Characterization of Watermelon (Citrullus lanatus var. citroides) Germplasm for Resistance to Root-knot Nematodes

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Abstract. Root-knot nematodes (Meloidogyne spp.) cause extensive damage to watermelon [Citrullus lanatus (Thunb.) Matsum. & Nakai var. lanatus], and resistance to root-knot nematodes has not been identified in any watermelon cultivar. Twenty-six U.S. Plant Introductions (PIs) of Citrullus lanatus (Thunb.) Matsum. & Nakai var. citroides (L. H. Bailey) Mans., one PI of C. lanatus var. lanatus, and three PIs of Citrullus colocynthis (L.) Schrad. were evaluated in greenhouse tests for resistance to Meloidogyne incognita (Kofoid & White) Chitwood race 3 and Meloidogyne arenaria (Neal) Chitwood race 2. Twenty-three of the C. lanatus var. citroides PIs and the C. lanatus var. lanatus PIs were previously identified as moderately resistant to M. arenaria race 1. Overall, the C. lanatus var. citroides PIs exhibited low to moderate resistance, and the C. lanatus var. lanatus and C. colocynthis PIs were susceptible to both M. incognita race 3 and M. arenaria race 2. The C. lanatus var. citroides PI 482303 was the most resistant PI with gall index (GI) = 2.88 and reproductive index (RI) = 0.34 for M. incognita race 3 and GI = 3.46 and RI = 0.38 for M. arenaria race 2 (1 = no galling; 5 = 26% to 38% root system galled; 9 = 81% to 100% root system galled). These results demonstrate that there is significant genetic variability within C. lanatus var. citroides for reaction to M. incognita and M. arenaria race 2, and several C. lanatus var. citroides PIs may provide sources of resistance to root-knot nematodes.

Watermelon (C. lanatus var. lanatus) is an important vegetable crop grown in the United States with an annual production of 2.1 million tons and a farm value of $435 million (U.S. Department of Agriculture [USDA], 2007). Root-knot nematodes (Meloidogyne spp.) cause extensive damage to watermelon throughout the southern United States (Sumner and Johnson, 1973; Thies, 1996; Thomason and McKinney, 1959; Winstead and Riggs, 1959) and increase the severity of Fusarium wilt in watermelon fields (Sumner and Johnson, 1973).

Root-knot nematodes are primarily controlled in watermelon by fumigation with methyl bromide. Approximately 6% of all methyl bromide treatments in vegetable crops throughout the world are for watermelon and melon (Cucumis melo L.) (USDA, 1993). However, use of methyl bromide is being phased out in the United States (U.S. Environmental Protection Agency, 2000). The loss of methyl bromide for preplant soil fumigation was predicted to result in annual yield losses of 15% to 20% for watermelon in Georgia and Florida (Lynch and Carpenter, 1999). The removal of methyl bromide from the U.S. market has raised great interest in developing an alternative approach for managing root-knot nematodes in vegetable crops. Host plant resistance would provide an economical and environmentally friendly alternative for managing root-knot nematodes in watermelon.

There are several reports describing the reactions of cultivated watermelon to root-knot nematodes. Seventy-eight watermelon cultivars and five breeding lines evaluated for response to root-knot nematode were all susceptible (Winstead and Riggs, 1959). Similarly, 10 watermelon cultivars evaluated in Puerto Rico were all susceptible to M. incognita (Montalvo and Esnard, 1994). Thomason and McKinney (1959) reported that the watermelon cultivar ‘Striped Klondike’ was susceptible to M. incognita acrita and M. javanica.

In a recent study, we optimized a procedure for evaluating U.S. PIs of Citrullus spp. for resistance to root-knot nematodes (Thies and Levi, 2003). In that study, moderate resistance to M. arenaria race 1 was identified among C. lanatus var. citroides PIs. The objective of this study was to evaluate the group of C. lanatus var. citroides PIs that showed moderate resistance to M. arenaria race 1 for resistances to M. incognita race 3 and M. arenaria race 2.

Materials and Methods

Inocula. Meloidogyne incognita race 3 and M. arenaria race 2 were cultured on ‘Kentucky Wonder 191’ pole bean (Phaseolus vulgaris L.) and Rutgers’ tomato (Lyco- persicon esculentum Mill.) in the greenhouse. Egg inocula were extracted from bean and tomato roots using 0.5% sodium hypochlorite (Hussey and Barker, 1973).

