Screening the Watermelon Germplasm Collection for Resistance to Gummy Stem Blight

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Abstract

All available accessions from the USDA watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) germplasm collection, including *C. lanatus* var. *citroides*, were screened for resistance to gummy stem blight (*Didymella bryoniae*, anamorph *Phoma cucurbitacearum*). The experiment was a randomized complete block with 1,325 cultigens (elite cultivars, obsolete cultivars, breeding lines, and PI accessions), two locations (field and greenhouse), and two or four replications. Isolates used were collected from cucurbits and verified for virulence on watermelon. The most resistant cultigens were significantly better than the check, 'Charleston Gray', and the most susceptible cultigens were significantly worse. The most resistant and most susceptible cultigens were retested, along with check cultivars (including a set of cucumber cultigens with known characteristics of resistance and susceptibility), to verify their reaction. The retest was a randomized complete block with 75 (38 in 2000) cultigens, two locations (field and greenhouse), and three or four replications. The most resistant cultigens were PI 279461, PI 254744, PI 482379, PI 244019, PI 526233, PI 482276, PI 164248, PI 482284, PI 296332, PI 490383, PI 271771, and PI 379243. The most susceptible cultigens were PI 226445, PI 534597, PI 525084, PI 223764, PI 169286, and PI 183398.

INTRODUCTION

Gummy stem blight (*Didymella bryoniae* (Auersw.) Rehm) is one of the most destructive diseases of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), a major vegetable crop in the U.S. (Schenck, 1962; Keinath, 1995).

Gummy stem blight causes crown blight, extensive defoliation and fruit rot, and can cause severe losses in the field. For example, in 1991 over 15% of the watermelon crop in South Carolina was abandoned before harvest (Power, 1992). The disease also causes loss of fruit during storage and transportation (Leupschen, 1961; Sowell and Pointer, 1962; Norton, 1978).

Didymella bryoniae is seed-borne (Lee et al., 1984), air-borne (Van Steekelenburg, 1983), and soil-borne (Keinath, 1996; Bruton, 1998). The fungus persists in crop residue even at extreme temperatures, such as –9°C for 14 days (Van Steekelenburg, 1983). *D. bryoniae* is a facultatively necrotrophic fungus (Svedelius, 1990); thus, wounding the leaves, particularly the old ones (Van Steekelenburg, 1985a), helps disease initiation because the production of exudates from the lesions favors the fungus in its growth and infection. High relative humidity and the presence of free water on the plants are required for the fungus to induce large lesions on leaves and stems (Van Steekelenburg, 1981, 1984, 1985a, b). Finally, there is no evidence of race specialization for this pathogen (St. Amand and Wehner, 1995).

Genetic resistance has received attention in the last 50 years. Differences in resistance to gummy stem blight have been demonstrated among cultivars of watermelon. 'Congo' was the least susceptible, 'Fairfax' was intermediate, and 'Charleston Gray' was the most susceptible (Schenck, 1962). PI 189225 was the most resistant accession of 439 evaluated from the USDA watermelon germplasm collection (Sowell and Pointer, 1962).

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Several years later, PI 271778 was identified as an additional source of resistance (Sowell, 1975). It had a disease response that was intermediate between PI 189225 and 'Charleston Gray'. A later screening effort of 138 watermelon accessions showed that PI 500335, PI 505590, PI 512373, PI 164247, and PI 500334 were resistant to gummy stem blight (Boyhan et al., 1994). Resistant cultivars were developed from two crosses ('Jubilee' x PI 271778 and 'Crimson Sweet' x PI 189225) by selecting disease-resistant seedlings from backcrossed families that produced high yield of excellent quality fruit (Norton et al., 1986). 'AU-Jubilant' and 'AU-Producer' (Norton et al., 1986), 'AU-Golden Producer' (Norton et al., 1993), and 'AU-Sweet Scarlet' (Norton et al., 1995) were released with moderate to high resistance to anthracnose, fusarium wilt, and gummy stem blight in greenhouse screening tests.

Seedling screening methods are the most commonly used among breeders to test new cultivars and lines for resistance to gummy stem blight in cucurbits (Zhang et al., 1995; Dias et al., 1996; Zhang et al., 1997; Wehner and Shetty, 2000). Tests involve spraying seedlings with a water suspension of spores collected from in vitro cultures of the pathogen. However, in field tests the gummy stem blight resistance of these cultivars has not proven useful. So far, no cultivars of watermelon (Sumner and Hall, 1993) have been released that have high resistance to gummy stem blight.

The objective of this study was to identify new and useful sources of resistance to gummy stem blight by screening the USDA watermelon germplasm collection. The ultimate objective was to develop resistant and adapted cultivars.

