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RESEARCH

Flowering Stage Resistance to Bacterial Fruit Blotch in the Watermelon Germplasm Collection

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ABSTRACT

Cucurbit bacterial fruit blotch caused by *Acidovorax avenae* subsp. *citrulli* is a significant threat to watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] production worldwide. In the United States, seedless cultivars are primarily used in watermelon production, which now relies largely on transplant production in greenhouses to ensure a high germination rate. Unfortunately, the warm and humid greenhouse environment provides ideal conditions for the spread of bacterial fruit blotch. Treatments designed to remove bacteria from the surface of the seed coat were investigated previously, but none eliminated the bacteria despite significant reductions reported in research studies. Resistant cultivars offer a solution to the problem if genetic resistance can be identified. The objectives of this study were to (i) identify germplasm resistant to bacterial fruit blotch using the available PI accessions in the USDA germplasm collection and (ii) improve the methods for screening in the field. Field evaluations on the basis of foliar disease symptoms at the flowering stage were conducted at Clinton, NC in 2011 to 2013. The field experiment was a randomized complete block with 1699 cultigens, 3 yr, and two replications of single-plant plots. Disease rating was on a 0 to 9 scale when the disease was uniformly distributed throughout the field (0 = no symptoms, 1–2 = trace, 3–4 = slight, 5–6 = moderate, 7–8 = severe, and 9 = dead). Plots were rated multiple times each year. Significant differences were found for disease resistance among accessions (P = 0.05). Rating dates having the greatest F ratio for differences among accessions were identified as best ratings for each year–block combination. Resistant accessions have the best ratings (<4.5), low standard deviation across replications, and multiple replication (≥4). The 23 most resistant cultigens originated from Africa, mainly Zimbabwe, Zambia, Republic of South Africa, and Nigeria, and they were either cultivated watermelon (*C. lanatus* var. *lanatus*) or citron (*C. lanatus* var. *citroides*).

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Watermelon belongs to the Cucurbitaceae family and is one of most economically important cucurbit crops. The watermelon industry has been threatened by bacterial fruit blotch commercially since 1989 in the United States (Hopkins, 1989). Bacterial fruit blotch is a seedborne disease. Disease incidence is 5 to 50%, with complete crop loss under ideal conditions, especially when the outbreaks occur early in the growing season (Latin and Hopkins, 1995). Bacterial fruit blotch has caused significant economic loss to the watermelon industry since the 1990s (Hodge, 1999). Most economic losses of bacterial fruit blotch have been reported in watermelon and melon (Burdman et al., 2005; Isakeit et al., 1997; Latin and Hopkins, 1995; O'Brien and Martin, 1999; Schaad et al., 2003; Somodi et al., 1991; Wall and Santos, 1988; Wall et al., 1990). Resistance resources have mainly been identified on these two crops (Bahar et al., 2009; Carvalho et al., 2013; Hopkins and Thompson, 2002; Hopkins et al., 1993; Somodi et al., 1991; Sowell and Schaad, 1979; Wechter et al., 2011). Attempts were made to increase watermelon resistance to bacterial fruit blotch (Hopkins and Levi, 2008).

Since bacterial fruit blotch is seedborne, contaminated seeds are the primary source of inoculum in both field and greenhouse (Hopkins and Thompson, 2002). Several treatments for external inoculum removal have been suggested and tested to decontaminate cucurbit seeds. Those include treatment with NaClO, dipping in HCl, use of biological control with antagonistic

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microorganisms, or seed fermentation at harvest (Hopkins et al., 1996; Rane and Latin, 1992; Sowell and Schaad, 1979). Seed fermentation became one of the routine seed treatments for removing bacterial fruit blotch for watermelon industry. Unfortunately, seed fermentation cannot be used for triploid watermelon seeds due to deleterious effects on germination. The chemical seed treatment of streptomycin sulphate, NaOCl, HCl, $CaOCl_2$, and peroxyacetic acid reduced bacterial fruit blotch transmission on watermelon seedlings with varying success depending on the study (Hopkins et al., 1996, 2003; Rane and Latin, 1992; Sowell and Schaad, 1979;). Dry heat treatment, chlorine gas exposure for 9 h, and acidic electrolyzed water were reported to be effective as well (Feng et al., 2009; Hopkins et al., 1996, 2003; Kubota et al., 2012; Shirakawa, 2003; Stephens et al., 2008). Despite those results, none of the seed treatments were able to eliminate bacterial fruit blotch from seeds reliably for day-to-day production, probably due to inoculum under the seed coat (Burdman and Walcott, 2012; Rane and Latin, 1992). To solve this problem; Johnson et al. (2011) developed a nonpathogenic *A. citrulli* strain as a biocontrol seed treatment to eliminate the pathogenicity of inoculum under the seed coat. The biocontrol method has not been tested commercially. Even if the seed treatments can remove the seed inoculum completely, contaminated volunteer watermelons, other cultivated cucurbits, wild cucurbits, and even weeds in the cucurbit family are able to introduce bacteria to the watermelon crop in the field (Isakeit et al., 1998; Hopkins and Thompson, 2002; Latin and Hopkins, 1995).

Once in the field, bacterial fruit blotch can only be controlled in the field using multiple applications of a copper-containing bactericide including cupric hydroxide, copper hydroxyl sulfate, or copper oxychloride (Hopkins, 1991; Hopkins and Thompson, 2002). It is not systemic, so good coverage and retention on the leaf surface is required (Ritchie, 2004). Those compounds are marginally successful in reducing *A. avenae* subsp. *citrulli* in the greenhouse and field, so their widespread use raises concerns of copper-resistant isolates of the bacterium (Latin and Hopkins, 1995; Walcott et al., 2004; Wechter et al., 2011).

