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Independence of the *mj* Nematode Resistance Gene from 17 Gene Loci in Cucumber

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Abstract. **Root knot caused by *Meloidogyne* spp. is an important disease of cucumber. Resistance to *M. javanica* in cucumber (*Cucumis sativus* L.) is conferred by the newly discovered *mj* gene. The objective of this research was to determine whether *mj* was linked to other genes controlling morphological or disease resistance traits in cucumber. Four inbred lines homozygous for *mj* (LJ 90430, 'Manteo', NCG-198, and NCG-199) were crossed with inbreds ('Coolgreen', M 21, NCG-101, WI 2757, and 'Wisconsin SMR 18') to form six families: NCG-101 x LJ 90430, WI 2757 x LJ 90430, NCG-199 x 'Wis. SMR 18', NCG-198 x M 21, 'Manteo' x M 21, and NCG-198 x 'Coolgreen'. F_2 progeny were evaluated in all families, and BC_1 progeny were evaluated only in the NCG-199 x 'Wis. SMR 18' family. *Meloidogyne javanica* resistance and the 17 other traits controlled by simple genes were evaluated in greenhouse or field tests. None of the 17 genes were linked with *mj*. Therefore, cucumber breeders interested in nematode resistance should be able to incorporate the trait into lines without having to break linkages with the 17 genes used in this study.**

Root knot caused by *Meloidogyne* spp. is an important disease of cucumber (*Cucumis sativus* var. *sativus* L.) in many production areas of the world (Netscher and Sikora, 1990). *Meloidogyne javanica* (Treub) Chitwood is a major problem in tropical areas. The use of resistant cultivars would be the most economical and environmentally acceptable method to control root knot. Resistance to *Meloidogyne javanica* was identified in LJ 90430, a line of *Cucumis sativus* var. *hardwickii* (Walters et al., 1993, 1996) and determined to be controlled by a single recessive gene, *mj* (Walters et al., 1997).

We know of no other references on linkage relationships of a gene for root-knot nematode resistance with other morphological or disease resistance genes in cucumber (Pierce and Wehner, 1990; Wehner, 1993). Identification of marker loci linked with the *mj* gene would permit marker-assisted selection, and identification of genes that would be difficult to recombine during selection would also be useful. The objective of this study was to determine the linkage relationships of the *mj* gene with several morphological and disease resistance loci in cucumber.

Materials and Methods

Crosses were made in the greenhouse using standard pollination techniques (Whitaker and Davis, 1962) between pairs of nine inbred lines to develop F_1, F_2, and BC_1 progeny in six families as follows: NCG-101 x LJ 90430, WI 2757 x LJ 90430, NCG-199 x 'Wis. SMR 18', NCG-198 x M 21, 'Manteo' x M 21, and NCG-198 x 'Coolgreen'. LJ 90430 is the only source of *M. javanica* resistance in cucumber (Walters et al., 1993). NCG-198 and NCG-199 were formed by backcrossing the *mj* gene into WI 2757 for five generations; 'Manteo' is resistant to *M. javanica* (Walters and Wehner, 1997). Most families were evaluated under greenhouse conditions, but all traits of NCG-199 x 'Wis. SMR 18' and NCG-198 x M 21 progeny were evaluated under field conditions at the Horticultural Crops Research Station, Clinton, N.C.

Progeny were evaluated for each of 17 traits controlled by single genes (Pierce and Wehner, 1990; Wehner, 1993) in each family where the parents differed. The traits evaluated were *B* (black spines), *B-3* (black spines-3), *B-4* (black spines-4), *Bt* (bitter fruit), *D* (dull fruit skin), *df* (delayed flowering), *de* (determinate habit), *F* (female sex expression), *lh* (long hypocotyl), *ns* (numerous spines), *pm-h* (powdery mildew resistance of the hypocotyl), *R* (red mature fruit), *ss* (small spines), *te* (tender fruit skin), *Tu* (tuberculate fruit), *u* (uniform immature fruit color), and *w* (white immature fruit color). Other commonly used marker traits contributed by WI 2757, such as bitterfree (*bi* gene), were not evaluated in this study.

