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Identification of sources of resistance to damping-off (*Rhizoctonia solani*) in two phenological phases of watermelon

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Abstract

Sources of resistance to damping-off caused by *Rhizoctonia solani* were researched in watermelon accessions, in which thirty accessions were inoculated in two phenological phases (at seeding and seedling stages). The completely randomized design with five replications per accession was used in both phenological stages. The inoculation in seeding stage was carried out at the time of sowing, while for seedling the inoculation was done 15 days after sowing. In both stages the evaluation occurred 15 days after inoculation using a rating scale. At seeding stage, six accessions were moderately resistant to CMM-2967 of *R. solani*. However, no accession resistant to CMM-1053 and CMM-1052 isolates was recognized. Three accessions were resistant to CMM-1053 at the seedling stage. Posteriorly, inoculation was carried out using only accessions classified as resistant in the previous evaluations using both phenological stages. For CMM-1053, factorial scheme of 2x4 were used (two stages and four accessions). For assessments against isolate CMM-2967 a factorial scheme of 2x12 (two stages and 12 accessions) was used. We confirmed the results of first two experiments for both isolates. At seeding stage, all accessions were classified as susceptible to CMM-1053. However, at the seedling stage, only Crimson Swett was susceptible. For the CMM-2967, inoculated at the seeding stage, the accessions were classified into two groups (resistant and susceptible) (Scott-Knott at 5%). At seedling stage there was no statistical difference among eleven accessions and they were all classified as resistant. Due to difficulty of finding sources of resistance to damping off, the method of inoculation at seedling stage showed more efficient for this pathosystem and could facilitate the work of breeders and plant pathologists.

Keywords: *Citrullus lanatus;* descriptive scale; genetic resistance; germplasm; method inoculation; wilt.
Abbreviations: CMM_Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes", Federal Rural University of Pernambuco (Recife, Pernambuco); BGCIA_Watermelon Germplasm Bank - Active Germplasm Bank of Cucurbitaceae from the Brazilian Northeastern, located at Embrapa Semiarid (Petrolina, Pernambuco).

Introduction

Watermelon [*Citrullus lanattus* (Thumb.) Matsum. & Nakai] is an economically important vegetable crop grown commercially in several countries and has a low cost of production and fast financial returns (Gama et al., 2013). Brazil is among the four largest producers of watermelon in the world, behind only China, Turkey and Iran (FAOSTAT 2016). The Southern and Northeastern regions of Brazil are the main national producers of this vegetable crop (IBGE 2014). Because of its excellent adaptation to the climatic conditions of the Northeast and its short growing cycle, watermelon has become one of the most cultivated vegetable crops in irrigated areas in the Northeast of Brazil (Lima et al., 2015; Saraiva et al., 2017).

However, during the crop cycle many factors can limit production. Among these factors is the occurrence of diseases, which can affect different stages of the crop causing a reduction in production and fruit quality. The pathogens such as some soil fungi can infect the watermelon (Michereff et al., 2005). One of the main representatives of this group is the fungus *Rhizoctonia solani* [teleomorph: *Thanatephorus cucumeris* (Frank) Donk], which is a basidiomycete that causes the disease known worldwide as damping-off. This pathogen has high competitive saprophytic ability and remains viable for long periods through sclerotia. With a wide host range, this pathogen can infect around 190 species of higher plants, distributed in 32 families, including several staple crops, ornamental plants and grasses (Lakshman et al., 2008). The fungus can attack watermelon at different stages causing rotten seeds, seedlings damping-off, stem cankers, rotten fruits and roots, and death (Andrade et al., 2005). These factors make the

management of *Rhizoctonia solani* a challenge for technicians and producers.

Genetic resistance is the most efficient method recommended for control of the disease because it has several advantages compared to conventional methods. Its benefits include economic gain, practicality of use, and the absence of the potential risk of chemical residues in fruits and environmental contamination. Despite the importance of genetic resistance to *Rhizoctonia solani* in watermelon, there are no studies of resistance evaluation in the current literature.

