

Identifying Resistance to Powdery Mildew Race 2W in the USDA-ARS Watermelon Germplasm Collection

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ABSTRACT

Powdery mildew caused by *Podosphaera xanthii* has recently become an important disease of watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] in the United States. The disease can be controlled with fungicides, but it would be more economical and environmentally safe to use genetic resistance. Here, we report sources of resistance to powdery mildew race 2W identified in the evaluation of the entire U.S. watermelon Plant Introduction collection made of four *Citrullus* Schrad. ex. Eckl. & Zeyh. species and *Praecitrullus fistulosus* (Stocks) Pangalo species. A total of 1654 PI accessions, cultivars, and breeding lines (hereafter collectively referred to as cultigens) were tested in the greenhouse using at least seven replications. From that, 54 cultigens including the 44 most resistant and 10 susceptible checks were retested in greenhouse and field experiments. All cultigens showed symptoms of powdery mildew. Resistance was identified in wild PI accessions. Eight cultigens had high resistance and 21 had intermediate resistance. Leaf and stem disease severity ratings were positively correlated ($r = 0.86$, $P < 0.0001$). Data were summarized from the screening and retest studies, and the most resistant cultigens were PI 632755, PI 386015, PI 189225, PI 346082, PI 525082, PI 432337, PI 386024, and PI 269365. The most susceptible cultigens were PI 222775 and PI 269677. Many of the resistant cultigens originated from Nigeria and Zimbabwe.

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Abbreviations: PI, plant introduction.

In the United States, watermelon is a major vegetable crop. Major production states are Florida, California, Arizona, Texas, and Georgia. In 2005, the total production of watermelon in the United States was 1.7 million Mg, with a farm value of \$410 million (USDA, 2006).

Powdery mildew is a fungal disease that affects a wide range of crops worldwide. On cucurbits, the disease is caused by *Podosphaera xanthii* (Castagne) Braun & Shishkoff [syn. *Sphaerotheca fuliginea* auct. p.p.] and *Golovinomyces cichoracearum* s.l. (D.C.) V.P. Heluta [syn. *Erysiphe cichoracearum* auct. p.p.] (Jahn et al., 2002). Presently, there are at least seven pathogenically distinct races of *P. xanthii* and these are differentiated using ten melon (*Cucumis melo* L.) differentials (McCreight et al., 1987; Pitrat et al., 1998; Hosoya et al., 1999). Recent work by McCreight (2006) showed that there may be as many as 28 races of *P. xanthii* on melon that include eight variants of race 1 and six variants of race 2.

In the past, watermelon was considered to be free of powdery mildew with the exception of a few isolated and mild cases of the disease (Ivanoff, 1957; Nagy, 1983; McLean, 1970; Robinson and Provvidenti, 1975). In recent years, powdery mildew outbreaks have been reported in the United States. Using melon (*Cucumis melo* L.) differential genotypes, the disease has been confirmed in South Carolina, Georgia, Florida, Maryland, Texas, Oklahoma, Arizona, New York, and California (Keinath, 2000; Davis et al.,

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2001; McGrath, 2001). To date, two races of *P. xanthii*, races 1W and 2W, have been identified on watermelon in the United States, (Davis et al., 2001; McGrath, 2001; Davis et al., 2007). Powdery mildew is manifested as chlorotic spots on leaves with or without white mycelial and/or conidial development on leaves and stem. In some highly susceptible cultigens the petioles and stem show water-soaked areas in addition to these symptoms (Davis et al., 2006b).

The consequences of powdery mildew on watermelon are a reduced plant canopy and a reduction in yield caused by reduced fruit size and number of fruit per plant. The few fruits that remain often develop sunscald due to reduced canopy, have poor fruit quality and flavor, and have a shorter storage life (Keinath and Dubose, 2004; McGrath and Thomas, 1996).

Powdery mildew on watermelon can be controlled with fungicides. However, there have been reports of resistance to the recommended fungicides, especially the strobilurins and myclobutanil in southeastern United States (McGrath et al., 1996). Moreover, there are reports of chlorothalonil injury to watermelons (Holmes et al., 2002). Adequate disease control often requires the use of systemic fungicides because spray application to the underside of leaves is difficult (McGrath and Thomas, 1996). Effective control of powdery mildew on watermelon is achieved with alternating preventative applications of mancozeb with azoxystrobin (Keinath and Dubose, 2004). The most effective and safe method to control powdery mildew in crops is to use resistant cultivars.

