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Reactions of black bean seedlings and adult plants to infection by *Fusarium oxysporum* f. sp. *phaseoli*

Marlon C. de Borba, Felipe R. Garcés-Fiallos, Marciel J. Stadnik*

Laboratory of Phytopathology, Agricultural Science Center, Federal University of Santa Catarina, Rod. Admar Gonzaga 1346, 88034-001 Florianópolis, SC, Brazil

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ABSTRACT

This study aimed to evaluate the resistance of 17 black bean genotypes artificially and naturally infected with *Fusarium oxysporum* f. sp. *phaseoli* under greenhouse and outdoor growing conditions, respectively, and compare the disease progress and bean yield components of the resistant line UFSC-01 and susceptible cv. Uirapuru. Five lines and one landrace were classified as resistant. Resistance classification of bean genotypes following root-dip inoculation in the greenhouse was tightly associated with the reactions in infected adult bean plants assessed on the 90th day. Disease severity rate in seedlings was positively correlated (0.93, $P \leq 0.01$) with Fusarium wilt under outdoor conditions. Fungus more efficiently colonized the susceptible aerial tissues of cv. Uirapuru, resulting in earlier and stronger disease symptoms. *Fop* reduced the bean yield by decreasing the number of pods per plant and weight of seeds even in the resistant genotype UFSC-01 but more dramatically by decreasing the number of seeds per pod in the susceptible cultivar.

1. Introduction

The common bean (*Phaseolus vulgaris* L.) plays an important social and economic role in Brazil, serving as the main source of dietary protein for millions of people. Brazil is one of the largest producers and consumers of dry beans in the world, with a consumption of approximately 17 kg per person per year (FAO, 2016). Although people's preferences for color and culinary traits of beans greatly vary among regions, black beans represent approximately 20% of the total Brazilian production. Furthermore, in the Southern states of Santa Catarina and Rio Grande do Sul, consumers prefer locally produced black beans (Costa et al., 2010). This scenario offers a big challenge for breeding programs searching for cultivars with high yields and other desirable agronomic traits without losing focus on local market demands.

Fusarium wilt (Fw), caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *phaseoli* JB Kendrick and WC Snyder (*Fop*), is one of the most important diseases of common bean, being present in all crop production areas worldwide (Xue et al., 2015; Niño-Sánchez et al., 2015). Fw symptoms include foliar chlorosis, premature defoliation of lower leaves, red-brown necrosis of vascular tissue, wilting, and plant death (Abawi and Pastor-Corrales, 1990; Buruchara and Camacho, 2000; Xue et al., 2015). Although the intensity of infection markedly varies among individual fields, in southern Brazil, Fw is considered one of the most important constraints for bean cultivation associated with intensive monoculture without crop rotation practices (Wordell Filho et al., 2013). Yield components of bean plants can be reduced by pathogenic soil-borne fungi such as *Fusarium oxysporum*, but data usually originated from field plants with mixed infections (Naseri, 2008), not allowing to exactly determine the effect of each one.

Fop infects bean plants through wounds, natural openings, or intact roots, preferentially at the junctions of the lateral roots and the taproot (Niño-Sánchez et al., 2015). During the infection, hyphae grow intracellularly in the root cortex until they reach the xylem vessels (Jiménez-Fernández et al., 2013; Niño-Sánchez et al., 2015). The development of disease symptoms and degree of colonization in xylem vessels differ greatly among genotypes (Pereira et al., 2013; Buruchara and Camacho, 2000). In fact, resistance to Fw has been associated with a slower fungal colonization (Pereira et al., 2013; Xue et al., 2015). Therefore, checking how far upward the pathogen is in the vessels from the roots might be useful to confirm resistance of inoculated bean plants.

Genetic resistance is a convenient and effective environmentally

* Corresponding author.
E-mail address: marciel.stadnik@ufsc.br (M.J. Stadnik).

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friendly management strategy to control soil-borne diseases because it requires no inputs during the production cycle and engenders no adverse environmental impacts (Gordon et al., 2015). However, the development and release of new bean cultivars with a broad spectrum of resistance to Fw has been difficult because of the high pathogen variability (Salgado and Schwartz, 1993). Although *Fop* race 2 is thought to be predominant in Brazil, an increasing number of highly virulent pathotypes have been found recently (Henrique et al., 2015). In this scenario, suitable sources of new plant resistance genes are scarce and not available against the majority of the pathogens (Xue et al., 2015). In southern Brazil, the cv. Uirapuru has been recommended and highly used by farmers because of its broad adaptation, high yield potential, and resistance to some foliar diseases such as common mosaic virus, rust, and powdery mildew (Moda-Cirino et al., 2001), but field observations have suggested that it is susceptible to Fw.

Bean genotypes resistant to Fw have been successfully selected through different methods under greenhouse conditions (van Schoonhoven and Pastor-Corrales, 1987; Buruchara and Camacho, 2000; Cavalcanti et al., 2002; Brick et al., 2006), but root-dipping inoculation has allowed to discriminate resistance levels more consistently than drenching seedlings with conidial suspension (Cavalcanti et al., 2002). Thus, the former method has been preferably applied in bean breeding programs to eliminate susceptible plants before field testing. Despite this, there is a lack of validation studies comparing the reactions of seedlings with those of adult plants, possibly because of methodological difficulties such as heterogeneous inoculum distribution in soil and environmental factors affecting disease development under natural conditions.

