Resistance to Fusarium Wilt and Root-knot Nematode in Watermelon Germplasm

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Watermelon is an important vegetable crop in the United States with close to 81,000 ha in production, which is concentrated in Texas, Georgia, and Florida (22). Root knot nematodes (*Meloidogyne* spp.) and Fusarium wilt (*Fusarium oxysporum* Schlechtend.:Fr. f.sp. *niveum* (E. F. Sm.) Snyd. & Hans.) race 2 can be serious problems in many areas, particularly in soils with a history of the diseases, which are persistent. Soil fumigants have proven to be effective in controlling these pathogens, but there is growing concern about their environmental and health effects as well as their costs. In addition, one of the most widely used fumigants, methyl bromide, is scheduled to be removed from the market in 2005 (23).

There is widespread interest in nematode and Fusarium wilt resistance in vegetable crops. Resistance to root-knot nematode has been reported in several different vegetable crops, however, there have not been widely accepted sources of root-knot nematode resistance with the exception of tomatoes (17). As an example, Khelu et al. (10) found tomato cv. Karla, cucumber cv. Capris, and pepper cv. Clovis to be resistant to root-knot nematodes (*M. arenaria* (Neal (Chitwood) and *M. javanica* (Treub) Chitwood)) compared to the susceptible entries.

Watermelons are attacked by several species of root-knot nematodes, and are susceptible to all 4 races of *M. incognita* (Kofoid & White) Chitwood), *M. javanica*, and both races of *M. arenaria* (16). Zhang et al. (24) found several watermelon lines to have resistance to root-knot nematodes including 'Crimson Sweet'. This finding is particularly interesting since 'Crimson Sweet' is generally considered susceptible. They used *M. incognita* race 2, *M. arenaria* race 2, and *M. javanica* in their screening. For resistance to be effective and widely adopted, it must apply to all species and races of root-knot nematode.

Boyhan et al. (3) found differences in susceptibility to *M. incognita* races 3 and 4 in watermelon, with subsequent testing showing some of this material retaining a high level of resistance at 7000 eggs/plant. These promising results suggest that additional sources of resistance may be found in the USDA germplasm collection.

There are at least 3 races (0, 1, 2) of Fusarium wilt that attack watermelon (5, 11). Race 2 has recently received the most intense scrutiny because there were no known sources of resistance until Netzer and Martyn (13) reported race 2 resistance in PI 296341. In addition, Dane et al. (6) reported resistance to Fusarium wilt race 2 in PI 271769. These results suggest that other sources of Fusarium wilt race 2 resistance may be present in the USDA germplasm collection.

Root-knot nematode infection of watermelon has been shown to enhance the susceptibility of watermelon to Fusarium wilt even in those lines showing Fusarium tolerance or resistance (8, 19, 20).

The objective of this study was to evaluate the USDA watermelon germplasm collection for resistance to Fusarium wilt race 2 and root-knot nematode race 3 with an emphasis on finding resistance to both diseases in a single accession.

Materials and Methods: All screening was conducted in the greenhouses at the Bamboo Farm and Coastal Gardens Extension-Research Center in Savannah, GA. Greenhouse temperatures during the screening ranged from 20-35 deg. C. Accessions from 58 countries were evaluated in two different groups for Fusarium wilt resistance with 1,034 in the first screening and 377 in the second screening. Flats (28 x 56 cm) were filled with soil mix (Metromix 300, Scotts-Serria Products Co., Marysville, OH) and nine seed were planted per replication with three replications in a randomized complete block design (RCBD). Fusarium wilt race 2 inoculum (culture 62939, Amer. Type Culture Collection, Manassas, VA) was obtained from Dr. Fenny Dane (Auburn University, Auburn, AL) and sufficient quantity was grown for 2 weeks in an agitated potato dextrose

broth at 20 deg. C. The inoculum was adjusted to $1.5x10^6$ microspores per ml with a hemacytometer. Seed were planted for the first screening on 7 October 1998 and each plant was inoculated during 26 to 28 October 1998 with 50 ul of inoculum injected into the plant's stem just above the soil line with a 50 unit insulin syringe (Becton Dickinson Co., Frankin Lakes, NJ). Plants were evaluated on 17 to 21 November 1998 on a 0-9 scale with 0, no sign of disease. The scale represents increasing levels of symptoms including discoloration and lesions on the stem and wilting of the plant culminating with death of the plant having a rating of 9. This scale was used to comport with the Germplasm Resource Information Network maintained by the USDA.