Greenhouse evaluations. Plants were grown in 150-mm-diameter (1.8-L) clay pots on benches in a greenhouse. Two seeds were sown in each pot, which contained a steam-sterilized mixture of 1 sand : 1 soil (by volume; 85% sand, 10% silt, 5% clay). Plants were thinned to one per pot at the first true leaf stage. Root-knot nematode eggs were extracted from roots of 'Rutgers' tomato (*Lycopersicon esculentum* Mill.) for 4 min using a 1% NaOCl solution, according to the method of Byrd et al. (1972). Plants were inoculated at the third true leaf stage by pouring an aqueous suspension of 5000 eggs onto the soil and supplied N at 200 $mg \cdot kg^{-1}$ twice daily by drip irrigation. Greenhouse temperatures averaged 35 °C day/27 °C night.

Plants were rated for resistance 10 weeks after inoculation using the gall index system (0% to 100% of roots galled) of Barker et al. (1986), and then classified as resistant (≤35% of root system galled) or susceptible (>35% of root system galled) based upon previous research (Walters et al., 1997). F_2 progeny of NCG-101 x LJ 90430, WI 2757 x LJ 90430, 'Manteo' x M 21, and NCG-198 x 'Coolgreen' were evaluated for *M. javanica* resistance as well as for other single-gene traits segregating in each family.

The F_2 progeny of NCG-122 x NCG-126 were inoculated with the powdery mildew pathogen [*Sphaerotheca fuliginea* (Schlecht. Fr.)] at the five- to seven-leaf stage by dusting with spores from infected plants for three consecutive days in the greenhouse. Plants were classified as resistant if powdery mildew did not develop on the stems or leaf petioles over a period of 3 weeks. Gynoecy was evaluated in the progeny of several F_2 families. Plants with continuous pistillate flowers after the ninth node were classified as gynoecious. Plants without flowers up to the fifth node were classified as delayed flowering.

Field evaluations. Seeds of the parents, F_1, F_2, and BC_1 were planted in 100-mm-diameter (0.45-L) plastic pots containing the same soil mix described previously. Two families were evaluated: NCG-199 x NCG-127 and NCG-198 x M 21. Two seeds were sown in each pot and thinned to one at the first true leaf stage. Pots were inoculated with *M. javanica* as described previously. Pots containing inoculated plants were placed 61 cm apart in rows 107 cm apart. Plants were evaluated for single-gene traits twice a week. Pots were removed from the field and roots were evaluated for resistance using the gall index system described previously.

Goodness-of-fit of observed to expected segregation ratios in the F_2 and backcross progeny was determined by chi-square tests in an SAS program for linkage analysis, SASGene (Liu et al., 1997).

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Table 1. Segregation of progeny for the *mj* gene and other genes in the F_2 and BC_1 of six families of cucumber.[z]

