

## Effect of the *Mi* Gene in Tomato on Reproductive Factors of *Meloidogyne chitwoodi* and *M. hapla*

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**Abstract:** The effect of the *Mi* gene on the reproductive factor of *Meloidogyne chitwoodi* and *M. hapla*, major nematode pests of potato, was measured on nearly isogenic tomato lines differing in presence or absence of the *Mi* gene. The *Mi* allele controlled resistance to reproduction of race 1 of *M. chitwoodi* and to one of two isolates of race 2. No resistance to race 3 of *M. chitwoodi* or to *M. hapla* was found. Variability in response to isolates of race 2 may reflect diversity of virulence genotypes heretofore undetected. Resistance to race 1 of *M. chitwoodi* could be useful in potato if the *Mi* gene were functional following transference by gene insertion technology into potato. Since the *Mi* gene is not superior to *R<sub>Mci</sub>* derived from *Solanum bulbocastanum*, the transference by protoplast fusion appears to offer no advantage.

**Key words:** Columbia root-knot nematode, isogenic lines, *Meloidogyne chitwoodi*, *Meloidogyne hapla*, nematode, northern root-knot nematode, potato, reproductive factor, resistance, tomato.

Root-knot nematodes (*Meloidogyne* spp.) are important pathogens of tomato (*Lycopersicon esculentum*). A source of resistance to several *Meloidogyne* species was discovered in an accession of the wild tomato *Lycopersicon peruvianum* (Bailey, 1941), which upon further characterization was found to be controlled by a single dominant factor (Gilbert and McGuire, 1956). This gene was denoted as *Mi* and mapped to a location on chromosome 6 (Medina Filho, 1980; Rick and Fobes, 1974). After introgression into advanced breeding lines, the *Mi* gene became widespread in modern-day cultivars, and is considered to be a necessary component for root-knot nematode control in many tomato-growing areas (Medina Filho and Stevens, 1980). Interest in the transfer of genes from tomato to potato has increased with the successful somatic fusion of the two species, successful backcrossing of the fusion product with potato as a recurrent parent, and the recovery of acceptable potato horticultural type (Jacobsen et al., 1994). Also, substantial progress has been made toward cloning the *Mi* gene from tomato (Williamson et al., 1994). Once the *Mi* is cloned it may be possible to transfer the

gene to tomato or potato via *Agrobacterium* transformation (Williamson et al., 1992).

With these possibilities it has become relevant to study the host suitability of isogenic tomatoes differing in *Mi* gene composition to two important *Meloidogyne* pests of potato. The purpose of this study was to assess the effectiveness of the *Mi* gene across races of *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley and to *M. hapla* Chitwood, both of which are pathogenic to potato and tomato. To accomplish this, a pair of nearly isogenic lines differing in presence or absence of *Mi* and a standard susceptible cultivar, 'Columbia,' were tested for host suitability with three races of *M. chitwoodi* and with *M. hapla*.

### MATERIALS AND METHODS

Sun 6082 (*Mi/Mi*) and Castlerock II (*mi/mi*), a nearly isogenic pair of tomato lines that differ in alleles at the *Mi* locus, originally were obtained from Sunseeds (Hollister, CA), and self seeds were produced in the Department of Nematology, University of California, Davis, CA. *Meloidogyne chitwoodi* race 1, isolate WAMc1, two separate isolates of race 2, ORMc8 and WAMc30 (Mojtahedi et al., 1988), one of race 3, CAMc2, and an isolate of *M. hapla* denoted as WAMh were maintained at the Washington State University Irrigated Agriculture Research and Extension Center, Prosser, Washington. All isolates of *M. chitwoodi* were first multiplied on wheat to prevent con-

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tamination by *M. hapla*, for which wheat is a non-host. *Meloidogyne chitwoodi* race 1 was then cultured on carrot (*Daucus carota* cv. Red Core Chantenay), race 2 was propagated on alfalfa (*Medicago sativa* cv. Thor) (Mojtahedi et al., 1988), and race 3 on a seedling of *Solanum bulbocastanum* selected from plant introduction accession 285187 (Brown, et al., 1989; Mojtahedi et al., 1994). *M. hapla* was cultured on pepper (*Capsicum annuum* cv. California Wonder). After propagative cycles on these differentials, all isolates were multiplied on tomato cv. Columbia.

