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RESEARCH ARTICLE / *ARTIGO*

Resistance of advanced common bean lines to Fusarium root rot

Alessandro Nicoli[1], Laércio Zambolim[1], Trazilbo J. Paula Júnior[2], Rogério F. Vieira[2], Hudson Teixeira[2] & José Eustáquio S. Carneiro[3]

[1]Departamento de Fitopatologia, Universidade Federal de Viçosa, 36570-000, Viçosa, MG, Brazil; [2]Agricultural Research Institute of the State of Minas Gerais (EPAMIG), Vila Gianetti 47, 36570-000, Viçosa, MG, Brazil; [3]Departamento de Fitotecnia, Universidade Federal de Viçosa, 36570-000, Viçosa, MG, Brazil

Author for correspondence: Alessandro Nicoli, e-mail: alessandro.nicoli@ufv.br

ABSTRACT

Fusarium root rot (FRR) is a distributed disease of common beans in Brazil. Our main aim was to verify if there are genotypes, mainly advanced lines from the common bean breeding program with levels of resistance to FRR similar to those of the line A-300. We also compared three developmental stages for FRR assessment. Genotypes of six classes were evaluated in greenhouse and field experiments. In greenhouse, substrate was infested with chlamydospores. In field, genotypes were screened in area infested with *Fusarium solani* f. sp. *phaseoli*. Plants were rated for disease at V3, R5 and R7 stages. Correlation among area under the disease progress curve (AUDPC) in greenhouse and in the field experiments was significant. Genotypes were ranked into four groups based on AUDPC. Sixteen advanced lines were as resistant to FRR as A-300. Correlation between the disease rating at R5 stages and AUDPC was higher than those among AUDPC and either disease rating at V3 or R7 in all experiments. Our results indicate that there are advanced lines with levels of FRR resistance similar to those of A-300 and that the FRR assessment at the R5 stage is more appropriated than at either V3 or R7.
Key words: *Phaseolus vulgaris*, assessment time, developmental stage, screening of genotypes.

INTRODUCTION

Fusarium root rot (FRR), caused by the fungi *Fusarium solani* f. sp. *phaseoli* (*Fsp*) W.C. Snyder & H.N. Hansen, is one of the most widely distributed diseases of common beans (*Phaseolus vulgaris* L.) in Latin America. In Brazil, the disease causes reduction of stand and plant growth and yield losses in the producing areas, especially in the irrigated fields of the cerrado region (Paula Júnior et al., 2006; Fernandes et al., 2010) and where Andean germoplasm are used (Beebe et al., 2001; Clare et al., 2010). The symptoms are initially characterized by longitudinal, narrow, bright-red streaks on hypocotyls and taproot surfaces; infected areas become reddish brown, lack definite margins, remain superficial, and may exhibit longitudinal fissures. The dark, thick-walled chlamydospores are the long-term survival structures in soil (Abawi & Pastor-Corrales, 1990).

Cultural practices that promote vigorous plant growth, biological control, and seed treatments are recommended to reduce the damage caused by FRR (Abawi & Pastor-Corrales, 1990; Paula Júnior et al., 2006). As these management practices alone may not be enough to keep the disease in low levels, the use by farmers of cultivars adapted to the region with high levels to FRR resistance might improve the management strategy to control the disease.

There is no source of complete resistance to FRR, but partial resistance in the common bean germplasm has been reported (Boomstra et al., 1977; Beebe et al., 1981; Silbernagel 1987; Abawi & Pastor-Corrales, 1990; Nascimento et al., 1999; Schneider & Kelly, 2000; Bilgi et al., 2008). The line A-300 is reported as a source of resistance to *Fsp* (Tu & Park, 1993; Schneider & Kelly, 2000). We believe that partial resistance to FRR is available in advanced common bean lines of the breeding program of Minas Gerais State because, in general, yield experiments are conducted with lines of Mesoamerican gene pool in areas with different levels of infestation by *Fsp*. FRR resistance has been associated with small seed size, especially the black market class (Clare et al., 2010).