Plant material. Twenty-six accessions of C. lanatus var. citroides, one accession of C. lanatus var. lanatus, and three accessions of C. colocynthis from the U.S. PI Citrullus germplasm collection were evaluated for resistance to M. incognita race 3 and M. arenaria race 2 in replicated greenhouse tests. The accessions were selected based on their reactions (both resistant and susceptible) to M. arenaria race 1 in previous greenhouse studies (Thies and Levi, 2003). ‘Charleston Gray’, ‘Crimson Sweet’, and ‘Dixie Lee’ (C. lanatus var. lanatus) were included as susceptible reference control cultivars in all tests.

Meloidogyne incognita tests 1 and 2. Thirty Citrullus spp. accessions and three watermelon control cultivars were evaluated for resistance to M. incognita in two greenhouse tests. The experimental design was a randomized complete block with 33 watermelon genotypes × four replicates × five plants per replicate (n = 20) in the first test and three replicates × five plants per replicate (n = 15) in the second test.

Meloidogyne arenaria race 2 tests 1 and 2. Thirty Citrullus spp. accessions and three watermelon control cultivars were evaluated for resistance to M. arenaria race 2 in two greenhouse tests. The experimental design, number of replicates, and numbers of plants per replicate for the M. arenaria race 2 tests were the same as for the M. incognita tests, previously described.

Greenhouse evaluation procedures. The seeds were sown in plastic trays containing 50 individual 0.2-L cells filled with Metro-Mix 360 (The Scotts Company, Marysville, OH) and placed in a greenhouse maintained between 26 °C and 31 °C. When seedlings were at the first true leaf stage, 3 mL distilled water containing ≈2500 eggs of either M. incognita race 3 or M. arenaria race 2 were pipetted into the rhizosphere soil of each plant at a 1-cm depth. Plants were fertilized 2 and 5 weeks after sowing with one-half strength 20N–20P–16K water-soluble fertilizer (Peter’s Fertilizer; United Industries Corp., St. Louis). Eight weeks later, the shoots of all plants were clipped at the crown, and the roots were removed from each cell and carefully washed. The root systems of each plant were then submerged in a 15% solution of McCormick’s...
red food color (Thies et al., 2002) for 15 to 20 min to stain the egg masses. The root systems were carefully rinsed under running tap water and evaluated for galling severity and egg mass production using a 1 to 9 scale in which 1 = 0, 2 = 1% to 3%, 3 = 4% to 12%, 4 = 13% to 25%, 5 = 26% to 38%, 6 = 39% to 50%, 7 = 51% to 65%, 8 = 66% to 80%, and 9 = 81% to 100% of root system galled or covered with egg masses, respectively (Thies and Fery, 1998). Ratings were 1 to 2.9 = high resistance, 3.0 to 4.0 = moderate resistance, 4.1 to 4.9 = low resistance, 5.0 to 6.9 = susceptible; and 7.0 to 9.0 = highly susceptible (Thies and Levi, 2003). Then, the entire root systems of all plants of each accession in a replicate were cut into 1- to 2-cm pieces, the total root weight was recorded, and root-knot nematode eggs were extracted from the root sample using 1.0% NaOCl (Hussey and Barker, 1973). The 0.5% NaOCl was used to extract eggs for inoculum recovery (Sasser et al., 1984). Eggs per gram fresh root and nematode reproductive index data were log_{10}(x + 1) transformed before analysis. Data were analyzed using the GLM procedure of SAS for Windows, v.8.0 (SAS Institute, Cary, NC), and means were separated using Fisher’s protected least significant difference test.

Results and Discussion

Overall, the C. lanatus var. citroides PI exhibited higher resistance to M. incognita and M. arenaria race 2 than the watermelon cultivars and C. colocynthis PI. The C. lanatus var. citroides root systems exhibited minimal to moderate galling; the root systems were more fibrous and had fewer and smaller galls than the root systems of the watermelon cultivars and the C. colocynthis PIs (Tables 1 and 2). Like in a previous study with M. arenaria race 1 (Thies and Levi, 2003), all three PIs of the desert species C. colocynthis also were highly susceptible to M. incognita race 3 and M. arenaria race 2 (Tables 1 and 2).