The second screening was conducted in the same fashion as the first. Seed were planted on 27 January 1999, inoculated 26 February 1999, and evaluated on 1-2 April 1999. Individual plants, which showed no sign of disease were self-pollinated to produce seed for later studies.

Root-knot nematode race 3 was obtained from Dr. Richard Davis (University of Georgia, Athens, GA) and were increased on okra [*Abelmoschus esculentus* (L.) Moench] planted on Ocilla-Pelham-Albany association (loamy fine sand) soil. Excess soil was rinsed from the okra roots, which were then agitated in a 10% bleach solution for 4 minutes. The resulting solution was passed through 180 um, and 75 um screens, and the nematode eggs were collected on a 25 um screen. The nematode inoculum was adjusted to 10,000 eggs per ml after counting them on a hemacytometer.

Seed of 1,235 accessions were sown in 28 x 56 cm flats with #809 inserts (8 packs of 9 cells, 3.8x3.8x6.4 cm) filled with field soil. The design was a RCBD with three plants per replication and three replications. One ml of inoculum was applied to each seed at the time of planting.

Seed were sown and inoculated on 30 August to 9 September 2000 and plants were evaluated 25 September to 11 October 2000 in the greenhouse. The soil was washed from the roots of each plant and the roots were visually evaluated on a 0-3 scale with 0, no sign of galling, 1, up to 25% of roots galled, 2, >25% to 50% roots galled, and 3, >50% galling. Individual plants with no signs of disease were self-pollinated for further testing.

Results and Discussion: There were 1,411 watermelon accessions evaluated for Fusarium wilt and 1,235 evaluated for root-knot nematode resistance. PIs 534536, 386522, 270524, 543212, 482273, 385964, 512383, 299378, 482308, 169233, and 482299 had mean ratings for Fusarium wilt resistance of 3.5 or less (Table 1). In addition, individuals without symptoms were saved from 63 accessions for self-pollination (Table 2). Overall, the majority of the tested accessions for Fusarium wilt had ratings between 5 and 8 (Figure 1).

There were 10 PIs with root-knot nematode resistance ratings of 1.5 or less (Table 3). The majority of accessions tested for root-knot nematodes had ratings of 2-3 (Figure 2).

None of the PIs tested exhibited resistance to both Fusarium wilt and root-knot nematodes. Many PIs have been reported as sources of resistance to a variety of diseases including root-knot nematodes, gummy stem blight, anthracnose, watermelon mosaic virus, and zucchini yellows mosaic virus (1, 4, 3, 6, 7, 12, 14, 15, 18, 21) Testing of PIs, with previously reported resistance to various pathogens, did not result in those materials having favorable ratings for resistance to Fusarium wilt or root-knot nematode (Table 5). PI 482299, which had previously had reported resistance to ZYMV (15) had a rating of 3.6 for Fusarium wilt. This was the only PI in this study with the promise of multiple disease resistance.

Breeding for resistance to Fusarium wilt has been problematic because of the complex interaction of the host, pathogen, and soil environment. Hopkins et al. (9) found in monoculture that the level of Fusarium wilt changed dramatically from one year to the next and the specific watermelon cultivar appeared to have a suppressive effect on disease incidence in subsequent watermelon plantings. In addition, testing conditions also appear to have an effect. In previous tests of PI 296341-FR, a selection with resistance to Fusarium wilt, it did not perform any better than other cultigens tested (2). Similarly in this screening, PI 296341 with a mean rating of 6.3 appeared quite susceptible to Fusarium wilt. The testing method might play an important role in these contradictory results. Although we used a peat based artificial media in our test, a testing method that excludes potential effects of the media might have been a better choice. To confirm their work with PI 296341-

Table 1. Fusarium wilt evaluation of accessions with mean evaluations of 3.5 or lower.

