

# Method for evaluating rhizoctonia resistance in melon germplasm<sup>1</sup>

## Metodologia para avaliação da resistência à rizoctoniose em germoplasma de meloeiro

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**ABSTRACT** - The intensive cultivation of melon, mainly in the Northeast of Brazil, has favored the occurrence of root diseases such as rhizoctonia, caused by the fungus *Rhizoctonia solani* Kuhn. The use of resistant cultivars is one of the most efficient strategic measures for integrated management of diseases in this crop. It is necessary to use an efficient inoculation method to evaluate rhizoctonia in melon germplasm with the aim of identifying sources of resistance. The objective of this study was to develop an efficient method to inoculate the pathogen to proceed in selection of *R. solani* resistant melon germplasm. Five experiments were conducted under greenhouse conditions to define the adequate container, growth environment, substrate for inoculum production, aggressiveness of the isolates, inoculum density, manner of inoculation, phenological stage of the plant, and time for evaluation after inoculation. The results obtained showed that for selection of *R. solani* resistant melon germplasm, the use of seedlings with roots cut during transplanting to pots is recommended. The pots should contain sand with organic inoculum obtained from rice grains at the concentration of 150 mg.kg<sup>-1</sup> of soil, associated with inoculation through a puncture in the plant stem by an infested toothpick, without the need for a moist chamber. The most aggressive isolate was CMM-1068.

**Key words:** *Cucumis melo*. *Rhizoctonia solani*. Genetic resistance. Damping off.

**RESUMO** - O cultivo intensivo do meloeiro, principalmente no Nordeste do Brasil, tem favorecido a ocorrência de doenças radiculares como a rizoctoniose, causada pelo fungo *Rhizoctonia solani* Kuhn. O uso de cultivares resistentes é uma das medidas estrategicamente mais eficiente para o manejo integrado de doenças na cultura. Para a avaliação da rizoctoniose no germoplasma de meloeiro, visando identificar fontes de resistência, é necessária a utilização de um método eficiente de inoculação. O objetivo desse trabalho foi desenvolver uma metodologia eficiente de inoculação do patógeno para a seleção de germoplasma de meloeiro resistente a *Rhizoctonia solani*. Cinco experimentos foram conduzidos em casa de vegetação, visando definir o recipiente, o ambiente de condução, o substrato para produção do inóculo, a agressividade dos isolados, a densidade do inóculo, a forma de inoculação, o estágio fenológico da planta e tempo para avaliação após a inoculação. Os resultados obtidos evidenciaram que para seleção de germoplasma de meloeiro quanto à resistência a *R. solani* recomenda-se o uso de mudas com raízes cortadas durante o transplante para vasos, contendo areia com inóculo orgânico, obtido a partir de grãos de arroz na concentração de 150 mg.kg<sup>-1</sup> de solo, associado à inoculação com furo no colo da planta por palito de dente infestado, sem necessidade do uso de câmara úmida. O isolado mais agressivo foi o CMM-1068.

**Palavras-chave:** *Cucumis melo*. Resistência genética. Tombamento. *Rhizoctonia solani*.

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## INTRODUCTION

Melon (*Cucumis melo* L.) is one of the cucurbits of greatest economic importance in the world, grown in more than 100 countries (ROCHA *et al.*, 2010). In 2018, around 28 million tons were produced on 1.1 million hectares, the largest producers being China, Turkey, Iran, Egypt, and India (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 2020). Brazil occupied 13th place, producing more than 580 thousand tons on 23 thousand hectares (IBGE, 2020).

The Northeast region is responsible for 95% of Brazilian production, especially the states of Rio Grande do Norte and Ceará, with 76% of the production of the Northeast region (IBGE, 2020). These states are also responsible for 99% of Brazilian melon exports (BRASIL, 2019). However, the intense and continuous cultivation of melon in the Brazilian semi-arid region favors the establishment of pests and diseases (OLIVEIRA *et al.*, 2017). Prominent among the diseases are those caused by soil-borne pathogens, such as rhizoctonia (*Rhizoctonia solani* Kühn), one of the most common in the region (MAIA; LIMA; LIMA, 2013) and which causes damage to melon throughout the world (EL-KOLALY; ABDEL-SATTAR, 2013; M'HAMDI *et al.*, 2016; SALARI *et al.*, 2012b).

*Rhizoctonia solani*, the most important species of the genus, is a pathogen with wide genetic diversity and a vast range of host plants (DAVID *et al.*, 2018), and it has been associated with injuries, damping off, and/or reduction in the stand of various plant species (DAVID *et al.*, 2018; YANG; LI, 2012). The use of resistant cultivars is the best strategy among control methods; however, success in introgression of this resistance depends on the source and genetic inheritance of the resistance, the nature of the pathogen, and the method used in selection (CUNHA *et al.*, 2019; OUMOULOUD *et al.*, 2013).