MATERIALS AND METHODS

Locations and Seed Sources

All experiments were run at the Plant Pathology greenhouses in Raleigh, N.C., and at the Horticultural Crops Research Station, Clinton, N.C. All *Citrullus* Plant Introduction (PI) accessions were obtained from the Southern Regional Plant Introduction Station, Griffin, Ga. The accessions originated from 66 different countries. The checks were 51 watermelon cultivars, along with a set of seven cucumber cultivars, to provide reference points for gummy stem blight resistance. The checks were obtained from public and private plant breeders in the U.S.

Inoculum Preparation

For all tests, *D. bryoniae* was increased on petri plates containing 15 ml potato dextrose agar (PDA). Inoculated plates were incubated for 2 to 3 weeks at 24 ± 2°C under alternating periods of 12 h fluorescent light (40 to 90 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ PPFD) and 12 h darkness until sporulating pycnidia formed. For all inoculations, a spore suspension was prepared by flooding the culture plates with 5 to 10 ml of sterile, distilled water and scraping the surface of the agar using a finger. The liquid from each plate was filtered through 4 layers of cheese-cloth to remove mycelia, pycnidia and dislodged agar. The final pH of the inoculum was unadjusted. Spore concentration was measured with a hemacytometer and adjusted to a concentration of $5x10^5$ spores/ml adding deionized water. Immediately before inoculation, Tween 80 (2 drops/L) was added to the inoculum.

Inoculation Procedure

In the greenhouse test, plants were inoculated at the second true leaf stage, after the leaf surface was damaged by brushing it with a wooden stake. Inoculum was applied using a hand-pumped spray bottle (EcoLogical, Sprayco, Mich.). Immediately after inoculation, plants were placed in a humidity chamber with clear-plastic walls (top open during the summer, top closed during the winter). Humidifiers were used in the chamber (Model 500, Trion, Sanford, N.C.) running continuously for the treatment time (1 day before inoculation through 3 days after inoculation) to keep the relative humidity close to 100% day and night. Plants in all treatments were watered daily using overhead sprinklers, except when humidifiers were running.

In the field test, plants were inoculated when they reached the fourth true leaf stage (usually on Thursdays). Before inoculation, field plots were watered with approximately 12 mm of overhead irrigation each on Monday and Wednesday to promote guttation on the day of inoculation, and the leaf surface was damaged by brushing plants with a wooden T-stake. Plants were inoculated 2 to 3 times (at two week intervals) by spraying the inoculum onto all upper leaf surfaces at dawn. It is important to inoculate actively guttating plants. The inoculum was delivered as a fine mist using a sprayer operated at 200 to 275 kP (30 to 40 psi). After inoculation, approximately 12 mm of irrigation was applied at 4 pm the same day to promote disease development with high relative humidity at night.

Isolates of *D. bryoniae* from muskmelon and cucumber were used in all screening tests. Isolates J-1, and F18 of *D. bryoniae* was used in a mixture to ensure virulence in the tests.

Experiment Design

Field and greenhouse tests were run in 1998, 1999, 2000, and 2001. Field plots were 1.5 m long with 1 plant (1998), 3 plants (1999, 2000), or 2 plants (3 for the retest) (2001) each. In the field, seeds were planted on raised, shaped beds 1.5 m apart (center to center), or 3 m apart in the retest (2000, 2001). Plots were separated at each end by 1.5 m alleys. Guard rows surrounded each test.

In the greenhouse, temperatures ranged 23 to 43°C (day) and 12 to 24°C (night) for the seasons when the experiments were run. Seeds were planted directly in plastic pots (100x100 mm size, 600 ml volume, Kord Corp., Lugoff, S.C.) filled with a soilless mix of peat, vermiculite, and perlite (Metromix 220, Grace/Sierra, Milpitas, Calif.). More than 1 seed per pot was planted to ensure a good plant stand, and then seedlings were thinned to reach the desired number of plants per pot (2 in the screenings and 1 in the retests) and pots were assembled to form the plots (2 pots per plot in the screenings and 3 pots per plot in the retests). A randomized complete block design was used in both locations (field and greenhouse) for all tests.

Disease Ratings

Plants were rated 3 weeks after inoculation in the greenhouse, and when symptoms began to appear on the leaves and stems of the susceptible check in the field. The rating system was general enough to allow for differences in growth rate in the PI accessions, but specific enough to distinguish resistant and susceptible plants. Plants were rated on a scale of 0 to 9 based on gummy stem blight symptoms where 0 = none, 9 = plant dead, and 0 to 5 = symptoms only on the leaves, 6 to 9 = symptoms on the leaves and stems.

