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Inheritance and Mapping of *Mj-2*, a New Source of Root-knot Nematode (*Meloidogyne javanica*) Resistance in Carrot

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Root-knot nematodes limit carrot production around the world by inducing taproot forking and galling deformities that render carrots unmarketable. In warmer climates, *Meloidogyne javanica* and *Meloidogyne incognita* are most prevalent. In F_2 and F_3 progeny from the cross between an Asian carrot resistant to *M. javanica*, PI 652188, and a susceptible carrot, resistance response was incompletely dominant with a relatively high heritability (H^2 = 0.78) and provided evidence for a single gene, designated *Mj-2*, contributing to resistance. Molecular markers linked to the previously described root-knot nematode resistance gene, *Mj-1* on chromosome 8 derived from "Brasilia," demonstrated that *Mj-2* does not map to that same locus but is on the same chromosome.

Key words: *Apiaceae,* Daucus carota, *molecular markers, SSRs*

Root-knot nematodes (*Meloidogyne* spp.) are serious root parasites of carrot, which adversely affect yield and quality of the crop around the world (Roberts and Mullens 2002). Among *Meloidogyne* species, *Meloidogyne javanica* and *Meloidogyne incognita* are most common and found in carrot production fields of tropical and subtropical regions (Peterson and Simon 1986; Stein and Nothnagel 1995). Root-knot nematodes infect behind root tips and at lateral root initiation sites, causing galling on lateral roots and galling and forking disfiguration of the tap root. Given the demand for high quality and lack of visible "cosmetic injury," even minor tap root damage results in severe economic losses. Control of the pathogen is difficult due to its persistence in soil, broad host range, and limited availability of environmentally safe nematicides (Hutchinson et al. 1999). One of the only reliable methods to manage nematode damage is the introduction of new resistant varieties. Conventional breeding protocols for developing root-knot nematode resistance are time consuming and labor intensive, often including both greenhouse and extensive field evaluations for phenotype. Resistance to *M. javanica* has been reported in "Brasilia" and found to be conditioned by 1 dominant factor, designated *Mj-1* (Simon et al. 2000). The *Mj-1* locus from Brasilia has been tagged with molecular markers (Boiteux et al. 2000) including high-resolution flanking sequence tagged site (STS) markers used in marker-assisted selection (Boiteux et al. 2004).

The discovery of *Mj-1* in "Brasilia" triggered a search for additional sources of resistance from a broad-based collection of carrot germplasm. In this communication, we describe a second source of resistance to *M. javanica* that is derived from Asian germplasm.

Materials and Methods

Segregating populations employed in this study were derived from a cross between a plant in an F_3 family derived from the (PI 652188 × B7262) cross that was true breeding for resistance to both *M. incognita* and *M. javanica*. This F_3 plant was used as the female parent and intercrossed with a nematode susceptible plant as the male parent. PI 652188 is a purple-rooted Asian cultivar designated "Ping Ding" in China and the ultimate source of the resistance in the resistant female

parent, because B7262 is also nematode susceptible. An individual F_1 plant from this cross was self-pollinated to produce the F_2 generation. In the F_2, 183 individuals were evaluated with *M. javanica* and 20 of the *M. javanica* screened F_2 plants were advanced by selfing for evaluation of resistance in F_3 families. For this, after resistance phenotype evaluation, the *M. javanica* screened F_2 roots were vernalized (4 °C for 40 days) and planted in the greenhouse at the University of Wisconsin-Madison, and self-pollinated in isolation for production of $F_{2:3}$ families. The F_3 family sizes evaluated for nematode resistance ranged from 17 to 42 individuals. In the F_2, 194 other individuals were also evaluated with *M. incognita* and F_3 families derived from *M. javanica* screened F_3 plants were also evaluated *M. incognita* resistance.

The parent lines, F_1, F_2, and F_3 populations, were evaluated for *M. javanica* and *M. incognita* resistance reaction under greenhouse conditions at University of California, Riverside, CA. Resistance screening was carried out using individual carrot seedlings cultivated in pots as described by Simon et al. (2000). Fibrous roots of individual plants were scored from 0 (no galls) to 8 (severely galled), using a modified version of the root-knot rating chart of Bridge and Page (1980). The resulting 0–8 scale was used for hypothesis testing. The F_1 and F_2 generations were also evaluated at the University of California, Kearney Agricultural Center on a field site uniformly infested with *M. javanica*. Nematode resistance scores were converted to an incomplete dominance scale in the F_2 and all F_3 families, designating plants with scores of 0–3.5 as "A," 4.0–5.5 as "H" (heterozygous), and 6.0–8.0 as "B."

