504	2.3809	3.54
C	230	226.50	72	75.50	302	0.2164	3.19
D	263	276.75	106	92.25	369	2.7327	2.48
E	85	87.00	31	29.00	116	0.1839	2.74
F	713	739.50	273	246.50	986	3.7985	2.61
G	1,252	1,079.25	187	359.75	1,439	110.6048†	6.69
H	190	184.50	56	61.50	246	0.6559	3.39
I	488	477.00	148	159.00	636	1.0147	3.30
J	1,824	1,798.50	574	599.50	2,398	1.4463	3.18
K	103	101.25	32	33.75	135	0.1209	3.22
L	308	307.50	102	102.50	410	0.0032	3.02
NC95‡	50						
402§			50				

*Plants were divided into 2 classes: 1) resistant, with clean roots; or 2) susceptible, with galled roots.
†Chi-squared values larger than 3.84 for one degree of freedom did not fit the 3:1 ratio at the $P = 0.05$ level.
‡'NC95' resistant check.
§'402' susceptible check.

was expected that not all of the 36 F_1 plants that were selfed for F_2 seed would be monosomics, because the estimated ovular monosomic transmission percentage associated with monosomic G was low at 6.4% (2). In fact, the presence of at least 16 monosomic F_1s in a population of 116 F_1 plants indicated that the rate of transmission in this case was more than double the rate reported by Clausen and Cameron (2).

These findings establish that the locus for resistance resides on chromosome G of the Tomentosae subgenome of 'NC95' resistant tobacco. This is consistant with other evidence that *M. incognita* resistance in tobacco is derived from a *Nicotiana* species in the Tomentosae subgenome, most likely *N. tomentosa.*

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Table 3. Numbers of resistant and susceptible plants and chi-square values for goodness-of-fit of 36 F_2 populations from the cross of 'Red Russian' monosomic G with *Meloidogyne incognita* resistant 'NC95' tobacco.

No. F_2 populations of Mono G x 'NC95'	Resistant*		Susceptible†		Total	χ^2 (3:1)	Actual ratio
	Observed	Expected	Observed	Expected			
20	609	597.00	187	199.00	796	0.9648	3.26:1
16	643	482.25	0	160.75	643	214.333‡	

*Resistant plants had no galls.
†Susceptible plant had severe galling.
‡A chi-square value above 3.84 was highly significant, $P = 0.05$.

Table 4. Probable inheritance scheme of 'Red Russian' monosomic G tobacco crossed with 'NC95' tobacco.

Generation	Probable genotype		
P	'Red Russian' ♀ root-knot susceptible tobacco (r—) monosomic for chromosome G, which carries the susceptible allele of the gene for resistance.	X	'NC95' ♂ root-knot resistant tobacco (RR) disomic for chromosome G which carries the dominant allele of the gene for resistance.
	↓		↓
F_1	(R—) selfed	or	(Rr) selfed
	↓		↓
F_2	RR, 29—, —— 3 : 0 Resistant disomic and monosomics survive; nullisomics do not survive.	or	RR, 2Rr, rr 3 : 1 Normal disomic plants with 3:1 segregation ratio.

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