Resistant cultivars would be an effective strategy for managing bacterial fruit blotch if they could be developed. In addition to cost effectiveness, resistance-based strategies are compatible with other integrated disease management approaches. So far, no cucurbit cultivars with resistance to bacterial fruit blotch have been developed (Hopkins and Levi, 2008; Hopkins and Thompson, 2002; Hopkins et al., 1993; Rane and Latin, 1992; Sowell and Schaad, 1979).

Screening 1344 *Citrullus* spp. and tinda [*Praecitrullus fistulosus* (Stocks) Pangolo] accessions revealed PI 482279 (Zimbabwe) and PI 494817 (Zambia) as the best sources of resistance to bacterial fruit blotch. In other studies, PI 500303 (Zambia), PI 500331 (Zambia), and PI 482246 (Zimbabwe) were recommended as sources of resistance. All of the resistant accessions are *C. lanatus* var. *citroides* (Hopkins and Thompson, 2002). Because of undesirable horticultural traits in PI 482279 and PI 494817, a project was initiated to incorporate resistance from PI 482279 and PI 494817 into 'Crimson Sweet' with desirable horticultural traits and to investigate the inheritance of resistance. By the third backcross, horticultural traits (fruit shape, flesh color, and flesh soluble solids) of selected lines were similar to Crimson Sweet. Resistance to bacterial fruit blotch from PI 482279 and PI 494817 was controlled by more than one gene. Quantitative inheritance of resistance in the accessions of citron made it difficult to maintain a useful level of resistance along with the fruit quality traits from Crimson Sweet (Hopkins and Levi, 2008). Thus, it would be useful to identify high resistance in watermelon accessions other than citron.

Bacterial fruit blotch strains with different virulence levels have been identified (Somodi et al., 1991). Study of fatty acid profiles, utilization of L-leucine and 2-amino ethanol, and DNA fingerprinting by pulse-field gel electrophoresis and repetitive extragenic palindromic polymerase chain reaction indicates that there are different groups of strains (O'Brien and Martin, 1999; Walcott et al., 2004). The bacterial strains have been identified as Group I or II (Walcott, 2005). Group II strains were more aggressive on watermelon, while Group I strains were more aggressive on other cucurbits.

The objectives of this study were (i) to develop screening methods for severe and uniform disease development and (ii) to screen the USDA watermelon germplasm collection for high resistance to bacterial fruit blotch from watermelon taxons other than citron.

MATERIALS AND METHODS

Plant Materials and Cultural Practices

A total of 1699 watermelon PI accessions from the USDA germplasm collection were screened for resistance at the flowering stage to bacterial fruit blotch isolates from Group II. The PI accessions originated from 73 countries as follows (number of accessions given from each): Turkey (308), Zimbabwe (157), Yugoslavia (184), India (137), United States (129), Spain (76), China (71), Zambia (69), South Africa (61), and Nigeria (48) were the major seed sources. Field tests were run in 2011 through 2013 at the Horticultural Crops Research Station in Clinton, NC (35.02° N, 78.28° E). All available USDA watermelon accessions were included, along with exotic *Citrullus* PI accessions and a diverse set of cultivars. Most accessions were *Citrullus lanatus*, but four other taxa were included: colocynth [*C. colocynthis* (L.) Schrad.], citron melon [*C. lanatus* (Thunb.) Mastum. and Nakai var. *citroides* (L.H. Bailey) Mansf.], wild species (*C. rehmii* De Winter), and *P. fistulosus*, constituting secondary or tertiary gene pools based on crossability with *C. lanatus* and studies of genetic diversity (Levi et al., 2011).

Seeds were seeded in single-plant hills using 2 to 10 seeds hill^{-1}, depending on seed availability and germination rate. In the field, raised beds were made up with drip irrigation tubes and covered with black polyethylene mulch. Rows were on 3.1-m centers with hills 1.2 m apart. We used recommended horticultural practices (Sanders, 2004). Soil type was an Orangeburg loamy sand. Plants were thinned to one plant hill^{-1} 3 wk after seeding (four to six true leaf stage). Overhead irrigation was applied to the field twice a week to encourage disease development and spread.

Experiment Design

Field screening. The experiment was a randomized complete block with 1699 cultigens, 3 yr (2011, 2012, and 2013) and two replications. One replication in 2013 was replanted due to problems of stand establishment. Planting dates for the two replications were 18 and 21 July in 2011, 18 and 25 June in 2012, and 28 May and 15 July in 2013.

Retest. Field resistance from the germplasm screening study was validated in a retest. In 2012, the 2011 germplasm screening data were used to choose the 17 most resistant and two most susceptible cultigens for the retest using a rating scale from 0 (healthy) to 9 (dead). In 2013, the 2012 germplasm screening data were used to choose the 20 most resistant cultigens and the most susceptible cultigen for the retest. Field plots were 3.7 m long with 6 plants per plot. Cultural practices were the same as for the germplasm screening study. The experiment was a randomized complete block design with four replications per year. The retest study was planted 18 June 2012 and 13 May 2013.

Inoculation Method

In 2011 and 2012, inoculum was spread through the field test from natural inoculum carried on the planted seeds. Diseased leaf samples were collected periodically from the field to confirm the presence of bacterial fruit blotch. Disease diagnosis and bacterial isolation were conducted by the Plant Disease and Insect Clinic at North Carolina State University. The disease was spread through the field using overhead irrigation and weekly vine training.