Since the evaluation of 590 (Robinson and Provvidenti (1975) and 266 (Thomas et al., 2005) PI accessions of *C. lanatus* for their reaction to powdery mildew, the USDA-ARS watermelon germplasm collection has expanded to more than 1600 PI accessions of *Citrullus*, making it possible to search a wider diversity of germplasm for powdery mildew resistance. Davis et al. (2006a) reported the screening of PI accessions for powdery mildew race 1W, which led to the release of a resistant selection PI 525088-PMR. Resistance to powdery mildew race 1W in this cultigen was reported to be multigenic. However, little information is available on resistance of *Citrullus* to powdery mildew race 2W.

The objective of this research was to evaluate the entire U.S. plant introduction collection of *Citrullus* along with U.S. watermelon cultivars, totaling 1654 cultigens, for resistance to powdery mildew race 2W.

MATERIALS AND METHODS

Germplasm

A powdery mildew screening experiment was conducted in the greenhouses of the Department of Horticultural Science, North Carolina State University during the spring, summer, and winter seasons of 2005 and 2006. A total of 1654 cultigens of *Citrullus* (Schrud. ex. Eckl. & Zeyh.) and *Praecitrullus fistulosus* (Stocks) Pangalo were evaluated. These cultigens were made

up of 1613 PI accessions and 41 cultivars and breeding lines, hereafter referred to collectively as cultigens. The PI accessions were obtained from the Plant Genetic Resources Conservation Unit, Southern Regional Plant Introduction Station, USDA-ARS, Griffin, GA. The cultigens originated from 72 countries, with the greatest numbers collected from Turkey, the former Yugoslavia, Zimbabwe, India, P.R. China, Spain, and Zambia.

Inoculum

A *Podosphaera xanthii* race 2W isolate that originated from an infected commercial watermelon field in South Carolina was maintained on cantaloupe (*Cucumis melo*) 'PMR 45' and squash (*Cucurbita maxima*) 'Gray Zucchini' plants in the greenhouse. Inoculum was prepared from freshly sporulating leaves of diseased Gray Zucchini plants. In an earlier study it had been established that the source of *P. xanthii* inoculum used to infect *Citrullus* did not affect the disease severity on watermelon (Tetteh, 2008). Squash plants were used because they provided a high quantity of inoculum. A spore suspension was prepared by detaching heavily sporulating leaves and washing them with a spray of 100 mL of water and filtered through a double layer of cheesecloth. The suspension was diluted to a spore concentration of 4×10^4 conidia mL⁻¹ determined by hemocytometer count. This was freshly prepared as needed.

Germplasm Screening

Greenhouse Test

Seeds of all cultigens were sown in 10-cm pots in 4P Fafard soilless media (Conrad Fafard Incorporated, MA) and placed on benches in a greenhouse set at 32/24°C temperatures and 70/40% RH day/night. The experiment was a randomized complete block design with seven (up to 12 for some cultigens which had to be replanted because of low seed viability) replications of single-plant plots. Plants were fertilized weekly with 150 mg kg⁻¹ Peters Professional 20-10-20 N-P-K (Scotts Sierra Horticultural Products Company, Marysville, OH). Seedlings were inoculated at 12, 19, and 26 d after seeding, with the first inoculation timed to the first to second true-leaf stage. Inoculation was performed by spraying each seedling to run-off with the spore suspension. Highly sporulating Gray Zucchini squash plants (*Cucurbita maxima* L., from Seminis Vegetable Seeds, Woodland, CA) were used as spreader plants. These were placed between rows as additional sources of powdery mildew infection (Ziv and Zitter, 1992). Plant Introduction 269677 was used as a susceptible control. To verify the race of *P. xanthii* present, melon differentials were included in the experiment. Eight of the differentials were 'Edisto 47', 'Iran H', 'MR 1', 'WMR 29', PI 124112, PI 313970, PI 414723, and 'PMR 5'. Seeds of these eight differentials were increased in the greenhouses of the Department of Horticultural Science, North Carolina State University, Raleigh, NC, by controlled self-pollination. Two additional differentials, Top Mark and PMR 45, were obtained from Hollar Seeds (Rocky Ford, CO).

Following the greenhouse germplasm screening experiment, those cultigens that did not have complete data in at least four replications were replanted in a replacement experiment and tested again in a randomized complete block design with four to eight replications. Seeding, thinning, and inoculation were done as before. Data from the replacement plantings were pooled with the germplasm screening.

