

# Inheritance of resistance to damping-off (*Rhizoctonia solani*) in watermelon

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**ABSTRACT:** This study aimed to find sources of resistance to damping-off and to determine the inheritance of resistance in watermelon. Firstly, 72 watermelon accessions were inoculated with CMM 1053 isolate of *Rhizoctonia solani*. Only two accessions (BGH 29 and BGH 76) were considered moderately resistant to the disease. The inoculated plants without symptoms from accession BGH-29 identified as resistant were self-fertilized and the inbred line S<sub>1</sub> was used for the crosses. The reaction to *R. solani* after inoculation of the segregating populations of the cross and backcrosses between susceptible cultivar Crimson Sweet and the inbred line BGH-29 was evaluated. In other experiment eight S1 lines of watermelon were inoculated with six different isolates of *R. solani* and the reaction was evaluated by partial diallel analysis using the Griffing's method IV to obtain estimates of general resistance ability (GRA), general aggressiveness ability (GAA), and specific interaction ability (SIA). According to ratings of the susceptible and resistant parents, generations F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub> (F<sub>1</sub> × P<sub>1</sub>) and BC<sub>2</sub> (F<sub>1</sub> × P<sub>2</sub>), resistance to damping-off was conferred by at least nine genes with additive effect and low heritability which indicates polygenic inheritance. The results of the diallel analysis corroborate with the analysis of segregating population, indicating inheritance of resistance was horizontal, since 46.1% of the total sum of squares of the variation observed in the crosses was due to GRA, while the SIA corresponded to only 20.39% of variation. This is the first report about inheritance of resistance to damping-off caused by *R. solani* in watermelon.

**Key words:** *Citrullus lanatus*, damping-off, genetic control, horizontal resistance, partial diallel.

## INTRODUCTION

Watermelon (*Citrullus lanatus* [Thunb.] Matsum & Nakai) stands out as one of the most produced vegetable crops in Brazil with total 2.28 million of tonnes produced in 2019 (FAO 2019). Northeast region is the main producing regions with 40.83 thousand hectares and 775.32 thousand ton (IBGE 2019). Diseases caused by fungi soil inhabitants, such as damping-off, caused by the fungus *Rhizoctonia solani*, are one of the factors that may limit watermelon production. *Rhizoctonia solani* has a great saprophytic capacity, can survive in the soil for many years using resistance structures, the sclerotia. Moreover, it is a fungus that has a wide host range and can reach up to 190 species (Lakshman et al. 2008). In Brazil, there are no registered products for chemical control of this disease in watermelon (MAPA 2021). Consequently, the most efficient method is breeding for resistant cultivars by introducing genes of resistance in commercial cultivars.

Thus, identification of sources of resistance in germplasm is an essential step in crop breeding. In melon, for example, Sales Júnior et al. (2015) found TA-09, immune accession to the isolate of *R. solani*. Michereff et al. (2008) searching resistance in commercial cultivars of melon, observed that the cultivar Sancho had high resistance to isolates of RS-09 and RS-10. Salari et al. (2012) found two commercial cultivars of melon highly resistant to damping-off. However, little is known

about resistance sources to damping-off in watermelon. Cunha et al. (2019) found only three accessions of watermelon with moderate resistance to damping off caused by most aggressive strain of *R. solani*.

In the Brazilian northeastern region, for several years, watermelon has been cultivated by traditional agriculture producers. In traditional farming, producers maintain a system of constant seed exchange between themselves which has promoted the conservation of variability for different characteristics of the crop (Romão 2000). Generally, there is no use of chemicals by traditional farmers, which makes it possible to select plants resistant to some pathogens (Costa et al. 2021; Romão 2000). In this context, in 2015, germplasm collect expeditions were carried out in the semiarid region, having established the Vegetable Germplasm Bank from Universidade Federal do Vale do São Francisco (BGH-Univasf), which is composed by collections of about 300 accessions from traditional agriculture and spontaneous plants that still under characterization.

Important information to succeed in the development of resistant cultivars is the genetic control of resistance. According to Van der Plank (1982), the resistance can be classified as vertical or horizontal. The vertical resistance is conferred by a few genes, having race specific resistance. On the other hand, the horizontal is conditioned by many genes, presenting nonspecific race resistance. For this pathosystem *Rhizoctonia* × watermelon, there are no reports about inheritance of resistance.

Number of genes involved in the expression of a character is important in the study of the inheritance of quantitative traits and in plants breeding, especially regarding the estimation of the probability of obtaining a certain genotype in a segregating population (Ramalho et al. 2012).

Melo and Santos (1999) developed an efficient methodology that can provide information about vertical and horizontal resistance of the host, as well as the aggressiveness of the isolates of pathogen. Data were analyzed by Griffing's model IV, which aims to evaluate the combinatorial ability of a group, estimating the specific and general combining ability using a partial diallel. Simulation was based on the expected severity with genotypes inoculated to 20 physiological races of the pathogen. A high correlation was observed between the general resistance ability (GRA) and the horizontal resistance of the host, as well as general aggressiveness ability (GAA) and aggressiveness of the isolate. The specific interaction ability (SIA) is an indicator of vertical resistance.

