Resistance of Watermelon Germplasm to the Peanut Root-knot Nematode

Judy A. Thies¹ and Amnon Levi²

U.S. Vegetable Laboratory, Agricultural Research Service, U.S. Department of Agriculture, 2700 Savannah Highway, Charleston, SC 29414

Additional index words. *Citrullus lanatus* var. *lanatus*, *C. lanatus* var. *citroides*, *C. colocynthis*, nematode resistance, *Meloidogyne arenaria* race 1

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 resistance 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 = ≥81% root system covered with galls). All *C. colocynthis* accessions were highly susceptible (GI range = 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; Winstead 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. f. sp. *niveum* (E.F. Sm.) W.C. Snyder & H.N. Hans. (Sumner and Johnson, 1973). In greenhouse experiments, Sumner and Johnson (1973) ob-

served 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 nematicides 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 Esnard, 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 will 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 (*Lycopersicon 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 galling 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 Fery, 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

Received for publication 15 Mar. 2002. Accepted for publication 22 May 2002. We thank S. Merrill, E.L. Corley, Jr. (retired), R. Reeves, P. Berland, and L. Pence for technical assistance in conducting the experiments. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Dept. of Agriculture and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

¹Research Plant Pathologist.

²Research Geneticist.

accessions to *M. arenaria* race 1. However, accessions were considered resistant only if both the root gall index and the egg mass index were ≤ 4.9 .

Plant material. All available accessions of *C. colocynthis* (21) and *C. lanatus* var. *citroides* (88), and $\approx 10\%$ 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*

PIs evaluated in this test represented at least 10% of the accessions from each country of origin. These accessions were selected in the following manner: First, the entire *C. lanatus* var. *lanatus* portion of the USDA *Citrullus* collection database was sorted in ascending order by PI accession number within each geographic origin (country) group. Then, the first PI (lowest accession number) and each successive 10th PI was selected in each country group, so that $\approx 10\%$ of each country group was represented. If there were fewer than 10 PIs per country group, then the last PI in the group was selected. Ten seeds of each of the 156 *C. lanatus* var. *lanatus* PIs were planted and the plants were evaluated for reaction to

M. arenaria race 1 in an unreplicated greenhouse test using evaluation procedures previously described.

Evaluation of C. lanatus var. citroides accessions. Ten seeds of each of the 88 *C. lanatus* var. *citroides* accessions were planted and the plants were evaluated for reaction to *M. arenaria* race 1 in an unreplicated test as previously described.

Evaluation of C. colocyntis accessions. Fifteen seeds of each of the 21 *C. colocyntis* accessions were planted in a randomized complete-block design with 3 replicates and 5 plants per replicate. Plants were evaluated for reaction to *M. arenaria* race 1 as previously described.

Table 1. Reactions of *Citrullus lanatus* var. *lanatus* U.S. Plant Introduction (PI) accessions and control cultivars to the peanut root-knot nematode, *Meloidogyne arenaria* race 1.²