For definition of a method that is effective and efficient in discrimination of the genotypes, precise determination of the reaction of the genotypes to the pathogen is necessary (MEDEIROS *et al.*, 2015). Although there are reports of sources of resistance to this pathogen in various crops, few have been identified in the melon germplasm (MICHEREFF; ANDRADE; SALES JÚNIOR, 2008; SALARI *et al.*, 2012a; SALES JÚNIOR *et al.*, 2015).

Diverse methods have been used with the aim of multiplying the pathogen and conditioning the host to its action. Production of the inoculum, for example, can be differentiated according to the substrate and the manner of application. The inoculum is produced through colonization in cereal grains or in liquid (suspension) and solid (mycelial discs) media, which are inoculated on the

seeds or incorporated in the soil (MICHEREFF FILHO *et al.*, 1996; SILVA *et al.*, 2016; SOUZA *et al.*, 2008). Another method consists of the use of a toothpick infested with the inoculum, which is introduced in the root collar of the seedlings (SCANDIANI *et al.*, 2011). In addition, for each method, there is variation in the concentration (SOUZA *et al.*, 2008) and in the period of growth and viability of the inoculum (MICHEREFF FILHO *et al.*, 1996).

Andrade *et al.* (2005), working with concentrations of an organic inoculum obtained in rice for use on common bean, noted that the occurrence of damping off in seedlings is directly related to the density of the inoculum, and that concentrations from 50 to 200 mg.kg<sup>-1</sup> of soil were adequate for selection of genotypes. Santos *et al.* (2005), adapting this method to cotton, with doses of up to 600 mg.kg<sup>-1</sup> of substrate, defined doses from 72 to 144 mg.kg<sup>-1</sup> as satisfactory.

In this context, the aim of this study was to develop an efficient and non-destructive method of inoculation of the pathogen for selection of melon germplasm regarding resistance to *R. solani*.

## MATERIAL AND METHODS

Five trials were conducted with the aim of defining the adequate container, growth environment, substrate for inoculum production, aggressiveness of the isolates, inoculum density, manner of inoculation, phenological stage of the plant, and time for evaluation after inoculation. The experiments were conducted in a greenhouse and in the Breeding and Plant Genetic Resources Laboratory, and the inocula of *R. solani* were produced in the Post-harvest Pathology Laboratory of Embrapa Agroindústria Tropical in Fortaleza, CE, Brazil (35°45' S, 38°34' W, 25 MASL, tropical rainy climate). The 'Goldex' melon hybrid was used as a control since it is widely grown in Brazil and has proven to be susceptible in preliminary trials (data not published).

The *R. solani* isolates used in this study are part of the "Prof. Maria Menezes - CMM" Phytopathogenic Fungal Culture Collection of the Phytopathology Department of the Universidade Federal Rural de Pernambuco (UFRPE). The isolates were first subcultured in Potato Dextrose Agar (PDA) culture medium + 0.1 g.L<sup>-1</sup> chloramphenicol and maintained under ambient temperature (28 °C ± 2 °C) and 12 -h photoperiod for seven days.

Before sowing, the melon seeds were disinfected with 70% alcohol for one minute and in a 1.5% NaClO solution for 5 min. After that, they were washed in abundant running water and placed to dry on paper toweling

under ambient temperature ( $\pm 28$  °C) (MICHEREFF; ANDRADE; SALES JÚNIOR, 2008). The sand used in all the experiments was previously autoclaved at 121 °C, 1 atm, for 1 h on 2 consecutive days.

The plants were evaluated regarding the severity of the disease through a scoring scale (NORONHA; MICHEREFF; MARIANO, 1995) adapted for the melon crop: 1 = without symptoms, 2 = small lesions on the hypocotyls, 3 = large lesions on the hypocotyls, but without constriction, 4 = hypocotyl fully constricted, and 5 = seeds not germinated (in experiments with seeds sown directly) or seedlings with symptoms of damping off (in experiments with seedlings). The disease severity index (MCKINNEY, 1923) was also calculated, using the expression:  $DSI = [\sum (\text{degree of the scale} \times \text{frequency}) / (\text{total number of units evaluated} \times \text{maximum degree of the scale})] \times 100$ , using the data obtained from the scoring scale.

The following five experiments were performed:

### 1 - Determination of the inoculum, incubation environment and growth container

The experiment was conducted in a completely randomized design (CRD) with four replications. Plots consisted of three containers with one plant each, analyzed in a factorial arrangement ( $3 \times 2 \times 2$ ), composed of three types of inoculum (organic, mycelial discs, and hyphal suspension), two types of containers (330-mL pot and tray with 72 100-mL cells), and two environments (with and without moist chamber). The inocula were added to the soil that had been previously sterilized in an autoclave (121 °C, 1 atm, 1 h, 2 consecutive days). The isolate CMM-1066 of *R. solani*, originating from the root collar of melon plants grown in Mossoró, RN, was used because it had the greatest mycelial growth *in vitro*.