Tomato seedlings, approximately 7 cm tall, were transplanted into 15-cm clay pots containing loamy sand soil (84% sand, 10% silt, 6% clay) previously fumigated with methyl bromide. At transplanting, 5,000 eggs in water were pipetted into holes made in the soil around the root system of each of three to five replicate pots per tomato genotype-nematode isolate combination. The pots were arranged in a completely randomized design on greenhouse tables. Plants were grown with regular watering and fertilization with slow-release pellets (Osmocote 14-14-14, Scotts, Marysville, OH) at  $24 \pm 3$  °C for 55 days, at which time eggs were extracted and counted (Hussey and Barker, 1973). Reproductive factor [ $R_f$  = final population ( $P_f$ )/initial population ( $P_i$ )] depends on the fecundity of a proportion of the primary inoculum and of intermediate generations that successfully infect the host and reach reproductive maturity.  $R_f$  is one measure of resistance of a plant species to *Meloidogyne* spp. (Oostenbrink, 1966, Sasser et al., 1984). This measurement was chosen as the primary resistance parameter in the screening. Host status was divided into three categories on the basis of  $R_f$  values as follows:  $R_f \geq 1.0$ , suitable host;  $0.1 < R_f < 1.0$ , poor host;  $R_f \leq 0.1$ , non-host (Sasser et al., 1984). A one-way analysis of variance was performed on the  $\ln(x + 1)$  transformed egg counts.  $R_f$  values were calculated from geometric means. Means were separated with Duncan's multiple range test ( $P < 0.05$ ) (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

The observation that the *Mi* gene does not control resistance to *M. hapla* (Medina Filho and Stevens, 1980) was confirmed in this study. The  $R_f$  values of *M. hapla* for *Mi*, *mi* and 'Columbia' were 15.3, 50.5, and 25.9, respectively, which indicates a "good host" status for all host genotypes. In contrast, the host suitability for races of *M. chitwoodi* represented a complex response (Fig. 1). The tomato line harboring *Mi* clearly expressed resistance to race 1 (isolate WAMc1) compared with *mi*, as evidenced by statistically different  $R_f$  values ( $R_f = 0.11$  vs.  $R_f = 1.21$ ). However, the two isolates of race 2 ORMc8 and WAMc30, reacted differently from each other. Reproduction of ORMc8 was poor ( $R_f = 0.33$ ) on the *Mi* isolate but was higher ( $R_f = 1.64$ ) on the *mi* isolate. In contrast, the  $R_f$  values on *Mi* (0.74) and *mi* (1.03) isolines were not significantly different when challenged with WAMc30. Although the  $R_f$  values of *Mi*-bearing plants fell into a poor host category while *mi* plants lay in the good host range when challenged by WAMc30, the lack of significance means that we cannot conclude that *Mi* and *mi* are different when challenged with this isolate of race 2. Race 3 was found to be a resistance-breaking type, showing "good host" responses on both *Mi* and *mi* isolines, although the  $R_f$  value was significantly lower on plants with *Mi* than on those without this gene. The  $R_f$  values of all *M. chitwoodi* races were in the "good host" category on Columbia tomato. The higher values on Columbia tomato may also indicate that both near-isogenic lines had additional resistance to the nematodes tested here, attributable to their different genetic contents.