Information about the *Rhizoctonia*-watermelon pathosystem is also scarce. Recently, the occurrence of pathogenic binucleate *Rhizoctonia*, responsible for root rots and associated with watermelon, was described in Italy (Aiello et al., 2012).

Therefore, the objective of this study was to evaluate watermelon accessions in the Germplasm Bank of *Citrullus* spp. from Embrapa Semi-Arid for their reaction to different isolates of *Rhizoctonia solani* inoculated in two phenological stages (seeds and seedlings). The main aim of study was identification of damping-off resistance sources.

Results

Reaction of the accessions inoculated at seeding stage

The inoculation with the CMM-2967 isolate at seeding revealed a total of six accessions (18.75%) with moderate resistance to the disease, namely BGCIA 115, BGCIA 227, BGCIA 811, BGCIA 812, BGCIA 849 and BGCIA 959. However, most accessions (81.25%) were classified as susceptible and highly susceptible to the CMM-2967 isolate (Table 1). For the CMM-1052 and CMM-1053 isolates, 96.87% and 100% of the accessions were classified as highly susceptible, respectively (Table 1). It was possible to detect that the most aggressive isolates were CMM-1052 and CMM-1053 using the Kruskal-Wallis test at a 5% level of significance, with mean scores of severity of 3.90 and 3.98, respectively. The CMM-2967 isolate was the least aggressive isolate with a mean score severity of 2.98.

Reaction of the accessions inoculated at seedling stage

Three accessions behaved like an immune plant, when inoculated with the isolate CMM-2967, namely BGCIA 012, BGCIA 036 and BGCIA 959. The results also showed that 24 accessions were classified as highly resistant and two accessions were classified as resistant (Table 2). In the evaluations using the isolate CMM-1053, accession BGCIA 843 was classified as resistant and BGCIA 064 and BGCIA 952 presented moderate resistance. It is also possible to note that from the 30 accessions, 93% were classified as highly susceptible or susceptible to the disease, when the CMM-1053 isolate was used. This shows that CMM-1053 is more aggressive than the isolate CMM-2967, with mean scores of severity of 3.62 and 0.63, respectively. This could be detected by the Wilcoxon test at a 5% significance level.

Accessions inoculated at the seedling stage showed higher resistance than those inoculated at the seeding stage. The accessions BGCIA 115, BGCIA 227, BGCIA 811, BGCIA 812, BGCIA 849 and BGCIA 959 were classified as moderately resistant, when inoculation occurred with the isolate CMM-2967 at seeding stage. Inoculation at seedling stage, classified BGCIA 115 as resistant, BGCIA 227, BGCIA 811, BGCIA 812 and BGCIA 849 as highly resistant and BGCIA 959 as immune to the isolate CMM-2967. For the isolate CMM-1053, the BGCIA 843 accession was classified as resistant, and the BGCIA 064 and BGCIA 952 accessions were classified as moderately resistant. In the first stage, they were all susceptible.

Reaction of accessions when simultaneously inoculated in the seeding and seedling stages

To confirm our hypothesis that inoculation at the seeding stage is more aggressive, a new experiment was carried out. According the results (Fig 2), there was interaction between the stage x accessions for both isolates CMM-2967($p=0.04$) and CMM-1053($p=0.003$) of *R. solani*.

For the CMM-2967 isolate inoculated at the seedling stage, all accessions were classified as resistant, with average scores less than 2.0, except for Crimson Sweet. At the seeding stage we confirmed our previous results, where accessions were classified into two groups by Scott-Knott (5%) with the most susceptible were BGCIA 12, BGCIA 115, BGCIA 849, BGCIA 952, BGCIA 959 and 'Crimson Sweet', and the most resistant BGCIA 36, BGCIA 64, BGCIA 227, BGCIA 811, BGCIA 812, and BGCIA 843.