The organic inoculum was produced according to the method described by Michereff Filho *et al.* (1996), with modifications. Parboiled rice (50 g) and distilled water (30 mL) were mixed in an Erlenmeyer flask and sterilized in an autoclave (121 °C, 1 atm, 30 min). Three mycelial discs of *R. solani* were then transferred to the Erlenmeyer flask containing the sterilized rice and stored in an incubator at a temperature of 25 °C with constant lighting for ten days. In the experiments, one Erlenmeyer flask with only the rice and disks of the culture medium without the fungus was used as a control, aiming to detect possible contaminations.

After 10 days, the colonized substrate was placed in paper bags that had previously been sterilized in an autoclave (121 °C, 1 atm, 20 min) for drying of the inoculum for 48 h at 30 °C, maintaining constant lighting. On the day of infestation, the substrate was ground in a blender until

forming a very fine and homogeneous powder, which was mixed with sterilized sand at a proportion of 50 mg.kg<sup>-1</sup>.

In the mycelial disc treatment, the pathogen was grown in PDA culture medium, kept under ambient temperature (28 °C  $\pm$  2 °C) and 12-h photoperiod for seven days. Three 4-mm diameter mycelial discs were removed from the colony and deposited in the substrate used in sowing. The seeds were sown at a depth of 1.5 cm.

The treatment of infestation through hyphal suspension consisted of cultivation of the fungus in PDA medium for seven days. One 5-mm diameter disk was transferred to an Erlenmeyer flask containing PD (potato dextrose) liquid medium. After five days, the suspension was prepared through triturating the fresh fungal mycelium at a concentration of 10 mg of mycelium per 100 ml of sterilized distilled water, with the aid of a mechanical shaker. Fifty (50) mL of suspension were added to each 10 g of seed. In this treatment, the seeds remained in contact with the suspension for 5 min under shaking, at which time all the liquid was drained and the seeds were placed to dry in a sterile environment (SOUZA *et al.*, 2008).

One seed was added per container (pot or cell) containing autoclaved sand; inoculation was then performed. For applicable treatments, a moist chamber was formed using a transparent plastic bag and cotton moistened with sterilized distilled water; the treatments were maintained within for 24 h. The severity of the disease was evaluated 15 days after sowing (DAS).

### 2 - Selection of the most aggressive isolate

This experiment was conducted in a CRD with four replications; plots consisted of three pots with one plant each. Five isolates of *R. solani* were used (Table 1), based on the method selected in the first experiment, including the control treatment. The organic method was used for preparation of the inoculum at a concentration of 50 mg.kg<sup>-1</sup> of autoclaved sand. The seeds were sown in 330 -mL-capacity pots.

The organic inoculum for each isolate was obtained as described in Experiment 1. The evaluations were performed at 15 DAS, observing the lesions at the root collar of the seedlings, death of the seed embryo, and damping off of the seedlings. The isolate selected in this experiment was used in the subsequent experiments.

### 3 - Determination of the ideal density of the inoculum

In this study, four densities of the inoculum CMM-1068 were tested: 50, 100, 150 and 200 mg of colonized organic substrate per kilogram of autoclaved sand. The inocula and the control were prepared as described in Experiment 1. A CDR was used with five replications;

**Table 1** - Isolates of *Rhizoctonia solani* (anastomosis group 4 - AG 4) used in the experiment, collected from melon plants

Code	Year of collection	Municipality	Region of isolation
CMM-1066	2005	Mossoró, RN	hypocotyl
CMM-1067	2005	Mossoró, RN	hypocotyl
CMM-1068	2005	Baraúna, RN	hypocotyl
CMM-2157	2008	Mossoró, RN	root
CMM-187	2011	Bragança Paulista, SP	not informed

plots consisted of three pots with one plant each. Melon was sown immediately after infestation of the soil. The severity of the disease was estimated at 15 DAS.

#### 4 - Determination of the phenological stage for evaluation

In this experiment, a CRD was used with five replications, consisting of three pots with one plant each. Seeds were sown in 200 cell plastic trays for the treatments with seedlings, and directly in the pots for treatments with seeds. For the treatments with seedlings, inoculation occurred when the seedlings were at 10 DAS; for the treatments with seeds, inoculation occurred at sowing. In both cases, the isolate CMM-1068 was used. On the day of transplanting / sowing in 330 mL pots, the organic inoculum, prepared as described for Experiment 1, was mixed with the previously autoclaved sand at the concentration of 150 mg.kg<sup>-1</sup>. For each phenological stage (treatment) tested, there was a control without infestation.

Considering seeds and seedlings, five manners of inoculation were evaluated: 1 - whole seedlings; 2 - seedlings with roots cut at transplanting (around 60% of the length of the roots was cut with the aid of a previously disinfected scissors); 3 - seedlings with a puncture in the root collar, performed with a syringe needle; 4 - seeds pre-germinated in a Gerbox® with blotting paper moistened with water at the proportion of 2.5 times the weight of the dry paper in a seed germinator at 25 °C (up to radicle emergence); and 5 - seeds sown directly in the substrate. Severity was evaluated at 15 days after sowing/transplanting.