The *Mi* gene appears to confer sufficient resistance to multiplication of *M. chitwoodi* race 1 that it might be useful if transferred into potato. It may provide protection equal to that of the  $R_{Mc1}$  gene derived from the wild species *Solanum bulbocastanum* (Brown et al., 1996). In such a circumstance, the main interest for potato improvement would rest in the potential availability of a

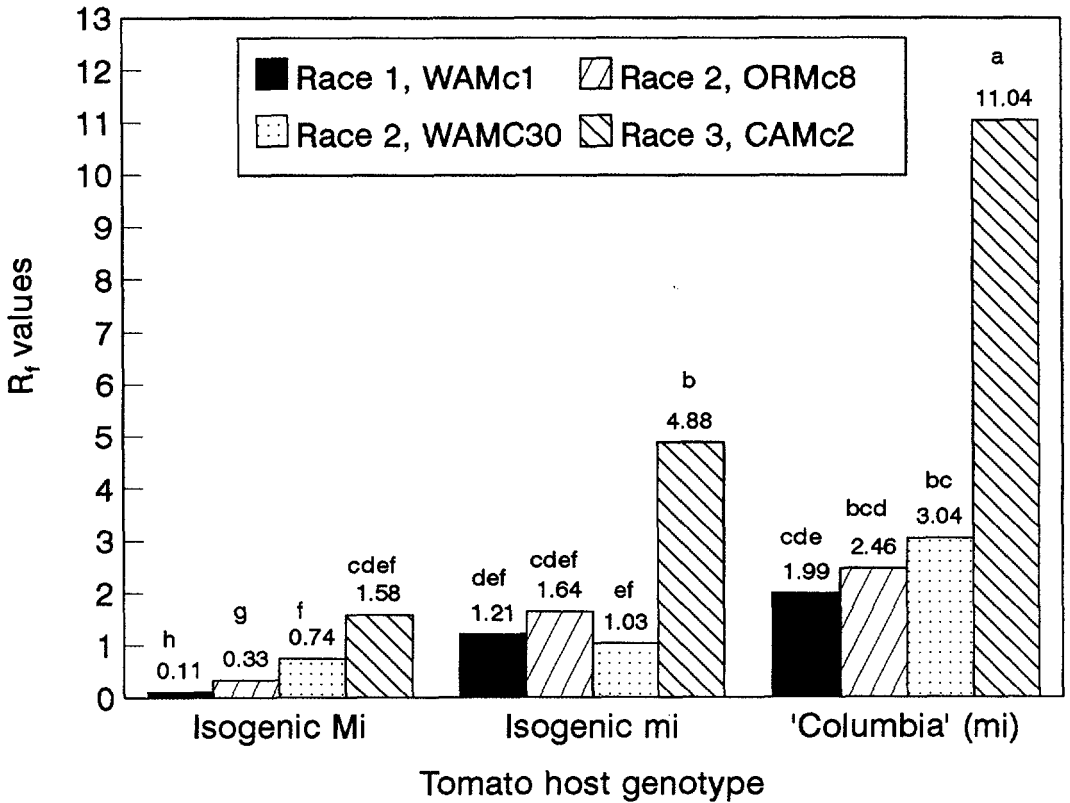


FIG. 1.  $R_f$  values of different races of *M. chitwoodi* on tomato lines Sun 6082 (isogenic *Mi*), Castlerock II (isogenic *mi*), and the cultivar Columbia, an *mi* genotype.  $R_f$  values are shown at the top of each bar; values not sharing the same letter are statistically different at  $P < 0.05$  according to Duncan's multiple-range test.  $R_f$  values are geometric means of 3 to 5 replicates.

cloned *Mi* gene for transformation of susceptible potato. Resistance to race 2 is less clear-cut, evidenced by differing results in the examination of two different isolates. These results suggest that the race designations of *M. chitwoodi* cannot be used to predict a response to the *Mi* gene. This distinction is not unique, as variability within races of root-knot nematodes is common when new differential hosts are tested (Roberts, 1995). As with the resistance to *M. chitwoodi* derived from *Solanum bulbocastanum* (Mojtahedi, et al., 1994; Brown et al., 1996), it appears that race 3 definitively overcomes the *Mi* gene. However, a statistically lower  $R_f$  value on *Mi* plants than on *mi* plants ( $R_f = 1.58$  vs.  $R_f = 4.88$ ) suggests that this gene provides partial protection and may be useful in combination with other resistance factors.

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