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Resistance to Fusarium Wilt in watermelon accessions inoculated by chlamydospores

Antonio Elton Silva Costa[a], Fábio Sanchez da Cunha[a], Alan da Cunha Honorato[a], Alexandre Sandri Capucho[a], Rita de Cássia Souza Dias[b], Jerônimo Constantino Borel[a], Francine Hiromi Ishikawa[a,*]

[a] Colegiado de Engenharia Agronômica, Campus de Ciências Agrárias, Universidade Federal do Vale do São Francisco, CEP 56300-990, Petrolina, PE, Brazil
[b] Embrapa Semiárido, CP23, CEP 56300-000 Petrolina, PE, Brazil

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ABSTRACT

The present study aimed to evaluate different inoculation methods of *Fusarium oxysporum* f. sp. *niveum* in watermelon and the reaction of accessions from this crop. Firstly, seven inoculation methods using conidia were tested on the susceptible cultivar Sugar Baby, including the standard dipping method. The methods initially tested were not efficient; therefore, we tested a new methodology using chlamydospores which are fungus survival structures. After the production of chlamydospores in vermiculite enriched with liquid culture media potato and sucrose (PS), the new method was tested on an experiment comparing inoculation by conidia and chlamydospores. For the conidia method, the plants were inoculated after their final leaf formed. For chlamydospores, inoculations were done in plants or sowed seeds. The use of chlamydospores in sowed seeds was effective for the inoculation of *Fusarium oxysporum* f. sp. *niveum* in watermelon and showed the highest severity scores in relation to the others methods. The inoculation method using chlamydospores also obtained the shortest means of root and shoot length in the cultivars Charleston Gray and Sugar Baby. Thus, using this methodology, 25 accessions from the watermelon germplasm were evaluated 21 days after inoculation with a grading scale. Eight accessions were classified as resistant, corresponding to 32% of accessions evaluated. Eight other accessions received the highest score of severity, proving the efficiency of the methology to evaluate the reaction to the disease.

1. Introduction

Watermelon cultivation is an activity practiced in different countries worldwide and it is a main vegetable grown in Brazil (FAO, 2015). This vegetable crop has relevant economic importance. Due to its short cycle, watermelon has attracted producers looking for quick financial returns. However, the main challenge in fruit cultivation is the occurrence of several diseases throughout the crop cycle (Romay et al., 2014).

Among the diseases that occur in watermelon is Fusarium Wilt, which is caused by the fungus *Fusarium oxysporum* f. sp. *niveum*. The pathogen produces two types of conidia, the macro and microconidia, and in the absence of a host, it can survive saprophytically for up to six years. The pathogen's survival is even more elongated and can be extended for more than 10 years when the production of survival structures, such as chlamydospores, occurs (Zhang et al., 2015). This is a common disease in several countries including the United States and China and it causes significant damage to watermelon production (Lü et al., 2011). The management of Fusarium Wilt has been a challenge to watermelon producers because it is caused by a soil pathogen. Because of this, producers have opted for planting in areas without disease occurrence, which is a less sustainable practice. The most common control method is chemical control, but there is a shortage of recommended products. Another commonly adopted measure is crop rotation, however, it becomes inapplicable when contamination occurs in the field due to the easy dissemination and survival characteristics of the pathogen. This makes genetic control, with the use of resistant cultivars, the most viable and efficient alternative for the management of Fusarium Wilt (Everts and Himmelstein, 2015).

In Brazil, the occurrence of Fusarium Wilt has been observed in fields of watermelon (Silva et al., 2016). However, there have been only a few studies in Brazil to evaluate the Fusarium Wilt resistance in cucurbits. The fungus inoculation is difficult and this is one of the reasons it is studied so rarely. All the methods used in several studies have used

* Corresponding author.
E-mail address: francine.hiromi@univasf.edu.br (F.H. Ishikawa).

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conidia, fungal reproduction structures, for inoculation.

The most used inoculation method on different *F. oxysporum* formae speciales has been dipping or the tray-dip method (Latin and Snell, 1986), which consists of the immersion of the roots in a suspension of conidia. Azevedo et al. (2015), using the dipping method of inoculation of *F. oxysporum* f. sp. *phaseoli* in bean genotypes, obtained variability for the reaction of the genotypes to disease. In addition, other studies prove that the dipping method has efficiency, such as on lettuce (Cabral et al., 2014) and tomatoes (Barboza et al., 2013).

An alternative to the standard method is inoculation using chlamydospores. The occurrence of disease in watermelon when planting in areas with disease registration could be related to the existence of these structures in the soil (Silva et al., 2016). According to Sanogo and Zhang (2016), the chlamydospores would be the primary inoculum under these conditions. However, there are no reports of the use of chlamydospores for evaluation of resistance in watermelon. Thus, the aim of this study was to evaluate different inoculation methods of *F. oxysporum* f. sp. *niveum* on watermelon using conidia and chlamydospores to identify the most efficient method to evaluate the reaction of Fusarium Wilt on watermelon accessions.

2. Materials and methods

2.1. Inoculation methodologies of F. oxysporum f. sp. niveum

The experiments were conducted in three steps in the laboratories and the greenhouse at the Federal University of the São Francisco Valley in Petrolina, Pernambuco, Brazil.