DNA was extracted from lyophilized leaves of 12 F_3 families segregating for resistance as described by Boiteux et al. (2000). DNA concentrations were estimated in 1% agarose gels and adjusted to 5.0 ng/μL. Random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) molecular markers were evaluated. For initial screening of primers by bulked segregant analysis, only F_2 and F_3 plants with a score of 0 were included in the resistant (R) bulk and only susceptible F_2 and F_3 plants with scores of 7 or 8 were included in the susceptible (S) bulk. DNA pools (bulks) were prepared by mixing equal amounts of total DNA of selected plants within each phenotypic group.

SSR markers (Cavagnaro et al. 2011; Iorizzo et al. 2011) were screened in both parents as well as in R and S bulks, and potentially useful polymorphisms were further characterized in F_3 families. For SSR evaluation, polymerase chain reaction conditions and fragment analysis by either agarose gel electrophoresis (for larger size differences) or separation of fluorescently labeled amplicons through an ABI 3730xl capillary sequencer were performed as described previously (Cavagnaro et al. 2011).

To identify RAPD segregation polymorphism, STS primers for RAPD Q1-850 identified as tightly linked to *Mj-1* by Boiteux et al. (2004), and 10-mer RAPD primers (AA to AX from Operon Technologies, Alameda, CA) were evaluated in 2 replicates using as template DNA samples from both parents as well as R and S bulks. Potentially useful polymorphisms were characterized further on each of the individuals from the F_3 lines that comprised the respective R and S bulks. Polymerase chain reaction was performed and amplicons scored as described by Boiteux et al. (2000, 2004). Markers were named by their primer designation.

The genotypes of codominant markers were recorded as "A" (homozygous maternal), "B" (homozygous paternal), and "H" (heterozygous), whereas dominant markers were scored as "A" and "C" (or "B" and "D") for absence and presence of a band, respectively. Marker segregation was evaluated in F_3 families segregating for resistance with the chi-square method to test expected ratios. For codominant molecular markers, segregation was tested against the expected 1:2:1 ratio for an F_2, and for dominant markers, segregation was tested against an expected 3:1 ratio. Those markers exhibiting significant segregation distortion ($P < 0.01$) were excluded from linkage analysis. For dominant RAPD markers, data codes 1 and 0 were transformed to A and C genotype codes according to presence or absence of the resistant or susceptible parent fragment, respectively. The mapping software MAPMAKER v. 3.0 (Lander et al. 1987) was used for linkage analysis. Linkage groups were obtained using a minimum log likelihood ratio or logarithm of the odds (LOD) score of 3.0 and a maximum recombination frequency of 0.3. Kosambi's mapping function (Kosambi 1944) was used to convert recombination frequencies to map distances in centiMorgans (cM). Linkages of <30 cM were included. The software program MapChart v. 2.2 (Voorrips 2002) was used to draw the genetic linkage map.

Broad sense heritability (H^2) was estimated based on parent (F_2 plant)–offspring (F_3 family mean) correlations as described by Frey and Horner (1957) and Nyquist (1991).

Results and Discussion

Resistance to *M. javanica* from the Asian carrot PI 652188 revealed an incompletely dominant pattern of inheritance in the F_2 population derived from a resistant × susceptible cross, as well as in F_3 families derived from this cross (Figure 1). This is in contrast to *Mj-1* from "Brasilia" where *M. javanica* resistance is nearly completely dominant (Simon et al. 2000). The parent–offspring (F_2–F_3) regression value was 0.78 indicating relatively high heritability. The *M. javanica* resistance score for the F_1 generation when evaluated in 3 screening tests in the infested field ranged from 5.0 to 7.0 and scores of the F_2 population ranged from 2.0 to 6.0 (mean score of 5.0) similar to the pattern of resistance segregation observed in the greenhouse test (Figure 1).