In 2013, plants were inoculated using a foliar spray when plants reached the four to six true leaf stage. The inoculum consisted of a bacterial suspension of Group II strains AAC 00–1 and AAC 94–21. The strains were obtained from R. Walcott and were collected in Georgia in 1990 and 1994, respectively (Walcott et al., 2004). The strain isolates were grown on nutrient agar (VWR, Radnor, PA) for 48 h and washed from the agar surface with deionized water. In the field, the suspension was diluted to 10^6 cfu ml^{-1}. Surfactant Islet L-77 (Momentive, Albany, NY) was added at 0.03% ratio before inoculation to lower the leaf surface tension. A dosage of 10 mL suspension was applied as a mist to each plant using a hand-sprayer.

Field Bacterial Isolate Identification with Biolog

Diseased leaf samples were collected from Clinton, NC in the summer of 2012. Pure colonies of *A. avenae* subsp. *citrulli* isolates were then grown on Biolog Universal Agar media (Biolog Inc. Hayward, CA) for 24 h at 30°C in an IsoTemp incubator (Fisher Scientific, Waltham, MA). A cell suspension was made of 1 × 10^8 cfu ml^{-1} using inoculating fluid (Biolog Inc. Hayward, CA) before measuring on a spectrophotometer. Then, 150 μL of cell suspension was transferred into a Biolog GN2 microtitre plate followed by incubation at 30°C for 20 to 24 h. During incubation, a purple color forms in each well where the substrate was used by the bacteria (the result of tetrazolium redox dye). The microtitre plate was loaded into a Biolog Microstation and the color pattern and intensity measured spectrophotometrically and matched to a library of known bacterial utilization patterns using Microlog software V.4.2 (Biolog Inc. Hayward, CA). Assignment to Group II was determined on the basis of the use of sole carbon substrates by *A. avenae* subsp. *citrulli* (Walcott et al., 2004). A preliminary study to test pathogenicity of bacterial strains of AAC 00–1 and AAC 94–21 was run on seedlings in the greenhouse before use in the large field tests (data not shown).

Data Collection and Analysis

Disease ratings were taken weekly on the basis of foliar disease symptom using a 0 to 9 scale for plant damage, where 0 = none, 1 to 2 = trace, 3 to 4 = slight, 5 to 6 = moderate, 7 to 8 = severe, and 9 = dead. In 2011 and 2013, the first, second, and third ratings were taken at the 8th, 9th, and 10th week after planting. In 2013, the first, second, and third ratings were taken at the third, fourth, and fifth week after planting, or 1, 2, and 3 wk after foliar inoculation. Of 1699 PI accessions, 96 were not included in the data analysis due to poor germination, emergence, or growth. Foliar disease ratings were subjected to analysis of variance using the GLM procedure of SAS 9.2 (SAS Institute, Cary, NC). Means were tested using Fisher's protected least significant difference with $P = 0.05$.

RESULTS

Germplasm Screening

In total, 1699 watermelon cultigens including wild accessions, related species, and elite cultivars were screened for field resistance at flowering-stage to the bacterial fruit blotch Group II strains at the Horticultural Crops Research Station in Clinton, NC from 2011 to 2013. As was expected, data were not obtained for all cultigens in all years and replications. In 2011, disease ratings were started 7 wk after planting. In 2012, disease ratings were started 6 wk after planting. In 2011 and 2012, disease was minimal in early ratings so the analysis was run using ratings at 8, 9, and 10 wk after planting. In 2013, artificial inoculation made it possible to use data from 3, 4, and 5 wk after planting or 1, 2, and 3 wk after inoculation. Hereafter, those ratings (as described above) for the 3 yr are referred to as Ratings 1, 2, and 3. The first replication in 2013 was removed from analysis due to poor emergence, although the data were used for the selection of cultigens having low variability over replication. A replacement replication was planted to balance the experiment. Therefore, a total of 3 yr with two replications and three ratings were used for the data analysis. The complete dataset (Ma, 2013) has been submitted to the USDA-ARS germplasm resources information network (www.ars-grin.gov, accessed 10 Dec. 2014).

Table 1. Statistics from ANOVA for bacterial fruit blotch ratings for watermelon germplasm screening for resistance to Group II strains.

Trait	Mean square	F ratio	R^2	CV
			%	
Mean of all ratings†	3.79	4.12***	81	19.69
Mean of first ratings‡	5.83	2.83***	76	39.67
Mean of second ratings§	4.18	2.95***	82	24.30
Mean of third ratings¶	5.17	3.94***	80	18.70

*** Significant at the 0.001 probability level.

† Mean of first, second, and third ratings from all 3 yr of 2011 to 2013 at Clinton, NC.

‡ The first rating for 2011 and 2012 started at the eighth week after planting and the first rating for 2013 started at the third week after planting, 1 wk after inoculation.

§ The second rating for 2011 and 2012 started at the ninth week after planting and the second rating for 2013 started at the fourth week after planting, 2 wk after inoculation.

¶ The third rating for 2011 and 2012 started at the 10th week after planting and the third rating for 2013 started at the third week after planting, 3 wk after inoculation.

Table 2. Analysis of variance for mean of all ratings and best ratings† on bacterial fruit blotch foliar symptoms in the watermelon germplasm screening from 2011 to 2013.

	Mean of all ratings			Mean of the best rating		
Source of variation	df	Mean square	F ratio	df	Mean square	F ratio
Year	2	3544.30	26.7*	2	3966.97	27.7*
Block (Year)	3	132.71		3	143.38	
Cultigen	1654	3.79	4.1***	1654	5.17	4.0***
Cultigen × Year	3000	1.66	1.8***	2992	2.33	1.8***
Error	4644	0.92		4575	1.31	

* Significant at the 0.05 probability level.