The seeds of the commercial cultivar Sugar Baby, which classified as susceptible for all races of *F. oxysporum* f. sp *niveum* (Zhou et al., 2010), were used for inoculation. The seeds were sowed on trays containing substrate for vegetables (compound of pine bark, peat, and expanded vermiculite enriched with macro and micronutrients). The trays containing seedlings were kept under a 50% shaded screen and irrigated daily until inoculation, which occurred 15 days after sowing.

The inoculations were carried out using a monosporic culture from one isolate of *F. oxysporum* f. sp. *niveum* (FON 10). The isolate was incubated in potato agar dextrose (PDA) and maintained in BOD at 25 °C for 10 days. After the growth, the conidial suspension was prepared with the addition of 10 mL of distilled water onto the plate and streaking using a Drigalski spatula. The conidial concentration in the inoculum suspension was adjusted using hemocytometer of Neubauer camera type to 1×10^6 conidia ml^{-1}.

The plants were inoculated when they presented three definitive leaves using seven methods (Table 1) and transplanted into 200 mL plastic cups containing commercial coconut powder substrate.

The evaluation occurred 45 days after inoculation (DAI) using the grading scale for hypocotyl lesions according to Dias et al. (2002). At the end of the experiment, the following development variables were evaluated: biomass weight, fresh root mass, fresh shoot mass, root dry mass and dry shoot mass.

2.2. Inoculation using chlamydospores and spores by the modified immersion method

The inoculum was obtained from the isolate FON10. First, the fungus was plated in Petri dishes containing PDA media. The plates were maintained under incubation in BOD at 25 °C for seven days. The liquid media PS (potato and sucrose) was used to produce the conidia (Dhingra et al., 2006). The PS media was prepared in Erlenmeyer 250 mL autoclaves for 15 min. After cooling, three mycelial discs about three millimeters in diameter were added into the PS media. Posteriorly, the Erlenmeyer was maintained with continuous rotation at 130 rpm at 25 °C for four days for the growth and sporulation of the fungus.

For the method using the chlamydospores, survival structures were obtained from an adaptation of the method proposed by Dhingra et al. (2006). In the present study, the chlamydospores were used with the objective of plant inoculation. The volume used to enrich the substrate and the incubation and drying times proposed by the authors were modified as follows: The substrate used for infestation was vermiculite enriched with PS media with the addition of two mL PS media for each gram of dry vermiculite. In total, 32 Liters of vermiculite were prepared and separated into 16 plastic bags with two liters of vermiculite in each bag. The bags were closed, homogenized and autoclaved for one hour on the first day and 30 min on the second day. The bags were refrigerated until a substrate infestation occurred four days after autoclaving.

The substrate was infested with an addition of 10 mL of conidial suspension per bag of vermiculite in an aseptic environment. The bags were closed for incubation and kept on benches at room temperature for 21 days. They were homogenized for better chlamydospore production. After this period, the bags were kept closed at temperatures between 26 and 30 °C for 19 days with paper towels to dry.

The inoculation tests were performed when the first definitive leaf developed for the two inoculation methods, in addition to the inoculation that occurred with the chlamydospore infested substrate method. The experiment was conducted with a completely randomized design with five replicates for each treatment and three inoculation methods: seedling with modified dipping method (TE1); seedling on substrate infested with chlamydospores (TE2); sowed seed on substrate infested with chlamydospores (TE3), further treatments on seedling transplant without inoculation (TE4) and sowed seed on non-infested substrate (TE5). The commercial cultivars Sugar Baby and Charleston Gray, plus the accession BGH 398 previously classified as resistant, were used in the inoculations tests.

The inoculated plants using conidia (TE1) were made according to modifications to the method proposed by Meru and McGregor (2016). The first modification was on the period of agitation for fungus growth in liquid media which was modified to four days. The second modification was the pot used for transplanting; in this study, disposable plastic cups of 200 mL containing expanded vermiculite were used. Cups with holes in their bases were kept inside the plastic tray containing a suspension of conidia for 30 min for substrate infestation by the fungus. The chlamydospore seedlings were transplanted onto substrate infested with chlamydospores (TE2) or seeds were sowed directly

Table 1
Inoculation methods tested for *F. oxysporum* f. sp *niveum* in watermelon.

Inoculation Methods	Description of Methods
Dipping with cut roots in conidial suspension	Wash the root system in water and cut 1/3 of the length with a subsequent dip in conidial suspension for 30 min
Dipping non-cut roots in the conidial suspension	Wash the root system in water, dip in conidial suspension for 30 min
Drop deposition of conidial suspension	Drop deposition of conidial suspension on plant stem followed by perforation
Injection of 0.1 mL conidial suspension	Inject conidial suspension by syringe in the stem of the plant with a volume of 0.1 mL
Digging and deposition of 10 mL of conidial suspension	Dig the substrate in the region around the hypocotyl with a pocketknife and deposit 10 mL of conidial suspension
Digging and deposition of 30 mL of conidial suspension	Dig the substrate in the region around the hypocotyl with a pocketknife and deposit of 30 mL of conidial suspension
Spray conidial suspension	Spray the conidial suspension on leaves until there is runoff