Therefore, this study aimed to evaluate watermelon accessions from BGH-Univasf, aiming at finding damping-off resistance sources, as well as determining the inheritance of resistance in watermelon, using segregating population and diallel method proposed by Melo and Santos (1999).

## MATERIAL AND METHODS

Experiments were conducted in three steps in the Laboratory of Phytopathology and greenhouse with a 50% shaded screen.

### Inoculum preparation of the *R. solani* isolates

*Rhizoctonia solani* isolates were obtained from the Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes" (CMM) from Universidade Federal Rural de Pernambuco (Table 1). The pathogenicity for all the isolates was restored by inoculation in watermelon plants.

**Table 1.** Description of *Rhizoctonia solani* isolates of watermelon obtained from the Phytopathogenic Fungal Culture Collection of Professora Maria Menezes (CMM) from Federal Rural University of Pernambuco.

Code	Year	Location
CMM-1053	2006	Quixeré-RN
CMM-2967	2007	Mossoró-RN
CMM-1052	2006	Quixeré-RN
CMM-2983	2007	Mossoró-RN
CMM-2971	2007	Baraúna-RN
CMM-3890	2006	Gama-DF

Isolates were grown on potato dextrose agar (PDA). After growth, three mycelium discs with 3 mm diameter were added in Erlenmeyer's flasks containing 50 g of previously autoclaved parboiled rice with the addition of 30 mL of distilled water (120 °C, 15 min, 1 atm). Erlenmeyer's flasks were stored in bio-oxygen demand (BOD) for 5 days at 25 °C with 12-h photoperiod. Inoculation was performed seven days post-incubation, colonized rice grains by *R. solani*. This same was used in the prepare and inoculation of all the isolates of *R. solani*. For the first and second experiment, the isolate of CMM 1053 was used. For the third experiment, all the isolates of *R. solani* were used (Table 1).

## Experiment I – Inoculation of germplasm bank accessions

In this experiment, 69 accessions from BGH-Univasf, two accessions from Embrapa Semiárido Germplasm Active Bank (BG CIA) and Crimson Sweet commercial cultivar (positive control) were used, totaling 72 genotypes with ten replicates, one plant per replicate, in a completely randomized design. BGH-Univasf is composed by collections of accessions from traditional agriculture and spontaneous plants that still under characterization.

The sowing of each watermelon accession was performed in trays of 200 cells. Seeds were previously disinfested in 1% sodium hypochlorite for 30 s and washed in distilled water. When the seedlings presented the first pair of true leaves, inoculation was performed with two rice grains colonized by CMM 1053 isolate of *R. solani*, which was considered the most aggressive in previous studies (Cunha et al. 2019). The seedlings were inoculated with the addition of one grain of rice colonized by the fungus close to the hypocotyl of the seedling. The negative control consisted of a seedling of each accession with rice not colonized by the pathogen. Evaluation occurred 7 days after inoculation. For this, a scale of scores proposed by Cunha et al. (2019) was adopted: 0 = no symptoms; 1 = small lesions on the roots or hypocotyl; 2 = lesions surrounding the hypocotyl without causing constriction; 3 = Initial constriction with partial destruction of tissues without damping-off; 4 = necrotic tissues with post emergence damping-off; 5 = pre-emergence damping-off. There were no plants in category 5 (diseases), because the plants were inoculated in the seedlings phase and class 5 is only when seeds were sowing in the infested substrate and did not germinate. The average score was used to classify each accession into five resistance classes as proposed by Michereff et al. (2008) with modifications: average score of 0 = similar to immune (SI); 0.1 to 1.0 = highly resistant (HR); 1.1 to 2.0 = resistant (R); 2.1 to 3.0 = moderately resistant (MR); 3.1 to 4.0 = susceptible (S); 4.1 to 5.0 = highly susceptible (HS).

## Experiment II – Inheritance of resistance by segregating population

For this experiment, seedlings of accession BGH 29 classified in the Experiment I as moderately resistant to damping-off were self-fertilized. Only plants that did not show symptom after inoculation were used to self-pollination. Population  $F_1$  were obtained using controlled, hand-pollination between 'Crimson Sweet', which is considered susceptible ( $P_1$  – male parent) and the inbred line S1 BGH 29 ( $P_2$  – female parent). 'Crimson Sweet' is characterized by circular fruit, clear rind with dark-green stripes, intense red flesh, sweet flavor (11.0 °Bx) and presence of few seeds. BGH 29 was classified as moderately resistant to damping-off, ellipsoid fruit, clear rind with dark-green stripes, pink flesh, little sweet (6.5 °Bx), and presence of excessive number of seeds. The  $F_1$  was self-pollinated and backcrossed to their respective parental lines, to obtain progenies  $F_2$ ,  $BC_1$  ( $F_1 \times P_1$ ) and  $BC_2$  ( $F_1 \times P_2$ ).