For the most aggressive isolate (CMM-1053) at the seeding stage, all accessions were susceptible, as shown in the first inoculation. However at seedling stage, they were all resistant, except for the control ('Crimsom Sweet').

Discussion

When the inoculation was occurred during the seeding stage, only six accessions were identified with moderate resistance to the isolate CMM-2967 which could potentially be regarded a source of resistance. But for the CMM-1052 and CMM-1053 isolates, which were considered as the most aggressive isolates, it was not possible to identify any accession classified as resistant or highly resistant to *Rhizoctonia solani* (Table 2). Mirmajlessi et al. (2011) evaluated 23 *Rhizoctonia solani* isolates in Cucurbitaceae (cucumber, melon, pumpkin and watermelon). For watermelon, disease severity was calculated using the McKinney index ranged from 16.6 to 91.6%, depending on the tested isolate.

The inoculation at seeding stage has already been used for the evaluation of resistance in others crops, such as melons (Michereff et al., 2008) and cowpeas (Noronha et al., 1995). However, for watermelon, we verified that it is a very aggressive method as many watermelon accessions did not germinate and received a score of 4 (the maximum score). To reduce this problem, we proposed a new methodology of inoculation, where only seedlings were inoculated (not the seeds). Chang et al. (2014) found that watermelon seedlings which exhibit resistance to *Fusarium* have thicker cell walls of xylem than susceptible seedlings. Furthermore, in the resistant plants there was an increase in lignin deposition in

Table 1. Reaction of 32 watermelon (*Citrullus lanatus*) accessions to CMM-2967, CMM-1052, and CMM-1053 isolates of *Rhizoctonia solani* inoculated during the seeding stage (first experiment).

Accession	Species	CMM-2967		CMM-1052		CMM-1053	
		Average Score[a]	Reaction[b]	Average Score	Reaction	Average Score	Reaction
BGCIA 002	*C. lanatus* var. *lanatus*	2.2	S	4	HS	4	HS
BGCIA 008	*C. lanatus* var. *lanatus*	3	S	4	HS	4	HS
BGCIA 012	*C. lanatus* var. *lanatus*	3.8	HS	4	HS	4	HS
BGCIA 028	*C. lanatus* var. *lanatus*	3.2	HS	4	HS	4	HS
BGCIA 034	*C. lanatus* var. *lanatus*	3.2	HS	4	HS	4	HS
BGCIA 036	*C. lanatus* var. *lanatus*	2.8	S	3.8	HS	4	HS
BGCIA 040	*C. lanatus* var. *lanatus*	3	S	4	HS	4	HS
BGCIA 064	*C. lanatus* var. *lanatus*	3.2	HS	4	HS	4	HS
BGCIA 115	*C. lanatus* var. *lanatus*	1.8	MR	4	HS	4	HS
BGCIA 219	*C. lanatus* var. *citroides*	2.8	S	2.6	S	4	HS
BGCIA 223	*C. lanatus* var. *citroides*	3.4	HS	4	HS	4	HS
BGCIA 225	*C. lanatus* var. *citroides*	2.8	S	4	HS	4	HS
BGCIA 226	*C. lanatus* var. *citroides*	2.6	S	4	HS	4	HS
BGCIA 227	*C. lanatus* var. *citroides*	2	MR	4	HS	4	HS
BGCIA 229	*C. lanatus* var. *citroides*	2.8	S	4	HS	4	HS
BGCIA 714	*C. lanatus* var. *lanatus*	2.2	S	4	HS	3.8	HS
BGCIA 811	*C. lanatus* var. *lanatus*	1.6	MR	4	HS	3.6	HS
BGCIA 812	*C. lanatus* var. *lanatus*	2	MR	3.8	HS	4	HS
BGCIA 821	*C. lanatus* var. *lanatus*	3.8	HS	4	HS	4	HS
BGCIA 843	*C. lanatus* var. *lanatus*	2.6	S	4	HS	4	HS
BGCIA 849	*C. lanatus* var. *lanatus*	1.8	MR	4	HS	4	HS
BGCIA 864	*C. lanatus* var. *lanatus*	3.2	HS	4	HS	4	HS
BGCIA 865	*C. lanatus* var. *lanatus*	3.6	HS	4	HS	4	HS
BGCIA 877	*C. lanatus* var. *lanatus*	3.2	HS	3.6	HS	3.8	HS
BGCIA 882	*C. lanatus* var. *lanatus*	3	S	4	HS	4	HS
BGCIA 947	*C. lanatus* var. *lanatus*	3.2	HS	4	HS	4	HS
BGCIA 952	*C. lanatus* var. *lanatus*	2.4	S	4	HS	4	HS
BGCIA 959	*C. lanatus* var. *lanatus*	1.8	MR	4	HS	4	HS
BGCIA 962	*C. lanatus* var. *citroides*	3	S	3.2	HS	4	HS
BGCIA 976	*C. lanatus* var. *lanatus*	4	HS	3.8	HS	3.8	HS
BGCIA 979	*C. lanatus* var. *lanatus*	3.8	HS	4	HS	4	HS
1.128.170.013	*C. lanatus* var. *citroides*	3	S	3.8	HS	4	HS