Disease Assessment

Powdery mildew on watermelon was rated as disease severity at 2 and 4 wk after inoculation to confirm disease progression. A preliminary assessment of disease progression had shown that powdery mildew on watermelon was manifested in two ways: plants which develop chlorotic spots, with or without mycelium, and plants which developed mycelia, with or without chlorotic spots. In plants which developed only chlorotic spots, physical examination of the stem showed that the most outstanding symptom on the stem was necrotic spots. Based on these symptoms, a disease rating scale was developed. Disease severity was rated on the leaves and stem of individual plants using a 0 to 9 rating scale (Tetteh, 2008) where 0 = no symptom; 1 = faint yellow speck on leaves and first appearance of necrotic spots on the stem; 2 = chlorotic lesions on leaves with 2 to 3 necrotic spots on the stem; 3 = chlorotic lesions covering 20% of leaves and necrotic spots covering less than 10% of stem; 4 = yellow chlorotic lesions on leaves turned to brown necrotic areas and first sign of active mycelium sporulation on leaves or stem; 5 = 2 to 3 healthy colonies of mycelium on leaves or stem; 6 = approaching 20% mycelium coverage; 7 = 20 to 50% mycelium coverage; 8 = 50 to 70% mycelium coverage with large necrotic areas; 9 = plant fully covered with powdery mycelium or plant dead. Total plant disease severity was calculated by averaging the leaf and stem ratings at 4 wk after inoculation. Disease severity ratings at 2 wk after inoculation were not used. Cultigens were classified based on their total plant disease rating from at least five replicates in the pooled germplasm screening and six replicates in the retest experiments. We considered them resistant if they had a total plant rating of ≤ 3 ; intermediate if the rating was 3.1 to 4; or susceptible if the rating was > 4.0 .

Germplasm Retest

Greenhouse Retest

A germplasm retest was performed in the greenhouse to verify the reaction of the resistant cultigens and cultivars from the germplasm screening. A total of 54 cultigens were chosen, made of 44 resistant and 10 susceptible cultigens. The 44 cultigens were chosen from the 70 cultigens having the lowest total plant rating and chosen to represent a wide geographical diversity. Ten countries and five species and subspecies (*C. lanatus* var. *lanatus*, *C. lanatus* var. *citroides*, *C. colocynthis*, *C. rehmanii* and *P. fistulosus*) were present. The experiment in the greenhouse was a randomized complete block design with four replications each of single-plant plots, and seedlings were inoculated and rated as in the first experiment. The final disease severity rating was used in data analysis.

Field Retest

A field retest was conducted at the Horticultural Crops Research Station, Clinton, NC, in the summer of 2006. The 54 cultigens chosen were planted in a randomized complete block design with four replications of three-plant plots. Seeds were planted on raised, shaped beds covered with black plastic mulch in rows spaced 3 m apart. Plots were 1.2 m long, and were planted with nine seeds and thinned to a uniform stand of 3 plants per plot. Recommended horticultural practices were used. Melon differentials Iran H, Top Mark, PMR 45, PMR 5, and MR 1 were planted in the front and rear of each row of 18 plots to verify the race of *P. xanthii* present

on the field. Rows were interplanted with spreader plants of squash that had previously been inoculated in the greenhouse and were showing heavy sporulation of *P. xanthii* race 2W (Ziv and Zitter, 1992). Rows were also inoculated with the spore suspension as before. Individual plants were rated on the 0 to 9 scale.

Data Analysis

Data were summarized as means for each cultigen in each study. Analysis of variance and correlation analysis were performed with PROC GLM and PROC CORR of SAS 9.1.3 statistical package (SAS Institute, Cary, NC). In the germplasm screening experiment, total plant disease severity was calculated as the average of final leaf and stem ratings. In the retest study, total plant disease severity was calculated as the average of final greenhouse and field ratings of leaf and stem.

RESULTS AND DISCUSSION

Germplasm Screening

In all experiments, the *P. xanthii* strain present was a variant of race 2 as defined by susceptibility of the melon differentials Iran H, Top Mark, PMR 45, and PI 414723 and resistance of PMR 5, WMR 29, Edisto 47, MR 1, PI 124112, and PI 313970 (McCreight, 2006; Pitrat et al., 1998, Tetteh, 2008), and confirming the absence of race 1, race 3, race 4, and race 5. Susceptibility of watermelon demonstrated that it was race 2W (race 2 that infects watermelon).

Powdery mildew race 2W was characterized by a wide range of reactions. Symptoms ranged from little sporulation to 100% coverage of mycelia on the plant; no blotching to moderate yellowing of many leaves; and no detectable water-soaked petioles to fully water-soaked petioles. A large number of the cultigens (93%) had total plant disease severity rating of > 4.0 , which means at least 20% mycelium coverage on leaves and stem. Seven percent of the cultigens had high or intermediate resistance to *P. xanthii*. There were 106 cultigens with total plant disease severity rating of 4.0 or less, which represents high or intermediate resistance (Table 1). Out of the 1654 cultigens, we got no data for seven due to nonviable seeds.