Origin	Accession (PI No.)	Gall index ^y	Egg mass index ^y	Origin	Accession (PI No.)	Gall index ^y	Egg mass index ^y	Origin	Accession (PI No.)	Gall index ^y	Egg mass index ^y
Afghanistan	207471	9.00	4.00	Japan	227202	8.75	5.33	Turkey	169287	8.20	5.60
Afghanistan	219906	8.57	3.71	Japan	279460	7.20	3.10	Turkey	169297	9.00	4.60
Algeria	222137	9.00	5.14	Kenya	385964	8.33	6.33	Turkey	171586	8.82	5.91
Angola	306367	9.00	3.25	Korea, South	161373	9.00	4.50	Turkey	172794	7.60	3.00
Argentina	162667	8.00	5.00	Lebanon	181740	9.00	5.00	Turkey	172804	8.40	4.80
Australia	504519	8.67	6.33	Liberia	164247	7.17	2.00	Turkey	174104	6.88	2.25
Belize	269676	9.00	1.78	Maldives	536448	9.00	4.00	Turkey	175654	7.75	5.00
Bolivia	543212	8.38	5.00	Maldives	536458	9.00	2.20	Turkey	175664	9.00	6.67
Botswana	459074	4.90	3.10	Mali	490375	8.75	3.38	Turkey	176493	8.75	5.25
Botswana	542119	5.50	4.00	Mauritania	549159	8.75	3.50	Ukraine	476325	8.78	5.67
Brazil	441722	9.00	5.33	Mexico	165448	9.00	4.20	United States	595202	9.00	4.25
Cameroon	535947	9.00	3.22	Moldova	506439	8.80	2.80	Uruguay	331106	9.00	8.00
Cameroon	535948	9.00	2.40	New Zealand	234603	9.00	2.00	Uzbekistan	476327	8.88	3.50
Chad	549160	9.00	5.00	Nigeria	184800	6.56	2.33	Venezuela	266028	7.44	3.44
Chile	241689	8.78	4.67	Nigeria	307609	8.56	5.78	Yugoslavia	190050	9.00	3.80
China	113326	9.00	5.80	Nigeria	559996	6.63	1.25	Yugoslavia	357663	8.67	3.00
China	192938	8.67	4.56	Nigeria	560006	7.78	2.00	Yugoslavia	357673	8.57	5.57
China	340615	9.00	4.63	Nigeria	560016	7.70	3.00	Yugoslavia	357683	8.60	4.40
China	435085	9.00	5.00	Pakistan	217937	7.89	2.44	Yugoslavia	357693	9.00	3.40
China	435991	9.00	5.20	Pakistan	426625	8.78	3.56	Yugoslavia	357703	8.50	2.00
China	505935	8.75	3.13	Pakistan	537273	8.86	3.71	Yugoslavia	357713	8.80	5.60
China	512332	9.00	6.50	Paraguay	458738	9.00	7.00	Yugoslavia	357723	9.00	5.11
China	532809	9.00	4.43	Philippines	188808	9.00	3.50	Yugoslavia	357733	9.00	5.33
China	532811	7.63	5.38	Portugal	234287	8.00	2.00	Yugoslavia	357743	9.00	3.50
Cuba	208740	9.00	6.89	Russian Federation	185606	8.50	4.50	Yugoslavia	357754	7.80	4.40
Egypt	183217	8.20	2.80	Senegal	246559	8.80	4.60	Yugoslavia	379224	8.20	2.70
Egypt	525090	9.00	4.60	Senegal	254744	9.00	5.22	Zaire	378612	9.00	2.70
El Salvador	200732	7.67	3.11	Somalia	271982	8.44	4.56	Zambia	494815	8.60	4.80
Ethiopia	193490	8.80	4.00	South Africa	255139	9.00	2.89	Zambia	500306	6.44	1.78
Former Soviet Union	345543	8.44	4.33	South Africa	296332	7.50	3.38	Zambia	500316	8.67	4.44
Former Soviet Union	518612	9.00	4.50	Spain	512339	9.00	4.33	Zambia	500329	5.89	3.56
Ghana	271750	8.60	2.60	Spain	512349	7.43	3.00	Zambia	500344	8.88	4.25
Greece	212208	7.80	4.40	Spain	512360	7.00	2.20	Zambia	505584	7.13	1.00
Guatemala	163572	8.50	5.20	Spain	512370	9.00	6.33	Zambia	505594	9.00	4.33
Honduras	415095	8.60	3.20	Spain	512382	6.67	3.11	Zimbabwe	225557	9.00	2.00
Hungary	270522	8.80	5.80	Spain	512393	8.75	3.75	Zimbabwe	482258	7.10	3.70
Hungary	507867	6.56	3.22	Spain	512404	8.80	3.80	Zimbabwe	482271	9.00	3.00
India	163202	8.56	4.78	Spain	537470	9.00	3.89	Zimbabwe	482288	6.22	3.89
India	164633	8.14	2.43	Sudan	254622	9.00	4.40	Zimbabwe	482305	8.78	3.33
India	164737	7.00	3.00	Syria	177330	9.00	4.00	Zimbabwe	482328	6.40	2.40
India	179878	6.90	1.50	Syria	534534	6.89	3.11	Zimbabwe	482349	7.00	3.22
India	180278	7.88	4.75	Syria	534591	9.00	5.25	Zimbabwe	482357	8.11	2.33
India	183124	8.63	4.00	Taiwan	306365	8.67	3.17	Zimbabwe	482371	8.90	3.20
Indonesia	470246	8.40	4.80	Thailand	249559	9.00	2.20	Zimbabwe	482378	8.22	2.78
Iran	211849	9.00	3.13	Tunisia	271132	8.50	2.50	Zimbabwe	526231	5.40	2.70
Iran	222713	8.70	3.60	Turkey	105445	8.56	3.44	Zimbabwe	526237	9.00	2.11
Iran	229604	7.67	4.78	Turkey	167124	8.40	3.50	Zimbabwe	276657	8.50	5.50
Iran	344395	7.89	3.56	Turkey	169237	8.80	5.00	<i>C. lanatus var. lanatus controls</i>			
Iraq	179241	8.56	3.89	Turkey	169242	7.67	3.33		Charleston Gray	8.93	4.41
Israel	177331	8.43	5.43	Turkey	169247	9.00	4.80		Crimson Sweet	8.86	4.15
Italy	525100	5.60	2.10	Turkey	169257	8.80	3.40		Dixie Lee	8.81	4.86
				Turkey	169267	9.00	4.60				
				Turkey	169277	8.20	2.80				

