CROP BREEDING AND APPLIED BIOTECHNOLOGY

NOTES

Assessment of resistance in common bean to *Fusarium oxysporum* f. sp. *phaseoli* using different inoculation and evaluation methods

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Abstract: *This study aimed to evaluate the resistance of three common bean genotypes (BRS Estilo, A211 and Mortiño) to the Fusarium oxysporum f. sp. phaseoli using different inoculation methods such as the root-dip, the colonized toothpick, and the agar-based disk-diffusion. After 35 days of inoculation, the disease severity was assessed by vascular discoloration on the stem of the plants by two methods: using a scoring scale and measuring the length of the discoloration with the aid of a millimeter ruler (cm). Results showed that the root-dip was the most effective inoculation method. As for the method of assessing the disease severity, the scoring scale was the best one, in addition to being easier for evaluating large amounts of common bean lines.*

Keywords: *Phaseolus vulgaris, Fusarium wilt, pathogenicity, disease severity.*

INTRODUCTION

Fusarium wilt caused by *Fusarium oxysporum* Schlecht. f. sp. *phaseoli* Kendrick & Snyder (*Fop*) is considered one of the main root diseases of common bean and has spread across all bean-producing areas of Brazil (Pereira et al. 2011). Decreased yield caused by this disease have been increasing, especially in areas with successive and irrigated crops (Toledo-Souza et al. 2012). The *Fop* classification belongs to the division *Ascomycota*, class *Sordariomycetes*, order *Hypocreomycetidae*, family *Nectriaceae*, genus *Fusarium* and species *Fusarium oxysporum*. The *F. oxysporum* species complex (FOSC) has suffered from multiple taxonomic classification systems and is composed of pathotypes classified into various *forma specialis* (*formae speciales*), based on pathogenic criteria (Lombard et al. 2018).

This fungus is ubiquitous in soils worldwide being able to grow saprophytically or colonizing plants and are responsible for the disease in more than 100 plant species (Michielse and Rep 2009, Pantelides et al. 2013). Each group of *forma specialis* of *F. oxysporum* is pathogenic to specific plant group, demonstrating the degree of host specificity. *Fop* infection starts through the roots, colonizing the xylem and causing leaf wilting, vascular discoloration, chlorosis, dwarfing and premature plant death (Niño-Sanchez et al. 2015, Garces-Fiallos et al. 2017). However, beans may be used to characterize the pathogenicity of *Fop*

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isolates, the race as well as the diversity of physiological races. This is important for the characterization of resistant bean genotypes to the fungus (Karimian et al. 2010, Henrique et al. 2015, Cruz et al. 2018, Wamalwa et al. 2018, Petkar et al. 2019, Sasseron et al. 2020).

Many factors such as natural root injuries, nematodes, soil pests and compacted and soaked soil can facilitate *Fop* infection under field conditions. Because of these, the evaluation of the resistant bean lines to the fungus in field is difficult and time consuming. The previous selection of resistant bean to the fungus through artificial inoculation and subsequent validation of only the most resistant in the field is the most appropriate and quick way (Cavalcanti et al. 2002, Batista et al. 2019). Some methods for *Fusarium* inoculation are used to evaluate resistance in controlled environments (Cruciol and Costa 2017). When evaluating *Fusarium* symptoms in plants, some signs, such as vascular discoloration on the stems, must be checked. The extent of this discoloration can be measured and used as a criterion, among others, to compose a grading scoring scale the disease severity in the crop (Borba et al. 2017). For common bean breeding programs, the *Fop* inoculation method must be efficient and easy to perform, causing disease in the plants, and must be followed by an appropriate method for evaluating the disease, detecting the resistance or susceptibility of the plants to the fungus.