When cultigens were ranked for resistance by their total plant disease severity rating, 68 had ratings ≤ 3.0 (Table 1). This number reduced to 48 cultigens when ranked by leaf rating only. Disease severity ratings of the stem were lower than leaf ratings. Within the resistant category, all cultigens that had total plant disease severity ratings of ≤ 3.0 also had stem ratings of ≤ 3.0 . All cultigens that showed leaf resistance also demonstrated stem resistance. However, of the 36 cultigens that showed intermediate resistance based on disease severity of total plant, 28 had stem resistance.

In the retest (Table 2), eight cultigens were resistant (PI 632755, PI 386015, PI 189225, PI 346082, PI 525082, PI 432337, PI 386024, and PI 269365). These cultigens also were rated as resistant in the germplasm screening. Twenty-one cultigens that were classified as resistant in the germplasm screening showed intermediate resistance in the retest

Table 1. Ranking of *Citrullus* and *Praecitrullus* PI accessions that were highly or moderately resistant to *P. xanthii* race 2W in the germplasm screening study; cultigens ranked by mean disease severity rating (minimum of five replications).[†]

Rank	Cultigen	No. of reps.	Mean disease severity rating (0–9 scale) [‡]					Seed source
			Total plant	Leaf	Stem	Species [§]		
1	PI 386015	6	0.5	0.7	0.2	O	Iran	
2	PI 346082	5	0.6	1.0	0.2	O	Afghanistan	
3	PI 482326	6	0.9	1.5	0.3	T	Zimbabwe	
4	Grif 14202	6	1.3	1.5	1.0	O	India	
5	PI 386024	9	1.4	1.9	0.8	O	Iran	
6	PI 482307	7	1.4	2.0	0.7	T	Zimbabwe	
7	PI 482311	7	1.6	2.1	1.0	T	Zimbabwe	
8	PI 560020	7	1.6	2.1	1.0	L	Nigeria	
9	PI 386016	9	1.8	2.1	1.4	O	Iran	
10	PI 482307	6	1.8	2.2	1.3	T	Zimbabwe	
11	PI 494531	7	1.9	2.3	1.4	L	Nigeria	
12	PI 432337	6	1.9	2.7	1.0	O	Cyprus	
13	PI 186489	6	1.9	2.3	1.5	L	Nigeria	
14	PI 500354	7	1.9	2.4	1.4	T	Zambia	
15	PI 560005	7	1.9	2.4	1.4	L	Nigeria	
16	PI 482288	6	1.9	2.5	1.3	L	Zimbabwe	
17	PI 482302	8	2.0	2.5	1.4	T	Zimbabwe	
18	PI 482298	6	2.0	2.5	1.5	T	Zimbabwe	
19	PI 525082	8	2.0	2.6	1.4	O	Egypt	
20	PI 482282	7	2.0	2.7	1.3	T	Zimbabwe	
21	PI 500329	6	2.0	2.8	1.2	L	Zambia	
22	PI 560003	7	2.1	3.0	1.1	L	Nigeria	
23	PI 482286	5	2.1	2.8	1.4	T	Zimbabwe	
24	PI 542616	8	2.1	2.9	1.3	O	Algeria	
25	PI 251244	6	2.1	2.3	2.0	F	India	
26	PI 482338	8	2.2	2.8	1.5	T	Zimbabwe	
27	PI 482322	7	2.2	3.0	1.4	T	Zimbabwe	
28	PI 482341	7	2.2	3.0	1.4	L	Zimbabwe	
29	PI 307608	8	2.2	2.9	1.6	L	Nigeria	
30	PI 386025	9	2.3	2.7	1.9	O	Iran	
31	PI 482259	7	2.3	2.6	2.1	T	Zimbabwe	
32	PI 482361	7	2.4	2.7	2.0	T	Zimbabwe	
33	PI 560024	6	2.4	3.0	1.7	L	Nigeria	
34	PI 560010	9	2.4	3.0	1.7	L	Nigeria	
35	PI 500332	7	2.4	3.0	1.7	T	Zambia	
36	PI 500331	7	2.4	3.0	1.7	T	Zambia	
37	PI 482318	7	2.4	3.0	1.7	L	Zimbabwe	
38	PI 225557	7	2.4	3.0	1.7	L	Zimbabwe	
39	PI 269365	6	2.4	2.8	2.0	O	Afghanistan	
40	PI 482299	7	2.4	2.9	1.9	T	Zimbabwe	
41	PI 381752	6	2.4	2.7	2.2	F	India	
42	PI 494528	7	2.5	2.9	2.0	L	Nigeria	
43	PI 482321	6	2.5	3.2	1.7	T	Zimbabwe	
44	PI 326516	7	2.5	2.9	2.1	L	Ghana	
45	PI 482319	7	2.5	3.4	1.6	T	Zimbabwe	
46	PI 560006	7	2.5	3.0	2.1	L	Nigeria	
47	PI 540911	7	2.6	2.9	2.3	F	Unknown	
48	PI 220778	8	2.6	2.9	2.3	O	Afghanistan	
49	PI 500303	7	2.6	2.6	2.6	T	Zambia	
50	PI 381750	6	2.6	2.7	2.5	F	India	
51	PI 482283	6	2.6	3.2	2.0	T	Zimbabwe	
52	PI 482377	6	2.6	3.2	2.0	L	Zimbabwe	
53	PI 386026	7	2.7	3.0	2.3	O	Iran	
54	PI 560023	7	2.7	3.3	2.0	L	Nigeria	
55	PI 560002	7	2.7	3.9	1.4	L	Nigeria	

(cont'd)

Table 1. Continued.