[a]Scores scale from 0 to 4 (Noronha et al., 1995). To assess the severity of disease caused by *Rhizoctonia solani* in watermelon where: 0 = no symptoms, 1 = hypocotyl with minor injuries; 2 = hypocotyl with large lesions, but without constriction; 3 = totally constricted hypocotyl and showing damping-off, 4 = non-germinated seeds or seedlings not emerged.
[b]Reaction classes to *Rhizoctonia* proposed by Michereff et al. (2008) where: average 0 - similar to immune; 0.1. -1.0 - highly resistant; 1.1-2.0 - moderately resistant; 2.1-3.0 - susceptible, and 3.1-4.0 - highly susceptible.

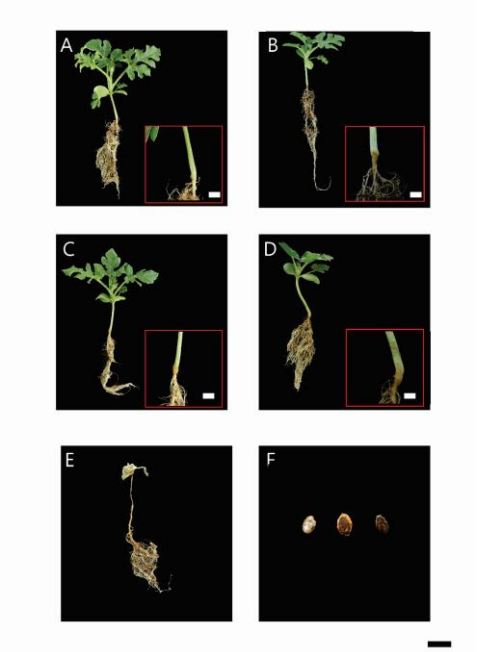

Fig 1. Descriptive scale developed for watermelon with scores from 0 to 5 used to severity of disease caused by *Rhizoctonia solani* where: A) score 0 = no symptoms, B) score 1 = small lesions on the roots or on the hypocotyls, C) score 2 = lesions circling the hypocotyl without constriction; D) score 3 = beginning of constriction and partial destruction of tissues without damping-off, E) score 4 = necrotic tissue with post-emergence damping-off, F) score 5 = pre-emergence damping-off. Bars: 1 cm

Table 2. Reaction of 30 watermelon accessions to CMM-2967 and CMM-1053 isolates of *Rhizoctonia solani* inoculated during the seedling stage (second experiment).