Seeds of all populations were sowing in 200-cell polystyrene trays containing commercial substrate. The number of emerged seedlings inoculated from each generation was 15 plants of each parent, 50 plants of  $F_1$ , 160 plants of  $F_2$ , 66 and 84 of  $BC_1$  and  $BC_2$ , respectively. Inoculation with *R. solani* CMM 1053 isolate was performed with grain of rice colonized by the fungus close to the hypocotyls of the seedling. After 7 days of inoculation, segregating populations were evaluated regarding their reactions to *R. solani* using descriptive scale proposed by Cunha et al. (2019). After evaluation, plants with symptoms were taken to the laboratory to re-isolation of the fungus from tissues with symptoms, aiming to closing of Koch's postulates. Observations of pathogen structures were also performed under a microscope.

## Experiment III – Inheritance of resistance using the diallel method proposed by Melo and Santos (1999)

Diallel method proposed by Melo and Santos (1999) was used to obtain information on the vertical and horizontal resistance of the hosts and the aggressiveness of the pathogen isolates. Six isolates of *R. solani* (Table 1) and eight inbred line S1 of watermelon from BGH-Univasf (BGH 76, BGH 128, BGH 174, BGH 185, BGH 210, BGH 321, BGH 395, BGH 398) were used for this experiment. These accessions were selected because they were previously self-fertilized (line S1) and showed resistance to other soilborne pathogen *Fusarium oxysporum* (Costa et al. 2018). The experiment was arranged in factorial design 8×6 (eight genotypes × six isolates) with seven replicates (one plant per repetition) per treatment. Isolates were inoculated 15 days after sowing. Evaluation was performed seven days after inoculation using descriptive scale proposed by Cunha et al. (2019).

### Statistical analyzes

For experiment II, the symptoms severity (scores) was used to obtain the variances of populations P<sub>1</sub>, P<sub>2</sub>, F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub> and BC<sub>2</sub>. Means and variances were calculated for each one of the six populations, in order to calculate genetic parameters. Environmental variance ( $\sigma^2E$ ) was estimated based of variances of P1, P2 and F1 generations (Eq. 1):

$$\sigma^2E = (\sigma^2_{P_1} + \sigma^2_{P_2} + 2\sigma^2_{F_1}) / 4 \quad (1)$$

Genetic variance ( $\sigma^2_G$ ), and its additive ( $\sigma^2_A$ ) and dominance ( $\sigma^2_D$ ) components, as well as broad sense heritability ( $h^2$ ) were estimated (Mather and Jinks 1977). All these parameters were obtained using GENES program (Cruz 2013). The additive [a] and nonadditive effects [d] of the gene(s) which controls the trait were estimated from generation means by the method of weighted least squares (Mather and Jinks 1977).

For the experiment III, the analysis of variance (Anova) in factorial scheme 8×6 (8 genotypes × 6 isolates) was performed using the scores attributed. For the partial diallel analysis, means, degrees of freedom and mean squared errors provided by Anova were used. Griffing's method IV was used to obtain estimates of GRA, GAA, and SIA. Diallel analysis followed the statistical model (Eq. 2):

$$Y_{ij} = \mu + r_i + a_j + s_{ij} + e_{ij} \quad (2)$$

in which,  $Y_{ij}$  is the disease severity presented by the host  $i$  when inoculated with the isolate  $j$ ,  $r_i$  is the effect of the general reaction capacity of host  $i$  (horizontal resistance),  $a_j$  is the effect of the general aggressiveness capacity of isolate  $j$  (isolate aggressiveness),  $s_{ij}$  is the effect of the specific reaction capacity of the host  $i$  inoculated with the isolate  $j$  (vertical resistance), and  $e_{ij}$  is the mean experimental error. GENES program was used to obtain these parameters (Cruz 2013).

## RESULTS AND DISCUSSION

### Identification of source of resistance by inoculation of germplasm bank accessions

Accessions BGH 29 and BGH 76 were moderately resistant to damping-off in the inoculation with the CMM 1053 isolate from *R. solani*. These two accessions from BGH-Univasf representing 2.7% of the total number of inoculated accessions (Table 2).

The results of the first experiment show there were few resistant accessions. This low frequency of resistant genotypes was also observed by Sales Júnior et al. (2015) working with *R. solani* isolates inoculated in melon and Cunha et al. (2019)

using accessions of watermelon from Embrapa Semiárid. In addition, the variation observed in the present studies through the standard deviation of the accessions shows how variable is the reaction within the accession. The accessions used in this study were collected from farmers, therefore, they were open pollination. This fact could justify this high variation observed.