²Means of 10 plants.

¹ 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.

Replicated evaluation of selected accessions. Selected accessions that represented the lowest and highest root gall indices were re-evaluated in a replicated greenhouse test. This test included 11 *C. lanatus* var. *citroides* accessions (482379, 482338, 282303, 542119, 189225, 500331, 244018, 512854, 244017, 248774, and 288316) that exhibited the least amount of root gall [gall index (GI) = 3.10 to 4.57], and two *C. lanatus* var. *citroides* accessions (299378 and 532666) and three *C. colocynthis* accessions (432337, 386015, and 525082) that exhibited the most root gall (GI = 7.30 to 9.00) in the initial tests. The three susceptible control cultivars Crimson Sweet, Dixie Lee, and Charleston Gray were also included in the test. Twenty seeds of each accession were planted and the plants were evaluated for reaction to *M. arenaria* race 1 as previously described. The experimental design was a randomized complete block with four replicates and five plants per replicate (one plant in each of five cells in the plastic growing tray). Each plant was rated for severity of root gall and egg mass production eight weeks after inoculation with *M. arenaria* race 1. 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

M. arenaria eggs were extracted from the root sample using 1.0% NaOCl (Hussey and Barker, 1973). Numbers of eggs were counted using a stereomicroscope. Nematode reproduction was assessed by calculating the reproductive index (RI) in which $RI = P_f/P_i$, where P_i = the initial inoculum level and P_f = final egg

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. 6.12 (SAS Inst., Cary, N.C.), and means were separated using Duncan's multiple range test.

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.²

Origin	Accession (PI No.)	Gall index ³	Egg mass index ³
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	537277	9.00	8.00
<i>C. lanatus</i> var. <i>lanatus</i> controls			
Charleston Gray		9.00	7.32
Crimson Sweet		8.83	6.90
Dixie Lee		9.00	7.53
LSD _{0.05}		NS	NS

²Means of three replicates of five plants per replicate (n = 15).

³1 to 9 scale where 1 = no gall 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.

^{NS}Nonsignificant.

Fig. 1. Root systems of (A) *Citrullus colocynthis* PI 432337, (B) *C. lanatus* var. *lanatus* 'Charleston Gray', and (C) *C. lanatus* var. *citroides* PI 189225 8 weeks after inoculation with the peanut root-knot nematode, *Meloidogyne arenaria* race 1. Roots of inoculated plants are shown on the left and roots of uninoculated control plants are shown on the right.

