Inhibition of Bacterial, Fungal, and Plant Growth by Testae Extracts of *Citrullus* Genotypes

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Abstract. Watermelon [*Citrullus lanatus* var. *lanatus* (Thunb.) Matsum & Nakai] seed and root exudates inhibit germination and seedling growth of plants and growth of pathogenic fungi and bacteria. This study was conducted to determine if extractable components in the testa (seedcoat) contribute to the inhibition previously reported. Testae of eight genetically diverse *Citrullus* genotypes were extracted first with dichloromethane to remove less polar components and then with 70% methanol to remove more polar components. The dichloromethane extracts were not inhibitory in a Proso millet radicle growth bioassay; however, they were highly inhibitory to the growth of the fruit blotch bacterial pathogen *Acidovorax avenae* subsp. *citrulli* (Aac). All dichloromethane extracts were highly inhibitory to Aac except those from a watermelon breeding line, 406-1-x 7 and a *C. lanatus* var. *citroides* accession, PI 500354. The more polar components extracted in 70% methanol inhibited Proso millet radicle and Aac growth and *Phytophthora capsici* zoospore germination. The greatest inhibition of radicle growth was found with 70% methanol extracts from two watermelon relatives, *C. lanatus* var. *citroides* [Bailey (Mansf.)] (PI 532738) and *C. colocynthis* [(L.) Scrad.] (PI 432337). They reduced radicle elongation by 90% at an extract concentration of 250 mg of tissue extracted per mL water. The 70% methanol extracts of several genotypes partially inhibited Aac colony formation, but the *C. lanatus* var. *citroides* accession, PI 532738, was the only genotype with 70% methanol extracts that completely inhibited the bacterium at 100 mg·mL⁻¹. The 70% methanol extracts of Charleston Gray, 406-1-x 7, PI 500354, PI 532738, and PI 167125 were highly inhibitory in a *Phytophthora capsici* zoospore germination bioassay. These results indicate that the testae of *Citrullus* genotypes contain at least two compounds that are inhibitory to microorganisms and plants in bioassay, and the amount of inhibition caused by the extracts varied among *Citrullus* genotypes.

Allelopathy in the Cucurbitaceae was first reported by Putnam and Duke (1974) who screened the U.S. PI collection of cucumber (*Cucumis sativus*) germplasm and identified accessions with varying allelopathic potentials. Lockerman and Putnam (1979, 1981) subsequently demonstrated that suppression of Proso millet (*Panicum miliaceum*) and white mustard (*Brassica alba*) by cucumber was largely the result of allelopathy and that the most allelopathic genotypes were competitive against weeds. Yu and Matsui (1994) found that cucumber root exudates contained a number of simple and complex phenolic compounds and organic acids that were inhibitory in a lettuce (*Lactuca sativus*) seedling growth bioassay. The reduced growth of

some cucurbit crops after repeated cropping was the result of the autotoxicity of compounds released into the soil in root exudates and by decomposing root tissues (Yu, 2001; Yu et al., 2000). The allelopathic potential of watermelon plants was attributed to phenolic compounds found in watermelon tissue extracts and root exudates (Hao et al., 2007). Hao et al. (2010) recently reported that although rice root exudates inhibited spore germination and sporulation of the phytopathogenic fungus, *Fusarium oxysporum* f. sp. *niveum*, watermelon exudates stimulated both growth parameters. The difference was the result of the greater concentration of phenolic compounds found in rice exudates.

Germinating seeds release many compounds that affect organisms in the spermosphere. The role of seed exudates in stimulating the germination and growth of beneficial and harmful microorganisms has been extensively investigated (Nelson, 1990, 2004). In some instances, constituents in seed exudates are inhibitory to microorganisms and protect the germinating seed against invasion by pathogens (Rose et al., 2006). Seed exudates may also be allelopathic against plants, and

there are several reports of phytotoxicity caused by seed exudates (Higashinakasu et al., 2004; Kushima et al., 1998; Ohno et al., 2001; Rose et al., 2006; Yamada et al., 1995). Kushima et al. (1998) reported that watermelon seed exudates were inhibitory to the root growth of several plant species in petri dish bioassays. They found that the exudates contained vanillic acid at a concentration that inhibited the growth of the test species in a bioassay. Otlewski et al. (1987) reported that watermelon seeds contained small peptides that function as protease inhibitors.