*** Significant at the 0.001 probability level.

† Best rating was defined as the third rating for each year.

The ANOVA indicated significant differences (P = 0.0001) in disease resistance among cultigens for all three ratings (Table 1). The best ratings for each replication were determined by ANOVA, with data summarized by year. In all 3 yr, Rating 3 had the largest F ratio for cultigen, so Rating 3 was used as the best rating for differentiating cultigen resistance. Mean rating (across the three ratings) was similar to the best rating in cultigen F ratio (4.12 vs. 3.94, respectively; Table 2). Of the total variance, 76, 82, and 80% was explained by Ratings 1, 2, and 3, respectively (Table 1).

The most resistant and most susceptible cultigens were chosen on the basis of rating and having few missing observations. Cultigens were considered highly susceptible if their Rating 3 had a mean value >7.0 (Table 3). Cultigens were considered resistant if Rating 3 was 5.5 or less, with a mean rating of 3.5 or less. The 54 most resistant cultigens originated from Zimbabwe (21), South Africa (17), Zambia (9), and Nigeria (7).

Germplasm Retest

The most resistant cultigens from the germplasm screening were retested in the following year to confirm their

Table 3. Most resistant and susceptible cultigens to bacterial fruit blotch Group II strains, with mean of best and overall ratings for 2011 to 2013 (ranking based on best rating).

		Bacterial fruit blotch rating			
Cultigen	Seed source	Best†	Ave.‡	SD	No.§
Resistant					
PI 482246	Zimbabwe	3.0	2.4	0.0	3
PI 482273	Zimbabwe	3.0	2.5	0.0	4
PI 482322	Zimbabwe	3.0	2.8	0.7	5
PI 596666	South Africa	3.2	2.8	1.4	5
PI 532670	Zimbabwe	3.3	2.9	0.0	3
PI 271770	South Africa	3.3	3.2	0.0	4
PI 482300	Zimbabwe	3.3	3.5	0.0	5
PI 482277	Zimbabwe	3.5	2.9	0.7	5
PI 482309	Zimbabwe	3.5	3.3	1.4	6
PI 596665	South Africa	3.5	3.2	0.0	6
PI 500331	Zambia	3.7	2.8	0.7	5
PI 596668	South Africa	3.7	3.3	0.7	5
PI 560006	Nigeria	3.7	2.8	3.5	6
PI 296337	South Africa	3.8	2.7	0.0	5
PI 482274	Zimbabwe	3.8	3.1	0.7	5
PI 500354	Zambia	3.8	3.3	0.7	5
PI 532664	Zimbabwe	3.8	3.3	0.0	5
PI 482318	Zimbabwe	3.8	3.0	0.0	6
PI 482333	Zimbabwe	3.8	3.5	1.4	6
PI 595201	United States	3.8	3.2	0.0	6
PI 482303	Zimbabwe	4.0	3.4	0.7	5
PI 482311	Zimbabwe	4.0	3.3	1.4	5
PI 595203	United States	4.2	2.8	0.7	5
PI 596696	South Africa	4.2	3.3	1.4	5
PI 296342	South Africa	4.2	2.8	0.7	6
PI 482367	Zimbabwe	4.2	2.9	0.0	6
PI 500328	Zambia	4.2	3.3	0.0	6
PI 500332	Zambia	4.2	3.3	1.4	6
PI 299379	South Africa	4.3	3.4	0.0	4
PI 271779	South Africa	4.3	3.4	0.7	5
PI 596656	South Africa	4.3	3.5	0.0	5
PI 596659	South Africa	4.3	3.4	1.4	5
Grif 15897	Russia	4.3	3.4	0.7	6
PI 248774	Namibia	4.3	3.4	0.7	6
PI 482355	Zimbabwe	4.3	3.2	1.4	6
PI 596653	South Africa	4.3	3.5	0.7	6
PI 295843	South Africa	4.3	3.1	0.7	3
PI 560901	China	4.5	2.9	0.0	4
PI 244017	South Africa	4.5	3.2	2.1	5
PI 532667	Zimbabwe	4.5	3.4	0.7	5
PI 560000	Nigeria	4.5	3.2	0.0	6
PI 482261	Zimbabwe	4.7	3.4	2.8	4
PI 482278	Zimbabwe	4.7	3.4	0.7	5
PI 500301	Zambia	4.7	3.3	0.7	5
PI 296343	South Africa	4.7	3.1	0.0	6
PI 482272	Zimbabwe	4.7	3.2	2.1	6
PI 482342	Zimbabwe	4.7	3.4	1.4	6
PI 494531	Nigeria	4.7	3.5	0.7	6
PI 500320	Zambia	4.7	3.4	2.8	6
PI 500321	Zambia	4.8	3.3	2.1	5
PI 500303	Zambia	4.8	3.4	0.0	6
PI 560023	Nigeria	4.8	2.9	0.7	6
PI 595202	United States	4.8	3.4	1.4	6

(cont'd)

Table 3. Continued.