**Table 2.** Mean score, standard deviation (SD) and reaction of the 71 accessions of watermelon and Crimson Sweet cultivar inoculated with CMM 1053 isolate of *R. solani*.

Accessions	Species	Mean score $\pm$ SD	Reaction	Accessions	Species	Average rating $\pm$ SD	Reaction
BGH 23	<i>C. lanatus</i> var. <i>lanatus</i>	3.20 $\pm$ 0.92	S <sup>1</sup>	BGH 239	<i>C. lanatus</i> var. <i>lanatus</i>	3.38 $\pm$ 0.74	S
BGH 26	<i>C. lanatus</i> var. <i>lanatus</i>	3.20 $\pm$ 1.03	S	BGH 319	<i>C. lanatus</i> var. <i>lanatus</i>	3.60 $\pm$ 0.55	S
BGH 27	<i>C. lanatus</i> var. <i>citroides</i>	3.10 $\pm$ 0.88	S	BGH 320	<i>C. lanatus</i> var. <i>lanatus</i>	3.44 $\pm$ 1.01	S
BGH 28	<i>C. lanatus</i> var. <i>lanatus</i>	3.00 $\pm$ 1.00	S	BGH 321	<i>C. lanatus</i> var. <i>lanatus</i>	3.33 $\pm$ 0.58	S
<b>BGH 29</b>	<b><i>C. lanatus</i> var. <i>lanatus</i></b>	<b>2.78 <math>\pm</math> 0.67</b>	<b>MR</b>	BGH 325	<i>C. lanatus</i> var. <i>lanatus</i>	3.33 $\pm$ 0.71	S
BGH 30	<i>C. lanatus</i> var. <i>lanatus</i>	3.44 $\pm$ 0.53	S	BGH 355	<i>C. lanatus</i> var. <i>lanatus</i>	3.20 $\pm$ 0.63	S
BGH 38	<i>C. lanatus</i> var. <i>lanatus</i>	3.56 $\pm$ 0.88	S	BGH 356	<i>C. lanatus</i> var. <i>lanatus</i>	4.00 $\pm$ 0.00	HS
BGH 40	<i>C. lanatus</i> var. <i>lanatus</i>	3.33 $\pm$ 0.71	S	BGH 357	<i>C. lanatus</i> var. <i>lanatus</i>	3.60 $\pm$ 0.70	S
BGH 50	<i>C. lanatus</i> var. <i>lanatus</i>	3.56 $\pm$ 0.88	S	BGH 359	<i>C. lanatus</i> var. <i>lanatus</i>	3.20 $\pm$ 0.63	S
BGH 65	<i>C. lanatus</i> var. <i>lanatus</i>	3.70 $\pm$ 0.48	S	BGH 368	<i>C. lanatus</i> var. <i>lanatus</i>	3.56 $\pm$ 0.73	S
BGH 66	<i>C. lanatus</i> var. <i>lanatus</i>	3.30 $\pm$ 0.67	S	BGH 389	<i>C. lanatus</i> var. <i>lanatus</i>	3.22 $\pm$ 0.67	S
BGH 67	<i>C. lanatus</i> var. <i>lanatus</i>	3.40 $\pm$ 0.52	S	BGH 390	<i>C. lanatus</i> var. <i>lanatus</i>	3.40 $\pm$ 0.52	S
BGH 68	<i>C. lanatus</i> var. <i>lanatus</i>	3.20 $\pm$ 0.79	S	BGH 391	<i>C. lanatus</i> var. <i>lanatus</i>	3.00 $\pm$ 0.47	S
BGH 69	<i>C. lanatus</i> var. <i>lanatus</i>	3.20 $\pm$ 0.63	S	BGH 392	<i>C. lanatus</i> var. <i>lanatus</i>	3.57 $\pm$ 0.79	S
BGH 70	<i>C. lanatus</i> var. <i>lanatus</i>	3.56 $\pm$ 0.53	S	BGH 393	<i>C. lanatus</i> var. <i>lanatus</i>	3.40 $\pm$ 0.70	S
<b>BGH 76</b>	<b><i>C. lanatus</i> var. <i>citroides</i></b>	<b>2.20 <math>\pm</math> 0.92</b>	<b>MR</b>	BGH 394	<i>C. lanatus</i> var. <i>lanatus</i>	3.33 $\pm$ 0.71	S
BGH 92	<i>C. lanatus</i> var. <i>lanatus</i>	3.50 $\pm$ 0.71	S	BGH 395	<i>C. lanatus</i> var. <i>lanatus</i>	3.60 $\pm$ 0.52	S