Gene pair	Generation	No. plants per class (based on phenotype) A_B_	A_bb	aaB_	aabb	Total	Expected ratio	Chi-square	*P* value
				NCG-101 x LJ 90430					
mj—lh	F_2	83	30	26	14	153	9:3:3:1	2.5	0.47
mj—w	F_2	78	31	29	8	146	9:3:3:1	0.9	0.82
mj—Bt	F_2	79	34	28	5	146	9:3:3:1	3.6	0.30
				WI 2757 x LJ 90430					
mj—pm-h	F_2	78	34	32	10	154	9:3:3:1	2.1	0.54
mj—F	F_2	81	36	29	8	154	9:3:3:1	2.4	0.49
mj—df	F_2	85	31	25	13	154	9:3:3:1	1.9	0.59
mj—ns	F_2	75	35	30	7	147	9:3:3:1	3.5	0.32
mj—ss	F_2	72	33	33	9	147	9:3:3:1	3.5	0.31
mj—te	F_2	69	34	36	8	147	9:3:3:1	6.5	0.08
mj—Tu	F_2	81	34	24	8	147	9:3:3:1	2.2	0.54
mj—u	F_2	84	34	21	8	147	9:3:3:1	3.2	0.35
mj—D	F_2	82	32	23	10	147	9:3:3:1	1.5	0.67
				NCG-199 x 'Wis. SMR-18'					
mj—df	F_2	71	27	30	8	136	9:3:3:1	1.3	0.73
mj—F	F_2	85	30	23	5	143	9:3:3:1	2.9	0.43
mj—B	F_2	73	27	35	8	143	9:3:3:1	3.6	0.33
mj—D	F_2	77	25	31	10	143	9:3:3:1	1.2	0.75
mj—ns	F_2	81	28	27	7	143	9:3:3:1	0.5	0.93
mj—ss	F_2	83	29	25	6	143	9:3:3:1	1.3	0.73
mj—Tu	F_2	75	23	33	12	143	9:3:3:1	3.4	0.35
mj—u	F_2	77	25	31	10	143	9:3:3:1	1.1	0.79
mj—R	F_2	74	27	35	7	143	9:3:3:1	3.4	0.35
mj—df	BC_1(NCG-199)	11	12	12	12	47	1:1:1:1	0.1	0.99
mj—B	BC_1(NCG-199)	12	12	13	12	49	1:1:1:1	0.1	0.99
mj—D	BC_1(NCG-199)	11	12	14	12	49	1:1:1:1	0.4	0.95
mj—ns	BC_1(NCG-199)	14	15	11	9	49	1:1:1:1	1.9	0.61
mj—ss	BC_1(NCG-199)	11	10	14	14	49	1:1:1:1	1.0	0.79
mj—Tu	BC_1(NCG-199)	12	10	13	14	49	1:1:1:1	0.7	0.87
mj—u	BC_1(NCG-199)	16	14	9	10	49	1:1:1:1	2.7	0.46
mj—R	BC_1(NCG-199)	12	12	13	12	49	1:1:1:1	0.1	0.99
				'Manteo' x M 21					
mj—de	F_2	84	38	28	9	159	9:3:3:1	2. 8	0.45
				NCG-198 x M 21					
mj—df	F_2	76	24	26	9	135	9:3:3:1	0.1	0.98
mj—F	F_2	81	24	24	10	139	9:3:3:1	0.6	0.89
mj—B-3	F_2	83	28	22	5	138	9:3:3:1	2.7	0.44
mj—ns	F_2	74	23	31	10	138	9:3:3:1	1.7	0.63
mj—ss	F_2	75	19	30	14	138	9:3:3:1	5.9	0.11
mj—Bt	F_2	81	25	23	8	137	9:3:3:1	0.5	0.91
mj—de	F_2	72	23	30	11	136	9:3:3:1	2.0	0.56
				NCG-198 x 'Coolgreen'					
mj—u	F_2	60	25	26	9	120	9:3:3:1	2.0	0.59
mj—Tu	F_2	68	25	18	9	120	9:3:3:1	1.5	0.70
mj—B-3	F_2	69	22	17	12	120	9:3:3:1	4.2	0.25
mj—Bt	F_2	59	24	27	10	120	9:3:3:1	2.9	0.43

[z]A_B_ denotes dominant expression for both genes; A_bb denotes dominant expression for the first gene and recessive for the second; aaB_ denotes dominant expression for the first gene and recessive for the second; aabb denotes recessive expression for both genes. The genes listed are: *B* (black spines), *B-3* (black spines-3), *B-4* (black spines-4), *Bt* (bitter fruit), *D* (dull fruit skin), *df* (delayed flowering), *de* (determinate habit), *F* (female sex expression), *lh* (long hypocotyl), *mj* (*Meloidogyne javanica* resistance), *ns* (numerous spines), *pm-h* (powdery mildew resistance of the hypocotyl), *R* (red mature fruit), *ss* (small spines), *te* (tender fruit skin), *Tu* (tuberculate fruit), *u* (uniform immature fruit color), and *w* (white immature fruit color).

Results and Discussion

The analysis of six F_2 families and one BC_1 family indicated no genetic linkage between the gene for *Meloidogyne javanica* resistance (*mj*) and the 17 morphological and disease resistance genes in cucumber (Table 1).