In a previous study we observed that watermelon seed exudates inhibited Proso millet radicle growth and *Phytophthora capsici* sporangia formation (Harrison et al., 2010). Exudates of some genotypes were highly inhibitory, whereas exudates of others did not inhibit growth. The objectives of this study were to determine if extractable components of watermelon testae contribute to the inhibitory properties of watermelon seed exudates and to assess the relative inhibitory potential of testa extracts from genetically diverse genotypes of watermelon and the related species *C. colocynthis*.

Materials and Methods

Testa tissue preparation. The genotypes included in this experiment were the watermelon cultivar, Charleston Gray, the U.S. Vegetable Laboratory experimental watermelon line, 406-1-x 7, a watermelon germplasm accessions, PI 167125, three citron melon, *C. lanatus* var. *citroides* germplasm accessions, PI 482246, PI 500354, and PI 532738, and two accessions from the related species *C. colocynthis*, PI 432334 and PI 432337. Seeds used in this experiment were obtained from ripe fruit produced on greenhouse-grown plants that were self-pollinated. Seeds were thoroughly rinsed and air-dried after harvest and stored at 5 °C. Seeds were bisected with scissors, and all tissues except the testa were removed with a dissecting needle. Testae were ground to pass through a 60-mesh screen using a Wiley mill and lyophilized. Dried and ground testae were stored under nitrogen at -25 °C until they were extracted.

Testa extraction. Ground testae were extracted first with dichloromethane to remove soluble nonpolar components and then with 70% methanol to extract the more polar soluble components. Extraction was accomplished by placing 3 g of the tissue in a round-bottomed flask with 45 mL solvent. The slurry was placed on a wrist action shaker at 50 rpm in the dark at room temperature for 24 h. Testae were extracted twice with both solvents and the two extractions with the same solvent were combined. Extracts were filtered through nylon 66 filters (0.8 µm), dried on a rotary evaporator, and stored at -25 °C under nitrogen until they were used in bioassay experiments.

Proso millet bioassay. The 70% methanol extracts were re-dissolved in 70% methanol

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to obtain an extract concentration equivalent to 250 mg of testa extracted per mL. Aliquots of the extract plus additional 70% methanol to equal 0.5 mL total volume were pipetted onto filter paper disks in 35-mm plastic petri dishes. The solvent was allowed to evaporate at room temperature. Ten Proso millet seeds and 0.5 mL of distilled water were subsequently added to each dish, and the seeds were incubated in the dark at 24 °C. After 72 h, the petri dishes were placed in a freezer to stop growth. The dishes were removed from the freezer, millet radicle lengths were measured with an electronic caliper, and average radicle length was determined for each dish. For the dichloromethane extract, the extracts were re-dissolved in dichloromethane to obtain a concentration of 250 mg testa extracted per mL. The extracts plus additional dichloromethane to bring the total volume to 1 mL were pipetted onto filter paper in 50-mm glass petri dishes. The solvent was evaporated at room temperature and 1 mL of distilled water and 20 Proso millet seeds were added to each dish. The seeds were incubated, frozen, and radicle lengths measured as described previously.

A preliminary experiment examined the effect of the sequential dichloromethane and 70% methanol extracts. The extract concentrations were equivalent to 100 mg testa extracted per mL water. The experiment was arranged in a completely random design with five replications and was repeated. Data were analyzed using the PROC GLM procedure of SAS Version 9.1 (SAS Institute, Cary, NC). No treatment-by-experiment interactions were observed; thus, the combined data from two repetitions of the experiments were subjected to analysis of variance, and genotype means within extraction solvents were separated by Tukey's honestly significant difference (HSD) test ($P = 0.05$).

