Resistance of Watermelon Germplasm to the Peanut Root-knot Nematode

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Abstract. Root-knot nematodes [Meloidogyne arenaria (Neal) Chitwood, Meloidogyne incognita (Kofoid & White) Chitwood, and Meloidogyne javanica (Treub) Chitwood] are serious pests of watermelon [Citrullus lanatus (Thunb.) Matsum. & Nakai var. lanatus] in the southern United States and worldwide. Watermelon cultivars with resistance to any of these nematode pests are not available. Therefore, we evaluated all accessions of Citrullus colocynthis (L.) Schrad. (21) and Citrullus lanatus (Thunb.) Matsum. & Nakai var. citroides (L.H. Bailey) Mansf.(88), and about 10% of C. lanatus var. lanatus (156) accessions from the U.S. Plant Introduction (PI) Citrullus germplasm collection for reaction to M. arenaria race 1 in greenhouse tests. Only one C. lanatus var. lanatus accession exhibited very low resistance [root gall index (GI) = 4.9] and 155 C. lanatus var. lanatus accessions were susceptible (GI ranged from 5.0 to 9.0, where 1 = no galls and 9 = 281% root system covered with galls). All C. colocynthis accessions were highly susceptible (GI ranged from 8.5 to 9.0). However, 20 of 88 C. lanatus var. citroides accessions were moderately resistant with a GI range of 3.1 to 4.0; overall GI range for the C. lanatus var. citroides accessions was 3.1 to 9.0. Resistance to M. arenaria race 1 identified in the C. lanatus var. citroides accessions was confirmed on a subset of accessions in a replicated greenhouse test. The results of our evaluations demonstrated that there is significant genetic variability within the U.S. PI Citrullus germplasm collection for resistance to M. arenaria race 1 and also identified C. lanatus var. citroides accessions as potential sources of resistance.

Watermelon is an important vegetable crop in the United States and throughout the world. Fresh market production of watermelons in the United States in 2000 was 37,152,000 hundredweight valued at $236,286,720 [U.S. Dept. of Agriculture (USDA), 2001]. Root-knot nematodes (Meloidogyne arenaria, M. incognita, and M. javanica) cause serious damage to watermelon throughout the southern United States (Sumner and Johnson, 1973; Thies, 1996; Thomason and McKinney, 1959; Winsted and Riggs, 1959). In addition, root-knot nematodes increased the severity of Fusarium wilt, a disease of watermelon that occurs throughout watermelon growing areas of the United States and the world, in both Fusarium wilt-resistant and susceptible cultivars (Sumner and Johnson, 1973). Greater percentages of watermelon plants wilted in field soils that were naturally infested with both M. incognita and Fusarium oxysporum Schlechtend.:Fr. L sp. niveum (E.F. Sm.) W.C. Snyder & H.N. Hans. (Sumner and Johnson, 1973). In greenhouse experiments, Sumner and Johnson (1973) observed that M. arenaria reduced foliage weights in both Fusarium wilt-resistant and susceptible watermelon cultivars and increased wilting in the moderately resistant ‘Charleston Gray’.

Currently, root-knot nematodes are controlled in watermelon by pre-plant fumigation with methyl bromide or with other nematicide treatments. About 6% of all methyl bromide used for pre-plant soil treatment is used for melons (Cucumis melo L) and watermelon to control nematodes, weeds, and soil-borne pathogens (USDA, 1993). The proposed removal of methyl bromide from the U.S. market in 2005 has focused significant interest in developing alternatives for managing plant-parasitic nematodes and other soil-borne crop pests in high value vegetable crops. Additionally, many other nematics are likely to be lost from the U.S. market because of prohibitive re-registration costs and environmental concerns. Alternative strategies must be developed for managing root-knot nematodes in the highly susceptible watermelon crop. Host plant resistance, if available, would provide an inexpensive and environmentally compatible alternative to methyl bromide, as well as other fumigant and non-fumigant nematicides, for managing root-knot nematodes in watermelon.