#### 5 - Determination of the inoculation method and of the ideal age for evaluation

The experiment was conducted in a CRD with five replications; plots consisted of three pots with one plant each, which were analyzed in a factorial arrangement (4 × 4) composed of four inoculation methods and four evaluation times.

The following inoculation methods were used in addition to the organic method (rice) defined in the first

experiment: 1 - addition of 10 ml of hyphal suspension after transplanting (suspension); 2 - immersion of roots in hyphal suspension for 5 min before transplanting (immersion); 3 - puncture in the root collar of the plant with infested toothpick (puncture in root collar), and 4 - only organic (rice) with 150 mg.kg<sup>-1</sup>. The plants were evaluated at 7, 14, 21 and 28 days after transplanting. With the aim of confirming the action of the fungus, a control treatment was added to each method, which consisted of the same method but without the presence of the fungus.

The suspension was prepared as described in Experiment 1. In method 1, after transplanting seedlings in sand containing organic substrate, each pot was irrigated with 10 ml of suspension. In method 2, a sample of 15 seedlings with cut roots was immersed in 750 ml of suspension for 5 min before transplanting in the sand and organic substrate.

In method 3 (the puncture in the root collar method), adapted from Medeiros *et al.* (2015), the isolate was previously subcultured in Petri dishes containing PDA culture medium with chloramphenicol (0.1 g/l) and kept for seven days in a BOD incubator at 28 ± 2 °C, with a 12-h photoperiod. The toothpicks were sterilized separately in an autoclave (121 °C, 1 atm for 30 min). At the time of inoculation, the tips of the sterilized toothpicks were infested in the colonies of *R. solani* and they were then inserted in the root collar of the plant at around 1.0 mm above the soil. In all the treatments, as a practical measure, seedlings with cut roots were used. Evaluation was performed as described in the fourth experiment.

#### Statistical analyses

The data of Experiment 1 were analyzed using non-parametric statistics through the Kruskal-Wallis test at the 5% probability level. Experiments 2 and 4 were analyzed through the Scott-Knott test at the 5% significance level.

Analysis of variance of the regression was performed on the data obtained from inoculum concentration in Experiment 3, and analysis of variance (ANOVA) was performed on the data for Experiment 5. The means of the inoculation methods were compared

through the Scott-Knott test at the 5% significance level.

## RESULT AND DISCUSSION

### Inoculum, incubation environment and growth container

There was no statistical difference for the containers and the environments studied (Table 2). Although the soil volume required by the cells of the trays (100 mL) is smaller than that required by the pots (330 mL), the individualization of the plants in pots facilitated both development, through having a larger volume of substrates, and the time of evaluation, through facilitating removal of the plant for evaluation.

In addition, facility in handling containers is important in plant breeding in trials regarding the response of accessions. The main aim is to allow plants identified as resistant to be transplanted in an easy and efficient manner. It is important that inoculation and removal of the plant from the container be easy at the time of analysis and that there be little damage to the roots. Pots are better in this regard because they facilitate these management practices.

From a practical and economic perspective, the lack of statistical differences in regard to the use of moist chambers suggests that selections for resistance be carried out without a moist chamber. Nevertheless, Goulart (2016), in tests using the same pathogen in cotton, found that the moist chamber was favorable to acceleration

of vigorous mycelial development, typical of *R. solani*. Such was also the case for Nechet and Halfeld-Vieira (2011), who used a moist chamber for 24 h to favor the development of *R. solani* in common bean.

In relation to the type of inoculum, the greatest severity was observed with the mycelial discs, which differed statistically from the other treatments (Table 2). Nevertheless, a large number of seeds under the disc treatment did not germinate, and it was necessary to re-isolate the pathogen from each ungerminated seed, for the purpose of clarifying if the lack of germination was due to the attack of the pathogen on the embryo or due to some seed physiological disfunction. According to Santos *et al.* (2005), inocula with density so high as to impede the presence of live seedlings are not ideal, because they do not allow visual comparison of the results.

It is important to observe that the inoculation method should ensure seed infection while maintaining germination and allowing the study of resistance to the pathogen (SOUZA, 2008). The pathogen inoculation techniques that use seeds reduce germination power in most cases. In this study, this reduction was evident when mycelial discs were used as inoculum. Thus, the use of mycelial discs on melon seeds is not recommended because, in addition to raising questions regarding selection, it requires later detection of the action of the pathogen.