Cultigen	Seed source	Bacterial fruit blotch rating			
		Best†	Ave.‡	SD	No.§
PI 500340	Zambia	5.0	3.1	0.7	6
PI 249008	Nigeria	5.2	3.5	0.0	5
PI 560014	Nigeria	5.2	3.3	0.7	6
PI 296341	South Africa	5.3	3.4	0.0	3
PI 296339	South Africa	5.3	3.3	1.4	4
PI 482264	Zimbabwe	5.5	3.3	1.4	5
PI 512348	Spain	5.5	3.3	0.7	5
PI 560010	Nigeria	5.5	3.4	0.0	5
PI 595200	United States	5.5	3.5	2.1	6
Check cultivars					
Jubilee	United States	3.5	2.0	0.7	2
Peacock Shipper	United States	5.5	4.4	0.0	5
Allsweet	United States	6.0	3.8	0.0	3
Georgia Rattlesnake	United States	6.3	4.1	0.7	3
Crimson Sweet	United States	6.6	5.7	0.7	4
Minilee	United States	6.6	5.3	0.7	5
Congo	United States	6.7	6.5	2.1	3
Mickylee	United States	6.7	5.0	2.1	6
Charleston Gray	United States	6.8	4.8	0.7	5
Calhoun Gray	United States	6.8	4.8	0.0	5
Golden Midget	United States	7.3	5.3	1.4	3
Black Diamond YB	United States	7.5	6.0	0.0	3
Sugar Baby	United States	7.8	6.1	0.0	4
Stone Mountain	United States	8.0	6.5	1.4	4
Susceptible					
PI 525090	Egypt	8.5	5.9	0.0	5
PI 536459	Maldives	8.5	7.4	0.0	5
PI 212288	Afghanistan	8.8	6.8	0.0	4
PI 357725	Yugoslavia	8.8	6.6	0.7	4
PI 357751	Yugoslavia	8.8	8.0	0.0	4
PI 536454	Maldives	8.8	6.7	0.0	4
PI 183217	Egypt	9.0	9.0	0.0	1
PI 278036	Turkey	9.0	9.0	0.0	1
PI 386021	Iran	9.0	4.7	0.0	1
PI 559995	Nigeria	9.0	9.0	0.0	1
PI 632751	Namibia	9.0	9.0	0.0	1
PI 536461	Maldives	9.0	9.0	_	2
PI 559994	Nigeria	9.0	7.7	0.0	2
PI 536462	Maldives	9.0	8.2	0.0	3
PI 536464	Maldives	9.0	7.3	0.0	3
PI 183398	India	9.0	6.8	0.0	4
PI 536463	Maldives	9.0	7.6	0.0	4

† Best rating was referred to third ratings, which for 2011 and 2012 started at the 10th week after planting, and the third rating for 2013 started at the fifth week after planting, 3 wk after inoculation, equals to the mean of best ratings during 3 yr from 2011 to 2013.

‡ Mean of all ratings during 3 yr from 2011 to 2013.

§ Number of replications in third ratings.

resistance. In the retest studies of 2012 and 2013 (Table 4 and 5), there were eight resistant cultigens (PI 271770, PI 482246, PI 482277, PI 482319, PI 482324, PI 482331, PI 482342, and PI 596666) that performed well in both years.

The most resistant cultigens ranged from 2.0 to 5.8 for Rating 3 in the 2012 retest and 2.0 to 5.0 for Rating 3 in the 2011 germplasm screening (Table 4). PI 271770 and PI 532670 were the most resistant in the 2011 germplasm screening. PI 271770 was the most resistant cultigen in the 2012 retest. PI 482342 had the worst rating (5.8) of all the resistant cultigens. Data were not obtained from PI 482246 and PI 532670 due to poor germination. PI 525100 and PI 164665 were consistently susceptible in the 2011 germplasm screening and the 2012 retest. The check cultivars Calhoun Gray, Mickylee, and Crimson Sweet were not as susceptible as the most susceptible accessions, but were more susceptible that the resistant accessions (except PI 482342) in the 2012 retest. All tested cultigens showed consistency in ratings in 2012 and 2013 screening except PI 532670, PI 482342 (6 in 2012 screening), and PI 596692 (6 in 2013 screening).

The most resistant cultigens ranged from 1.0 to 2.5 for Rating 3 in the 2013 retest and 1.3 to 6.0 for rating 3 in the 2012 germplasm screening (Table 5). PI 482246 was the most resistant cultigen in the 2012 germplasm screening, while PI 482322 and PI 596666 were the most resistant cultigens in the 2013 retest. The resistant cultigens from the 2012 germplasm screening were more resistant than the check cultivar Charleston Gray. PI 635598 was consistently susceptible in both the 2012 germplasm screening and the 2013 retest with Rating 3 values of 6.0 and 4.9, respectively.

Data Validation

The 23 most resistant cultigens were identified in 2011 to 2013 (Table 6). Of those, five were in the 2012 and 2013 retest studies (Table 4 and Table 5): PI 271770, PI 482246, PI 482277, PI 596666, and PI 596668. Another five cultigens were in a single retest study: PI 296342, PI 482309, PI 482322, PI 500354, and PI 596665. Several of the resistant cultigens had similar origins: PI 271770 and PI 271779 from South Africa, PI 482273 and PI 482277 from Zimbabwe, PI 500331 and PI 500332 from Zambia, and PI 596665, PI 596666, PI 596668, and PI 596696 from South Africa. Most of them were from South Africa, Zimbabwe, and Zambia, with the exception of PI 560006 (Nigeria) and PI 595201 (United States selection from PI 189317, origin Zaire). Even though not selected as the most resistant cultigens, PI 560000, PI 560010, PI 560014, PI 560023, and PI 595200 that was developed from PI 189317 (Zaire) had good resistance (Table 3).

PI accessions are not maintained as inbred lines, and may be sampled in the wild from segregating populations. We were interested in the amount of variability from plant to plant, especially for the resistant cultigens. Compared with elite inbreds (Charleston Gray and Mickylee), the resistant cultigens were similar in variability across years and replications within year (Table 6).