The critical period for infection of *Fsp* is between V0 (germination) and V3 (first trifoliate leaf) (Fernandes et al., 2010). In general the disease severity is assessed in greenhouse between 10 and 31 d after planting (DAP) (Schneider & Kelly, 2000; Chaudhary et al., 2006; Miranda et al., 2007; Bilgi et al., 2008) and between flowering and maturity in field (Schneider & Kelly, 2000; Román-Avilés & Kelly, 2005; Bilgi et al., 2008; Clare et al., 2010). That means that in greenhouse the common bean is assessed during the vegetative phase whereas in field the evaluation is made during the reproductive phase. There is a lack of information regarding the relationship

of disease severity in greenhouse and in field experiments when disease is assessed at the same developmental stage.

Our objective was to verify if there are genotypes of common bean, especially advanced breeding lines from the breeding program of Minas Gerais State, Brazil, with levels of resistance to FRR similar to those reported for A-300. We also compared three developmental stages for FRR assessment in both greenhouse and field experiments.

MATERIAL AND METHODS

Genotypes

In total 96 genotypes were evaluated for resistance to FRR. Sixty-five advanced breeding lines (beginning with BJ, BP, CNF, MA, MN, RP, VC, Vi, VP and VR) from the common bean breeding program of Minas Gerais State, 23 cultivars under cultivation in the State (Bambuí, BRS Esplendor, BRS Estilo, IAC Bico de Ouro, Jalo EEP 558, Jalo MG 65, Ouro Negro, Ouro Vermelho, Pérola, Pitoco, Roxo 90, Vermelho 2157 and those beginning with BRS and followed by names), and seven sources of resistance to some important foliar diseases of common bean (AB-136, AFR 188, BP-9116396, BAT 332, Costa Rica and DOR 371) (Table 1) were compared to A-300. This line was obtained from International Centre for Tropical Agriculture, at Cali, Colombia. Besides its resistance to FRR, it is also resistant to *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *phaseoli*, and *Pythium ultimum* (Tu & Park, 1993). The Minas Gerais common bean breeding program and the field experiments are coordinated by the Agricultural Research Institute of the State of Minas Gerais (EPAMIG), Federal University of Viçosa (UFV), Federal University of Lavras (UFLA) and

TABLE 1 - Advanced breeding lines (beginning with CNF, VP, MN, RP, VC, BP, MA, Vi, VR and BJ), cultivars and sources of resistance to some important foliar diseases of common bean tested for reaction to the Fusarium root rot

Genotype	Market class	Genotype	Market class	Genotype	Market class
CNFP 9328	black	MN-34-46	black	AFR 188[b]	red
CNFP 7994	black	MN-34-44	black	Vi 16-3-4	red
CNFP 10798	black	Costa Rica[b]	black	Ouro Vermelho[a]	red
CNFP 7966	black	CNFC 10720	carioca[c]	DOR 371[b]	red
CNFP 8096	black	CNFC 9504	carioca	Vermelho 2157[a] (BAT 94)	red
CNFP 10773	black	CNFC 9506	carioca	CNFRX 8144	violet
CNFP 10117	black	CNFC 10764	carioca	CNFRX 10535	violet
CNFP 10180	black	CNFC 10722	carioca	CNFRX 10531	violet
CNFP 8108	black	CNFC 9500	carioca	CNFR 8149	violet
CNFP 7726	black	BRS Estilo[a]	carioca	CNFR 7847	violet
CNFP 7677	black	BRS Cometa[a]	carioca	VR-3	violet
CNFP 10047	black	Pérola[a]	carioca	VR-12	violet
BRS Esplendor[a]	black	RP-2	carioca	BRS Pitanga[a]	violet
BRS Supremo[a]	black	BRSMG Madrepérola[a]	carioca	Roxo 90[a]	violett
BRS Valente[a]	black	VC-13	carioca	BRS Timbó[a] (FEB 163)	violet
BRS Campeiro[a]	black	VC-14	carioca	Bambuí[a]	mulatinho[d]
VP-14	black	VC-15	carioca	BAT 332[b]	mulatinho
VP-15	black	CVIII-39-24	carioca	IAC Bico de Ouro[a]	mulatinho
VP-16	black	BP-31	carioca	CNFRJ 10571	jalo[e]
VP-17	black	BRSMG Talismã[a]	carioca	CNFRJ 10564	jalo
VP-18	black	BRSMG Majestoso[a]	carioca	CNFRJ 10556	jalo
VP-19	black	VC-16	carioca	Jalo EEP 558[a]	jalo
VP-20	black	BRSMG Pioneiro[a]	carioca	BJ-1	jalo
VP-21	black	MAII-2	carioca	BJ-2	jalo
VP-22	black	MAII-16	carioca	BJ-3	jalo
VP-23	black	MAII-22	carioca	BRSMG União[a]	jalo
Ouro Negro[a]	black	RP-1	carioca	BJ-5	jalo
CNFP 10802	black	CVIII-85-11	carioca	BJ-6	jalo
MN-37-2	black	CVIII-119-4	carioca	BJ-7	jalo
MN-34-20	black	Pitoco[a]	carioca	BJ-8	jalo
MN-34-66	black	AB-136[b]	red	Jalo MG 65[a]	Jalo
MN-34-53	black	BP-9116396[b]	red		