The methods with mycelial suspension or organic substrate (in rice) were statistically equal and showed uniformity in severity. However, the method of inoculation through organic substrate has been reported in discrimination of genotypes in crops such as melon

**Table 2** - Means of the subjective score and of the disease severity index (DSI) in different containers, environmental conditions, and type of inoculum of *Rhizoctonia solani* in melon

Factor	Severity	
	Subjective score <sup>1</sup>	DSI <sup>2</sup>
Container		
Tray	3.5 ± 0.3 a	72.5 ± 8.9 a
Pot	3.2 ± 0.3 a	66.7 ± 9.4 a
Moist chamber		
Without	3.8 ± 0.4 a	76.7 ± 6.5 a
With	3.0 ± 0.3 a	62.5 ± 10 a
Inoculum		
Disk	4.3 ± 0.3 a	87.5 ± 6.3 a
Organic (rice)	3.2 ± 0.4 b	67.5 ± 9.9 b
Suspension	2.7 ± 0.4 b	53.8 ± 9.7 b

<sup>1</sup>Mean values followed by the same letter do not differ from each other by the Kruskal-Wallis test; <sup>2</sup>Mean values followed by the same letter do not differ from each other by the Scott-Knott test at the 5% significance level

(MICHEREFF; ANDRADE; SALES JÚNIOR, 2008; SALES JUNIOR *et al.*, 2015), zucchini, cucumber, and watermelon (MIRMAJLESSI *et al.*, 2012), and cowpea (NEREY *et al.*, 2010). Thus, this method was used in the subsequent experiments.

### Most aggressive isolate

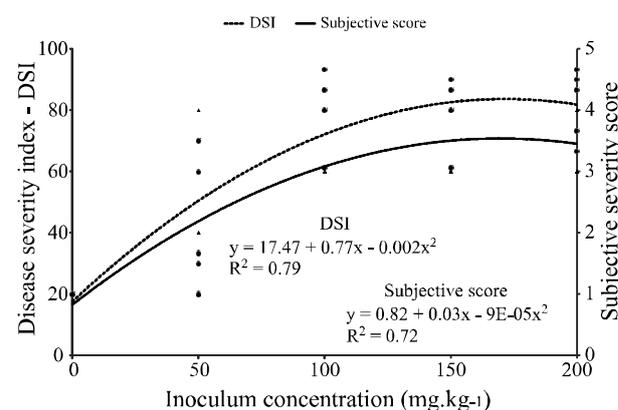
All the isolates tested caused damage to the root collar/roots, with variable severity (Table 3) that was statistically different. The CMM-1068 isolate was the most aggressive (four times more than the control). The CMM-2157 and CMM-1066 isolates were moderately aggressive and did not differ statistically from each other. The CMM-187 and CMM-1067 isolates were less aggressive and did not differ statistically from the control.

Thus, the CMM-1068 isolate was selected for the rest of the tests performed, aiming to adjust the resistance evaluation method to rhizoctonia in melon. However, due to the variability exhibited by the fungus, it is essential that new studies be performed with new isolates, always seeking success in selection of genotypes. It should be emphasized that genetic variability of pathogens creates difficulties in conducting plant breeding studies that seek to obtain disease resistant plant varieties.

### Ideal inoculum density

There were significant differences among the concentrations used (Figure 1), that is, the different inoculum densities used led to different responses in regard to the ability to cause rhizoctonia symptoms in melon seedlings at 15 days after inoculation. The severity progress curve in accordance with the concentration of the inoculum fit a quadratic model, with a coefficient of determination of 71.88%. The inoculum concentrations of 150 and 200 mg.kg<sup>-1</sup> were similar and provided the highest levels of severity, with severity score estimates of 3.60 and 3.62, respectively (Figure 1).

**Figure 1** - Subjective score and disease severity index in melon in accordance with the concentration of *R. solani* inoculum in melon



The results of this study corroborate those reported by Andrade *et al.* (2005), who, evaluating the effect of *R. solani* inoculum density on melon, found that the levels of severity increased with the increase in inoculum concentration. Inoculum densities of 5 and 25 mg.kg<sup>-1</sup> led to moderate levels of disease severity, 12.2% and 47.2%, respectively. For the high densities of 50 and 200 mg.kg<sup>-1</sup>, the severity levels were 58.3% and 81.7%, respectively.

The inoculum concentration has an important effect on the severity of the disease brought about by *R. solani* in melon, and a direct relationship was found between inoculum density and disease severity. Thus, the density of 150 mg.kg<sup>-1</sup> is recommended for evaluation of rhizoctonia in melon.

### Phenological stage for evaluation

The phenological stage of melon also affected the severity of rhizoctonia. The treatments of seedlings with cut roots, seedlings with root collar puncture, and directly sown seeds were superior and did not differ significantly from

**Table 3** - Mean values of the subjective score and of the disease severity index (DSI) as a result of different isolates of *Rhizoctonia solani*

Isolate	Severity	
	Subjective score <sup>1</sup>	DSI <sup>2</sup>
CMM - 1068	4.1 ± 0.3 a	75.0 ± 8.8 a
CMM - 2157	2.8 ± 0.4 b	55.0 ± 7.4 b
CMM - 1066	2.8 ± 0.5 b	55.0 ± 3.2 b
CMM - 187	2.0 ± 0.4 c	36.7 ± 8.4 c
CMM - 1067	1.5 ± 0.2 c	30.0 ± 1.9 c
Control	1.0 ± 0.0 c	20.0 ± 0.0 c

<sup>1</sup>Mean values followed by the same letter do not differ from each other by the Scott-Knott test; <sup>2</sup>Mean values followed by the same letter do not differ from each other by the Scott-Knott test at the 5% significance level

each other. In contrast, the treatments with pre-germinated seeds and whole seedlings was statistically inferior to the treatment of seedlings with cut roots (Table 4).