Resistance to M. arenaria, M. javanica, and M. incognita has been difficult to identify in Citrullus spp. However, cultivated watermelon, C. lanatus var. lanatus, is considered a poor host for M. hapla (Sumner and Johnson, 1973). Seventy-eight watermelon cultivars and five breeding lines evaluated by Winstead and Riggs (1959) were susceptible to M. incognita, M. incognita acrita, M. arenaria arenaria and M. javanica, but were resistant to M. hapla Chitwood. Thomason and McKinney (1959) reported that the watermelon cultivar Striped Klondike was susceptible to M. incognita acrita and M. javanica, but resistant to M. hapla. In Puerto Rico, 10 watermelon cultivars evaluated for their responses to M. incognita were all susceptible (Montalvo and Ensard, 1994). There are no reports of resistance in watermelon cultivars or Citrullus germplasm to root-knot nematode species that are known to cause serious damage to watermelon. Therefore, our objectives were to determine the range of genetic variation within the U.S. Plant Introduction (PI) Citrullus germplasm collection for reaction to M. arenaria race 1 and to identify accessions that may be potentially useful sources of resistance to M. arenaria race 1.

Materials and Methods

Inoculum. Meloidogyne arenaria race 1 (obtained from S. Lewis, Clemson Univ., Clemson, S.C.) was cultured on ‘Rutgers’ tomato (Lycocepinium esculentum Mill.) and ‘Kentucky Wonder 191’ pole bean (Phaseolus vulgaris L.) in isolated soil benches in the greenhouse. Egg inocula were extracted from infected tomato and bean roots using 0.5% sodium hypochlorite (Hussey and Barker, 1973).

Greenhouse evaluation procedures. Seeds of each watermelon genotype were sown in the greenhouse in plastic growing trays containing 50 individual 0.2-L cells filled with Metro-Mix 360 (The Scotts Co., Marysville, Ohio). Ten days later, an aliquot of 3-mL distilled water containing ≥2,500 eggs of M. arenaria race 1 was pipetted into the soil surrounding each root system at a 1-cm depth. Plants were fertilized 2 and 5 weeks after sowing with N at 83 mg L-1 (2% nitrate, 18% ammoniacal) from a 20N–20P–16K water soluble fertilizer (Peter’s Fertilizer, United Industries Corp., St. Louis). The greenhouse air temperature was maintained between 26°C and 31°C. Eight weeks after inoculation, the shoots of all plants were severed at the crown (junction of roots and shoots), and the roots were removed from the plastic cells and carefully washed. The roots of each plant were then immersed in a 15% solution of McCormick’s red food color (McCormick & Co., Hunt Valley, Md.) (Thies et al., 2002) for ≤15 min to stain the egg masses, rinsed gently under running tap water, and evaluated for gall severity and egg mass production using a 1 to 9 scale where 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 Ferry, 1998). Ratings of 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. The primary criterion for designating resistance and susceptibility of the accessions was the root gall severity index. Egg mass data was also presented because it provides additional information about the reaction of the
accessions to *M. arenaria* race 1. However, accessions were considered resistant only if both the root gall index and the egg mass index were ≤3.9.

**Plant material.** All available accessions of *C. colocynthis* (21) and *C. lanatus* var. *citroides* (88), and ≥10% of the *C. lanatus* var. *lanatus* (156) accessions from the U.S. PI Citrullus germplasm collection were evaluated for resistance to *M. arenaria* race 1 in greenhouse tests. ‘Charleston Gray’, ‘Crimson Sweet’, and ‘Dixie Lee’ (*C. lanatus* var. *lanatus*) were included as susceptible reference check cultivars in all tests.