[a] Cultivars under cultivation in Minas Gerais State, Brazil.
[b] Sources of resistance to some important foliar diseases.
[c] Small cream-striped seeds.
[d] Small beige seeds.
[e] Large yellow seeds from Andean gene pool.

Brazilian Agricultural Research Corporation (EMBRAPA). Genotypes belong to Mesoamerican (34 black, 27 "carioca", 10 violet, seven red and four "mulatinho") or Andean gene pool (13 "jalo"). Carioca is a class widely cultivated in Brazil and has cream-striped seeds. Jalo class has large yellow seeds and mulatinho, small beige seeds.

Greenhouse experiment

Two isolates of *Fsp* was obtained from common bean roots with symptoms of FRR in a field at EPAMIG, located in Oratórios, Minas Gerais. A test of pathogenicity was performed with these isolates. The most aggressive isolate was grown on potato-dextrose-agar (PDA) and stored at 10°C in the dark. Chlamydospores of *Fsp* were produced according to Zambolim et al. (1983) with some modifications as follow. Eight 8-mm-diameter *Fsp* discs from a 10-d-old PDA culture were placed into 250-mL Erlenmeyer flasks with 50 mL of soil extract. Five hundred grams of this soil were autoclaved in 1 L of tap water for 30 min. Then, the extract was filtered through filter paper and adjusted to the pH 6.5 with $CaCO_3$, before been autoclaved at 121°C for 15 minutes. After incubation for 20 days at 26°C, the contents of the flasks were poured out into a 37-µm sieve and gently rinsed with tap water to separate chlamydospores and mycelia from the extract. The fungal mat was ground in a mixer for 30 s. After dilution in series, the chlamydospores were mixed with sterilized dry sand in the concentration of 2 x 10^6 chlamydospores per gram of sand. They were maintained at 4ºC.

The chlamydospores were mixed to the substrate Plantmax HT® with a concrete mixer for 40 min to obtain a rate of 4000 chlamydospores per gram of substrate. Plantmax HT® is made of pine bark, processed peat and vermiculite, with a pH of 5.8 and with the following nutrient composition, in g kg^{-1}: N, 5.8; P, 0.9; K, 4.3. The genotypes were sown in 1.0 L pots with the infested substrate. Five seeds were sown per pot and subsequently thinned to three plants at the end of seedling emergence. A randomized block design with five replicates was used. Each replicate was a pot. All treatments were irrigated once a day and received approximately the same amount of water.