A second experiment was conducted to assess the concentration response to the 70% methanol testa extracts. The methods of extraction are described previously. Test concentrations were equivalent to 0, 31, 63, 125, and 250 mg·mL⁻¹. The experiment was arranged in a completely randomized design with five replications. Radicle lengths were analyzed using the PROC GLM procedure of SAS Version 9.1. No treatment-by-experiment interaction was observed; thus, data from the two repetitions of the experiment were combined for analysis. Genotype means within extract concentrations were separated using Tukey's standardized range HSD ($P = 0.05$). Nonlinear regression analyses (proc nlin; SAS System; SAS Institute) using the parallel dose-response curves procedure described by Seefeldt et al. (1995) were used to estimate the 70% methanol extract concentration required to cause a 50% reduction in radicle length (GR₅₀) for each genotype.

Acidovorax avenae bioassay. The bacterial fruit blight pathogen, Aac, isolate 531 was obtained under USDA, APHIS permit from Dr. Ron Walcott, University of Georgia, Athens, GA. Isolate 531 was originally isolated from infected watermelon fruit from Georgia and has been identified as a Group I

isolate (Wechter et al., 2011). The bacterium was started from -80 °C glycerol freezer stocks for each experiment. The isolate was streaked onto Difco™ nutrient agar (NA) medium (Becton, Dickinson and Company, Sparks, MD) and grown for 24 h at 27 °C. A single colony from the 24-h-old plates was again transferred to an NA plate and grown for an additional 24 h. A 10-μL loop of cells from this plate was inoculated into 100 mL of King's B broth and placed on a gyratory shaker at 150 RPM until midlog phase growth (≈ 0.8 OD₆₀₀) as determined by Biophotometer (Eppendorf, Westbury, NY). Cells were pelleted by centrifugation at 10,000 RCF. Supernatant was discarded and cell pellet washed once with sterile 0.01 M phosphate-buffered saline (sPBS), again pelleted by centrifugation, and then resuspended in sPBS to a concentration of $\approx 1 \times 10^8$ colony-forming units (CFU)/mL as determined by optical density at a wavelength 600 nm.

Bacterial assays were performed in 96-well polystyrene microtiter plates. One milliliter aliquots of re-solubilized testa extracts were pipetted into polypropylene microcentrifuge tubes and placed, uncapped, into a 50 °C dry-block heater in a sterile hood until all trace of solvent was gone. In addition, 1 mL of solvent (either methanol or dichloromethane, depending on the test) was pipetted into a microcentrifuge tube and evaporated to dryness as in a similar fashion. One milliliter of sterile King's B broth was added to each microcentrifuge tube. Tubes were capped and vortexed until contents were fully suspended. Three hundred-microliter aliquots of each resuspended extract as well as the solvent controls were pipetted in triplicate into individual wells of the microtiter plate. Six additional empty wells also received 300 μL of King's B to be used as with and without bacteria controls. All wells then received 25 μL of the 1×10^8 CFU/mL Aac described previously with the exception of the "without-bacteria" control wells, which received 25 μL sPBS. Plates were covered and placed on a rotary shaker (50 RPM) in a 28 °C incubator. After 24 h of growth, 10-μL aliquots were removed from each well and added to 990 μL of King's B broth in a microcentrifuge tube. The contents of these tubes were serially diluted by 10-fold and 100 μL of each dilution was spread-plated onto two petri plates of *Pseudomonas* agar F. Plates were incubated at 28 °C for 36 h and colonies were tallied. Each test was performed twice. The dilution that allowed all treatments to be scored (i.e., the lowest dilution at which colonies were not too numerous to count) was used for counts. An average CFU was taken for the two plates at the chosen dilution and used in the statistical analysis. Data were analyzed using the PROC GLM procedure of SAS Version 9.1 (SAS Institute). Significant experiment-by-treatment interactions were found for both the dichloromethane and methanol extracts; thus, the data from each test were analyzed separately. Genotype means within extraction solvents were separated using Tukey's standardized range HSD ($P = 0.05$).