Rank	Cultigen	No. of reps.	Mean disease severity rating (0–9 scale) [‡]				Seed source
			Total plant	Leaf	Stem	Species [§]	
56	PI 189225	10	2.7	3.1	2.2	T	Zaire
57	PI 494532	7	2.7	3.4	2.0	L	Nigeria
58	PI 596696	7	2.7	3.7	1.7	T	South Africa
59	PI 388770	6	2.8	3.2	2.3	O	Morocco
60	PI 500312	6	2.8	3.3	2.2	L	Zambia
61	PI 482277	6	2.8	3.3	2.3	T	Zimbabwe
62	PI 270545	7	2.9	3.6	2.1	L	Sudan
63	PI 482246	7	2.9	3.6	2.1	T	Zimbabwe
64	PI 560019	6	2.9	3.8	2.0	L	Nigeria
65	PI 632755	7	2.9	3.1	2.7	R	Namibia
66	PI 500327	7	3.0	3.6	2.3	L	Zambia
67	PI 482355	7	3.0	3.3	2.7	T	Zimbabwe
68	Grif 5596	7	3.0	3.3	2.7	L	India
69	PI 482360	5	3.1	3.8	2.4	L	Zimbabwe
70	PI 595203	6	3.1	3.2	3.0	L	United States
71	PI 532726	7	3.1	3.6	2.6	L	Zimbabwe
72	PI 560001	7	3.1	3.6	2.6	L	Nigeria
73	PI 532722	7	3.2	3.3	3.0	L	Zaire
74	PI 500302	6	3.2	3.8	2.5	L	Zambia
75	PI 482262	6	3.2	3.8	2.5	L	Zimbabwe
76	PI 560011	7	3.2	4.0	2.3	L	Nigeria
77	PI 560014	7	3.2	4.0	2.3	L	Nigeria
78	PI 459074	7	3.2	3.7	2.6	L	Botswana
79	PI 559997	7	3.2	3.7	2.6	L	Nigeria
80	PI 525084	6	3.2	3.7	2.7	L	Egypt
81	PI 482308	7	3.2	3.7	2.7	T	Zimbabwe
82	PI 549161	7	3.2	3.7	2.7	O	Chad
83	PI 512828	6	3.3	3.3	3.2	L	Spain
84	PI 559994	6	3.3	3.7	2.8	L	Nigeria
85	PI 532730	7	3.3	3.9	2.6	L	Zimbabwe
86	PI 560008	7	3.3	3.9	2.6	L	Nigeria
87	PI 500345	6	3.3	3.8	2.8	L	Zambia
88	PI 500318	7	3.3	3.9	2.7	L	Zambia
89	PI 482368	7	3.3	4.0	2.6	L	Zimbabwe
90	PI 381743	7	3.4	4.0	2.7	F	India
91	PI 482257	7	3.4	4.0	2.7	T	Zimbabwe
92	PI 254738	7	3.4	4.0	2.7	L	Senegal
93	PI 249009	7	3.4	3.9	2.9	L	Nigeria
94	PI 482312	7	3.5	3.9	3.0	T	Zimbabwe
95	PI 381748	7	3.5	4.0	2.9	F	India
96	PI 560004	7	3.5	4.0	2.9	L	Nigeria
97	PI 482273	6	3.5	3.8	3.2	T	Zimbabwe
98	PI 485583	7	3.5	4.0	3.0	T	Botswana
99	PI 482276	7	3.7	4.0	3.3	T	Zimbabwe
100	PI 386019	9	3.7	4.0	3.3	O	Iran
101	PI 525081	7	3.7	4.0	3.4	T	Egypt
102	PI 482372	7	3.8	4.0	3.6	L	Zimbabwe
103	Grif 5602	7	3.9	4.0	3.7	F	India
104	PI 306782	7	3.9	4.0	3.7	L	Nigeria
105	PI 500307	7	4.0	4.0	4.0	L	Zambia
106	PI 250145	7	4.0	4.0	4.0	F	Pakistan

[†]Data were pooled from germplasm screening and replacement experiments.