The present study aimed at testing different methods for *F. oxysporum* f. sp. *phaseoli* (*Fop*) inoculation in common bean plants with the criteria: a) traditional – root dipping in an inoculum suspension (root-dip method); b) easy and fast – employing the introduction of toothpicks infested with pathogen into the hypocotyl (colonized toothpick method) and c) easy and fast – employing a disk of culture medium with the pathogen colonized into the injured hypocotyl (agar-based disk-diffusion method) To assess the disease severity, vascular discoloration on the stem was quantified by two methods: a) with a scoring scale; b) measuring the lenght of the discoloration with the aid of a millimiter ruler (cm).

MATERIAL AND METHODS

Plant material

Three bean accessions were used, namely A221 and Mortiño, which are classified as *Fop*-differentiating genotype, according to Woo et al. (1996), and a common bean genotype with carioca grain type, BRS Estilo, which is considered susceptible to *Fop* (Batista et al. 2016). The accessions were sown in 128-cell seed trays containing Biomix® substrate for vegetables and kept in a greenhouse for germination and seedling growth until stage V2 (fully expanded primary leaf).

Reaction to *Fop*

The isolate used in this assay was *Fop* UFV01 collected from plants of the common bean with typical *Fusarium* wilt symptoms, in Coimbra (Minas Gerais, Brazil), and concluded that this isolate is a new race of the species (Pereira et al. 2013). After the pathogenicity tests, the monosporic culture was obtained and preserved in distilled and autoclaved water (Castelani 1939). Three methods for fungal isolate inoculation in common bean were tested, a traditional method and two other methods, easy and fast.

The traditional method adopted was the root-dip (Pastor Corrales and Abawi 1987). Seedlings of the genotypes were carefully removed from the trays, their roots were washed with tap water, and 1/3 of their length was cut with sterile scissors. Immediately after cutting, the seedling roots were immersed for 20 minutes in 10 mL of spore suspension of the *Fop* UFV01 isolate (1×10^6 conidia mL^{-1}). The roots of the control plants were immersed in autoclaved distilled water under the same conditions described.

In the second inoculation method - colonized toothpick method (Coelho Neto and Dhingra 1996), the inoculum of the *Fop* UFV01 isolate was prepared in Petri dishes containing a thin layer of PDA medium poured under the filter paper with toothpicks. After the medium solidified, a PDA disk containing the fungus isolate was transferred to the center of the plate and then incubated in a BOD chamber for 14 days at 25°C with 12 hour photoperiod. After, colonized toothpicks were directly inserted into the stems of the plants, more specifically, into hypocotyl collar region. Control plants were inoculated with toothpicks without the fungus in the same region.

In the third inoculation method - the agar-based disk-diffusion method (Fischer et al. 2010), Petri dishes containing PDA medium with the *Fop* UFV01 isolate at the center were kept in a BOD chamber for 14 days at 25°C with 12 hour photoperiod. After, a PDA disk containing the fungus (5 mm diameter) was removed and placed under adhesive tape and, then, inserted into hypocotyl collar region wounded using a sterile scalpel. The control treatment consisted of plants inoculated in the same region only with a PDA disk, which was not colonized with the fungal isolate.

After inoculation, the seedlings were transplanted to plastic pots with dimensions (11 x 8 x 9 cm) with Biomix® substrate and kept in a Phytotron plant growth chamber at a 12-hour photoperiod, photosynthetic photon flux density (PPFD) of 600 µmol $m^{-2}s^{-1}$, and temperature of 25/20 °C (day/night) with control of irrigation (2.0 mm dia^{-1}). After 10 days after inoculation (DAI), each pot received 0.5 g of urea as a nitrogen source.

The experiment was performed in a completely randomized 3 (genotypes) × 3 (inoculation methods) factorial design with 8 replicates inoculated and 8 replicated control. Each replicate consisted of a pot with 2 plants. The average of the data from the two plants was considered for statistical analysis.