Phytophthora capsici bioassay. An isolate of the plant pathogen *Phytophthora capsici* was obtained from Dr. A.P. Keinath, Clemson University. This isolate of *P. capsici* is highly aggressive on watermelon (Kousik et al., unpublished data). The isolate had been maintained on V8 juice agar in the laboratory (Keinath, 2007; Keinath and Kousik, 2011). Zoospore germination assays were conducted using methods described by Keinath and Kousik (2011) with slight modifications as follows. One hundred microliters of re-solubilized 70% methanol testa extracts were pipetted into 2-mL polypropylene microcentrifuge tubes and placed, uncapped, into a 50 °C dry-block heater in a sterile hood until all trace of solvent was gone. In addition, 100 μL of 70% methanol was pipetted into a microcentrifuge tube and evaporated to dryness in a similar fashion. Once the tubes were completely dry of all traces of solvent, 100 μL of a zoospore suspension in sterile distilled water, prepared as described by Keinath and Kousik (2011) was pipetted into the 2-mL microcentrifuge tubes. The tubes were capped and vortexed vigorously until contents were completely suspended. This action also enables the zoospores to encyst (Keinath, 2007; Keinath and Kousik, 2011). The tubes were placed in a rack and shook mildly for 1 h at 26 °C. Then, the suspensions were plated on 1% water agar plates as described previously (Keinath, 2007; Keinath and Kousik, 2011) and placed in an incubator at 26 °C for another 90 min after which germinated and ungerminated zoospores were counted. Each testa extract was tested on four plates, which served as replications, and two germination counts per replication were recorded. The experiment was repeated once. Data were arcsine transformed and analyzed using the PROC GLM procedure of SAS Version 9.1 (SAS Institute). Significant treatment-by-experiment interactions were observed; thus, the data from each experiment were analyzed separately. Genotype means were separated using Tukey's standardized range HSD ($P = 0.05$).

Results and Discussion

Proso millet bioassay. The dichloromethane extracts contained fatty material that changes the absorptive properties of the filter paper in comparison with the control that was treated with dichloromethane only; however, they did not inhibit radicle growth at 100 mg·mL⁻¹ (Table 1). Given the high inhibition of the methanol extract of some genotypes, we concluded that the soluble nonpolar components of watermelon testae do not contribute greatly to the inhibition of Proso millet radicle growth observed with seed exudates (Harrison et al., 2010). The 70% methanol extracts of four genotypes, one watermelon genotype ('Charleston Gray'), one citron melon genotype (PI 532738), and both of the *C. colocynthis* genotypes inhibited radicle growth at 100 mg·mL⁻¹ (Table 1). The concentration response experiment also indicated differences between *Citrullus* genotypes in inhibitory potential of 70% methanol extracts

Table 1. Response of Proso millet radicle growth to dichloromethane and 70% methanol extracts^z of testa of eight *Citrullus* genotypes.

Genotype	<i>Citrullus</i> species and variety	Radicle length (mm)	
		Dichloromethane extract ^y	70% methanol extract ^y
Charleston Gray	<i>lanatus</i> var. <i>lanatus</i>	18.8	15.1 bc
406-1-x 7	<i>lanatus</i> var. <i>lanatus</i>	19.2	17.5 ab
PI 167125	<i>lanatus</i> var. <i>lanatus</i>	21.1	20.0 ab
PI 482246	<i>lanatus</i> var. <i>citroides</i>	23.4	17.2 ab
PI 500354	<i>lanatus</i> var. <i>citroides</i>	22.9	22.4 a
PI 532738	<i>lanatus</i> var. <i>citroides</i>	19.0	9.56 cd
PI 432334	<i>colocynthis</i>	19.4	15.1 bc
PI 432337	<i>colocynthis</i>	24.5	5.2 d
Control		20.5	21.4 a
<i>P</i> value ^x		0.0103	<0.0001

^zExtracts were tested at a concentration equivalent to 100 mg testa tissue extracted per mL.