Therefore, the present work aimed to evaluate the resistance of black bean genotypes to *Fop* under greenhouse and outdoor pot conditions and compare the reactions in both environments. Furthermore, we also examined the disease progress and bean yield components of resistant (UFSC-01) and susceptible (cv. Uirapuru) genotypes under natural infection conditions.

2. Material and methods

2.1. Biological material

Seventeen black common bean genotypes (*P. vulgaris*) were obtained from the Plant Genetic Resources Program (RGV-UFSC) of the Federal University of Santa Catarina. They included the lines AL 9021332, CF 22, CF 128, CHP 97-04, CI 96712V, CP 9310635, FT 84113, FT 991159, LP 97-04, MD 841, TB 9401, UFSC-01, and UFSC-02; the cv. Uirapuru (IPR88 Uirapuru; Moda-Cirino et al., 2001); and the landraces Becker Bela Vista, Negro Bola, and Sogro Daniel.

The highly aggressive monosporic strain (MANE 174) of *Fop* was used in this study.

2.2. Inoculum obtaining

For greenhouse assays, inoculum was obtained by incubating *Fop*-infected bean stems on two layers of moistened paper towels in plastic boxes at 25 °C and 12-h photoperiod. After 15 days, the stems were transferred to tubes, flooded with 10 mL of distilled water, and strongly vortexed for 1 min, and the conidial suspension was collected and filtered twice to remove mycelial fragments. Conidial concentration was determined using a Neubauer's counting chamber and adjusted to 1×10^6 macroconidia mL^{-1} with distilled water.

In outdoor pot experiments, plants were naturally inoculated by growing them in a substrate infested with *Fop* at 1.3×10^3 colony forming units (CFU) g^{-1} of soil.

2.3. Plant growing conditions and inoculation

For greenhouse assays, bean plants were cultivated during spring (from August to September and from October to November) 2014. Bean seeds were sown in 128-cell plant trays (each cell of 45 cm^3) containing vermiculite as substrate. One-week-old bean seedlings were removed from the vermiculite at the first leaf stage, and their roots were carefully washed under running tap water. Root tips were cut off at ¼ of their length and immediately inoculated by dipping in the conidial suspension for 20 min. Seedlings dipped in distilled water served as control. After inoculation, the seedlings were transplanted to 0.2-L pots containing a mixture of vermiculite and organic compost (1:1; v/v) and kept under greenhouse conditions (22 ± 3 °C and 12-h photoperiod). Plants were irrigated according to their water requirements and fertilized at the first trifoliate leaf growth stage with 25 mL of a nutritive solution containing N, P_2O_5, Fe, Mn, and Zn at 10.8, 8.8, 0.1, 7.0, and 2.5 mg L^{-1}, respectively.

For outdoor growing experiments, bean plants were cultivated during spring (from August to December) 2012 and 2013 (27°34.958′S; 48°30.313′W) in 20-L plastic pots containing a substrate composed of *Fop*-infested clay soil and organic compost (3:1; v/v). Twelve days after sowing (DAS), *i.e.*, at the first leaf growth stage, seedlings were thinned out to four per pot. Fertilizers were added twice, *i.e.,* at the first trifoliate leaf and preflowering growth stage, with 100 mL of the nutrient solution previously described. Insects were controlled by spraying Tiametoxan 250 g kg^{-1} (Actara 250 WG®, Syngenta, Brazil) at 35 (third trifoliate leaf growth stage) and 50 (pod filling growth stage) DAS. Plants were irrigated according to their water requirements.

2.4. Disease assessment

For disease assessments in greenhouse, disease symptoms were assessed on the 30th day after inoculation (DAI). Disease severity rating (DSR) of individual plants was scored according to a scale varying from 1.0 (no visible symptoms) to 9.0 (approximately 75% or more of the leaves and branches exhibiting wilt, chlorosis, and defoliation, eventually with plant death) (van Schoonhoven and Pastor-Corrales, 1987). Plants that had an average DSR of 1.0 to 3.0 were considered resistant, 3.1 to 6.0 susceptible, and 6.1 to 9.0 highly susceptible. Moreover, the number of wilting and dead plants was counted. Plant height was determined using a ruler by measuring the distance from the surface of the soil until the highest growth point. Finally, stems were longitudinally cut, and the vascular necrosis in the hypocotyl region was percentually estimated.

In the outdoor experiments, the incidence of wilt symptoms was assessed at intervals of 4 days, from their first appearance at pod filling until grain filling growth stage (nine evaluations in total). Incidence of wilt symptoms and number of dead plants were recorded for each pot. The area under the disease progress curve (AUDPC) was calculated from the data of wilt incidence using the formula $AUDPC = \sum[((y1 + y2)/2)*(t2-t1)]$, where y1 and y2 are two consecutive assessments of wilt incidence at time points t1 and t2, respectively (Campbell and Madden, 1990).

2.5. Determination of colony forming units

Two bean genotypes exhibiting the most contrasting reactions to *Fop* (UFSC-01 and cv. Uirapuru) were chosen from the greenhouse and outdoor experiments and used in follow-up tests to compare the fungal colonization. Plants grown in greenhouse had their roots, hypocotyls, and epicotyls taken at the third trifoliate leaf growth stage (30 DAI), whereas for those grown outdoor, only