[‡]PI accessions were classified into resistant, intermediate, or susceptible classes based on their total plant mean disease severity rating at 4 wk after inoculation. Accessions were resistant if their total plant mean disease severity rating was ≤3.0; intermediate if 3.1–4; susceptible if >4.0.

[§]L = *Citrullus lanatus* var. *lanatus*; T = *Citrullus lanatus* var. *citroides*; O = *Citrullus colocynthis*; R = *Citrullus rehmii*; F = *Praecitrullus fistulosus*.

Table 2. Ranking of the 54 *Citrullus* and *Praecitrullus* PI accessions that demonstrated highly or moderately resistant reactions to *P. xanthii* race 2W in the retest greenhouse and field study, including checks.

Rank	Cultigen	Rep	Total plant	Mean disease severity rating on the 0–9 scale [†]							
				Greenhouse				Field			
				Leaf	Stem	SD [‡] leaf	SD stem	Leaf	Stem	SD leaf	SD stem
1	PI 632755	5	0.6	1.3	0.6	0.6	2.0	0.3	0.2	1.0	0.0
2	PI 386015	7	1.1	1.0	0.5	0.8	0.8	2.0	0.9	0.8	0.2
3	PI 189225	7	1.1	1.7	0.5	1.0	0.4	–	–	–	–
4	PI 346082	7	1.5	2.0	1.1	0.6	1.0	2.3	0.6	0.0	0.0
5	PI 525082	7	1.7	2.7	1.5	1.0	1.0	2.3	0.6	0.5	0.6
6	PI 432337	5	1.8	2.5	1.5	1.3	0.8	2.3	1.0	0.6	0.3
7	PI 386024	8	2.1	3.3	2.6	0.8	1.0	2.0	0.6	0.5	0.0
8	PI 269365	8	2.4	3.3	1.7	1.3	1.3	3.3	1.4	1.3	0.5
9	PI 482283	7	3.2	4.0	2.7	0.8	1.0	4.0	2.0	1.0	0.2
10	PI 494523	8	3.4	5.0	2.9	0.8	0.8	4.0	1.6	0.6	0.3
11	PI 482319	7	3.5	4.0	2.1	0.6	0.5	5.5	2.3	0.5	0.2
12	PI 270545	7	3.5	5.3	4.3	0.6	0.0	2.3	1.2	0.6	0.3
13	PI 482246	8	3.6	4.3	2.3	0.6	0.5	5.5	2.3	0.0	0.2
14	PI 500354	7	3.6	4.5	2.7	0.5	0.6	5.3	2.0	0.5	0.5
15	PI 560010	8	3.7	5.8	3.8	1.3	0.5	3.5	1.6	0.6	0.2
16	PI 251244	7	3.7	5.3	3.9	1.4	0.5	4.0	1.9	0.8	0.2
17	PI 186489	8	3.7	5.0	2.7	1.0	1.6	4.5	2.5	0.8	0.8
18	PI 482326	5	3.7	5.0	3.6	1.0	1.0	4.3	2.0	0.5	0.2
19	PI 560003	8	3.7	5.5	3.8	1.4	0.8	4.0	1.6	0.6	0.2
20	PI 482338	7	3.8	4.5	2.0	0.0	0.0	6.0	3.0	0.0	0.3
21	PI 482259	8	3.8	4.5	2.0	0.6	1.0	5.5	3.2	0.0	0.2
22	PI 307608	5	3.8	6.7	5.1	0.0	0.7	1.0	0.5	0.0	0.3
23	PI 500329	8	3.9	5.0	3.6	1.0	0.5	5.3	1.6	0.5	0.4
24	PI 500331	6	3.9	5.0	5.0	0.5	0.8	4.3	2.5	0.5	0.3
25	PI 482307	7	3.9	5.3	3.0	0.0	0.5	5.0	2.5	0.0	0.6
26	PI 494531	8	4.0	5.8	3.6	0.8	1.6	4.0	2.7	0.6	0.6
27	PI 560020	8	4.0	5.3	3.6	1.0	1.6	4.8	2.5	0.8	0.3
28	PI 560024	8	4.0	5.3	3.4	1.3	0.6	4.8	2.7	0.8	0.8
29	PI 560006	7	4.0	5.3	3.8	0.0	0.6	4.0	2.7	0.6	0.0
30	PI 482302	5	4.1	7.0	4.5	0.5	0.0	5.3	2.0	0.5	0.2
31	PI 560005	8	4.1	5.3	4.3	1.3	0.5	4.5	2.3	0.8	0.0
32	PI 482311	8	4.1	6.3	3.4	0.5	0.5	4.8	2.0	0.5	0.2
33	PI 482377	6	4.1	6.8	4.7	0.5	0.5	3.8	1.4	0.5	0.3
34	PI 482361	7	4.3	5.8	4.1	0.0	1.0	5.0	2.3	1.0	0.2
35	PI 560023	3	4.3	6.5	4.5	0.5	0.8	4.8	1.8	0.5	0.2
36	PI 482341	8	4.4	7.0	5.0	0.0	1.0	4.0	1.8	0.5	0.2
37	PI 482318	8	4.6	6.8	5.4	1.0	1.8	4.5	1.6	0.8	0.4
38	PI 482322	6	4.6	6.0	4.1	1.4	1.4	5.0	2.3	0.7	0.0
39	PI 494528	8	4.6	6.5	5.4	1.2	1.2	3.7	2.1	1.2	0.8
40	PI 482298	8	4.7	5.5	4.5	1.0	0.5	5.8	2.9	0.6	1.0
41	PI 381750	8	4.9	6.5	4.7	0.5	0.6	5.3	2.9	0.0	1.6
42	PI 225557	7	4.9	5.8	4.3	0.0	0.6	6.0	3.3	0.6	0.5
43	PI 540911	7	4.9	6.3	5.1	0.6	0.5	5.5	2.9	1.0	1.3
44	PI 482286	5	5.0	6.3	4.5	0.7	0.0	5.5	3.2	0.0	0.3
45	Tastigold	4	5.6	8.0	6.3	0.5	0.6	5.3	2.7	0.5	0.2
46	Charleston Gray	8	5.6	8.3	6.1	0.5	1.0	5.8	2.5	0.5	0.7
47	Peacock Shipper	8	5.7	8.3	5.9	0.6	0.5	5.5	3.4	0.5	0.7
48	Hopi Red Flesh	6	5.9	8.3	6.3	0.5	0.5	6.3	2.7	0.5	0.9
49	Florida Favorite	8	5.9	8.0	6.0	0.0	0.6	6.0	3.6	0.6	0.7
50	Charlee	6	6.0	8.3	6.8	0.5	0.5	5.8	3.4	0.6	0.7
51	Navajo Sweet	8	6.6	9.0	8.6	0.5	0.6	6.3	2.7	0.0	0.5
52	Chubby Gray	8	6.7	9.0	9.0	0.0	1.0	6.0	2.9	0.5	0.7
53	Moon & Stars	6	6.7	9.0	7.9	0.6	0.6	5.7	3.3	0.6	0.7
54	PI 269677	8	7.0	9.0	9.0	0.5	0.6	6.8	3.2	0.0	0.9