All traits were inherited as single genes except for spine color in two crosses: NCG-101 x LJ 90430 and WI 2757 x LJ 90430. Spine color in those two families fit a ratio of 9 black spine : 7 white spine, with 27 black spine/*M. javanica* susceptible : 21 white spine/*M. javanica* susceptible : 9 black spine/*M. javanica* resistant : 7 white spine/*M. javanica* resistant (data not shown). The fruit bitterness of the WI 2757 x LJ 90430 cross also fit a ratio of 9 bitter : 7 nonbitter, with 27 bitter/*M. javanica* susceptible : 21 nonbitter/*M. javanica* susceptible : 9 bitter/*M. javanica* resistant : 7 nonbitter/*M. javanica* resistant (data not shown). Cowen and Helsel (1983) reported that black spine in LJ 90430 was controlled by two dominant genes, *B-3* and *B-4*. The *Bt* gene was inherited as a single gene when *bi* was not segregating in the cross (Table 1). However, when *bi* and *Bt* were in the same genetic background, *Bt* was inherited in a 9:7 ratio. This interaction has not been reported previously. The *Bt* gene was segregating in a 3:1 ratio when the *bi* gene was not present in the genetic background (F_2 progeny of NCG-101 x LJ 90430, NCG-198 x M 21, and NCG-198 x 'Coolgreen'), indicating interaction.

Since no linkages were found between *mj* and the 17 genes in this study, it may not be located in linkage groups I, III, IV, or V involving those genes (Pierce and Wehner, 1990). The genes *B-3* or *B-4*, *df*, *de*, and *F* are part of linkage group I, and linkage group III consists partially of the genes *D*, *ns*, *pm-h*, *ss*, *te*, *Tu*, and *u*. However, one end of linkage group III consists of several disease resistance genes that were not evaluated in this study, and *mj* might be in that region. The genes *B* and R are in linkage group IV, and *lh* is in linkage group V. The locations of *w* and *Bt* have not been determined, although *w* is independent of *sp* (short petiole), *bi*

(bitterfree), and *B* (black spines) (Wehner and Liu, 1997), and *Bt* is independent of *F*, and *B-3* and *B-4* (Cowen and Helsel, 1983).

Cucumber breeders should be able to incorporate the *mj* gene into elite inbred lines, since there are no known linkages with traits of interest. Further studies should focus on the relationships of the *mj* gene with genes on other linkage groups not described in this study, as well as a search for molecular markers that are linked with *mj* that could be used in screening seedlings for nematode resistance.

Literature Cited

Barker, K.R., J.L. Townshend, G.W. Bird, I.J. Thomason, and D.W. Dickson. 1986. Determining nematode population responses to control agents, p. 283–287. In: K.D. Hickey (ed.). Methods for developing pesticides for control of plant pathogens, APS Press, St. Paul, Minn.

Byrd, D.W., Jr., H. Ferris, and C.J. Nusbaum. 1972. A method for estimating numbers of eggs of *Meloidogyne* spp. in soil. J. Nematol. 4:266–269.

Cowen, N.M. and D.B. Helsel. 1983. Inheritance for spine color and linkage in a cucumber cross. J. Hered. 74:308–309.

Liu, J.S., T.C. Wehner, and S.B. Donaghy. 1997. SASGENE: A SAS computer program for genetic analysis of gene segregation and linkage. J. Hered. 88:253–254.

Netscher, C. and R.A. Sikora. 1990. Nematode parasites of vegetables, p. 237–283. In: M. Luc, R.A. Sikora, and J. Bridge (eds.). Plant parasitic nematodes in subtropical and tropical agriculture. CAB Intl. Wallingford, U.K.

Pierce, L.K. and T.C. Wehner. 1990. Review of genes and linkage groups in cucumber. HortScience 25:605–615.

Walters, S.A., T.C. Wehner, and K.R. Barker. 1993. Root-knot nematode resistance in cucumber and horned cucumber. HortScience 28:151–154.

Walters, S.A., T.C. Wehner, and K.R. Barker. 1996. NC-42 and NC-43: Root-knot nematode-resistant cucumber germplasm. HortScience 31:1246–1247.

Walters, S.A., T.C. Wehner, and K.R. Barker. 1997. A single recessive gene conditioning resistance to *Meloidogyne javanica* in cucumber. J. Hered. 88:66–69.

Walters, S.A. and T.C. Wehner. 1997. 'Lucia', 'Manteo', and 'Shelby' root-knot nematode–resistant cucumber inbred lines. HortScience 32:1301–1303.

Wehner, T.C. 1993. Gene list update for cucumber. Cucurbit Genet. Coop. Rpt. 16:92–97.

Wehner, T.C. and J.S. Liu. 1997. Independent segregation among 11 gene loci in cucumber. Cucurbit Genet. Coop. Rpt. 20:1–2.

Whitaker, T.W. and G.N. Davis. 1962. Cucurbits. Leonard Hill, London.