^yMeans within 70% methanol extracts followed by the same letter are not different by Tukey's honestly significant difference ($P = 0.05$) test. Means within the dichloromethane extracts were not different by the test.

^xSignificance level of the F test for genotype effect on Proso millet radicle growth.

Table 2. Concentration^z response of Proso millet radicle growth to 70% methanol extracts of testa from eight *Citrullus* genotypes.

Genotype	<i>Citrullus</i> species and variety	Radical length ^y (mm)				GR ₅₀ ^x (mg·kg ⁻¹)
		31 mg·mL ⁻¹	63 mg·mL ⁻¹	125 mg·mL ⁻¹	250 mg·mL ⁻¹	
Charleston Gray	<i>lanatus</i> var. <i>lanatus</i>	25.8 ab	24.6 ab	16.9 abc	7.9 b	171 ± 18
406-1-x 7	<i>lanatus</i> var. <i>lanatus</i>	21.9 b	21.6 bc	16.1 abc	10.0 b	173 ± 18
PI 167125	<i>lanatus</i> var. <i>lanatus</i>	24.4 b	24.8 ab	19.5 ab	9.0 b	196 ± 20
PI 482246	<i>lanatus</i> var. <i>citroides</i>	25.8 ab	24.7 ab	17.0 abc	10.4 b	191 ± 20
PI 500354	<i>lanatus</i> var. <i>citroides</i>	30.7 a	29.1 a	20.0 ab	9.6 b	211 ± 21
PI 532738	<i>lanatus</i> var. <i>citroides</i>	26.4 ab	19.8 bc	10.0 cd	1.5 c	102 ± 11
PI 432334	<i>colocynthis</i>	24.7 ab	24.0 ab	12.9 bcd	5.5 bc	134 ± 14
PI 432337	<i>colocynthis</i>	22.6 b	16.6 c	7.0 d	1.7 c	80 ± 9
Control		22.3 b	22.3 abc	22.3 a	22.3 a	—
<i>P</i> value ^w		0.0041	0.0001	<0.0001	<0.0001	

^zConcentrations are expressed as weight of testa extracted per mL water.

^yMeans within concentrations followed by the same letter are not different by Tukey's honestly significant difference ($P = 0.05$).

^xThe concentration of extract that caused a 50% reduction in shoot length (GR₅₀) was estimated for each genotype using the log-logistics analysis described by Seefeldt et al. (1995). GR₅₀ estimates ± the approximate SE of the estimate are presented.

^wSignificance level of the F test for genotype effect on Proso millet radicle growth.

(Table 2). At 31 mg·mL⁻¹, no genotypes inhibited radicle growth in comparison with the control. At this concentration, means were different as a result of an apparent stimulation of radicle growth by the PI 500354 extract that resulted in radicle lengths greater than the control. At 63 mg·mL⁻¹, none of the extracts were different from the control; however, there were differences between genotypes as a result of the apparent stimulation of radicle growth by PI 500354 and slight inhibition by other genotypes. Neither stimulated nor inhibited radicle lengths were different from the control, but they were different from each other. At 125 mg·mL⁻¹, extracts of three genotypes, the *C. lanatus*, var. *citroides* accession PI 532738 and both *C. colocynthis* accessions, inhibited radicle growth compared with the control. At 250 mg·mL⁻¹, extracts of all genotypes inhibited radicle growth in comparison with the control. Two genotypes (PI 532738 and PI 432337) that were inhibitory in the preliminary experiment (Table 1) caused the greatest inhibition of radicle growth at this concentration. Seed exudates of these genotypes were also highly inhibitory in a Proso millet bioassay (Harrison et al., 2010). GR₅₀ estimates indicated that

the extracts of PI 432337 were over twice as inhibitory as the extracts of PI 500354.