Intrafamily variation was broad in the F_2 and most F_3 families. Three F_3 families were more uniformly resistant, with family means ranging from 1.8 to 3.9 and resistance scores among individuals ranging no higher than 5.0 (standard deviation [SD] = ±<1) (e.g., C-162, Figure 1). Four F_3 families were more uniformly susceptible, with family means ranging from 5.0 to 6.8 and resistance scores typically ranging no lower than 3.0 (e.g., C-102, Figure 1). However, only 1 of these 4 families (C-141, not shown) was uniformly susceptible, the other three (e.g., C-102, Figure 1) having some individuals expressing moderate resistance. In contrast to these more uniformly resistant and susceptible families, the

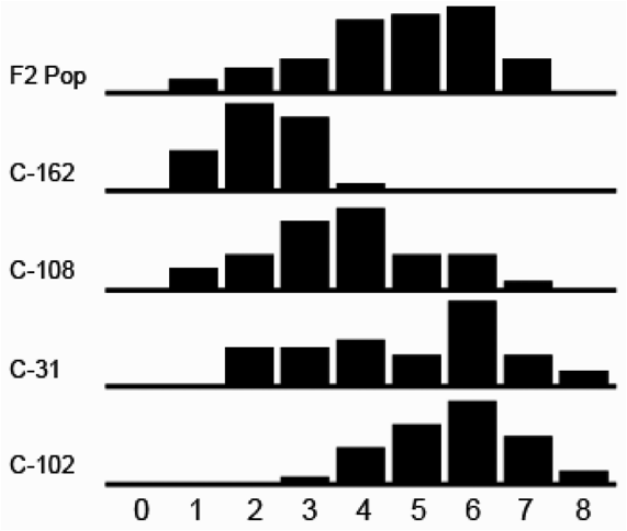

Figure 1. Resistance to *Meloidogyne javanica* in a resistant (PI 652188) × susceptible F_2 population (top) and selected F_3 families (C-162, C-108, C-31, C-102, below). Individual plants were scored from 0 (no galls) to 8 (>88% galling). Bar height indicates relative number of plants in each resistance rating. The F_2 scores of F_3 families C-162, C-108, C-31, and C-102 were 1, 3, 5, and 6, respectively.

12 remaining F_3 families had nematode resistance ratings with family means that ranged more widely (from 2.5 to 6.0; SD from ±1.02 to ± 2.15) (e.g., C-31 and C-108, Figure 1). By assigning A_1A_1, A_1A_2, and A_2A_2 genotypes to plants with nematode resistance scores of 0–3.5, 4.0–5.5, and 6.0–8.0, respectively, a monogenic inheritance model of incomplete dominance was confirmed in 9 of these 12 families ($P > 0.95$ for 1:2:1 ratio). The wide range of scores among plants in a given category of resistance suggests possible interactions of this major resistance locus with other genes that may affect the resistance response. In addition, nongenetic variables encountered in evaluating nematode resistance may have influenced phenotypes.

Given these results, this study led us to the hypothesis that a single major incompletely dominant gene acting together with modifier genes conditions resistance to *M. javanica* in derivatives of PI 652188. Based on this hypothesis, we utilized the genotypes assigned with this approach to map the primary nematode resistance locus and in particular to determine whether the new nematode resistance from PI 652188 is allelic to *Mj-1*. To that end, we evaluated SSR, sequence characterized amplified region (SCAR), and RAPD markers in segregating F_3 families (e.g., C-31 and C-108, Figure 1). The new resistance mapped to chromosome 8, which also carries *Mj-1*. A total of 6 SSRs, the Q1-850 SCAR located 0.1 cM from *Mj-1* (Boiteux et al. 2004), and SCAR 21A fit expected ratios and were mapped to chromosome 8 (Figure 2). Linkages were observed among markers previously found to be linked, including Q1-850, thereby validating the marker relationships in this genetic cross. The new source of root-knot nematode resistance