Meloidogyne incognita race 3 tests 1 and 2. The C. lanatus var. citroides PI 482303 exhibited high resistance in both tests with M. incognita race 3; gall index (GI) was 2.80 and GI = 2.97 in tests 1 and 2, respectively, with 1535 M. incognita eggs per gram fresh root and RI = 0.34 (Table 1). An additional 21 C. lanatus var. citroides PIs exhibited low to moderate resistance to M. incognita race 3 based on root gall severity (GI ranges: 3.07 to 4.80 in test 1 and 3.00 to 4.25 in test 2). However, some of these PIs had relatively high numbers of eggs per g fresh root (greater than 5000) and RIs greater than 1.0 (Table 1). For example, PI 189225 supported 16,508 eggs of M. incognita per gram fresh root and RI = 2.17. The PIs 248774, 288316, and 532666 exhibited susceptible reactions to M. arenaria race 1 in previous tests (Thies and Levi, 2003) and were also susceptible to M. incognita race 3 in the current tests (Table 1). The PI 542114 exhibited low resistance to M. arenaria race 1 in a previous

Table 1. Gall indices, egg mass indices, numbers of Meloidogyne incognita race 3 eggs per gram fresh root, and reproductive indices for selected PIs of Citrullus lanatus var. citroides, C. lanatus var. lanatus, and C. colocynthis and control watermelon cultivars inoculated with M. incognita race 3 in replicated greenhouse tests.1

<table>
<thead>
<tr>
<th>Accession (PI no.)</th>
<th>Gall indexy</th>
<th>Egg mass indexy</th>
<th>Gall indexy</th>
<th>Egg mass indexy</th>
<th>Eggs/g fresh rooty</th>
<th>Reproductive indexy</th>
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<tr>
<td>Citrullus lanatus var. citroides</td>
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<tr>
<td>482259</td>
<td>3.15 a–c</td>
<td>2.85 ab</td>
<td>4.00 a</td>
<td>3.40 ab</td>
<td>6,343 b–h</td>
<td>1.12 d–i</td>
</tr>
<tr>
<td>244017</td>
<td>4.75 e–h</td>
<td>3.05 a–d</td>
<td>4.07 a</td>
<td>3.47 ab</td>
<td>100,577 i</td>
<td>8.45 k</td>
</tr>
<tr>
<td>482338</td>
<td>3.47 a–d</td>
<td>2.11 a</td>
<td>3.39 ab</td>
<td>2.11 a</td>
<td>12,873 e–h</td>
<td>1.96 f–i</td>
</tr>
<tr>
<td>248774</td>
<td>5.67 g–i</td>
<td>3.93 b–g</td>
<td>4.50 a</td>
<td>3.78 a–c</td>
<td>4,080 a–g</td>
<td>0.80 b–g</td>
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<tr>
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<td>9.00 j</td>
<td>6.57 i–l</td>
<td>5.75 c–f</td>
<td>3.07 a</td>
<td>4,080 a–g</td>
<td>1.18 e–i</td>
</tr>
<tr>
<td>432337</td>
<td>8.80 j</td>
<td>4.50 c–h</td>
<td>8.42 h</td>
<td>5.93 de</td>
<td>8,327 d–h</td>
<td>1.62 e–i</td>
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<td>459074</td>
<td>5.81 g–i</td>
<td>4.79 e–h</td>
<td>4.82 a–e</td>
<td>2.97 a</td>
<td>10,278 d–h</td>
<td>2.96 h–k</td>
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<td>4,080 a–g</td>
<td>1.18 e–i</td>
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<td>7.67 gh</td>
<td>7.67 e</td>
<td>100,577 i</td>
<td>8.45 k</td>
</tr>
<tr>
<td>432337</td>
<td>8.80 j</td>
<td>4.50 c–h</td>
<td>8.42 h</td>
<td>5.93 de</td>
<td>8,327 d–h</td>
<td>1.62 e–i</td>
</tr>
<tr>
<td>C. lanatus var. lanatus controls</td>
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<td>Charleston Gray</td>
<td>7.85 j</td>
<td>6.80 j–l</td>
<td>7.53 f–h</td>
<td>6.87 de</td>
<td>9,658 d–h</td>
<td>5.37 jk</td>
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<tr>
<td>Crimson Sweet</td>
<td>7.83 j</td>
<td>7.27 kl</td>
<td>6.25 g</td>
<td>5.25 b–d</td>
<td>5,530 b–g</td>
<td>1.08 d–i</td>
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<tr>
<td>Dixie Lee</td>
<td>8.37 j</td>
<td>5.80 h–k</td>
<td>6.09 g</td>
<td>5.49 cd</td>
<td>3,497 a–f</td>
<td>0.57 a–e</td>
</tr>
</tbody>
</table>

1Means of four replicates of five plants per replicate (n = 20) in test 1 and means of three replicates of five plants per replicate (n = 15) in test 2.

1To 9 scale in which 1 = no galling or visible egg masses present, 2 = 1% to 3%, 3 = 4% to 10%, 4 = 11% to 25%, 5 = 26% to 35%, 6 = 36% to 50%, 7 = 51% to 65%, 8 = 66% to 80%, and 9 = 81% to 100% of root system galled or covered with egg masses, respectively.