Entry	Specie	CMM-2967		CMM-1053	
		Average score[a]	Reaction[b]	Average score	Reaction
BGCIA 002	*C. lanatus* var. *lanatus*	0.6	HR	4	HS
BGCIA 008	*C. lanatus* var. *lanatus*	0.4	HR	4	HS
BGCIA 012	*C. lanatus* var. *lanatus*	0	I	3.2	S
BGCIA 028	*C. lanatus* var. *lanatus*	0.6	HR	3.6	S
BGCIA 034	*C. lanatus* var. *lanatus*	3.2	S	3.4	S
BGCIA 036	*C. lanatus* var. *lanatus*	0	I	3.2	S
BGCIA 040	*C. lanatus* var. *lanatus*	0.4	HR	3.4	S
BGCIA 064	*C. lanatus* var. *lanatus*	0.4	HR	3	MR
BGCIA 115	*C. lanatus* var. *lanatus*	1.6	R	4	HS
BGCIA 219	*C. lanatus* var. *citroides*	1	HR	4	HS
BGCIA 223	*C. lanatus* var. *citroides*	0.6	HR	3.2	S
BGCIA 225	*C. lanatus* var. *citroides*	0.6	HR	4	HS
BGCIA 226	*C. lanatus* var. *citroides*	1	HR	3.8	S
BGCIA 227	*C. lanatus* var. *citroides*	0.6	HR	4	HS
BGCIA 229	*C. lanatus* var. *citroides*	0.8	HR	3.8	S
BGCIA 811	*C. lanatus* var. *lanatus*	0.6	HR	4	HS
BGCIA 812	*C. lanatus* var. *lanatus*	0.4	HR	3.25	S
BGCIA 821	*C. lanatus* var. *lanatus*	0.8	HR	3.6	S
BGCIA 843	*C. lanatus* var. *lanatus*	0.4	HR	2	R
BGCIA 849	*C. lanatus* var. *lanatus*	0.5	HR	3.75	S
BGCIA 864	*C. lanatus* var. *lanatus*	1	HR	3.8	S
BGCIA 865	*C. lanatus* var. *lanatus*	0.7	HR	4	HS
BGCIA 882	*C. lanatus* var. *lanatus*	0.6	HR	3.8	S
BGCIA 947	*C. lanatus* var. *lanatus*	0.6	HR	4	HS
BGCIA 952	*C. lanatus* var. *lanatus*	0.2	HR	2.8	MR
BGCIA 959	*C. lanatus* var. *lanatus*	0	I	3.6	S
BGCIA 962	*C. lanatus* var. *citroides*	0.75	HR	4	HS
BGCIA 976	*C. lanatus* var. *lanatus*	0.75	HR	3.8	S
BGCIA 979	*C. lanatus* var. *lanatus*	2	MR	4	HS
Sugar Baby	*C. lanatus* var. *lanatus*	0.8	HR	4	HS

[a] Score scale developed for watermelon with scores from 0 to 5 used to assess the severity of the disease caused by *Rhizoctonia solani* where: 0 = no symptoms, 1 = small lesions on the roots or on the hypocotyls, 2 = lesions circling the hypocotyl without constriction; 3 = beginning of constriction and partial destruction of tissues without damping-off, 4 = necrotic tissue with post-emergence damping-off, 5 = pre-emergence damping-off. [b] reaction classes to *Rhizoctonia* proposed by Michereff et al. (2008) adapted to watermelon where: mean value= 0 - similar to immune; 0.1-1.0 - highly resistant; 1.1-2.0 resistant; 2.1-3.0 - moderately resistant; 3.1-4.0 - susceptible; and 4.1-5.0 - highly susceptible.

