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 (IJ 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 IJ 90430, WI 2757 x IJ 90430, NCG-199 x ‘Wis. SMR 18’, NCG-198 x M 21, ‘Manteo’ x M 21, and NCG-198 x ‘Coolgreen’. F1 progeny were evaluated in all families, and BC1 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 IJ 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 F1, F2, and BC1 progeny in six families as follows: NCG-101 x IJ 90430, WI 2757 x IJ 90430, NCG-199 x ‘Wis. SMR 18’, NCG-198 x M 21, ‘Manteo’ x M 21, and NCG-198 x ‘Coolgreen’. IJ 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), BI (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), T (tender fruit skin), Tu (tuberous fruit), u (uniform immature fruit color), and w (white immature fruit color). Other commonly used marker traits contributed by WI 2757, such as bitterfree (bf 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% NaCl 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-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 (<5% of root system galled) or susceptible (>5% of root system galled) based upon previous research (Walters et al., 1997). F1 progeny of NCG-101 x IJ 90430, WI 2757 x IJ 90430, ‘Manteo’ x M 21, and NCG-198 x ‘Coolgreen’ were evaluated for M. javanica resistance as well as for other single-locus traits segregating in each family.

The F2 progeny of NCG-122 x NCG-126 were inoculated with the powdery mildew pathogen [Sphaerotorhca 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 F2 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, F1, F2, and BC1, 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-locus 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 F1 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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<tr>
<th>Gene pair</th>
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<th>No. plants per class (based on phenotype)</th>
<th>Expected ratio</th>
<th>Chi-square</th>
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<td>mj–lh</td>
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| mj–df | F<sub>2</sub> | A<sub>B</sub> 71 A<sub>bb</sub> 27 aA<sub>B</sub> 30 aabb 8 | 136            |            |        |
| mj–F  | F<sub>2</sub> | A<sub>B</sub> 85 A<sub>bb</sub> 30 aA<sub>B</sub> 23 aabb 5 | 143            |            |        |
| mj–B  | F<sub>2</sub> | A<sub>B</sub> 73 A<sub>bb</sub> 27 aA<sub>B</sub> 35 aabb 8 | 143            |            |        |
| mj–D  | F<sub>2</sub> | A<sub>B</sub> 77 A<sub>bb</sub> 25 aA<sub>B</sub> 31 aabb 10 | 143            |            |        |
| mj–ns  | F<sub>2</sub> | A<sub>B</sub> 81 A<sub>bb</sub> 28 aA<sub>B</sub> 27 aabb 7 | 143            |            |        |
| mj–ss  | F<sub>2</sub> | A<sub>B</sub> 83 A<sub>bb</sub> 29 aA<sub>B</sub> 25 aabb 6 | 143            |            |        |
| mj–Tu  | F<sub>2</sub> | A<sub>B</sub> 75 A<sub>bb</sub> 23 aA<sub>B</sub> 33 aabb 12 | 143            |            |        |
| mj–u  | F<sub>2</sub> | A<sub>B</sub> 77 A<sub>bb</sub> 25 aA<sub>B</sub> 31 aabb 10 | 143            |            |        |
| mj–R  | F<sub>2</sub> | A<sub>B</sub> 74 A<sub>bb</sub> 27 aA<sub>B</sub> 35 aabb 7 | 143            |            |        |

| mj–df | BC<sub>1</sub>(NCG-199) | A<sub>B</sub> 11 A<sub>bb</sub> 12 aA<sub>B</sub> 12 aabb 12 | 47             |            |        |
| mj–B  | BC<sub>1</sub>(NCG-199) | A<sub>B</sub> 12 A<sub>bb</sub> 13 aA<sub>B</sub> 12 aabb 49 | 49             |            |        |
| mj–D  | BC<sub>1</sub>(NCG-199) | A<sub>B</sub> 11 A<sub>bb</sub> 12 aA<sub>B</sub> 14 aabb 49 | 49             |            |        |
| mj–ns  | BC<sub>1</sub>(NCG-199) | A<sub>B</sub> 14 A<sub>bb</sub> 15 aA<sub>B</sub> 11 aabb 9 | 49             |            |        |
| mj–ss  | BC<sub>1</sub>(NCG-199) | A<sub>B</sub> 11 A<sub>bb</sub> 10 aA<sub>B</sub> 14 aabb 49 | 49             |            |        |
| mj–Tu  | BC<sub>1</sub>(NCG-199) | A<sub>B</sub> 12 A<sub>bb</sub> 10 aA<sub>B</sub> 13 aabb 49 | 49             |            |        |
| mj–u  | BC<sub>1</sub>(NCG-199) | A<sub>B</sub> 16 A<sub>bb</sub> 14 aA<sub>B</sub> 9 aabb 10 | 49             |            |        |
| mj–R  | BC<sub>1</sub>(NCG-199) | A<sub>B</sub> 12 A<sub>bb</sub> 12 aA<sub>B</sub> 13 aabb 49 | 49             |            |        |

| mj–de | F<sub>2</sub> | A<sub>B</sub> 84 A<sub>bb</sub> 38 aA<sub>B</sub> 28 aabb 9 | 159            |            |        |

Results and Discussion

The analysis of six F<sub>2</sub> families and one BC<sub>1</sub> family indicated no genetic linkage between the genes 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 the two families fit a ratio of 9 black spine: 7 white spine, with 27 black spine/M. javanica susceptible: 21 white spine/M. javanica resistant: 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 9:2:1, with 27 bitter/M. javanica susceptible: 21 nonbitter/M. javanica susceptible: 9 bitter/M. javanica resistant: 7 nonbitter/M. javanica resistant (data not shown). Coven 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<sub>2</sub> 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 Bt are in linkage group IV, and Bt 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 Br is independent of F,
and B-3 and B-4 (Cowen and Helsel, 1983).
Cucumber breeders should be able to in-
corporate 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 mark-
ers that are linked with mj that could be used in
screening seedlings for nematode resistance.

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