1Data were log_{10}(x + 1) transformed before analysis. Nontransformed data are shown.

1Mean separation within columns by Fisher’s protected least significant difference test, P ≤ 0.05.
unreplicated test (Thies and Levi, 2003), but was moderately susceptible to *M. incognita* in the previous studies.

The PI 459074 was the only one of 156 *C. lanatus* var. *latus* PIs evaluated that exhibited any resistance to *M. arenaria* race 1 (Thies and Levi, 2003). However, in this study, PI 459074 was susceptible to *M. incognita* race 3 on both root gall severity and nematode reproduction (Table 1). The three *C. lanatus* var. *latus* watermelon cultivars (‘Crimson Sweet’, ‘Dixie Lee’, and ‘Charleston Gray’) were also susceptible to *M. incognita* race 3 (Table 1), like in a previous study with *M. arenaria* race 1 (Thies and Levi, 2003). The GIs ranged from 7.85 to 8.37 in test 1 and 6.09 to 7.53 in test 2; numbers of eggs per gram fresh root ranged from 3497 to 9658 and RIs ranged from 0.57 to 5.37. Numbers of eggs per gram fresh root and RIs for ‘Crimson Sweet’ and ‘Dixie Lee’ were somewhat lower than expected as a result of a reduction in fibrous root systems associated with root-knot nematode damage.

The three *C. colocynthis* PIs were highly susceptible to *M. incognita* race 3 (Table 1), similar to their reactions to *M. arenaria* race 1 in previous tests (Thies and Levi, 2003). The root gall severity indices for the *C. colocynthis* accessions ranged from 8.80 to 9.00 in test 1 and from 5.75 to 8.42 in test 2; numbers of eggs per gram fresh root ranged from 4080 to 100,577 and RIs ranged from 1.18 to 8.45.

Meloidogyne arenaria race 2 tests 1 and 2. Ten *C. lanatus* var. *citroides* accessions (PI 482303, PI 482338, PI 482326, PI 482319, PI 255137, PI 482259, PI 485583, PI 482301, PI 482319, and PI 482324) were highly susceptible to *M. arenaria* race 2, exhibiting low to moderate resistance to *M. arenaria* race 2 in both tests 1 and 2 (GI ranges: 3.70 to 4.98 in test 1 and 2.67 to 3.47 in test 2; numbers of *M. arenaria* race 2 eggs per gram fresh root ranged from 106 to 4320 and RIs ranged from 0.02 to 0.64) (Table 2).

The CI test 1 var. *latus* PI 459074 was susceptible to *M. arenaria* race 2 (Table 1). In contrast, this accession (PI 459074) exhibited low resistance to *M. arenaria* race 1 in a previous study (Thies and Levi, 2003). The three *C. colocynthis* PIs were highly susceptible to *M. arenaria* race 2 (Table 2), similar to their reactions to *M. arenaria* race 1 in past tests (Thies and Levi, 2003). The root gall severity indices for the *C. colocynthis* accessions ranged from 7.31 to 9.00 in test 1 and from 5.71 to 7.40 in test 2; numbers of *M. arenaria* race 2 eggs per gram fresh root ranged from 8592 to 15,758 and RIs ranged from 1.77 to 3.27 (Table 2). These three watermelon cultivars were also susceptible to *M. arenaria* race 1 in an earlier study (Thies and Levi, 2003).

The three *C. colocynthis* PIs were highly susceptible to *M. arenaria* race 2 (Table 2), similar to their reactions to *M. arenaria* race 1 in past tests (Thies and Levi, 2003). The root gall severity indices for the *C. colocynthis* accessions ranged from 7.31 to 9.00 in test 1 and 5.80 to 9.00 in test 2; the numbers of eggs per gram fresh root ranged from 8082 to 19,665 and RIs ranged from 1.06 to 2.40. In general, the *C. lanatus* var. *citroides* PIs exhibited low to moderate resistance to both *M. incognita* race 3 and *M. arenaria* race 2. The *C. lanatus* var. *citroides* PI 482303 was the most resistant with average GIs of 2.88 and 3.46 for *M. incognita* and *M. arenaria* race 2, respectively; RIs were less than 0.40 for both Meloidogyne species. These results demonstrate that there is significant genetic
variability within \textit{C. lanatus} var. \textit{citroides} for reaction to \textit{M. incognita} race 3 and \textit{M. arenaria} race 2, and several \textit{C. lanatus} var. \textit{citroides} PIs may provide sources of resistance to root-knot nematodes for the development of resistant watermelon cultivars.

\textbf{Literature Cited}