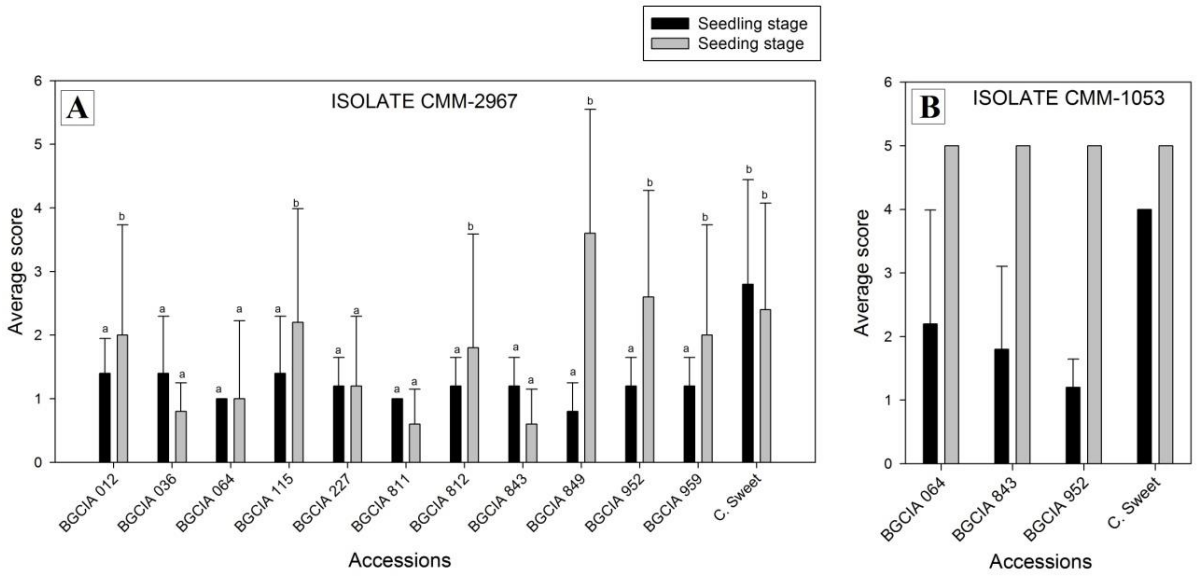

Fig 2. Average score of two inoculation methods (Seedling and seeding stage) evaluated for two *Rhizoctonia solani* isolates: (A) Twelve watermelon accessions inoculated by the CMM-2967 isolate; (B) Four watermelon accessions inoculated by CMM-1053. Means followed by the same letter within the method do not differ statistically by Scott-Knott at the 5% of significance. Bars: standard deviation

the cell wall of xylem three days after inoculation. Thus, the inoculation during the seedling stage allows the defense mechanism to occur, facilitating the identification of some resistant genotypes and the proposal of some measures for the management of the culture.

The development of a descriptive scale for the watermelon pathosystem to *Rhizoctonia* was necessary for inoculation at seedling stage because the scale from Noronha et al. (1995) was proposed to evaluate accessions at the time of seeding and not at the seedling stage. Moreover, the Noronha scale (1995) was developed for the cowpea and adapted for melons (Michereff et al., 2008; Sales-Junior et al., 2015) and cotton (Abd-Elsalamet al., 2010). The scale proposed in this experiment was adequate for evaluations, as it was possible to identify three accessions with resistance to the attacks of the isolates CMM-2967 and CMM-1053 of *R. solani*. For selection of accessions for resistance to participate in breeding programs, the priority should be given to the information of phenotypes inoculated with the most aggressive isolates. As highlighted in this study, the BGCIA 843, BGCIA 064 and BGCIA 952 showed moderate resistance against the most aggressive isolate CMM-1053 in all of the experiment at the seedling stage (Table 2 and Fig 2). These results demonstrate that inoculated seedlings may have post-formed resistance mechanisms.

Comparison of methods showed that when the accessions were inoculated at the seedling stage, they had higher resistance than those inoculated at the seed stage. This was proved in the last experiment, where both methods were tested simultaneously. This can have a direct involvement in the management of the watermelon crop in the field by replacing the direct sowing with seedling transplanting. Plants use the continuous increase of lignin and phenolic compounds in cells as a defense mechanism to pathogen attacks (Nicholson and Hammerschmidt 1992). Thus, the genotypes that present resistance after germination may have a greater chance of success in the field, even in the presence of the pathogen, due to substance accumulation in tissue cells that make unfavorable conditions for the development of the fungus.