Table 4. List of resistant cultigens (with seed source and species and variety) from 2011 screening and their mean of best ratings in 2012 retest and in 2011 to 2013 screening.

Cultigen	Seed source	Species and variety	2011 mean	2012 retest†	2012 mean	2013 mean
Resistant						
PI 271770	South Africa	*C. lanatus var. citroides*	2.0	2.0	6.0	2.0
PI 296342	South Africa	*C. lanatus var. citroides*	4.5	3.7	3.5	4.5
PI 482246	Zimbabwe	*Citrullus lanatus*	2.0	–	3.0	4.0
PI 482272	Zimbabwe	*Citrullus lanatus*	3.5	4.3	5.5	5.0
PI 482274	Zimbabwe	*Citrullus lanatus*	3.0	4.0	5.5	3.0
PI 482277	Zimbabwe	*C. lanatus var. citroides*	4.0	3.8	3.5	3.0
PI 482293	Zimbabwe	*C. lanatus var. citroides*	4.5	4.5	5.0	4.0
PI 482319	Zimbabwe	*C. lanatus var. citroides*	3.5	4.0	3.5	5.0
PI 482324	Zimbabwe	*C. lanatus var. citroides*	3.0	3.5	3.5	4.0
PI 482331	Zimbabwe	*C. lanatus var. citroides*	3.0	3.0	4.5	3.0
PI 482342	Zimbabwe	*C. lanatus var. citroides*	3.5	5.8	6.0	4.5
PI 482367	Zimbabwe	*Citrullus lanatus*	3.5	4.0	5.0	4.0
PI 532670	Zimbabwe	*C. lanatus var. citroides*	2.0	–	6.0	2.0
PI 560019	Nigeria	*Citrullus lanatus*	5.0	4.3	–	–
PI 596666	South Africa	*C. lanatus var. citroides*	2.5	3.0	3.0	4.0
PI 596668	South Africa	*C. lanatus var. citroides*	3.5	3.8	3.5	4.0
PI 596692	South Africa	*C. lanatus var. citroides*	3.0	4.0	–	6.0
Check cultivars						
Calhoun Gray	United States	*Citrullus lanatus*	8.5	5.3	8.5	4.0
Mickylee	United States	*Citrullus lanatus*	8.5	5.5	6.5	5.0
Crimson Sweet	United States	*Citrullus lanatus*	7.0	5.0	7.5	4.0
Susceptible						
PI 164665	India	*Citrullus lanatus*	9.0	6.5	8.5	6.0
PI 525100	Italy	*Citrullus lanatus*	9.0	6.3	6.5	6.5
LSD (0.05)			2.0	1.3	2.1	2.0

† The correlation coefficients between 2012 retest and 2011, 2012, 2013 screening were 0.83, 0.65, and 0.71, respectively (significant at the 0.001 level of probability).

Table 5. List of resistant cultigens (with seed source and species and variety) from 2012 screening, their mean of besting ratings in 2013 retest and in 2011 to 2013 screening, and plant size measured in 2013.

Cultigen	Seed source	Species and variety	2011 mean	2012 mean	2013 mean	2013 retest†
PI 271770	South Africa	*C. lanatus var. citroides*	2.0	3.3	2.0	2.5
PI 482246	Zimbabwe	*Citrullus lanatus*	2.0	1.3	4.0	1.5
PI 482252	Zimbabwe	*C. lanatus var. citroides*	3.0	5.0	3.5	1.8
PI 482265	Zimbabwe	*C. lanatus var. citroides*	3.5	3.5	5.0	1.8
PI 482277	Zimbabwe	*C. lanatus var. citroides*	4.0	3.5	3.0	2.0
PI 482283	Zimbabwe	*C. lanatus var. citroides*	4.0	4.5	–	1.3
PI 482284	Zimbabwe	*Citrullus lanatus*	2.0	4.5	5.0	1.8
PI 482309	Zimbabwe	*C. lanatus var. citroides*	2.5	3.0	5.0	2.0
PI 482319	Zimbabwe	*C. lanatus var. citroides*	3.5	3.5	5.0	2.0
PI 482322	Zimbabwe	*C. lanatus var. citroides*	2.0	4.5	2.5	1.0
PI 482324	Zimbabwe	*C. lanatus var. citroides*	3.0	3.5	4.0	2.3
PI 482331	Zimbabwe	*C. lanatus var. citroides*	3.0	4.5	3.0	1.3
PI 482342	Zimbabwe	*C. lanatus var. citroides*	3.5	6.0	4.5	1.3
PI 500354	Zambia	*C. lanatus var. citroides*	4.0	3.5	4.0	1.8
PI 485583	Botswana	*C. lanatus var. citroides*	2.5	5.0	3.5	1.8
PI 532738	Zaire	*C. lanatus var. citroides*	4.5	3.0	5.0	2.3
PI 596665	South Africa	*C. lanatus var. citroides*	4.0	3.0	3.5	2.3
PI 596666	South Africa	*C. lanatus var. citroides*	3.0	4.0	3.5	1.0
PI 596668	South Africa	*C. lanatus var. citroides*	3.5	3.5	4.0	1.5
PI 596669	South Africa	*C. lanatus var. citroides*	4.0	2.5	4.0	2.3
Charleston Gray	United States	*Citrullus lanatus*	8.0	6.5	5.0	3.0
PI 635598	United States	*Citrullus lanatus*	9.0	8.0	6.0	4.8
LSD (0.05)			2.0	2.1	2.0	1.1

† The correlation coefficients between 2013 retest and 2011, 2012, 2013 screening were 0.82, 0.49, and 0.61, respectively (significant at the 0.001 level of probability).