Thirty-five days after inoculation, the inoculated plants of each genotype and the control were removed and the vascular discoloration of the roots until insertion of the last leaf was evaluated by two methods: 1) measuring the vascular discoloration in the hypocotyl (VDH) with the aid of a millimeter ruler (cm) and 2) disease severity rating (DSR) using a scoring scale adapted from Pastor Corrales and Abawi (1987), ranging from 1 to 9: 1 = no symptoms; 3 = light vascular discoloration on only one side of the stem and symptoms of chlorosis, wilt and necrosis restricted to the first leaves of the plant; 5 = traces of intermediate vascular discoloration throughout the length of the stem and symptoms of chlorosis, wilt and necrosis in the leaves below the pointer; 7 = dark vascular discoloration throughout the length of the stem and severe symptoms of wilt and necrosis generalized in the aerial part and 9 = dead plant. The genotypes with scores 1.0 to 3.0 were classified as resistant; from 3.1 to 6.0 as intermediate and from 6.1 to 9.0 as susceptible (Pastor Corrales and Abawi 1987, Elena and Papas 2002).

Statistical analyses

After verification of homogeneity of the variances of the data sets, data were subjected to analysis of variance (ANOVA). Box-cox method was used before analysis to meet ANOVA assumptions followed by a Tukey test with 5% significance to compare means between treatments (Cruz 2016).

RESULTS AND DISCUSSION

The parameters VDH and DSR per genotype (G), per assessment method (M), and G x M interaction were significant ($p<0.05$) (Table 1). For the VDH parameter, the genotypes showed different susceptibility in relation to *F. oxysporum* f. sp. *phaseoli* (*Fop* UFV01 isolate) by inoculation method. In method 1, the most susceptible genotype was BRS Estilo. In methods 2 and 3, the susceptible ones were A211 and BRS Estilo. According to the response of the genotypes to the inoculation methods, it was found that method 1 stood out, regardless of the genotypes. This method was the only one that caused the longest vascular discoloration in genotypes (Figure 1). Pereira et al. (2008) tested inoculation methods different of *F. oxysporum* f. sp. *phaseoli* in common beans and found that the root dipping method in a suspension of conidia with cut of the roots was the best method too.

For the DSR parameter, the genotypes resistance in relation to *F. oxysporum* f. sp. *phaseoli* (*Fop* UFV01 isolate) was different by inoculation method. For method 1, the susceptible genotypes were BRS Estilo and A211. For method 2, the susceptible genotype was A211, while for method 3 no susceptible genotype was detected in relation to the fungus isolate (Table 2).

According to the scoring scale of the current study, genotypes with an average above 6.1 are considered susceptible in relation to *Fop*. Focusing on the inoculation method 1, the genotypes BRS Estilo and A211 were considered susceptible and the genotype Mortiño as intermediate to *F. oxysporum* f. sp. *phaseoli* (*Fop* UFV01 isolate). The results validate previous studies in which the BRS Estilo and A 211 genotypes were evaluated as susceptible in relation to *Fop* (Pastor-Corrales and Abawi 1987, Batista et al. 2016). Thus, the most highly effective method for assessing the disease severity is the scoring

Table 1. Analysis of variance of the vascular discoloration of the hypocotyl (VDH) length (cm) and disease severity rating (DSR) measured in common bean genotypes inoculated with *Fusarium oxysporum* f. sp. *phaseoli* (*Fop* UFV01 isolate) using different inoculation methods

Sources of variation	df	Mean Square	
		VHD	DSR
Genotypes (G)	2	7.65**	4.78**
Methods (M)	2	179.35**	65.33**
G x M	4	17.68**	2.78**
Error	16	0.18	0.19
Mean		3.87	4.11
CV (%)		11.10	10.72