Previous results in the literature point the difficulty of finding watermelon genotypes that present qualitative resistance to *Rhizoctonia* damping-off. The results of this study are consistent with studies of other hosts (Michereff et al., 2008; Mirmajlessi et al., 2011) with resistance to *Rhizoctonia* in watermelon, indicating that it could be horizontal or quantitative due to the variations of symptoms and scores presented. However, more studies are necessary.

Michereff et al. (2008) evaluated the resistance of 20 melon genotypes to two *Rhizoctonia solani* isolates. Six genotypes were obtained as highly resistant (30%) to both tested isolates. Sales-Junior et al. (2015) studied melon, inoculated with three isolates (RS-21, RS-22, and RS-23) of fungus *Rhizoctonia solani* in seedlings phase and detected only three suitable phenotypes, one immune to one of the isolates and two with high resistance to two isolates. Our results also show the presence of moderate to high resistance in accessions inoculated with the isolate CMM-1053 and CMM-2967 with both of these inoculations performed in the seedling stage. Several authors have reported the difficulty of obtaining genotypes with immunity or high resistance to *Rhizoctonia,* probably due the aggressive pathogenesis process of the fungus *Rhizoctonia solani* (Zhang et al., 2016; Melo et al., 2018).

In this study, we identified the method of inoculation at the seedling stage as the most promising for identifying resistance sources to *Rhizoctonia solani* in watermelon. The descriptive scale developed for the pathosystem was adequate for evaluation of accessions since it allowed the identification of different levels of resistance.

Materials and Methods

Local and experimental design

The experiment was carried in three steps in greenhouse of the Plant Pathology Laboratory at University of the Valley of the São Francisco (UNIVASF) in Petrolina, PE, Brazil. The design plotted in all stages of the experiment was completely randomized with five replications per accession. The experimental unit consisted of five seedlings or seeds. In the first experiment, the inoculation was carried out during the seed stage. At this stage, the inoculation of *Rhizoctonia solani* isolates was occurred at the sowing time with deposition of two rice grains that previously colonized by the pathogen plus one clean seed. In the second experiment, inoculation was carried out during the seedling stage, where the inoculation was done in plants 15 days after sowing using one rice grain also colonized close to hypocotyl.

Plant material and R. solani isolates

The accessions provided by the Germplasm Bank of *Citrullus* spp. from Embrapa Semi-Arid (Table 1 and 2). These accessions were from traditional agriculture producers or forage watermelons (*C. lanatus* var. *citroides*) collected in previous studies.

Rhizoctonia solani isolates were obtained from the Culture Collection of Phytopathogenic Fungi “Prof. Maria Menezes” (CMM) from Universidade Federal Rural de Pernambuco. The isolates were obtained from watermelon stems in Quixeré County in the state of Ceará (CMM-1052 and CMM-1053) and Mossoró county in the state of Rio Grande do Norte (CMM-2967). The isolates were stored in Petri dishes containing potato dextrose agar (PDA) and Erlenmeyers flasks with grains of colonized parboiled rice. The pathogenicity for all of the isolates was restored by inoculation in watermelon and re-isolation.

Inoculation at seeding stage

In this experiment, inoculation was carried out in 32 accessions of *Citrullus* spp. using three *R. solani* isolates (CMM-2967, CMM-1052 and CMM-1053) at the time of seeding. The isolates were incubated in Petri dishes containing PDA in BOD at 25 °C for 10 days in the dark. The inoculum of each isolate was prepared independently in Erlenmeyer flasks of 250 ml. For this purpose, 50 g of parboiled rice and 30 ml of distilled water were autoclaved. A mycelium disc with a diameter of 5 mm was added to each isolate per flask. After incubation at room temperature for a period of 10 days, all rice grains were colonized by the fungus. The sowing of each watermelon accession was performed in trays of 200 cells with each tray containing coconut fiber substrate, which was prepared by hand. In

each cell, a seed was added from the accession and two grains of rice colonized by the pathogen. The negative control consisted of a seed of each accession inoculated with rice in the same conditions, but not colonized by the pathogen. The trays containing the inoculated seeds were then placed on greenhouse bench underscreens (Sombrite®) that retained 50% brightness and irrigated daily until the evaluations. This took place 15 days after inoculation. For evaluations, a descriptive scale adapted from cowpea research was used. This scale has the following scores: 0 = no symptoms; 1 = hypocotyl with small lesions; 2 = hypocotyl with large lesions, but without constriction; 3 = hypocotyl totally constricted and showing damping-off; 4 = non-germinated seeds and / or seedlings not emerged (Noronha et al., 1995).