[†]PI accessions were classified into resistant, intermediate, or susceptible based on their total plant mean disease severity rating at 4 wk after inoculation, providing that there were at least six replications: resistant ≤ 3.0 ; intermediate 3.1–4; and susceptible if > 4.0 .

[‡]SD = standard deviation.

Table 3. Number and percentage of resistant and intermediate resistant accessions within species.

Species	No. of resistant accessions [†]	% of all resistant accessions [‡]	% of resistant accessions relative to total species tested	Species as % of PI collection
<i>C. lanatus</i> var. <i>lanatus</i>	50	47	3.4	88.9
<i>C. lanatus</i> var. <i>citroides</i>	32	30	24.6	7.9
<i>C. colocynthis</i>	15	14	75.7	1.2
<i>C. rehmii</i>	1	1	100	<1
<i>P. fistulosus</i>	8	8	26.8	1.8
Total	106	100	–	99.8

[†]Number of cultigens within species that had a total plant disease severity rating of ≤ 4.0 on a 0–9 scale: resistant ≤ 3.0 ; intermediate 3.1–4; and susceptible if > 4.0 .

[‡]Percentage of resistant and intermediate resistant cultigens within each species with a total plant disease severity rating of > 4.0 .

(PI 482283, PI 494532, PI 482319, PI 270545, PI 482286, PI 500354, PI 560010, PI 251244, PI 560003, PI 186489, PI 482326, PI 482338, PI 482259, PI 307608, PI 500329, PI 500331, PI 482307, PI 494531, PI 560020, PI 560024, and PI 560006). Fifteen cultigens that were rated as resistant in the germplasm screening were susceptible in the retest. Thus, there were cultigens that appeared resistant in the germplasm screening that were actually escapes. Combining data from the pooled germplasm screening and retest, cultigens that were most resistant to race 2W were PI 632755, PI 386015, PI 189225, PI 346082, PI 525082, PI 432337, PI 386024, and PI 269365. PI 386015, PI 525082, and PI 270545, which are resistant to powdery mildew race 1W (Davis et al., 2007), also were resistant to race 2W. All watermelon cultivars in our study were susceptible to powdery mildew race 2W.