**Evaluation of *C. lanatus* var. *lanatus* accessions.** The 156 *C. lanatus* var. *lanatus* accessions were evaluated for reactivity to *M. arenaria* race 1 in an unrepli...
Table 2. Reactions of Citrullus colocynthis U.S. Plant Introduction (PI) accessions and control cultivars to the peanut root-knot nematode, Meloidogyne arenaria race 1 in greenhouse tests.  

| Origin       | Accession (PI No.) | Gall index | Egg mass index |
|--------------|--------------------|------------|----------------|
| Afghanistan  | 220778             | 9.00       | 9.00           |
| Afghanistan  | 269365             | 9.00       | 7.00           |
| Algeria      | 542616             | 9.00       | 9.00           |
| Chad         | 549161             | 9.00       | 9.00           |
| Cyprus       | 432337             | 8.93       | 7.60           |
| Egypt        | 525082             | 9.00       | 7.75           |
| Egypt        | 525083             | 9.00       | 5.00           |
| Ethiopia     | 195927             | 8.78       | 5.17           |
| India        | Grif 14201         | 9.00       | 9.00           |
| India        | Grif 14202         | 9.00       | 9.00           |
| Iran         | 386014             | 9.00       | 7.00           |
| Iran         | 386015             | 9.00       | 8.11           |
| Iran         | 386016             | 8.67       | 7.76           |
| Iran         | 386018             | 9.00       | 7.67           |
| Iran         | 386019             | 9.00       | 7.17           |
| Iran         | 386021             | 9.00       | 8.33           |
| Iran         | 386024             | 8.50       | 8.00           |
| Iran         | 386025             | 9.00       | 7.78           |
| Iran         | 386026             | 9.00       | 7.78           |
| Morocco      | 388770             | 9.00       | 9.00           |
| Pakistan     | 337277             | 9.00       | 8.00           |
| C. lanatus var. lanatus controls | | | |
| Charleston Gray | 9.00     | 7.32       |                |
| Crimson Sweet     | 8.83          | 6.90       |                |
| Dixie Lee        | 9.00          | 7.53       |                |

LSD<sub>0.05</sub> NS NS

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Means of three replicates of five plants per replicate (n = 15).

1 to 9 scale where 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.

Nonsignificant.
Results and Discussion

*Citrullus lanatus* var. *lanatus*. All but one of the 156 *C. lanatus* var. *lanatus* accessions were susceptible (Table 1). Root gall severity indices ranged from 4.90 to 9.00 and egg mass indices ranged from 1.00 to 8.00. Only PI 459074 from Botswana had a reaction that could be classified as low resistance (GI = 4.90 and EMI = 3.10). The control cultivars (Charleston Gray, Crimson Sweet, and Dixie Lee) were highly susceptible; root gall severity indices ranged from 8.81 to 8.93 and egg mass indices from 4.15 to 4.86.

*Citrullus colocynthis*. All of the *C. colocynthis* accessions evaluated were highly susceptible (Table 2). The root gall severity indices ranged from 8.50 to 9.00 and egg mass indices ranged from 8.60 to 9.30. The control cultivars also were highly susceptible (gall indices ranged from 8.83 to 9.00 and egg mass indices from 6.90 to 7.53). Although the gall severity indices of the control cultivars were numerically similar to those of the *C. colocynthis* accessions, the galls on roots of the *C. colocynthis* accessions were much larger than those of the control cultivars (Fig. 1 A and B).

*Citrullus lanatus* var. *citroides*. Twenty of the *C. lanatus* var. *citroides* accessions appeared to be moderately resistant (GI = 3.1 to 4.0), but none were highly resistant (GI = 1.0 to 2.0). Root gall severity indices for the *C. lanatus* var. *citroides* accessions ranged from 3.1 to 9.0 and egg mass indices ranged from 4.0 to 3.1.
from 1.3 to 9.0. Accessions with gall severity indices ≤0.00 originated from: Botswana (2 of 4 PI), India (1 of 2), South Africa (6 of 24), Spain (1 of 2), Zaire (1 of 1), and Zimbabwe (9 of 41). All of the check cultivars, Charleston Gray, Crimson Sweet, and Dixie Lee, were highly susceptible; root gall severity ratings ranged from 8.0 to 8.16 and egg mass indices ranged from 4.6 to 6.18.

