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Water deficit increases the susceptibility of yellow passion fruit seedlings to Fusarium wilt in controlled conditions

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ABSTRACT

Fusarium wilt is considered the main fungal disease of yellow passion fruit plants in Brazil. There is ample anecdotal evidence of greater intensity of Fusarium wilt after water shortage in field conditions, but this association needs scientific confirmation. There is also a need to increase the efficiency of inoculation with *Fop* (*Fusarium oxysporum* f. sp. *passiflorae*) under controlled conditions for research purposes. Therefore, in this study we evaluated the effect of propagation method (from cuttings or seedlings) associated with controlled water deficit on the incidence of Fusarium wilt in yellow passion fruit plants. The artificial inoculation with *Fop* involved application of a spore suspension of 10^6 conidia mL^{-1} and infestation of the potting media with *Fop* grown in sand and cornmeal substrate. For anatomical analysis, root segments were used from inoculated and non-inoculated plants (control). Seedlings that were submitted to water deficit presented the highest incidence of Fusarium wilt, 75.0%, while in the irrigated control the incidence was below 40.0%. The mortality associated with *Fop* in cutting-propagated plants did not differ from non-inoculated plants. The plants subjected to water stress had greater presence of hyphae and chlamydospores and reduced starch concentration in the root cortex region. Propagation by seeds associated with controlled water stress can be used to screen accessions of *P. edulis* for resistance to Fusarium wilt.

1. Introduction

The yellow passion fruit vine (*Passiflora edulis* Sims) is susceptible to various diseases that affect the aerial part and root system. Among these, Fusarium wilt, caused by the fungus *Fusarium oxysporum* f. sp. *passiflorae* (*Fop*), stands out as one of the main diseases (Ortiz and Hoyos-Carvajal, 2016), and can cause production losses greater than 80% (Fischer and Rezende, 2008; Freitas et al., 2016). In the absence of *Fop*, the plants can remain productive for at least three years, but the presence of this fungus usually reduces the productive lifespan to at most one year.

The vascular system of infected plants presents a rusty coloration in the roots, stems and branches, due to the oxidation of phenolic compounds, necrosis of the root and collapse of the xylem. These alterations are caused by the physical structures of the pathogen, such as hyphae and spores, besides fungal toxins either the defense structures produced by the plant (Stangarlin and Leite, 2008; Fischer et al., 2010; Ortiz et al., 2014).

Control of this disease is very complex, because application of chemical pesticides alone does not result in significant suppression of the disease, and the pathogen can remain in the soil for several years in the form of chlamydospores, preventing planting in previously infected areas (Fischer and Rezende, 2008). The use of resistant varieties is considered the best strategy to minimize the damages caused by the disease, although truly resistant cultivars are not yet available (Freitas et al., 2016).

The response of host plants to pathogens often depends on the developmental stage of the host when challenged by the pathogen (Whalen, 2005; Del Ponte et al., 2007). Fusarium wilt is favored by high air temperatures during the seedling stage, even though plants become more susceptible at flowering in field conditions (Ahmad et al., 2010). In tomato, the presence of root exudates stimulates the microconidia germination of *F. oxysporum*, and the specific stimulation level depends on physiological changes during the plant development (Steinkellner

Abbreviations: Fop, *Fusarium oxysporum* f. sp. *passiflorae*; DI, disease index; DAI, days after inoculation

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et al., 2005). Another group also reported that seedlings exhibited greater susceptibility to disease compared to cuttings among the diff ;erent fungi evaluated including the *Fusarium solani* (Ahmad et al., 2013).

In the yellow passion fruit, the highest percentage of dead plants by Fusarium wilt occurs in the reproductive phase of the crop after drought events followed by a period with high rainfall index associated with high temperatures, as these last two conditions favor the development of the fungus (Cavichioli et al., 2011). The water stress affects the susceptibility of various plant species to the pathogen (Choi et al., 2013; Ramegowda and Enthil-Kumar, 2015; Nejat and Mantri, 2017; Daranas et al., 2018), which can be associated with damage to the roots (Berta et al., 2005), alteration of the soil microbiota (Rolli et al., 2015), physiological changes (Ghaemi et al., 2010), quantity of available proteins (Ramegowda and Enthil-Kumar, 2015; Pandey et al., 2017), and alteration in the defense mechanisms (Pandey et al., 2017). In this way, the effects of biotic or abiotic stresses cannot be overlooked, since they both contribute to the manifestation of the symptoms of the pathogen, exerting a direct influence on the infection according to the susceptibility or resistance of the host plants (Ramegowda and Senthil-Kumar, 2015).

The identification and the selection of more resistant genotypes are usually carried out in field conditions in areas infested by the pathogen. However, the long period required for evaluation and the high cost of cultivation make the selection process burdensome, and the influence of other diseases and environmental factors can hamper the diagnosis.

Some studies have been conducted on passion fruit to establish efficient inoculation protocols to identify resistant individuals under controlled conditions (Fischer et al., 2010; Flores et al., 2012; Ortiz and Hoyos-Carvajal, 2016). The results obtained by these researchers are divergent, indicating the need for adjustments of the method for a more accurate identification of sources of resistance within *Passiflora* germplasm in a short time interval. Furthermore, this will enable assessment of various passion fruit genotypes simultaneously, facilitating the development of *Fop* resistant cultivars.