Table 3. Reactions of *Citrullus lanatus* var. *citroides* U.S. Plant Introduction (PI) accessions and controls to the peanut root-knot nematode, *Meloidogyne arenaria* race 1 in greenhouse tests.^z

Origin	Accession (PI No.)	Gall index ^y	Egg mass index ^y	Origin	Accession (PI No.)	Gall index ^y	Egg mass index ^y	Origin	Accession (PI No.)	Gall index ^y	Egg mass index ^y
Afghanistan	346082	9.00	9.00	South Africa	296343	6.50	4.70	Zimbabwe	482299	5.20	2.20
Botswana	485583	4.00	1.33	South Africa	299378	7.55	4.55	Zimbabwe	482300	5.12	2.00
Botswana	542114	4.20	3.30	South Africa	299379	5.40	3.10	Zimbabwe	482301	4.10	1.80
Botswana	542119	3.20	2.10	South Africa	532659	4.33	2.67	Zimbabwe	482302	4.70	4.00
Botswana	542123	5.00	5.00	Spain	512385	6.70	4.70	Zimbabwe	482303	3.50	1.60
Egypt	525081	6.33	6.67	Spain	512854	3.80	2.90	Zimbabwe	482307	3.89	2.11
India	179881	6.33	3.44	Swaziland	532664	7.30	4.50	Zimbabwe	482308	4.40	2.40
India	288316	3.50	1.50	Swaziland	532666	7.30	1.40	Zimbabwe	482309	5.00	2.89
Namibia	248774	4.57	3.86	Swaziland	532667	7.33	5.67	Zimbabwe	482311	5.00	3.00
Namibia	485579	6.00	5.50	Swaziland	532668	7.67	4.17	Zimbabwe	482312	4.90	3.30
South Africa	244017	3.50	2.50	Yugoslavia	379243	6.33	4.22	Zimbabwe	482315	4.30	2.50
South Africa	244018	3.20	2.60	Zaire	189225	3.10	1.40	Zimbabwe	482316	4.60	1.70
South Africa	244019	3.60	2.00	Zambia	500331	4.30	2.20	Zimbabwe	482319	3.80	2.20
South Africa	255137	4.00	2.44	Zambia	500332	4.30	3.30	Zimbabwe	482321	4.20	1.30
South Africa	270562	9.00	5.00	Zambia	505604	4.63	3.88	Zimbabwe	482322	4.70	2.10
South Africa	270563	3.70	1.90	Zimbabwe	482246	5.33	3.44	Zimbabwe	482324	3.80	1.90
South Africa	271767	5.29	3.43	Zimbabwe	482252	4.67	3.30	Zimbabwe	482326	4.27	2.27
South Africa	271769	5.50	4.50	Zimbabwe	482257	5.90	3.40	Zimbabwe	482331	5.11	3.67
South Africa	271770	4.29	1.86	Zimbabwe	482259	3.70	2.70	Zimbabwe	482333	4.00	1.88
South Africa	271771	4.50	3.00	Zimbabwe	482261	5.10	3.70	Zimbabwe	482334	6.00	4.46
South Africa	271773	3.88	3.38	Zimbabwe	482265	4.70	3.10	Zimbabwe	482335	5.43	3.71
South Africa	271775	4.30	3.30	Zimbabwe	482273	5.50	3.00	Zimbabwe	482336	4.20	1.80
South Africa	271779	5.13	3.88	Zimbabwe	482276	4.80	3.00	Zimbabwe	482338	3.56	2.33
South Africa	295850	7.00	6.00	Zimbabwe	482277	5.11	3.33	Zimbabwe	482342	4.00	2.40
South Africa	296334	4.11	2.90	Zimbabwe	482279	5.10	3.00	Zimbabwe	482379	3.22	1.44
South Africa	296335	4.29	3.43	Zimbabwe	482282	6.00	4.00	Zimbabwe	532624	4.00	1.70
South Africa	296337	6.43	4.86	Zimbabwe	482283	5.00	3.44	<i>C. lanatus</i> var. <i>lanatus</i> controls			
South Africa	296339	5.00	4.00	Zimbabwe	482286	4.89	2.56	Charleston Gray		8.15	5.29
South Africa	296341	5.86	4.57	Zimbabwe	482293	4.88	3.11	Crimson Sweet		8.16	4.66
South Africa	296342	5.10	2.60	Zimbabwe	482298	4.90	1.70	Dixie Lee		8.08	6.18