Field experiment

Two field experiments were carried out (May/June and July/August of 2009) in an experimental area (680 m^2) at EPAMIG, Oratórios, Minas Gerais (20°24'11" S, 42°49'08" W, elevation of 478 m). Plants with symptoms of FRR in this area have been observed in the past six years. A previous test showed that the experimental area had a homogeneous distribution of *Fsp*: 90% of plants of a susceptible cultivar had FRR symptoms at 25 d after emergence (DAE).

Each plot was two rows 0.5 m apart and 2 m long, with 15 plants per meter. A randomized complete block design with three replicates was used. A commercial fertilizer (4.0N:6.1P:6.6K) was applied at 600 kg ha^{-1} in bands below the seeds. Weed control was performed weekly by hand hoeing. The area was irrigated with overhead sprinklers positioned 1.5 m above ground level. Irrigation was provided as needed to promote good seedling emergence, and a rate of approximately 40 mm of water per week was applied thereafter, as is generally used in the region. Insects were controlled with monocrotophos (400 mL ha^{-1}).

Disease evaluation and statistical analyses

Either one plant per plot in greenhouse or 10 plants per plot in field were rated for disease severity at 15, 30 and 45 DAE, corresponding to V3 (first trifoliate leaf), R5 (preflowering) and R7 (beginning of pod formation) developmental stages, respectively. Plants of each plot were carefully removed from soil by hand and rated for FRR according the scale of Table 2 based on Van Schoonhoven & Pastor-Corrales (1987). The disease severities, expressed as the exact percentage of the disease on hypocotyls and

TABLE 2 - Fusarium root rot rating scale used in the experiments[a]

Score	Phenotypic description
1	No visible disease symptoms.
2	Approximately 5% of the hypocotyls and root tissues covered with lesions.
3	Light discoloration either without necrotic lesions or with approximately 10% of the hypocotyls and root tissues covered with lesions.
4	Approximately 17.5% of the hypocotyls and root tissues covered with lesions.
5	Approximately 25% of the hypocotyls and root tissues covered with lesions but tissues remain firm with deterioration of the root system and heavy discoloration symptoms may be evident.
6	Approximately 37.5% of the hypocotyls and root tissues with lesions.
7	Approximately 50% of the hypocotyls and root tissues covered with lesions combined with considerable softening, rotting, and reduction of the root system.
8	Approximately 62.5% of the hypocotyls and root tissues covered with lesions.
9	Approximately 75% or more of the hypocotyls and root tissues affected with advanced stages of rotting combined with a severe reduction in the root system.

[a] Adapted from Van Schoonhoven & Pastor-Corrales (1987).

root tissues described for each score in Table 2, were used to estimate the area under the disease progress curve (AUDPC) for each experiment.

The AUDPC data were submitted to analysis the homogeneity of variance with Bartlett`s test and for normality with Lilliefor`s test, both at the level of 5% probability and using the software program "Sistema para Análise Estatística" (SAEG) (Ribeiro Júnior, 2001). The AUDPC did not meet the normality assumption in the two field experiments. Thus, it was transformed using log 10. After, the analysis of variance was performed and means were grouped by Scott-Knott test ($P < 0.05$). A Pearson correlation was used to evaluate the relationship among the disease ratings at the developmental stages and the AUDPC and among disease ratings of the experiments at the three stages.

RESULTS

Correlation coefficients among AUDPC in greenhouse and in the first (r = 0.72) and the second (r = 0.73) field experiments were significant (P < 0.001). Significant genetic variation for root rot ratings occurred for greenhouse and field experiments. FRR ratings ranged from 2.6 (at V3 stage) to 7.1 (R7) in greenhouse, from 3.2 (V3) to 6.7 (R7) in the first field experiment, and from 3.3 (R5) to 7.0 (R5) in the second field experiment.

Significant correlations between the AUDPC and the disease ratings in the three developmental stages occurred for all experiments, in higher magnitude between AUDPC and R5 stage (Table 3). Also, the correlation coefficients between disease ratings in greenhouse and each field experiment were higher at R5 (r = 0.57*** and r = 0.66***), compared with V3 (r = 0.52*** and r = 0.41***) and R7 (r = 0.34** and r = 0.39***). Thus, just results of FRR rating of genotypes at R5 stage are showed in this study.