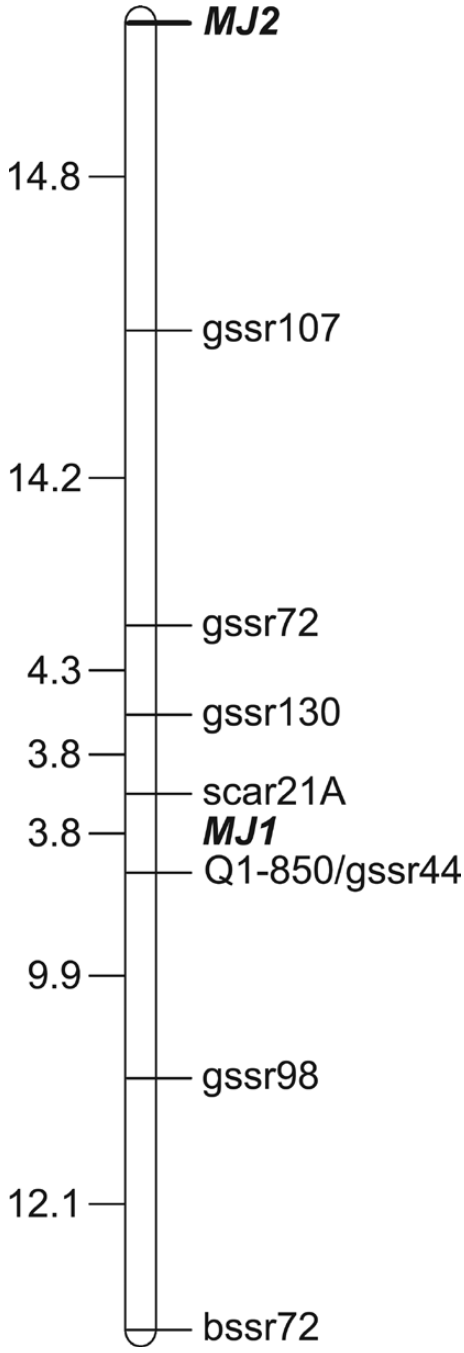

Figure 2. Linkage relationships among *Mj-2*, a new source of resistance to the root-knot nematode *Meloidogyne javanica*, SCARs Q1-850 and scar21A flanking *Mj-1*, and 6 SSRs on chromosome 8 of carrot as determined in segregating F3 families derived from a resistant (PI 652188) × susceptible F2 population. Distances are indicated in centiMorgans on the left with marker names on the right. The linkage map was oriented based on common markers with the carrot reference map (Cavagnaro et al. 2011).

in this study was ~41 cM from *Mj-1* and we propose the gene symbol *Mj-2* be used to designate this new source of resistance. To determine the contribution of genes beyond

Mj-2 that account for the observed variation in resistance scores in the PI 652188 derivatives, a genome-wide analysis of more polymorphic markers in larger families will be necessary. The complete linkage between gssr44 and Q1/850, a SCAR tightly linked to *Mj-1* in a Brasilia derivative F_2 (Boiteux et al. 2000), suggests that gssr44 can be used as an additional marker for assisting selection for *Mj-1* resistance in backgrounds where Q1/850 is monomorphic. Marker was highly polymorphic across several carrot F_2 populations (Cavagnaro et al. 2011).

The identification of an additional *M. javanica* resistance gene, *Mj-2*, sets the stage for combining this resistance with *Mj-1* to determine their combined effects in conferring resistance to root-knot nematodes. In addition, inbred lines derived from PI 652188 revealed *M. incognita* resistance scores as low as 1 in recent field testing, in comparison to scores of 6–8 in susceptible control cultivar "Imperator 58." In 2 greenhouse tests, mean root scores of this parental line to *M. javanica* were 1.2 and 3.3 (susceptible control was scored as 5.5 and 8.0, respectively, in 2 tests), whereas with *M. incognita*, mean root score was less ($P > 0.01$) for this line (6.5) than on the susceptible control (7.5). In field tests, the PI 652188–derived parental line mean score with *M. javanica* was 0 (susceptible control score 5.0) and with *M. incognita* was 3.0 (susceptible control score 7.0). A score of 3.0 represents a moderate level of resistance in these tests. Additional evidence for the resistance to *M. javanica* in the PI 652188–derived parental line also conferring resistance to *M. incognita* was found in the positive correlation ($y = 2.36 + 0.47x$; $P = 0.024$) between the *M. javanica* single root scores of the PI 652188–derived parental line and the *M. incognita* mean scores of families derived from those single roots. Whether *Mj-1*/__ *Mj-2R*/*Mj-2R* plants have improved levels and durability of resistance to the diverse range of root-knot nematode species attacking carrots remains an interesting question that is being pursued.

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