In regard to sowing or transplanting, given the problematic situation observed in the first experiment and with the possibility of a low or non-uniform germination reducing the time of seedling exposure to the pathogen, the methods that use seedlings were more efficient. Furtado *et al.* (2009), affirm that the phenological stage of the plants is an important factor and that these stages are fundamental in development of diseases. In addition, depending on the pathosystem, the tissues of the host plant may become more susceptible or less susceptible over time (STANGARLIN *et al.*, 2011).

In regard to cutting of roots or puncture in the root collar of the plants, it should be emphasized that both the phytopathogenic fungi and the necrotrophic fungi have a large quantity of cell-wall-degrading enzymes that break down plant defenses (DAVID *et al.*, 2018). Thus, the methods that damage the plant tissue facilitate the infection process; however, the reliability of the method is preserved.

#### Inoculation method and age for evaluation

There was no significant interaction between the combinations of inocula and evaluation time. In contrast,

the inoculation methods affected the severity and the DSI of rhizoctonia in melon (Table 5). The method of puncturing the root collar with an infested toothpick was statistically superior to the others, and was also the most uniform (Figure 2A), which allows more accurate evaluation of a large number of genotypes regarding resistance to *R. solani*. As the organic method by itself led to the lowest speed in the emergence of symptoms, using it together with the direct inoculation method through a puncture in the root collar led to greater aggressiveness of the pathogen, in all the periods evaluated (Figure 2B).

In regard to the period for evaluation of rhizoctonia severity, an increase in the severity of the disease was observed over time for all the inoculation methods within the interval studied, recording the maximum severity level at 28 days (Figure 2B). Thus, it may be that studies that evaluated the severity of *R. solani* in melon at 15 days after infestation may have found different results with a longer evaluation time (MICHEREFF; ANDRADE; SALES JÚNIOR, 2008; SALARI *et al.*, 2012a).

Sales Junior *et al.* (2015), using seedlings of 14 days of age in a study on reaction of melon accessions to *R. solani*, evaluated the severity at 45 days after inoculation. Nevertheless, considering that the melon cycle in the region is around 65 days, a late evaluation could compromise the self-fertilization of the genotypes

**Table 4** - Mean values of the subjective score and of the disease severity index (DSI) at different phenological stages of melon inoculated with *Rhizoctonia solani*

Treatment	Severity	
	Subjective score <sup>1</sup>	DSI <sup>2</sup>
Pre-germinated seeds	2.1 ± 0.3 b	46.7 ± 4.2 b
Directly sown seeds	3.0 ± 0.5 a	60.0 ± 8.5 a
Whole seedlings	2.6 ± 0.1 b	52.7 ± 2.2 b
Seedlings with puncture in root collar	2.9 ± 0.2 a	58.7 ± 6.5 a
Seedlings with cut roots	3.4 ± 0.2 a	65.3 ± 6.5 a

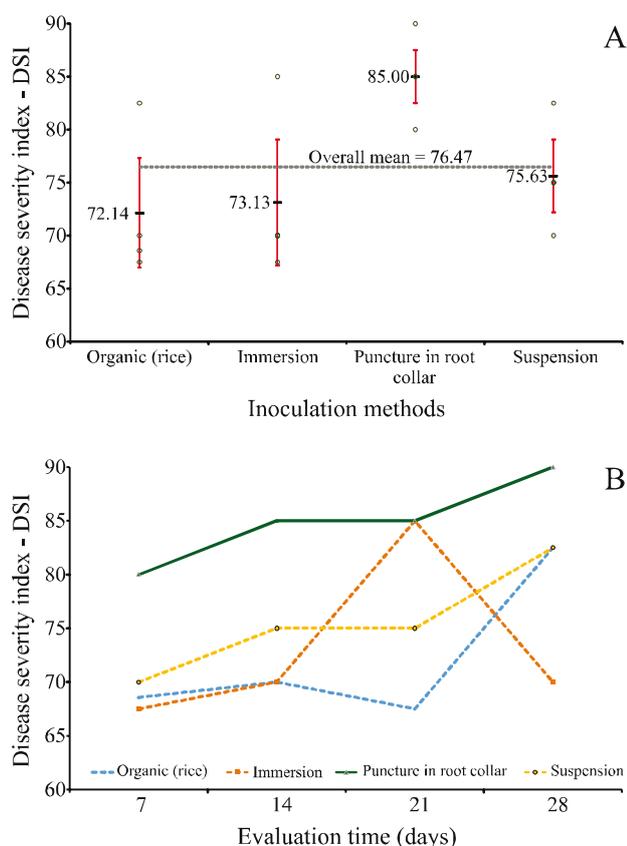
<sup>1</sup>Mean values followed by the same letter do not differ from each other by the Scott-Knott test; <sup>2</sup>Mean values followed by the same letter do not differ from each other by the Scott-Knott test at the 5% significance level