Replicated evaluation of selected accessions. Ten accessions of *C. lanatus var. citroides* that exhibited low to moderate resistance in the initial unreplicated test and one accession, PI 299378, that appeared to be susceptible in the initial test (Table 3), exhibited low to moderate resistance to *M. arenaria* race 1 in the replicated greenhouse test (Table 4). The gall indices of the 11 resistant accessions ranged from 2.05 to 1.48. Two accessions of *C. lanatus var. citroides* (PI288316, moderately resistant in the unreplicated test, and PI 532666, susceptible in the unreplicated test) exhibited intermediate to susceptible reactions in the replicated test. Gall indices for PI 288316 and PI 532666 were 4.99 and 3.55, numbers of eggs per gram fresh root ranged from 16,696 and 8,770, and RI ranged from 2.53 and 1.04, respectively.

Galling of root systems of the *C. lanatus* var. *citroides* accessions was minimal to moderate, and generally the root systems were more fibrous than those of the *C. lanatus* var. *lanatus* and *C. colocythis* accessions (Fig. 1, A–C). Eight of the *C. lanatus* var. *citroides* accessions had RI <1, suggesting that they are resistant to *M. arenaria* race 1. In general, the three *C. lanatus* var. *citroides* accessions from Zimbabwe and the *C. lanatus* var. *citroides* accession from Botswana (PIs 482379, 482338, 482303, and 542119, respectively) had lower (*P* ≤ 0.05) root gall severity indices (mean = 3.21) and egg mass indices (mean = 2.40), supported fewer (*P* ≤ 0.05) *M. arenaria* race 1 eggs per gram fresh root (mean = 2.635), and had lower (*P* ≤ 0.05) RI (mean = 0.50) than the *C. colocythis* accessions and the *C. lanatus* var. *lanatus* control cultivars.

All of the re-evaluated *C. colocythis* accessions were highly susceptible to *M. arenaria* race 1 (Table 4). Gall indices ranged from 7.73 to 8.12 and egg mass indices ranged from 6.79 to 8.67. Galls on root systems of the *C. colocythis* accessions were larger than galls on roots of the *C. lanatus* var. *citroides* accessions and the *C. lanatus* var. *lanatus* control cultivars (Fig. 1A–C). Numbers of eggs per gram fresh root ranged from 9,649 to 43,033 and reproductive indices (RI) ranged from 1.79 to 3.94.

The three control cultivars (‘Crimson Sweet’, ‘Dixie Lee’, and ‘Charleston Gray’) were also susceptible (Table 4). Gall indices ranged from 8.00 to 8.56, egg mass indices ranged from 5.44 to 7.46, numbers of eggs per gram fresh root ranged from 7,475 to 19,209, eggs per gram fresh root, and RI ranged from 1.80 to 5.43 for the control cultivars.

The resistance to *M. arenaria* race 1 observed in *C. lanatus* var. *citroides* in the present study may be attributed to the greater genetic divergence in this *Citrullus* subspecies compared to that found in *C. lanatus* var. *lanatus* (Jarret et al., 1997; Levi et al., 2002; Navot and Zamir, 1987). Several of the resistant *C. lanatus* var. *citroides* accessions identified in this study may contain root-knot nematode resistance genes that can be introgressed into cultivated watermelon (*C. lanatus* var. *lanatus*). However, in order to make significant gains in developing resistant watermelon cultivars, it will be necessary to determine the modes of inheritance of resistance to root-knot nematodes.

**Conclusions**

The results of the present studies demonstrate that there is significant genetic variability within the USDA *Citrullus* germplasm collection for reaction to *M. arenaria* race 1. Numerous *Citrullus lanatus* var. *citroides* accessions were identified as potential sources of resistance to *M. arenaria* race 1. Thus, future germplasm evaluations for resistance to *M. arenaria* race 1 and to other *Meloidogyne* species should initially focus on *C. lanatus* var. *citroides* accessions.

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