In addition, an adequate inoculation protocol will support studies to better understand the genetic mechanisms of the plant-pathogen interaction, and histochemical and histopathological analyses for in loco evaluation of distinct patterns of infection between resistant and susceptible species, will allow the identification of chemical markers associated with resistance (Flores et al., 2012; Marques et al., 2013; Ortiz et al., 2014). Based on these considerations, the objective of this work was to evaluate an inoculation protocol of *Fop* using two methods of propagation (cuttings obtained in adult plants during the reproductive stage and seeds harvested in young plants) associated with controlled water deficit on the expression of *Fop* symptoms, as well as provide information about the anatomical alterations of the root of yellow passion fruit after the infection by *Fop*.

2. Materials and methods

2.1. Location of the experiment

The study was conducted in a greenhouse at Embrapa Cassava and Fruits, located in the municipality of Cruz das Almas, Bahia state, Brazil (12°39′25″S, 39°07′27″W, 222m). ′25″ S, 39°07′27′ the air temperature inside the greenhouse was maintained at 28 ± 2 °C and the relative humidity was 60%. The genotype evaluated was the cultivar 'BRS Gigante Amarelo' (*P. edulis*), which is considered susceptible to diseases caused by *Fusarium* species.

2.2. Plant material and growing conditions

Two propagation methods of the passion fruit plants were evaluated, seedlings and cuttings, regarding the manifestation of symptoms of Fusarium wilt. BRS Gigante Amarelo seeds were obtained from a commercial nursery and the cuttings of the same variety were collected from two mother plants (10 months old) that had been kept in greenhouse. These source plants were near the start of flowering and were vigorous, well-nourished and free of pests and symptoms of diseases. The cuttings, presenting two buds without leaves with length of 17 ± 2 cm, were taken from mature and lignified branches with length of approximately 1.5 m. A segment of about 20 cm was removed from the apical region, and the resulting cuttings were placed for rooting in expanded vermiculite.

2.3. Formation of plantlets and inoculation with Fop

The yellow passion fruit plantlets (BRS Gigante Amarelo) were obtained from seeds planted or cuttings rooted in small tubes with volume of 75 mL, containing vermiculite with medium granulometry. Thirty days after emergence of the seedlings (when they had six leaves) or rooting of the cuttings (with six leaves), the roots of all plants were injured with a scalpel to facilitate the penetration of the pathogen at the moment of inoculation (Supplementary Material, Fig. A).

The monosporic isolate used for inoculation was *Fop* 05 (*Fusarium oxysporum* f. sp. *passiflorae* - *Fop*), obtained from the collection of the Phytopathology Laboratory of Embrapa Cassava and Fruits. The inoculation was made by the immersion of the roots for 10 min in a suspension adjusted to 10^6 conidia mL^{-1}. The plantlets were then transplanted to polyethylene pots containing 1.2 L of washed and sterilized sand, previously infested with 50 g of substrate containing the same isolate (*Fop* 05) in the concentration of 10^6 CFU g^{-1}. The substrate for inoculum production was prepared by mixing washed fine sand + cornmeal + water in a 9:1:2 proportion (m:m:m). Two hundred grams of this mixture was distributed in plastic bags with 1 kg capacity and autoclaved twice for 1.5 h. Then 20 disks (diameter of 5 mm) of potato dextrose medium (PDA) containing the *Fop* 05 isolate were added, and were cultured as proposed by Flores et al. (2012). The plastic bags were agitated every three days to obtain homogeneous colonization of the substrate by the fungi.

2.4. Water deficit after inoculation with Fop

After the inoculation with *Fop*, the soil in all treatments was kept at field capacity for 15 days. This condition was maintained for the control treatments, while the water deficit was applied to the other treatments, with and without *Fop*. The moisture of the substrate was monitored on alternating days by the time domain reflectometry technique (TDR), utilizing probes with length of 10 cm (Coelho et al., 2006).

Irrigation was suspended until the manifestation of partial wilting of the leaves, which occurred at relative moisture of around 0.12 ± 2 $m^3 m^{-3}$, and was resumed until full recovery of leaves turgidity, with moisture of 0.25 ± 2 $m^3 m^{-3}$, followed by another cycle of controlled water deficit until reaching the same levels mentioned. This process was repeated during the experiment period until the plants presented visual symptoms of the Fusarium wilt, which was set as the permanent wilting even after rehydration. In contrast, the plants in the control group (absence of *Fop*) submitted to water deficit regained turgidity of the leaves (Supplementary Material, Fig. B).

2.5. Experimental design and phytopathological variables studied

The experimental design was completely randomized in a 2 × 2 × 2 triple factorial scheme (propagation method × water regime × pathogen inoculation), comprising eight treatments with four replications and 10 plants in each plot. For the anatomical analyses, at least four plants were collected per treatment.

2.6. Evaluation of incidence and severity of Fusarium wilt

To assess the Fusarium wilt in the stems and roots, visual