^yMeans of 10 plants.^z1 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.

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 5.00 to 9.00. 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 2.9) (Table 3). Root gall severity indices for the *C. lanatus* var. *citroides* accessions ranged from 3.1 to 9.0 and egg mass indices ranged

Table 4. Gall indices, egg mass indices, numbers of *Meloidogyne arenaria* race 1 eggs per gram fresh root, and reproductive indices of *M. arenaria* race 1 for selected *Citrullus* spp. Plant Introduction (PI) accessions and control cultivars inoculated with *M. arenaria* race 1, in a replicated greenhouse test.^z

Taxon/Origin	Accession (PI No.)	Gall index ^y	Egg mass index ^y	Eggs/g fresh root	Reproductive index ^x
<i>Citrullus lanatus</i> var. <i>citroides</i>					
Zimbabwe	482379*	3.05 a ^y	2.00 a	1,880 a ^y	0.33 a ^y
Zimbabwe	482338*	3.21 ab	2.17 ab	2,901 a-c	0.59 a-c
Zimbabwe	482303*	3.30 ab	2.50 ab	2,261 ab	0.40 ab
Botswana	542119*	3.29 ab	2.94 ab	3,500 a-d	0.70 a-c
Zaire	189225*	3.74 a-c	3.01 ab	4,716 a-e	0.69 a-c
Zambia	500331*	3.95 a-d	3.55 a-c	6,488 c-f	1.13 b-d
South Africa	244018*	4.15 a-d	3.25 ab	6,738 b-e	0.80 a-c
Spain	512854*	4.15 a-d	3.65 a-c	6,530 c-f	1.19 b-d
South Africa	299378**	4.35 a-d	3.45 a-c	4,724 a-e	0.95 a-d
South Africa	244017*	4.45 b-d	3.55 a-c	6,624 c-f	0.91 a-d
Namibia	248774*	4.70 cd	3.95 b-d	11,262 e-h	1.48 c-e
India	288316*	4.99 cd	5.04 cd	16,696 f-h	2.53 d-f
Swaziland	532666**	5.17 d	3.55 a-c	8,770 d-g	1.04 b-d
<i>Citrullus colocynthis</i>					
Cyprus	432337**	7.73 e	6.79 ef	9,649 e-g	1.79 c-e
Iran	386015**	7.83 e	8.67 g	43,033 i	3.72 ef
Egypt	525082**	8.12 e	7.25 fg	26,940 i	3.94 ef
<i>C. lanatus</i> var. <i>lanatus</i> controls					
Crimson Sweet		8.00 e	5.44 de	11,424 e-h	2.78 d-f
Dixie Lee		8.17 e	7.46 fg	7,475 c-g	1.80 c-e
Charleston Gray		8.56 e	7.36 fg	19,209 g-i	5.43 f

^zMeans of four replicates of five plants per replicate (n = 20).^y1 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.^xReproductive index = P_f/P_i , where P_i = initial inoculum level and P_f = final egg recovery.^yMean separation within columns by Duncan's multiple range test, $P \leq 0.05$.^zData were $\log_{10}(x+1)$ transformed before analysis. Back transformed data are shown.

*Accessions with gall indices = 3.10 to 4.57 in the unreplicated test.