Acidovorax avenae bioassay. The dichloromethane methanol extracts of all but two genotypes, 406-1-x and PI 500354, were highly inhibitory to CFU formation by Aac (Table 3). The 406-1-x7 extract inhibited CFU counts compared with both controls in Expt. 1, counts with the PI 500354 extract were lower than the solvent control in Expt. 1, but counts with the two extracts were not different from the controls in Expt. 2. Aac CFU counts in dichloromethane extracts of the six highly inhibitory genotypes were lower than both controls and 406-1-x and PI 500354 extracts in both experiments. Overall, inhibition of Aac growth was greater for the dichloromethane extracts than for the 70% methanol extracts. Averaged over genotypes and experiments, CFU counts were 22% of control in dichloromethane extracts and 37% of control in 70% methanol extracts. The 70% methanol extracts of the watermelon genotypes, 'Charleston Gray', 406-1-x, and PI 167125, and the citron melon genotype, PI 532738, inhibited Aac growth in comparison with both controls and PI 482246, PI 500354, PI 432334,

and PI 432337 extracts in both experiments. The PI 532738 extract was the only 70% methanol extract that completely inhibited Aac growth.

Phytophthora capsici bioassay. *P. capsici* zoospore germination was high (greater than 70%) in the water and the solvent control. The 70% methanol extracts of 'Charleston Gray', 406-1-x 7, PI 167125, PI 500354, and PI 532738 were highly inhibitory to *P. capsici* zoospore germination in both tests (Table 4) in which germination was lower than both of the controls. The testa extracts of the *C. colocynthis* accessions were not inhibitory to zoospore germination. It is interesting to note that the testa extract of PI 500354 was inhibitory to zoospores of *P. capsici*, although the fruit rind is highly susceptible to fruit rot (Kousik, unpublished data). This may indicate that the inhibitory substances extracted from testa are not involved in resistance to the fruit rot disease or that they or not present in inhibitory concentrations in the fruit rind of this genotype. The results of this experiment do not correspond closely to the inhibition of sporangial formation by seed exudates observed in a previous experiment (Harrison et al., 2010) where the *C. colocynthis* genotypes were highly inhibitory and *C. lanatus* genotypes were not. The reasons for this discrepancy are not known; however, it may be the result of differences in the *P. capsici* growth parameters that were measured in the two bioassays or a more thorough removal of inhibitors by extraction in comparison with the seed leachate. The dichloromethane extracts were not tested against *P. capsici* as a result of difficulties with the procedure, and the seed leachate may have contained inhibitors found in these extracts, but not in the 70% methanol extracts.

These experiments demonstrate that the inhibitory properties of watermelon seed exudates observed in previous studies (Harrison et al., 2010) are at least partially the result of extractable components in the testa. This nonliving, maternal tissue may serve as a storage site for inhibitory compounds that protect the embryo from predation or infection before germination and during the early stages of seedling growth. Extract concentrations of 100 mg of testa extracted per mL medium caused strong inhibition of pathogenic microorganisms (Tables 3 and 4). This extract concentration is approximately equivalent of a 1:10 (w:w) dilution of the extractable material relevant to its concentration within the testae. Inhibitors extractable in 70% methanol probably leach out of the testa during seed imbibition and may reach microorganism growth-inhibiting concentrations that protect the germinating seedling. Testae components could not cause the allelopathic potential of watermelon seedlings and roots that has been demonstrated in greenhouse and field experiments (Hao et al., 2007; Kushima et al., 1998). However, it is possible that the compounds found in testa are synthesized in living tissues and released into the soil to cause plant inhibition. High levels of pest resistance are reported in some *C. lanatus* var. *citroides* and

Table 3. Growth of *Acidovorax avenae* subsp. *citrulli* after incubation in King's B broth amended with dichloromethane and 70% methanol extracts^z of testae of eight *Citrullus* genotypes.