Plant Introduction 189225, which is resistant to gummy stem blight (Sowell and Pointer, 1962; Gusmini et al., 2005) and anthracnose (Sowell et al., 1980; Winstead et al., 1959), had high resistance to race 2W. It was thoroughly tested in the germplasm screening with a total of 10 replications, where it was ranked among the 60 cultigens most resistant to race 2W (Table 1). In the greenhouse retest, PI 189225 had a total of seven replications and was ranked among the eight most resistant cultigens (Table 2). However, due to constraint of seed availability we could not test it in the field. Since powdery mildew severity in the greenhouse was greater than in the field (Table 2), we established that greenhouse testing was enough to evaluate the reaction of watermelon to *P. xanthii* race 2W.

Variability of Resistance within Cultigens

The majority of the PI accessions were heterogeneous for powdery mildew in plants rated resistant using the total plant severity rating with standard deviations ranging from 0 to 70% of the mean and an average of 35%. Individual replicates for some resistant cultigens had ratings ranging from 0 to 8. Plant Introduction accessions are often heterogeneous when collected, and seed increases are often by open pollination, so this reaction was not unexpected. Although variable, they may carry useful resistance for breeding purposes. Variability was lower for susceptible than for resistant PI accessions. Final disease severity ratings among the highly susceptible PI

accessions varied from 6 to 9 in all experiments. This sort of variability was also reported by Davis et al. (2007).

Correlation of Leaf and Stem Disease Severity Ratings

Correlation analysis of the disease severity ratings of leaf and stem of all 1647 cultigens which were rated was performed. There was significant and positive correlation between leaf and stem ratings ($r = 0.86$; $P < 0.0001$). There were significant and positive correlations between leaf and stem ratings for greenhouse retests ($r = 0.94$; $P < 0.0001$) and for field retests ($r = 0.84$; $P < 0.0001$). Likewise, significant and positive correlations were observed between greenhouse leaf and field leaf ratings ($r = 0.075$; $P < 0.0001$) and between greenhouse stem and field stem ratings ($r = 0.79$; $P < 0.0001$). For most of the cultigens, disease severity was higher in the greenhouse than in the field (Table 2). Though stems had lower disease severity ratings than leaves, the high correlation suggests that resistance in stem and leaf may be controlled by the same gene. For race 1W resistance, a significant but weak correlation was observed between leaf and stem (Davis et al., 2007).

Resistance to *P. xanthii* Race 2W in *Citrullus* and *Praecitrullus*

Five species within those two genera were evaluated for resistance to *P. xanthii* race 2W. A small percentage of *C. lanatus* var. *lanatus* (3.4%) was resistant. A large percentage of cultigens of *C. lanatus* var. *citroides* (30%) and *C. colocynthis* (14%) were represented in the 106 most resistant cultigens (Table 3). Because those two taxons make up less than 8% and 2% of the total PI collection, respectively, the high percentage of resistant cultigens in those species demonstrates that resistance to race 2W is more common in the wild PI accessions (Table 3). Fewer resistant cultigens were represented by *P. fistulosus*.

Analysis of the data by geographical origin of the cultigens indicated that 33, 21 and 10% of the 106 cultigens that were most resistant to race 2W were from Zimbabwe, Nigeria, and Zambia, respectively, although these make up only 9.5, 4, and 3% of the U.S. *Citrullus* and *Praecitrullus* germplasm collection. Thus, majority of the resistant cultigens originated in Africa. Watermelon is indigenous to tropical Africa where it grows wild (De Candolle, 1882)

and therefore it is not unexpected to find powdery mildew resistant cultigens in this center of origin.

From the screening and retest experiments, cultigens were selected and self-pollinated to produce lines having uniform and high resistance to powdery mildew race 2W. Those inbred lines will be used to determine inheritance of resistance and to develop resistant cultivars.

CONCLUSIONS

We have identified resistance to powdery mildew race 2W in the watermelon germplasm collection. Among the 1654 *Citrullus* cultigens tested, 22 *C. lanatus* var. *citroides* had resistance to powdery mildew race 2W. High-level resistance was identified in only *C. lanatus* var. *citroides* and *C. colocynthis* cultigens. Intermediate resistance was identified in *C. lanatus* var. *lanatus* cultigens. We considered this type of resistance to be commercially useful. For breeding purposes, *C. lanatus* var. *lanatus* ($2n = 2x = 22$) PI accessions are preferred because they are more closely related to cultivated watermelon than. *C. lanatus* var. *citroides* which is considered a wild progenitor of the cultivated watermelon. *Citrullus lanatus* var. *citroides* cultigens represent an important source of powdery mildew resistance genes, although they do not have the desirable horticultural characteristics of *C. lanatus* var. *lanatus*. We also concluded that screening watermelon for powdery mildew resistance in the greenhouse alone and using leaf disease severity were enough to identify and confirm resistant, intermediate resistant, and susceptible cultigens.

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