Table 6. Variability for bacterial fruit blotch rating for selected resistant and susceptible PI accessions compared with inbred line cultivars across six single-plant hills (3 yr and two replications).

Cultigen	Seed source	Bacterial fruit blotch rating¶								
		Mean†	Best‡	Max§	1	2	3	4	5	6
Resistant										
PI 482246	Zimbabwe	2.4	3.0	3.3	2	2	3	3	4	4
PI 482273	Zimbabwe	2.5	3.0	3.8	5	5	4	4	0	0
PI 482322	Zimbabwe	2.8	3.0	4.3	2	2	4	5	3	2
PI 596666	South Africa	2.8	3.2	4.5	4	1	2	4	4	4
PI 271770	South Africa	3.2	3.3	4.7	2	2	6	6	2	2
PI 482300	Zimbabwe	3.5	3.3	4.3	5	4	3	3	2	3
PI 532670	Zimbabwe	2.9	3.3	4.7	2	2	6	6	2	2
PI 482277	Zimbabwe	2.9	3.5	4.0	5	3	4	3	3	3
PI 596665	South Africa	3.2	3.5	4.3	4	4	3	3	4	3
PI 500331	Zambia	2.8	3.7	4.0	4	5	4	3	3	3
PI 560006	Nigeria	2.8	3.7	4.2	2	6	2	7	2	3
PI 596668	South Africa	3.3	3.7	4.3	4	3	3	4	4	4
PI 296337	South Africa	2.7	3.8	3.8	5	4	5	5	2	2
PI 482318	Zimbabwe	3.0	3.8	4.3	7	3	4	4	5	0
PI 500354	Zambia	3.3	3.8	4.3	5	3	4	3	4	4
PI 595201	United States	3.2	3.8	4.5	3	4	4	4	4	4
PI 482311	Zimbabwe	3.3	4.0	4.5	5	5	4	2	4	4
PI 296342	South Africa	2.8	4.2	4.3	5	4	3	4	4	5
PI 482367	Zimbabwe	2.9	4.2	4.7	6	1	5	5	4	4
PI 500328	Zambia	3.3	4.2	4.3	5	8	4	4	3	1
PI 500332	Zambia	3.3	4.2	5.0	6	3	5	3	3	5
PI 596696	South Africa	3.3	4.2	4.5	4	6	2	4	5	4
PI 271779	South Africa	3.4	4.3	4.3	5	5	4	5	4	3
Checks										
Charleston Gray	United States	5.1	6.7	6.7	9	7	7	6	5	6
Mickylee	United States	5.0	6.7	6.7	8	9	8	5	5	5
Susceptible										
PI 536463	Maldives	7.6	9.0	9.0	9	9	9	9	9	9
PI 183398	India	6.8	9.0	9.0	9	9	9	9	8	9
PI 357725	Yugoslavia	6.6	8.8	8.8	9	9	9	8	8	9
PI 222715	Iran	7.1	8.5	8.5	9	7	9	9	8	9

† Mean of the best ratings during 2011 to 2013.

‡ Mean of all the ratings during 2011 to 2013.

§ Mean of the maximum ratings during 2011 to 2013.

¶ Replications for 2011 to 2013. The correlation between ratings of 2011 and 2012, 2011 and 2013, and 2012 and 2013 were 0.31, 0.15, and 0.23, respectively (significant at the 0.001 level of probability).

DISCUSSION

Germplasm Screening

The watermelon germplasm collection was screened for resistance to bacterial fruit blotch in the field from 2011 to 2013. The study was successful in differentiating highly resistant, moderately resistant, and susceptible watermelon cultigens. The retest study in 2012 and 2013 was used to confirm the results, and the most resistant cultigens were selected for those interested in developing cultivars or studying the trait further.

The ANOVA showed a significant effect for both cultigen and cultigen by year interaction. The field screening for resistance to bacterial fruit blotch based on foliar symptoms was effective in revealing differences in resistance among individual plants. Overall, the ratings in 2011 were higher than the ratings in 2012 and 2013. We were able to identify cultigens that had resistance that was consistent across years and replications. Of the five species and botanical varieties in the watermelon germplasm collection, only *C. lanatus* and *C. lanatus* var. *citroides* had accessions showing resistance. Certain PI accessions of *C. lanatus* var. *citroides* also have resistance to important diseases of watermelon, including gummy stem blight (Gusmini et al., 2005), *Fusarium* wilt (Martyn and Netzer, 1991), and root-knot nematode (Thies and Levi, 2007). Most of the *Praecitrullus fistulosus* and *C. colocynthis* accessions in our study were susceptible to bacterial fruit blotch. Two of the accessions of *C. rehmii* had slight resistance to bacterial fruit blotch in 2013, but the data for the other two was incomplete.

Resistance Resources

South Africa, Zimbabwe, Zambia, and Nigeria were the sources of many of the resistant PI accessions. Those are also in the primary and secondary centers of diversity for watermelon and colocynth. Citron is indigenous to the arid and sandy regions of southern Africa (Bates and Robinson, 1995). Citron is considered the progenitor of cultivated watermelon and the Tsamma watermelon (*C. lanatus* var. *citroides*). Similarly, most of the resistant accessions identified by Hopkins and Thompson (2002) were from Zimbabwe or Zambia.