**Accessions with gall indices = 7.30 to 9.00 in the unreplicated test(s).

from 1.3 to 9.0. Accessions with gall severity indices ≤ 4.00 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 8.08 to 8.16 and egg mass indices ranged from 4.66 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 3.05 to 4.70, egg mass indices ranged from 2.00 to 3.95, numbers of eggs per gram fresh root ranged from 1,880 to 11,262, and reproductive indices (RI) ranged from 0.33 to 1.48. Two accessions of *C. lanatus* var. *citroides* (PI 288316, 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 5.17, egg mass indices were 5.04 and 3.55, numbers of eggs per gram fresh root were 16,696 and 8,770, and RI were 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. colocynthis* 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 ($P \leq 0.05$) root gall severity indices (mean = 3.21) and egg mass indices (mean = 2.40), supported fewer ($P \leq 0.05$) *M. arenaria* race 1 eggs per gram fresh root (mean = 2,635), and had lower ($P \leq 0.05$) RI (mean = 0.50) than the *C. colocynthis* accessions and the *C. lanatus* var. *lanatus* control cultivars.

All of the re-evaluated *C. colocynthis* 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. colocynthis* 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.

Literature Cited

- Hussey, R.S. and K.R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis. Rpt. 57:1025–1028.
- Jarret, R.L., L.C. Merrick, T. Holms, J. Evans, and M.K. Aradhya. 1997. Simple sequence repeats in watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]. Genome 40:433–441.
- Levi, A., C.E. Thomas, A.P. Keinath, and T.C. Wehner. 2002. Genetic diversity among watermelon (*Citrullus lanatus* and *Citrullus colocynthis*) accessions. Genetic Resources and Crop Evol. 48:559–566.
- Montalvo, A.E. and J. Esnard. 1994. Reaction of ten cultivars of watermelon (*Citrullus lanatus*) to a Puerto Rican population of *Meloidogyne incognita*. Suppl. to J. Nematol. 26(4S):640–643.
- Navot, N. and D. Zamir. 1987. Isozyme and seed protein phylogeny of the genus *Citrullus* (Cucurbitaceae). Plant Syst. Evol. 156:61–67.
- Sasser, J.N., C.C. Carter, and K.M. Hartman. 1984. Standardization of host suitability studies and reporting of resistance to root-knot nematodes. Crop Nematode Resistance Control Project, N.C. State Univ., U.S. Agency for Intl. Dev., Raleigh, N.C.
- Sumner, D.R. and A.W. Johnson. 1973. Effect of root-knot nematodes on Fusarium wilt of watermelon. Phytopathology 63:857–861.
- Thies, J.A. 1996. Diseases caused by nematodes, p. 56–58. In: T.A. Zitter, D.L. Hopkins, and C.E. Thomas (eds.). Compendium of cucurbit diseases. APS Press, St. Paul, Minn.
- Thies, J.A. and R.L. Fery. 1998. Modified expression of the *N* gene for southern root-knot nematode resistance in pepper at high soil temperatures. J. Amer. Soc. Hort. Sci. 123:1012–1015.
- Thies, J.A., S.B. Merrill, and E.L. Corley, Jr. 2002. Red food coloring stain: New, safer procedures for staining nematodes in roots and egg masses on root surfaces. J. Nematol. 34:179–181.
- Thomason, I.J. and H.E. McKinney. 1959. Reaction of some Cucurbitaceae to root-knot nematodes (*Meloidogyne* spp.). Plant Dis. Rpt. 43:448–450.
- U.S. Dept. of Agriculture. 1993. USDA workshop on alternatives for methyl bromide. 29 June–1 July 1993. Crystal City, Va.
- U.S. Dept. of Agriculture. 2001. Agricultural Statistics 2001. p. IV-37. Natl. Agr. Stat. Serv. U.S. Govt. Printing Office. Washington: 2001.
- Winstead, N.N. and R.D. Riggs. 1959. Reaction of watermelon varieties to root-knot nematodes. Plant Dis. Rpt. 43:909–912.