BGH 105	<i>C. lanatus</i> var. <i>lanatus</i>	3.89 $\pm$ 0.33	S	BGH 396	<i>C. lanatus</i> var. <i>lanatus</i>	3.50 $\pm$ 0.71	S
BGH 107	<i>C. lanatus</i> var. <i>lanatus</i>	3.78 $\pm$ 0.67	S	BGH 397	<i>C. lanatus</i> var. <i>lanatus</i>	3.67 $\pm$ 0.71	S
BGH 108	<i>C. lanatus</i> var. <i>lanatus</i>	3.50 $\pm$ 0.53	S	BGH 398	<i>C. lanatus</i> var. <i>lanatus</i>	3.67 $\pm$ 0.50	S
BGH 109	<i>C. lanatus</i> var. <i>lanatus</i>	3.80 $\pm$ 0.42	S	BGH 399	<i>C. lanatus</i> var. <i>lanatus</i>	3.90 $\pm$ 0.32	S
BGH 116	<i>C. lanatus</i> var. <i>lanatus</i>	3.44 $\pm$ 1.01	S	BGH 400	<i>C. lanatus</i> var. <i>lanatus</i>	4.00 $\pm$ 0.00	HS
BGH 117	<i>C. lanatus</i> var. <i>lanatus</i>	3.40 $\pm$ 0.52	S	BGH 401	<i>C. lanatus</i> var. <i>lanatus</i>	3.37 $\pm$ 0.74	S
BGH 118	<i>C. lanatus</i> var. <i>lanatus</i>	3.20 $\pm$ 0.84	S	BGH 402	<i>C. lanatus</i> var. <i>lanatus</i>	3.80 $\pm$ 0.42	S
BGH 121	<i>C. lanatus</i> var. <i>lanatus</i>	3.22 $\pm$ 1.30	S	BGH 403	<i>C. lanatus</i> var. <i>lanatus</i>	4.00 $\pm$ 0.00	S
BGH 128	<i>C. lanatus</i> var. <i>citroides</i>	3.90 $\pm$ 0.32	S	BGH 404	<i>C. lanatus</i> var. <i>lanatus</i>	3.37 $\pm$ 0.52	S
BGH 147	<i>C. lanatus</i> var. <i>lanatus</i>	3.78 $\pm$ 0.44	S	BGH 405	<i>C. lanatus</i> var. <i>lanatus</i>	3.70 $\pm$ 0.48	S
BGH 169	<i>C. lanatus</i> var. <i>lanatus</i>	3.33 $\pm$ 0.71	S	BGH 406	<i>C. lanatus</i> var. <i>lanatus</i>	3.56 $\pm$ 0.73	S
BGH 174	<i>C. lanatus</i> var. <i>lanatus</i>	3.90 $\pm$ 0.32	S	BGH 407	<i>C. lanatus</i> var. <i>lanatus</i>	3.89 $\pm$ 0.33	S
BGH 177	<i>C. lanatus</i> var. <i>lanatus</i>	4.00 $\pm$ 0.00	HS	BGH 408	<i>C. lanatus</i> var. <i>lanatus</i>	3.70 $\pm$ 0.48	S
BGH 180	<i>C. lanatus</i> var. <i>lanatus</i>	3.57 $\pm$ 1.13	S	BGH 409	<i>C. lanatus</i> var. <i>lanatus</i>	3.86 $\pm$ 0.38	S
BGH 185	<i>C. lanatus</i> var. <i>citroides</i>	3.60 $\pm$ 0.55	S	BGH 411	<i>C. lanatus</i> var. <i>lanatus</i>	4.00 $\pm$ 0.00	HS
BGH 190	<i>C. lanatus</i> var. <i>lanatus</i>	3.50 $\pm$ 0.97	S	BGH 412	<i>C. lanatus</i> var. <i>lanatus</i>	3.80 $\pm$ 0.42	S
BGH 210	<i>C. lanatus</i> var. <i>lanatus</i>	3.60 $\pm$ 0.52	S	BGCIA 843	<i>C. lanatus</i> var. <i>lanatus</i>	3.90 $\pm$ 0.32	S
BGH 218	<i>C. lanatus</i> var. <i>lanatus</i>	4.00 $\pm$ 0.00	HS	BGCIA 952	<i>C. lanatus</i> var. <i>lanatus</i>	3.00 $\pm$ 0.93	S
BGH 232	<i>C. lanatus</i> var. <i>lanatus</i>	3.30 $\pm$ 1.06	S	Crimson Sweet	<i>C. lanatus</i> var. <i>lanatus</i>	4.00 $\pm$ 0.00	HS