The most resistant accessions identified by Hopkins and Thompson (2002) were PI 482279 (Zimbabwe) and PI 494817 (Zambia). PI 500303 (Zambia), PI 500331 (Zambia), and PI 482246 (Zimbabwe) were also resistant. Our results showed that PI 482273, PI 482277, and PI 4822246 from Zimbabwe and PI 500328 and PI 500331 from Zambia were resistant to bacterial fruit blotch. PI accessions in the USDA germplasm collection come from all around the world, and often sequential PI numbers were assigned to accessions collected from the same locality. According to latitude and longitude data from the germplasm resources information network database, PI 482273 and PI 482277 were collected in the same location, close to where PI 482279 was collected (Germplasm Resources Information Network, 2013). Thus, their bacterial fruit blotch resistance may originate from the same population.

Since Zimbabwe, Zambia, and South Africa are in southern Africa, the accessions collected from Nigeria (in West Africa) may have nonallelic genes for resistance. Some of those accessions are the egusi type (*C. lanatus* var. *lanatus*) from Nigeria: PI 560000, PI 560006, PI 560010, PI 560014, and PI 560023. They are in the primary gene pool of watermelon and may be easier to use in the development of resistant cultivars. A previous study incorporating resistance to bacterial fruit blotch from *C. lanatus* var. *citroides* PI 494817 and PI 482279 into Crimson Sweet was not successful (Hopkins and Levi, 2008). Introgression of favorable alleles from wild watermelon, such as *C. lanatus* var. *citroides,* into cultivars is difficult because of linkage or chromosome pairing problems (Levi et al., 2011). However, since only one of the egusi type accessions (PI 560019) was in the retest, additional testing is needed to confirm resistance of the other egusi accessions.

We did not find an association of dark rind color with resistance to bacterial fruit blotch as reported by Hopkins et al. (1993), although most of the resistant cultigens did have more vigorous vine growth as reported by Levi et al. (2011).

The susceptible checks in the 2011 and 2012 retest (PI 164665, PI 525100, and PI 635598) developed large leaf spots but were not killed by the bacterial pathogen. Similarly, the check cultivar Charleston Gray was susceptible compared with most of the PI accessions but was not killed by bacterial fruit blotch. Sugar Baby was reported to be one of the more resistant cultivars to bacterial fruit blotch (Carvalho et al., 2013; Hopkins and Thompson, 2002) but we found it to be susceptible. Plants of many accessions died in our tests. However, they may have died from something other than bacterial fruit blotch, and we were often unable to diagnose the cause of death.

Most of the selected resistant accessions had disease ratings that were consistent across replications. However, it is possible that resistance in some accessions was segregating (Hopkins and Thompson, 2002). Seeds of most PI accessions were collected from open-pollinated populations around the world, and watermelon is naturally cross-pollinated. The original variability is maintained at plant introduction stations using sib-mating for seed increase. Therefore, PI accessions are often heterogeneous and heterozygous. In addition, accessions identified as resistant may have occasional susceptible plants in them. Thus, researchers should self-pollinate and select the most resistant plants within the resistant accessions to develop inbred lines for further use.

Screening Methodology

In addition to genetic heterogeneity of the accessions, variation of disease ratings in our tests may be due to environmental variation. All check cultivars, including Charleston Gray and Mickylee, are inbred lines, but their disease ratings varied across year and replication. Test uniformity was improved using field inoculation and overhead irrigation, but more work is needed to improve the screening tests.

Careful study of symptoms may also improve test results. Leaf symptoms vary among accessions and may differ from the descriptions developed to assist growers by Mullin and Schench (1963) and Webb and Goth (1965). Plants of some accessions had extended water-soaked lesions with a greasy look, while others only developed chlorosis. Plants of other accessions developed leaf lesions in a cluster, while others developed spots only sporadically.

The time to symptom development was variable among PI accessions, and the appearance of visible symptoms was dependent on the availability of free moisture on the leaves, as previously reported (Panagopoulos and Crosse, 1964). In our study, natural inoculation required 8 wk for the first symptoms to appear and 10 wk for the majority of PI accessions to show symptoms as the result of rain, wind, vine training, and overhead irrigation (Walcott, 2005). However, artificial inoculation shortened the time to symptom development to only 3 wk after inoculation. The shortened time also reduced the chance for accessions to be exposed to other diseases that often appeared in late summer, such as anthracnose, powdery mildew, and downy mildew. In this study, it was important to take multiple ratings in the field throughout the season for comparison across years.

Future Studies

There were 104 PI accessions that had data missing from at least four replications in this study. To make the screening for bacterial fruit blotch data more complete, these accessions need to be tested using a better seed source along with other accessions that were not available from the USDA due to limited seed supplies. Out of the 62 resistant PI accessions, we chose 23 for future studies because of their consistent response over 3 yr and also because of their diverse seed sources. Most of them were only tested in single-plant hills, so their resistance needs to be confirmed in larger tests.

Inbred lines should be developed by self-pollination and selection of the accessions for several generations. Those lines could then be used in crosses with susceptible cultivars to study the inheritance of resistance. The horticultural traits in the resistant cultigens were not desirable so improvement by backcrossing to elite watermelon cultivars will be needed.

CONCLUSIONS

Screening the watermelon germplasm collection for resistance to bacterial fruit blotch has resulted in the identification of 23 accessions for further research. Similar to other watermelon disease resistance studies, *C. lanatus* var. *citroides* contributed many of the 23 resistant accessions. Zimbabwe and Zambia were common origins for accessions resistant to bacterial fruit blotch; South Africa and Nigeria were also origins. The accessions from Nigeria may be of importance for cultivar improvement because they are the more closely related to *C. lanatus* var. *lanatus*.

Additional tests of resistant accessions will be needed to confirm their resistance. Finally, artificial inoculation using a mix of virulent strains helps shorten the time for disease development and may improve the uniformity of the test.

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