**Table 5** - Mean values of the subjective score and of the disease severity index (DSI) of different methods of inoculation of *Rhizoctonia solani* in melon

Inoculation method	Severity	
	Subjective score <sup>1</sup>	DSI <sup>2</sup>
Suspension	3.8 ± 0.1 b	72.1 ± 3.5 b
Immersion	3.7 ± 0.2 b	73.1 ± 4.0 b
Puncture in root collar	4.3 ± 0.2 a	85.0 ± 2.1 a
Organic (rice)	3.6 ± 0.1 b	75.7 ± 2.6 b

<sup>1</sup>Mean values followed by the same letter do not differ from each other by the Scott-Knott test; <sup>2</sup>Mean values followed by the same letter do not differ from each other by the Scott-Knott test at the 5% significance level

**Figure 2** - Disease severity index (DSI) according to the *Rhizoctonia solani* inoculation method in melon (A) and according to evaluation time (B)



selected, making it impossible to obtain melon lines resistant to rhizoctonia. In contrast, in the present study, it was possible to accurately select plants while still allowing them to be used for formation of new generations.

Definition of the evaluation method for melon in regard to rhizoctonia resistance will allow advances in plant breeding programs in respect to making resistant genotypes available to the melon production chain. In addition, we emphasize the importance both of evaluating germplasm in search of sources of resistance and of continually collecting new isolates of *R. solani*, above all in production areas with greater occurrence of the disease and of importance for the crop.

## CONCLUSION

For selection of melon germplasm regarding resistance to *R. solani*, recommendations are as follows: use of seedlings with roots cut during transplanting to pots containing sand with organic inoculum, obtained

from rice grain at the concentration of 150 mg.kg<sup>-1</sup> of soil, associated with inoculation through a puncture in the root collar of the plant with an infested toothpick, without the need to use a moist chamber. The most aggressive isolate was CMM-1068.

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## REFERENCES

- ANDRADE, D. E. G. T. *et al.* Influência da densidade do inóculo e de isolados de *Rhizoctonia solani* na severidade da rizoctoniose do meloeiro. *Revista Caatinga*, v. 18, n. 3, p. 164-168, 2005.
- BRASIL. Ministério da Indústria, Comércio Exterior e Serviços. **Comex Stat**: sistema para extração de relatórios personalizados sobre os dados do comércio exterior brasileiro. 2019. Retrieved from: <http://comexstat.mdic.gov.br/>. Accessed on: 13 Jun. 2019.
- CUNHA, F. S. *et al.* Identification of sources of resistance to damping-off (*Rhizoctonia solani*) in two phenological phases of watermelon. *Australian Journal of Crop Science*, v. 13, n. 4, p. 628-634, 2019.
- DAVID, G. Q. *et al.* *Rhizoctonia* como fitopatógeno no agroecossistema brasileiro. In: LOPES, U. P.; MICHEREFF, S. J. **Desafios do manejo de doenças radiculares causadas por fungos**. Recife: EDUFRPE, 2018. p. 46-67.
- EL-KOLALY, G. A. A.; ABDEL-SATTAR, M. A. The etiology of sudden wilt disease syndrome on melon in Egypt. *Nature and Science*, v. 11, n. 11, p. 79-87, 2013.
- FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. Faostat. **Statistics Database**. 2020. Retrieved from: <http://www.fao.org/faostat/en/#home>. Accessed on: 11 Jun. 2020.
- FURTADO, G. Q. *et al.* Influência do estágio fenológico e da idade dos trifólios de soja na infecção de *Phakopsora pachyrhizi*. *Tropical Plant Pathology*, v. 34, n. 2, p. 118-122, 2009.
- GOULART, A. C. P. Reaction of cotton cultivars to *Rhizoctonia solani* at seedling stage and benefits of fungicide seed treatment. *Summa Phytopathologica*, v. 42, n. 4, p. 308-312, 2016.
- IBGE. **Sistema IBGE de recuperação automática - SIDRA**. 2020. Retrieved from: <http://www.sidra.ibge.gov.br> Accessed

on: 11 Jun. 2020.

M'HAMDI, N. *et al.* Evaluation of local watermelon and melon rootstocks resistance to six soilborne plant pathogenic fungi in Tunisia. **Tunisian Journal of Plant Protection**, v. 11, p. 191-206, 2016.

MAIA, L. K. R.; LIMA, R. E. M.; LIMA, J. S. Importância do meloeiro e aspectos relacionados à resistência a *Rhizoctonia solani*. **Enciclopédia Biosfera**, v. 9, n. 17, p. 1609-1622, 2013.