The results of the AUDPC in greenhouse were used to show the reaction of the genotypes to FRR because Scott-Knott test distinguished more groups of genotypes in this experiment (Table 4). Twenty-one genotypes belonged to the group A (most susceptible) and 19 to the group D (most resistant). The genotypes belonging to jalo market class were in general in the most susceptible group. Their FRR ratings varied from 5.2 to 6.7 at the R5 stage. On the other hand, black beans were among the most resistant genotypes with FRR ratings varying from 3.4 to 5.6. Eighteen genotypes

TABLE 3 - Pearson correlation coefficients (CC) between Fusarium root rot (FRR) ratings on 96 genotypes of common bean assessed at the plant developmental stages V3 (first trifoliate leaf), R5 (preflowering) and R7 (pod formation) and area under disease progress curve (AUDPC) in three experiments

Variables	correlated[a]	Greenhouse[b]	First field experiment[b]	Second field experiment[b]
V3	AUDPC	0.844	0.611	0.500
R5	AUDPC	0.967	0.873	0.936
R7	AUDPC	0.870	0.574	0.563

[a] V3, R5 and R7 were the developmental stages when FRR ratings were assessed.
[b] Correlation coefficients were significant (P < 0.001).

TABLE 4 - Genotypes grouped by Scott-Knott test ($P < 0.05$) according to the area under disease progress curve (AUDPC) obtained in greenhouse

Groups[a]	Genotypes[b]
A	BJ-8, BRSMG Majestoso, BJ-2, CNFRJ 10564, CNFRJ 10556, BJ-5, BJ 1, Jalo EEP 558, Jalo MG 65, Roxo 90, VC-16, BRS Estilo, CNFRJ 10571, CNFP 10773, CNFP 10117, CNFC-10722, CNFP 7966, VP 20, BJ-7, IAC Bico de Ouro, Costa Rica, CNFC-9500
B	BJ-3, BRSMG União, BRS Esplendor, BRS Supremo, CNFP 10798, Cometa, Pérola, CNFP 8096, VP 14, VP 19, VC-13, VC -14, VC -15, VP 21, BRS Pitanga, BRSMG Talismã, BRSMG Pioneiro, MAII -2, MAII -16, AFR 188, CNFRX 8144, Bambuí, BAT 332, Pitoco, CNFP 8108, CNFP 7677, BJ-6, CNFRX 10535, DOR 371, BRS Timbó, CNFP 7726, CNFP 10047, CVIII-39-24, VP 22, Ouro Negro, MN-34-53, CNFR 8149
C	BP-31, VR 3, CNFRX 10531, Ouro Vermelho, CVIII-85-11, MAII-22, CNFC-9504, CNFC-9506, CNFP 10802, BRS Valente, CNFP 10180, CNFP 7994, CNFR 7847, AB -136, BRSMG Madrepérola, CNFC -10764, VP 23, BRS Campeiro
D	CVIII-119-4, VP 15, Vermelho 2157, VR 12, Vi 16-3-4, CNFC-10720, MN-34-46, CNFP 9328, VP 16, VP 17, VP 18, MN-34-44, BP-9116396, MN 37-2, MN-34-20, A-300, MN-34-66, RP-2, RP-1

[a] A – genotypes with AUDPC from 945 to 1256 and Fusarium root rot rating at R5 from 5.6 to 6.6;
B – 776 to 926 and 4.4 to 5.6; C – 660 to 769 and 4.2 to 5.2; and D – 409 to 630 and 3.4 to 4.5.
[b]Genotypes in each group are presented in order from the higher AUDPC to the lower AUDPC.

were as resistant to FRR as the line A-300 (group D). These genotypes belong to the following market classes: 10 black, four "carioca", three red, and one violet. Except for one source of resistance to foliar diseases, BP-9116396, and one cultivar, Vermelho 2157, the others were advanced breeding lines. The cultivars under cultivation in Minas Gerais Ouro Vermelho, BRS Valente, BRSMG Madrepérola, and BRS Campeiro presented some resistance to FRR (group C). However, most of the cultivars were susceptible to FRR, mainly BRSMG Majestoso, Jalo EEP 558, Jalo MG 65, Roxo 90, and IAC Bico de Ouro.