Accession Number	Seed Source	Evaluation Mean[a]	Number of Plants Evaluated
PI 534536	Syria	0.9	9
PI 368522	Yugoslavia	2.7	26
PI 270524	Israel	2.9	25
PI 543212	Bolivia	3.0	35
PI 482273	Zimbabwe	3.0	18
PI 385964	Kenya	3.1	21
PI 512383	Spain	3.2	29
PI 299378	South Africa, Transvaal	3.3	23
PI 482308	Zimbabwe	3.4	27
PI 169233	Turkey	3.5	35
PI 482299	Zimbabwe	3.5	24

[a]Scale: 0-9 with 0-no symptoms, 9-plant death.

Table 2. Plant introductions (Pls) from which plants without Fusarium wilt symptoms were saved.

PI 255137	PI 482350	PI 278010
PI 500327	PI 482273	Grif 1732
PI 385964	PI 438671	PI 278031
PI 167126	PI 438671	PI 368493
PI 482299	PI 521383	PI 357720
PI 190050	PI 482329	PI 182934
PI 181937	PI 482350	PI 368493
PI 482265	PI 249008	PI 183300
PI 487458	PI 482318	PI 344298
PI 534588	PI 482286	PI 370434
PI 482265	PI 249008	PI 306365
PI 357695	PI 378613	PI 357661
PI 181937	PI 378613	PI 278050
PI 181937	PI 299379	PI 278004
PI 532809	PI 378611	PI 368498
PI 490378	PI 482324	PI 532809
PI 378617	PI 457916	PI 270144
PI 490378	PI 482252	PI 482265
PI 487459	PI 378616	PI 482265
PI 482273	PI 295850	PI 482265
PI 326515	PI 512384	PI 378617

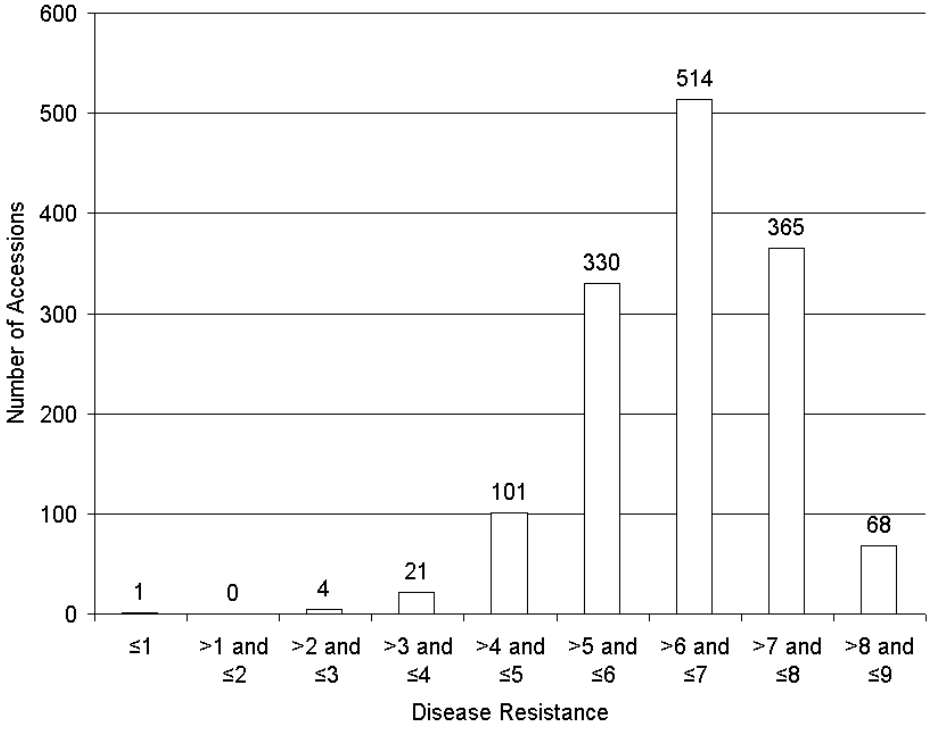

Figure 1. Frequency distribution of Fusarium wilt ratings of watermelon germplasm.