Accession	<i>Citrullus</i> species	Dichloromethane extract ^y		70% methanol extract ^y	
		Expt. 1	Expt. 2	Expt. 1	Expt. 2
		----- (CFU × 10) -----			
406-1-x 7	<i>lanatus</i> var. <i>lanatus</i>	205.8 c	417.5 a	70.5 de	26.5 d
PI 167125	<i>lanatus</i> var. <i>lanatus</i>	1.0 d	0.3 b	89.8 de	3.5 d
PI 482246	<i>lanatus</i> var. <i>citroides</i>	31.8 d	49.2 b	350.7 b	357.5 b
PI 500354	<i>lanatus</i> var. <i>citroides</i>	282.0 bc	450.5 a	290.5 bc	185.2 c
PI 532738	<i>lanatus</i> var. <i>citroides</i>	0.8 d	0.7 b	0.0 e	0.0 d
PI 432334	<i>colocynthis</i>	35.2 d	35.8 b	296.7 bc	393.8 b
PI 432337	<i>colocynthis</i>	44.5 d	7.3 b	396.7 ab	444.5 ab
Charleston Grey	<i>lanatus</i> var. <i>lanatus</i>	0.0 d	0.0 b	198.3 dc	86.2 cd
Control ^x		432. a	440.0 a	510.7 a	562.2 a
Solvent control ^w		357.5 ab	559.7 a	530.0 a	370.8 b
Negative control ^v		0.0 d	0.0 b	0.0 e	0.0 d
<i>P</i> value ^u		<0.0001	<0.0001	<0.0001	<0.0001

^zExtracts were tested at a concentration of 100 mg testa tissue extracted per mL.

^yMeans within columns followed by the same letter are not significantly different based on Tukey's honestly significant difference ($P = 0.05$).

^xKing's B broth plus bacterium.

^wKing's B broth added to evaporated dichloromethane or 70% methanol tube then used in microtitre assay plus bacterium.

^vKing's B with no added bacterium.

^uSignificance level of the F test for genotype effect on *A. avenae* growth.

CFU = colony-forming unit.

Table 4. Effect of 70% methanol extracts^z of testae of eight *Citrullus* genotypes on *Phytophthora capsici* zoospore germination.

Genotype	<i>Citrullus</i> species	Germination (%) ^y	
		Expt. 1	Expt. 2
PI 482246	<i>lanatus</i> var. <i>citroides</i>	88.9 a	78.8 b
PI 432334	<i>colocynthis</i>	88.9 a	74.0 b
PI 432337	<i>colocynthis</i>	76.1 a	77.0 b
PI 500354	<i>lanatus</i> var. <i>citroides</i>	12.2 b	16.6 d
406-1-X 7	<i>lanatus</i> var. <i>lanatus</i>	5.9 bc	31.8 c
PI 167125	<i>lanatus</i> var. <i>lanatus</i>	2.3 bc	4.6 e
PI 532738	<i>lanatus</i> var. <i>citroides</i>	0.5 c	8.1 e
Charleston Gray	<i>lanatus</i> var. <i>lanatus</i>	0.1 c	28.0 c
Solvent control		72.8 a	82.4 ab
Water control		70.5 a	88.6 a
<i>P</i> value ^x		<0.0001	<0.0001

^zTesta extracts were tested at a concentration equivalent to 100 mg testa extracted per mL distilled water.

^yMeans followed by the same letter in column are not significantly different based on Tukey's honestly significant difference ($P = 0.05$).

^xSignificance level of the F test for genotype effect on zoospore germination.

C. colocynthis genotypes (Boyhan et al., 1994; Levi et al., 2001; Simmons and Levi, 2002; Thies and Levy, 2007). Further research is warranted to identify biologically active compounds in testae and other tissues of watermelon and related species and investigates their role in pest resistance and allelopathy.

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