Statistical Analysis

Data were analyzed using the MEANS, ANOVA, and CORRELATION procedures of SAS (SAS Institute, Cary, N.C.). Rating data were summarized as mean, number of blocks (year, season, replication combinations that were not missing), and standard deviation over blocks. Data then were standardized (mean = 4.5, standard deviation = 1.5) using the STANDARD procedure of SAS to reduce variability over years, locations, and the person doing the disease ratings.

RESULTS AND DISCUSSION

The most resistant cultigens were identified as those that had a low mean (<3.0), a low standard deviation (<2.5), and data from many replications (>10). Based on those criteria, the most resistant cultigens were PI 279461, PI 254744, PI 482379, PI 244019, PI 526233, PI 482276, PI 164248, PI 482284, PI 296332, PI 490383, PI 271771, and PI 379243 (Table 1). These 12 resistant cultigens were generally more resistant than PI 189225, the most resistant cultigen in previous screening studies (Sowell and Pointer, 1962; Norton, 1979). The only gummy stem blight resistant cultivars available are AU-

Producer (Norton et al., 1986), AU-Sweet Scarlet (Norton et al., 1995), and AU-Golden Producer (Norton et al., 1993). In our studies, those cultivars were more similar to the susceptible cultigens than to the resistant ones. Thus, our experiment confirmed the need expressed by plant breeders for new and better sources of resistance to *D. bryoniae*.

The most susceptible cultigens were chosen based on the same criteria as above but having a high mean (>6.0). They were: PI 226445, PI 534597, PI 525084, PI 223764, PI 169286, and PI 183398 (Table 1), and could be used as susceptible checks in future gummy stem blight tests.

CONCLUSIONS

New sources of genetic resistance to gummy stem blight have been identified as well as highly susceptible checks. Future steps for breeding for resistance to gummy stem blight in watermelon could include the development of resistant inbreds by selection and self pollination of the most resistant plants within each of the most resistant cultigens, study of the inheritance of the resistance by crossing resistant inbreds with susceptible inbreds, and the development of gummy stem blight resistant cultivars by crossing resistant inbreds with adapted cultivars. It may also be useful to develop molecular markers linked to gummy stem blight resistance to make it easier to backcross resistance into adapted cultivars.

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Tables

Table 1. Standardized average gummy stem blight rating for the 12 most resistant and 6 most susceptible watermelon PI accessions along with 19 check cultivars.

Cultigen	Mean	SD	No.reps	Cultigen	Mean	SD	No.reps
Resistant accessions				*Cultivars*			
PI 279461	2.3	1.3	14	Dixielee	3.3	0.8	25
PI 254744	2.5	1.8	20	Cr. of Saskat.	3.3	1.4	18
PI 482379	2.6	0.9	15	Allsweet	3.3	2.2	23
PI 244019	2.7	2.0	33	Peacock WR60	3.4	1.6	12
PI 526233	2.7	1.1	12	Tendersweet OF	3.5	1.1	21
PI 482276	2.7	1.0	17	Navajo Sweet	3.6	1.6	21
PI 164248	2.8	2.0	15	Calhoun Gray	4.0	1.2	28
PI 482284	2.9	1.5	23	Crimson Sweet	4.1	1.0	24
PI 296332	2.9	1.2	13	Regency	4.1	1.1	28
PI 490383	2.9	1.5	18	AU-Gold. Prod.	4.1	1.1	18
PI 271771	2.9	2.2	19	AU-Jubilant	4.2	1.5	18
PI 379243	2.9	1.8	16	YF Black Diam.	4.3	1.6	16
Susceptible accessions				Black Diamond	4.6	1.9	15
PI 226445	6.1	1.4	17	Fairfax	4.7	1.0	16
PI 534597	6.1	1.0	12	Congo	4.7	1.7	42
PI 525084	6.1	0.9	13	Sugar Baby	4.9	1.2	24
PI 223764	6.2	1.0	30	AU-Producer	5.0	1.6	20
PI 169286	6.3	1.1	33	Charleston Gray	5.1	1.5	61
PI 183398	6.3	1.9	14	NH Midget	5.1	0.9	15
Checks accessions				AU-Sweet Scar.	5.1	1.7	19
PI 189225	3.8	1.2	34	Tendergold	5.2	0.9	24
PI 271778	4.2	1.4	17	Golden Honey	5.7	1.2	22

Statistics
LSD (5%) = 0.32
Mean (1,325 cultigens) = 4.50 (Non-standardized mean = 6.30)
F ratio (cultigen) = 3.80 (P = 0.0001)