<sup>1</sup> Classes of reactions to damping-off: 0 = similar to an immune plant (I); 0.1–1.0 = highly resistant (HR); 1.1–1.9 = resistant (R); 2.0–2.9 = moderately resistant (MR); 3.0–3.9 = susceptible (S); 4.0–5.0 = highly susceptible (HS).

## Inheritance of resistance by segregating populations

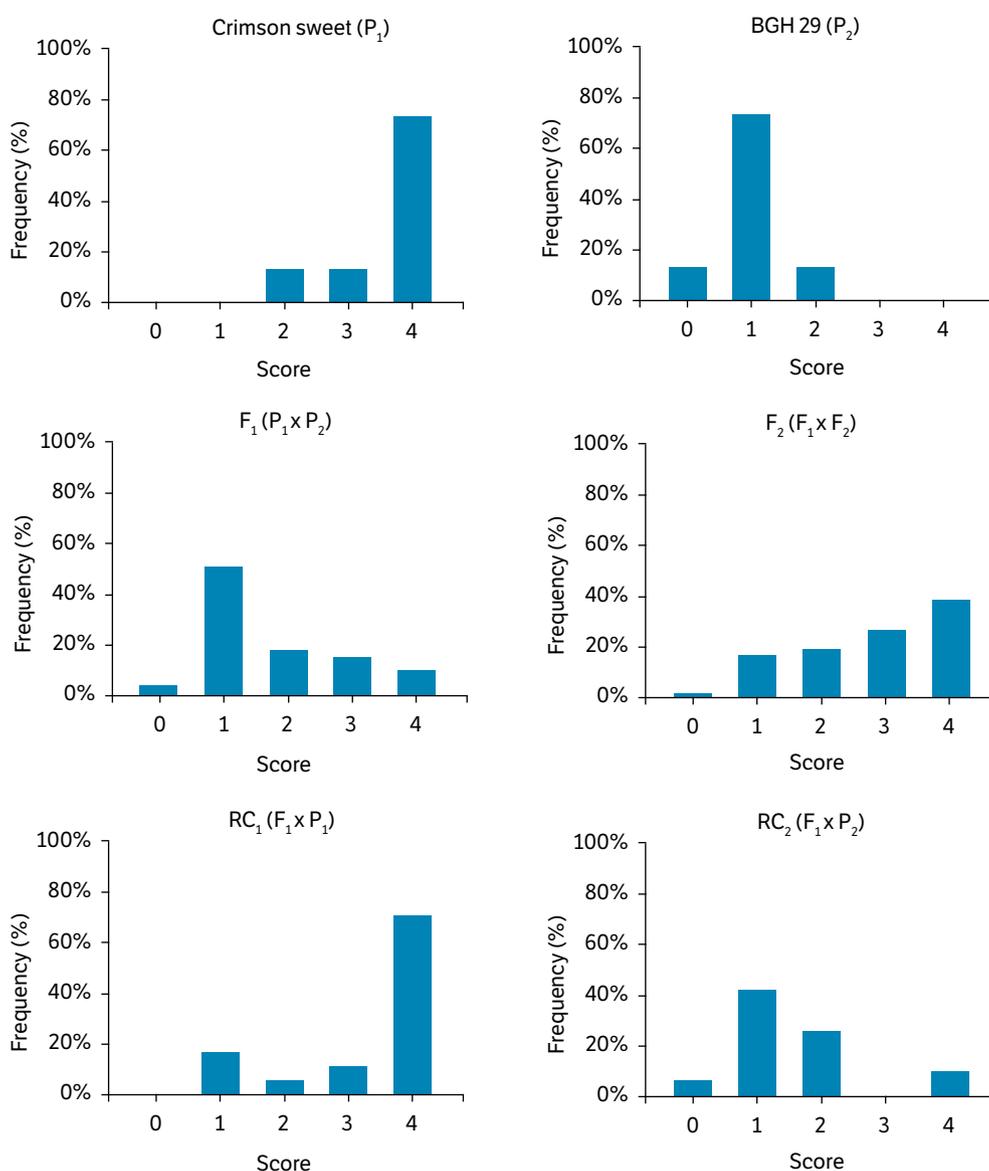
Two sources with moderate resistance to CMM 1053 isolate of *R. solani* (accessions BGH 29 and BGH 76) were observed in the first experiment. Only BGH 29 was self-fertilized, BGH76 (*Citrullus lanatus* var. *citroides*) was not used for the crossing, because of difficulty to self-fertilization.

Analysis of the crossing ‘Crimson Sweet’  $\times$  BGH 29 allowed to estimate the genetic parameters. Analyzing the segregating populations of this crossing, parents have contrasting means and low variance for the resistant parent

BGH 29 ( $P_2$ ) ( $\sigma^2_F = 0.28$ ) and superior to the susceptible parent ‘Crimson Sweet’ ( $P_1$ ) ( $\sigma^2_F = 0.54$ ). The estimated variances for each population show that the largest variance found was in the  $F_2$  population ( $\sigma^2_F = 1.32$ ) (Table 3). It is also possible to see that the grade frequencies between parents are quite contrasting as well as BC 1 and 2 (Fig. 1).

**Table 3.** Rating for resistance and the variance to damping-off in crosses of watermelon ‘Crimson Sweet’ ( $P_1$ ) and line BGH 29 ( $P_2$ ).