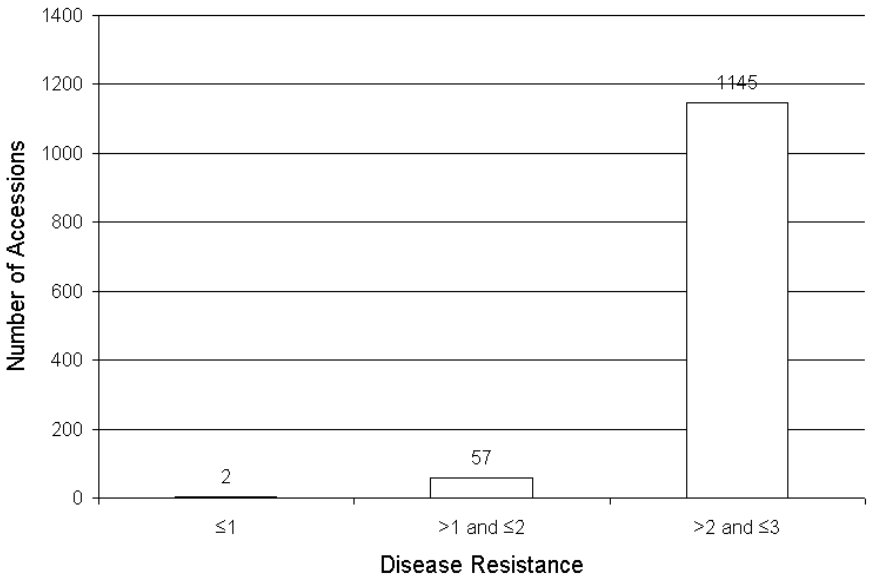

Figure 2. Frequency distribution of root-knot nematode ratings of watermelon germplasm.

Table 3. Root-knot nematode evaluation of accessions with mean evaluations of 1.5 or lower.

Accession Number	Seed Source	Evaluation Mean[a]	Number of Plants Evaluated
PI 482271	Zimbabwe	1.0	4
PI 512833	Spain	1.0	3
PI 169276	Turkey	1.4	5
PI 169248	Turkey	1.5	4
PI 214316	India	1.5	3
PI 271770	South Africa, Transvaal	1.5	3
PI 278000	Turkey	1.5	4
PI 295845	South Africa, Transvaal	1.5	3
PI 357738	Yugoslavia	1.5	4
PI 482309	Zimbabwe	1.5	6

[a]Scale: 0-3 with 0-no root galling, 3-severe root galling.

Table 4. Pls from which plants without root-knot nematode symptoms were saved.

PI 278057	PI 392291	PI 254743	PI 500347
PI 379246	PI 179878	PI 270140	PI 192937
PI 278057	PI 368511	PI 512342	PI 525096
PI 500307	PI 169299	PI 534598	PI 426625
PI 278057	PI 357705	PI 177322	PI 357697
PI 177329	PI 490382	PI 254743	PI 164247
PI 379233	PI 500336	PI 270140	PI 180426
PI 559993	PI 212094	PI 512342	PI 525096
PI 559993	PI 481871	PI 172791	PI 368527
PI 482269	PI 482331	PI 357738	PI 357697
PI 487458	PI 177325	PI 254743	PI 500335
PI 269680	PI 181936	PI 270140	PI 678615
PI 165448	PI 175659	PI 482248	PI 500336
PI 542114	PI 512393	PI 381708	PI 181937
PI 482300	PI 169250	PI 172801	PI 181937
PI 559993	PI 171582	PI 370423	PI 357667
PI 169262	PI 179234	PI 164685	PI 512342
PI 500319	PI 482282	PI 482281	PI 559992
PI 476329	PI 212209	PI 500312	PI 512833
PI 174106	PI 482291	PI 357709	PI 379255
PI 172786	PI 595203	PI 254623	PI 207473

Table 5. Reaction of watermelon germplasm to Fusarium wilt and root-knot nematodes with previous reports of disease resistance.