To classify each accession into the five resistance classes, the average score of each accession was used, as proposed by Michereff et al. (2008) as follows: Average of 0 - plants immune; average score between 0.1 and 1.0 - highly resistant; between 1.1 and 2.0 - moderately resistant; between 2.1 and 3.0 - susceptible; and between 3.1 and 4.0 - highly susceptible.

Inoculation at seedling stage

In this experiment inoculation was carried out in 30 accessions of watermelon (*Citrullus* spp.) at the seedling stage using the isolates of *R. solani* CMM-2967 and CMM-1053. The inoculum of each isolate was prepared as previously described. The sowing of each entry was performed in trays of 200 cells containing a mixture of 50% coconut fiber substrate and 50% expanded vermiculite type prepared by hand. In each cell tray a seed was added and, 15 days after sowing, when the plants presented three definitive leaves, 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. As in the first inoculation, the trays were kept in a greenhouse and irrigated until evaluations were done. For the evaluation of this experiment, a specific descriptive scale for this pathosystem was developed (Fig 1), since there was no specific scale for *Rhizoctonia solani* in watermelon. For adequacy of the scale, seedlings were collected with symptoms at different phenological phases of the host. These symptoms were grouped according to the following notes: 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. 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 – 2.0- resistant (R); 2.1 – 3.0- moderately resistant (MR); 3.1 - 4.0 susceptible (S); 4.1 – 5.0- highly susceptible (HS).

Inoculations of resistant accessions simultaneously in the seeding and seedling stages

To confirm the results, a third experiment was carried out using the resistant accessions for both methods (at the seeding and seedling stages). For the CMM-1053 isolate, three resistant accessions (BGCIA 064, 843 and 952) plus the commercial cultivar Crimson Sweet (control treatment) were used in a factorial scheme of 2 x 4 (two methods and four accessions). For the CMM-2967 isolate, 11 accessions (BGCIA 012, 036, 064, 115, 227, 811, 812, 843, 849, 952 and 959) were used plus the control (using a factorial scheme of 2 x 12). The inoculation was carried out as described before. For the evaluation of this experiment, the specific scale of scores for this pathosystem was used.

Statistical analysis

After inoculation at seeding stage, scores were submitted to the Shapiro-Wilk test, with a 5% significance level to verify the normality of the data. Due to a non-normal distribution, data were submitted to the nonparametric Kruskal-Wallis test to verify differences among the isolates. For the inoculation at seedling stage, only two isolates were compared, CMM-1053 and CMM-2967, using the non-parametric Wilcoxon test at a 5% significance level to differentiate the aggressiveness of the isolates. Data from the last part of experient were submitted to ANOVA in the factorial scheme. For CMM-1053, there was a factorial of 2 x 4 and for CMM-2967, there was a factorial of 2 x 12. Statistical analyses were performed using the statistical program R Core Team (2015).

Conclusion

The accessions BGCIA 843, BGCIA 064 and BGCIA 952 showed resistance when inoculated with both isolates. These accessions could be used in breeding programs to determine the genetic control of resistance to *Rhizoctonia solani* as well as source of resistance. Thus, we developed an efficient method of inoculation at the seedling stage as well a specific scale for this pathosystem that allows us to identify the source of resistance and to facilitate the breeder's and plant pathologist work in developing resistance to damping-off.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical standard

This article does not contain any studies with human participants or animals performed by any of the authors.

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