** Significant with 5% by the F test.

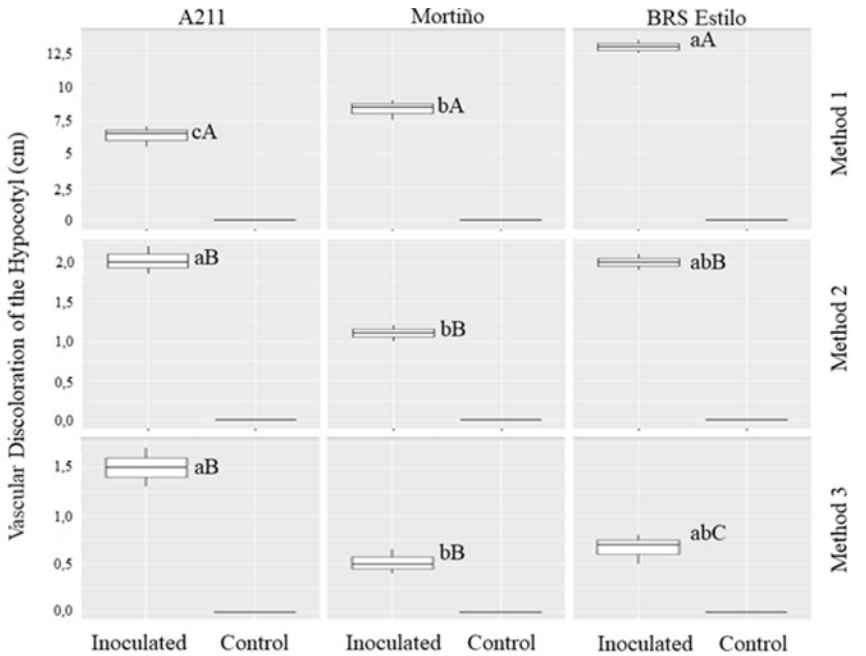

Figure 1. Boxplots of length of the vascular discoloration of the hypocotil (VDH) of common bean genotypes caused by *Fop* (UFV01 isolate) using different inoculation methods: Root-dip (method 1); Colonized toothpick (method 2); and Agar-based disk-diffusion (method 3). Different lower-case letters show significant differences in accessions in relation to each inoculation method and different upper-case letters indicate significant effects of the methods in each accession (genotype), according to the Tukey test with 5% significance.

Table 2. Disease severity rating (DSR) of common bean genotypes caused by *Fusarium oxysporum* f. sp. *phaseoli* (*Fop* UFV01 isolate) inoculated with different methods

Genotype	Method[1]		Method[2]		Method[3]	
	DSR (1-9)	Reaction*	DSR (1-9)	Reaction	DSR (1-9)	Reaction
BRS Estilo	8.00 ± 1.00	Susceptible	3.00 ± 0.00	Resistant	1.00 ± 0.00	Resistant
Mortiño	6.00 ± 0.58	Intermediate	3.00 ± 0.00	Resistant	1.00 ± 0.00	Resistant
A211	6.67 ± 0.58	Susceptible	5.00 ± 0.00	Susceptible	3.00 ± 0.00	Resistant

[1] Root-dip method; [2] Colonized toothpick method; [3] Agar-based disk-diffusion method. Resistant – Score: 1.0 to 3.0; Intermediate – 3.1 to 6.0 and Susceptible – 6.1 to 9.0 (Pastor Corrales and Abawi 1987).

scale, in addition to being easy and rapid for assessing large amounts of common bean inbred lines. Pereira et al. (2008) tested different inoculation methods of *F. oxysporum* f. sp. *phaseoli* in common beans and used a rating scoring scale to assess disease severity like the current study.

In susceptible plants, the fungus mycelium colonizes upward the xylem vessels of the stem causing extensive vascular discoloration and consequently symptoms in the aerial part of the plant (Nino-Sanchez et al. 2015, Borba et al. 2017, Garcés-Fiallos et al. 2017). This fact reinforces the need for a scoring scale to contemplate, in addition to the symptoms of the aerial part as wilt, the vascular discoloration on the stem.

The results obtained in the present study demonstrate that the using the inoculation method of the root dipping in a spore suspension with cut roots in associate with the evaluation of the disease severity with a scoring scale is efficient for screening common bean germplasm in the search for sources of resistance to *F. oxysporum* f. sp. p*haseoli*.

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