Generations	Number of seedlings	Average resistance rating	Variance
$P_1$	15	3.60	0.54
$P_2$	15	1.00	0.28
$F_1$	50	1.70	1.11
$F_2$	160	2.82	1.32
$BC_{11}$	66	3.35	1.24
$BC_{12}$	84	1.76	1.17



**Figure 1.** Distribution of frequencies in relation to the scores attributed to the reaction to *Rhizoctonia solani* in the segregating generations from crossings of ‘Crimson Sweet’ ( $P_1$ ) and BGH 29 ( $P_2$ ).

Only the estimate of the additive effect parameters was significant, indicating that each allele contributes with a small effect on the resistance. The average degree of dominance was  $-0.46$ , being an indicative of dominance in increasing resistance to damping-off. At least nine genes were estimated to control the resistance, which indicates polygenic inheritance (Table 4).

**Table 4.** Estimates of mean components, average degree of dominance (ADD) and number of genes ( $\eta$ ) in the control of resistance to damping-off in watermelon from the crossing between Crimson Sweet and BGH 29.

M	2.37
[a]	1.30**
[d]	-0.35ns
ADD	-0.46
$\eta$	9.05

M: Overall mean; [a] additive effects; [d] dominance effects

From the estimates of the phenotypic variance components, it was observed that the environmental variance presented greater magnitude, decreasing heritability, which estimates were smaller than 50% (Table 5). Usually, quantitative character has a great influence of the environment in the phenotype, what was observed in this case. The additive variance was 34% lower than the dominance variance. According to Bernardo (2010), when using the least squares method to estimate the variance components, it is expected that there is a predominance of additive variance, unless there is epistasis, linkage or overdominance for the character.

**Table 5.** Estimates of genetic ( $\sigma^2_G$ ), environment ( $\sigma^2_E$ ), phenotypic ( $\sigma^2_P$ ) and heritability parameters of watermelon reaction to *Rhizoctonia solani* for the F2 of the crossing between 'Crimson Sweet' ( $P_1$ ) and BGH 29 ( $P_2$ ).

Crossing	Variance components					Heritabilities (%)	
	$\sigma^2_G$	$\sigma^2_E$	$\sigma^2_P$	$\sigma^2_A$	$\sigma^2_D$	$h^2_a$	$h^2_r$
P1 x P2	0.556	0.763	1.319	0.221	0.335	42.14	16.74

It is fundamental to perform the evaluation of the accession reaction with an aggressive and efficient isolate to avoid escape. As well as the parents who will originate the segregating population should be as homogeneous and contrasting as possible (Alves et al. 2014). Thus, the results of experiment II proved to be efficient in having homogenous and contrasting parents, which allowed determining that the inheritance is polygenic and controlled by at least nine genes with additive effect and low heritability. The additive effect means each allele contributes with a small effect on the phenotype, or each allele contributes slightly to disease resistance, characterizing a horizontal resistance (Ramalho et al. 2012; Van der Plank 1968). This is the first report about genetic resistance to damping off caused by *R. solani* in watermelon. For other disease in watermelon, resistance to *F. oxysporum* f. sp. *niveum* race 2 (FON 2) in PI 296341-FR is thought to be polygenic and controlled by at least a pair of recessive genes in epistasis with other minor genes and some QTLs markers were identified in some studies (Meru and McGregor 2016; Ren et al. 2015). Little is known about QTLs markers to *R. solani*.

Narrow-sense heritability considers only the additive genetic variance, which is the one that is fixed by selection, which is the most important for the breeder (Ramalho et al. 2012). Thus, the magnitude of heritability was low, as seen by Alves et al. (2014) working with genetic inheritance to the PRSV virus reaction in watermelon. Thus, breeders should seek recurrent selection as a method to gradually increase the frequency of desirable alleles for the quantitative characteristic, through repeated selection cycles, which favors the increase of narrow-sense heritability (Borém and Miranda 2009).

## Inheritance of resistance using the diallel method

Based on the results obtained in the diallel analysis (Table 6), it was observed that all sources of variation were statistically significant. It was verified there was a predominance of the GRA, corresponding to 46.1% of the total sum of squares of

the variation observed in the combinations, while the SIA corresponded to only 33.51% of the variation observed in the combinations, indicating the presence of horizontal resistance to the disease. The GAA also presented high magnitude corresponding to 20.39% of the variation.

**Table 6.** Summary of the analysis of variance of the partial diallel for the evaluation of damping-off in watermelon accessions inoculated with different *Rhizoctonia solani* isolates.

SV	DF	MS	SS (%)
Crossings	47	6.9741**	100
GRA(RH)	7	21.5710**	46.10
GAA (AH)	5	21.9672**	20.39
SIA (RV)	35	1.9128**	33.51
Error	288	0.4890	-
Mean	2.6994	-	-

\*\* Significant at 1 % probability the F test. SV = source of variation; DF = degrees of freedom; MS = medium square; SS = % of the total sum of squares; GRA (RH) = general resistance ability; GAA (AH) = general aggressiveness ability; SIA (RV) = specific interaction ability.