Accession Number	Previous Published Resistance	Fusarium Wilt Race 2[a] Mean	Number of plants evaluated	Root-knot Nematode Race 3[b] Mean	Number of plants evaluated
PI 164247	nematodes	7.5	23	2.2	5
PI 189225	GSB/Anthracnose	5.9	26	3.0	7
PI 189316	WMV-2	6.4	34	2.9	8
PI 189317	WMV-2	7.2	16	2.9	9
PI 248178	WMV-2	7.2	22	2.6	5
PI 271769	Fusarium race 2	6.6	19	3.0	4
PI 271775	Anthracnose	5.0	35	3.0	7
PI 271778	Anthracnose	6.4	24	2.8	9
PI 296341	Fusarium race 2	6.3	23	2.0	2
PI 299379	Anthracnose	5.2	26	2.9	8
PI 326515	Anthracnose	4.3	12	3.0	2
PI 386025	ZYMV Resistance	5.8	12	1.8	5
PI 386026	ZYMV Resistance	4.9	15		
PI 482261	ZYMV Resistance	5.7	25	2.3	7
PI 482299	ZYMV Resistance	3.6	24	3.0	5
PI 494815	nematodes	7.6	25	2.5	6
PI 500327	nematodes	4.9	26	2.9	8
PI 500329	nematodes	4.5	27	2.9	8
PI 500335	nematodes	6.0	26	2.6	7
PI 506439	nematodes	5.9	25		
PI 512385	Anthracnose	6.4	44	3.0	6
PI 532811	nematodes	4.9	25	3.0	2

[a]Fusarium Reaction: 0-9; 0-no symptoms, 9-dead plant

[b]Nematode Reaction: 0-3; 0-no symptoms, 3-severe galling

FR (12) used three different methods of testing, root dip, tray dip, and infested microplots. Considering the number of accessions tested, it was not feasible for us to use multiple testing methods. In addition, we did not have the facilities to fumigate a large volume of media, which may have helped prevent possible interactions. However, even with fumigation, organic media have large numbers of high molecular weight organic compounds whose interaction with the plant and the pathogen are not clearly understood.

We did recognize some limitations with the root-knot nematode screening as well. Because our inoculum consisted of primarily *M. incognita* race 3, any PI exhibiting resistance would require further testing against other races and species to be truly useful. Because of the size of the experiment we had to increase inoculum under field conditions, which can result in the very real possibility of other races and/or species of root-knot nematodes being present. This we felt was not a problem because watermelon in general is known to be susceptible to all races and species of root-knot nematode. This study was to be an overall characterization of the collection for root-knot nematode resistance rather than specifically addressing a particular root-knot nematode species or race reaction. In addition, we did not have the facilities to sterilize a large quantity of field soil at the particular site for this evaluation, which resulted in the possibility of contamination by other pathogens. We did not see any specific additional disease problem in the root-knot nematode evaluation and we did see well developed galling across most of the material indicating the inoculation was successful.

PIs with previously reported root-knot nematode resistance did not exhibit resistance in this screening. Overall, we were very conservative in our assessment of the accessions, not wishing to erroneously identify a PI with resistance or tolerance that did not exist, which may in part explain these results. In addition, environmental conditions could have played a role as well as the material itself. As these accessions are grown out for increasing seed stocks, cross-pollination, inadvertent selection, and the small populations involved, all can contribute to changes in the collection over time.

The results of these screenings may indicate that genetic drift or a high level of heterozygosity is present in the PI collection. Our results were with relatively few individuals, which makes it difficult to draw any conclusive statements about genetic drift or heterozygosity but in light of our results compared to other studies suggest these may be factors. Researchers have been concerned about this for years. How stable is the collection, how much seed should be used to increase and maintain the collection, are we loosing diversity over time? Studies such as these in the aggregate may indicate genetic drift or a greater level of heterozygosity than previously thought. Modern tools for studying the genetic complexity and diversity will have to be employed to answer these questions.

In conclusion, our testing showed a great deal of diversity in the collection to Fusarium wilt and root-knot nematodes. Several PIs showed resistance to either Fusarium wilt or root-knot nematode; none, however, exhibited dual resistance.

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