MCKINNEY H. H. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. **Journal of Agricultural Research**, v. 26, p. 195-218, 1923.

MEDEIROS, A. C. *et al.* Métodos de inoculação de *Rhizoctonia solani* e *Macrophomina phaseolina* em meloeiro. **Summa Phytopathologica**, v. 41, n. 4, p. 281-286, 2015.

MICHEREFF FILHO, M. *et al.* Influência de tipos de solo do estado de Pernambuco na intensidade da doença induzida por *Rhizoctonia solani* em feijoeiro. **Fitopatologia Brasileira**, v. 21, n. 1, p. 19-25, 1996.

MICHEREFF, S. J.; ANDRADE, D. E. G. T.; SALES JÚNIOR, R. Reaction of melon genotypes to *Rhizoctonia solani*. **Horticultura Brasileira**, v. 26, n. 3, p. 401-404, 2008.

MIRMAJLESSI, S. M. *et al.* Genetic diversity among crown and root rot isolates of *Rhizoctonia solani* isolated from cucurbits using PCR-based techniques. **African Journal of Agricultural Research**, v. 7, n. 4, p. 583-590, 2012.

NECHET, K. L.; HALFELD-VIEIRA, B. A. Efeito do inóculo, período de molhamento foliar e do estágio fenológico do feijão-caupi no desenvolvimento da mela. **Tropical Plant Pathology**, v. 36, n. 2, p. 104-109, 2011.

NEREY, Y. *et al.* *Rhizoctonia* spp. causing root and hypocotyl rot in *Phaseolus vulgaris* in Cuba. **Journal of Phytopathology**, v. 158, n. 4, p. 236-243, 2010.

NORONHA, M. A.; MICHEREFF S. J.; MARIANO R. L. R. Efeito do tratamento de sementes de caupi com *Bacillus subtilis* no controle de *Rhizoctonia solani*. **Fitopatologia Brasileira**, v. 20, n. 2, p. 174-178, 1995.

OLIVEIRA, F. I. C. *et al.* A cultura do melão. In: FIGUEIRÊDO, M. C. B.; GONDIM, R. S.; ARAGÃO, F. A. S. **Produção de**

**melão e mudanças climáticas**: sistemas conservacionistas de cultivo para a redução das pegadas de carbono e hídrica. Brasília: Embrapa, 2017. cap. 1, p. 17-31.

OUMOULOU, A. *et al.* Breeding melon for resistance to *Fusarium* wilt: recent developments. **Euphytica**, v. 192, n. 2, p. 155-169, 2013.

ROCHA, R. H. C. *et al.* Caracterização morfoanômica do melão Gália no ponto de colheita. **Revista Brasileira de Fruticultura**, v. 32, n. 2, p. 375-385, 2010.

SALARI, M. *et al.* Reaction of melon cultivars to soilborne plant pathogenic fungi in Iran. **African Journal of Biotechnology**, v. 11, n. 87, p. 5324-15329, 2012a.

SALARI, M. *et al.* Screening of *Cucumis melo* L. cultivars from Iran for resistance against soilborne fungal pathogens. **Journal of Plant Pathology & Microbiology**, v. 2, 2012b.

SALES JÚNIOR, R. *et al.* Caracterização morfológica de fontes de resistência de meloeiro a *Rhizoctonia solani*. **Horticultura Brasileira**, v. 33, p. 196-202, 2015.

SANTOS, F. S. *et al.* Ajuste do inóculo de *Rhizoctonia solani* em substrato para estudos de Rizoctoniose em algodoeiro e feijoeiro. **Summa Phytopathologica**, v. 31, n. 4, p. 373-375, 2005.

SCANDIANI, M. M. *et al.* Comparison of inoculation methods for characterizing relative aggressiveness of two soybean sudden-death syndrome pathogens, *Fusarium virguliforme* and *F. tucumaniae*. **Tropical Plant Pathology**, v. 36, n. 3, p. 133-140, 2011.

SILVA, R. N. O. *et al.* Métodos de inoculação de *Rhizoctonia bataticola* (Taub.) (*Macrophomina phaseolina* (Tassi) Goid) em sementes de feijão. **Revista Verde de Agroecologia e Desenvolvimento Sustentável**, v. 11, p. 7-11, 2016.

SOUZA, M. V. *et al.* Métodos de inoculação e efeitos de *Fusarium oxysporum* f. sp. *vasinfectum* em sementes de algodoeiro. **Tropical Plant Pathology**, v. 33, n. 1, p. 41-48, 2008.

STANGARLIN, J. R. *et al.* A defesa vegetal contra fitopatógenos. **Scientia Agraria Paranaensis**, v. 10, n. 1, p. 18-46, 2011.

YANG, G.; LI, C. General description of *Rhizoctonia* species complex. In: CUMAGUN, C. J. R. **Plant Pathology**. Rijeka: Intech, 2012. p. 41-52.



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