The results of the diallel analysis corroborate results of the second experiment showing the inheritance is polygenic. According to Melo and Santos (1999), if the general reaction capacity of the accessions is high, it indicates that there is a predominance of horizontal resistance. As shown by Pereira et al. (2015) when evaluating commercial cultivars, differential cultivars, and bean lines to four isolates of the fungus *Pseudocercospora griseola*, which found 72.9% of the total sum of squares was due to the general reaction capacity, showing that the resistance is horizontal to the angular spot. Therefore, the efficiency of using this method to determine the resistance inheritance was verified also in this pathosystem, in a faster and easier way than by the method using the segregating populations.

Accessions BGH 128, BGH 185, BGH 210 and BGH 398 were the most resistant when inoculated with several isolates of *R. solani*, while accessions BGH 76, BGH 174, BGH 321 and BGH 395 were the most susceptible, presenting the highest estimate values for the GRA (Table 7).

**Table 7.** Estimates of the general reaction ability (GRA) for the reaction of the inoculated accessions with different isolates of *Rhizoctonia solani*. Negative values imply there was decreasing of average severity and accessions were more resistant when inoculated with six isolates.

Accession	GRA	Accession	GRA
BGH 76	0.25	BGH 210	-0.51
BGH 128	-1.17	BGH 321	1.16
BGH 174	0.09	BGH 395	0.63
BGH 185	-0.08	BGH 398	-0.36

Estimates of the GAA (Table 8) show the difference between aggressiveness of *R. solani* isolates. The most aggressive isolates were CMM 1053 and CMM 2983 because the estimates were positive, while the others were less aggressive because they obtained negative estimates, thus there was decreasing of average severity ratings.

**Table 8.** Estimates of the general aggressiveness ability (GAA) for *Rhizoctonia solani* isolates inoculated in different accessions of the watermelon. Positive values imply an increase in severity average and isolates were more aggressive.

Isolate	GAA	Isolate	GAA
CMM 1053	1.08	CMM 2983	0.39
CMM 2967	-0.54	CMM 2971	-0.48
CMM 1052	-0.25	CMM 3890	-0.19

In addition, this method provided information on the resistance of the accessions in wide range of isolates. BGH 128, BGH 185, BGH 210 and BGH 398 were the most resistant accessions, while BGH 76, BGH 174, BGH 321 and BGH 395 were the most susceptible. It also provided information on the aggressiveness of the isolates indicating CMM 1053 and CMM 2983 as the most aggressive isolates. Cunha et al. (2018; 2019) in other studies also found CMM 1053 as more aggressive. BGH 76 accessions, classified as moderately resistant to CMM 1053 in experiment I, was classified as most susceptible in the diallel analysis experiment, as well as the BGH 128, BGH 210 and BGH 398 accessions were susceptible in the first experiment, were more resistant in the third experiment. This can be explained by the magnitude of SIA that was 33.51%, showing the interaction between accessions  $\times$  isolates.

Resistance to damping off showed to be polygenic and controlled by at least nine genes, with additive effect and low heritability. The partial diallel was adequate for determining the type of damping-off resistance, as well as showing that the isolate used for the inheritance study, CMM1053, was the most aggressive, making it difficult to identify resistance sources. However, the use of only one isolate for search resistance sources is not appropriate according to the results found in this study, because of the interaction between accessions  $\times$  isolates.

## CONCLUSION

The use of genetic resistance to damping off caused by the fungus *R. solani* proved to be complex due to the difficulty of finding resistant genotypes, in addition to the fact that the resistance is polygenic. In addition, there is interaction between accessions  $\times$  isolates, requiring the evaluation of resistance for different isolates, seeking the most durable resistance.

## AUTHORS' CONTRIBUTION

**Conceptualization:** Cunha F. S. and Ishikawa F. H.; **Methodology:** Cunha F. S., Ishikawa F. H. and Lima Neto I. S.; **Data collection,** Cunha F. S., Costa A. E. S., Araújo K. M. G. and Ishikawa F. H.; **Analyses:** Cunha F. S., Ishikawa F. H. and Lima Neto I. S.; **Investigation:** Cunha F. S., Costa A. E. S., Araújo K. M. G. and Ishikawa F. H.; **Writing – Original Draft:** Cunha F. S. and Ishikawa F. H.; **Writing – Review and Editing:** Cunha F. S., Costa A. E. S., Araújo K. M. G., Lima Neto I. S., Capucho A. S. and Ishikawa F. H.; **Funding Acquisition:** Ishikawa F. H.; **Resources:** Ishikawa F. H., Lima Neto I. S. and Capucho A. S.; **Supervision:** Ishikawa F. H., Lima Neto I. S.

## DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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