DISCUSSION

The levels of FRR pressure were high in the three experiments, which contributed for the highly significant correlation coefficients among the disease severity in greenhouse and in the field experiments. Our results in greenhouse indicated that 16 advanced common bean breeding lines, 10 of them black, have levels of FRR resistance similar to those of the line A-300. Most of the bean genotypes that have been reported to be resistant to FRR are small-seeded, black in color, and originating from the Mesoamerican gene pool (Clare et al., 2010). These sources of resistance could be promptly used (as cultivar) or be used by breeding programs improving resistance in commercial varieties. One cultivar of red seeds, Vermelho 2157, used especially in the region of Zona da Mata of Minas Gerais, showed high levels of resistance to FRR. However, the cultivar Pérola (carioca market class), the most widely grown in Brazil, had low levels of FRR resistance. The carioca lines CVIII-119-4, CNFC-10720, RP-1 and RP-2 are options with high levels of FRR resistance to replace the cultivar Pérola. The carioca lines RP-1 and RP-2 are from a recurrent program for erect plants. The line RP-1, which has showed resistance to Fusarium wilt (*F. oxysporum* f. sp. *phaseoli*) in field (results not published), will be soon included in the official bean cultivar recommendation for Minas Gerais. The line CNFC 10720 has also showed in field less symptoms of white mold (*Sclerotinia sclerotiorum*) than other carioca cultivars and resistance to Fusarium wilt (results not published).

Six of the genotypes used as genitors in the crosses related to the above-mentioned resistant genotypes are known for their resistance to *Fsp*: Aporé, Iapar 81 (Molin et al., 1999; Miranda et al., 2007), IPR Uirapuru, BRS Timbó (Miranda et al., 2007), Pintado, and Carioca (Pastor-Corrales & Schwartz, 1994). In our study, however, BRS Timbó (FEB 163) was in the group B, with 30% of the hypocotyls and root tissues with lesions at R5 stage and AUDPC of 855. Our results corroborate the results of Miranda et al. (2007), in which the cultivar BRSMG Talismã was susceptible to FRR. Our results suggest that the jalo (large yellow seeds) class should not be used in areas infested with *Fsp*, as well as the genotypes belonging to the Andean germplasm (Beebe et al., 2001; Clare et al., 2010). Clare et al. (2010) evaluated reaction of 147 common beans to FRR. These authors found that disease resistance was highest among cultivars previously selected for resistance to Fusarium wilt and Pythium root rot indicating presence of quantitative trait loci modifying resistance to more than one root pathogen in some lines.

We found significant correlation coefficients among disease severity in greenhouse (using chlamydospores as inoculum) and in field experiments. Others authors found the same results, but using spore suspension as inoculum in greenhouse (Schneider & Kelly, 2000; Chaudhary et al., 2006; Bilgi et al., 2008; Clare et al., 2010). That means that either chlamydospores or spores are both effective methods for screening the genotypes. These significant correlations reinforce the conclusion drawn previously (Schneider & Kelly, 2000; Bilgi et al., 2008) that greenhouse experiments could be used to identify new sources of resistance to FRR while reducing environmental variation and demands upon limited resources.

The AUDPC was estimated based on percentage of disease on hypocotyls and root tissues of three development stages. The disease rated at R5 stage (preflowering) had higher correlation coefficients with AUPDC than V3 and R7 stages in all experiments. Also, at R5 stage the highest correlation coefficients among disease severity in greenhouse and field experiments were obtained. These results suggest that FRR assessment in greenhouse should be done also at the beginning of reproductive phase. In general the disease severity in greenhouse is assessed between 10 and 31 DAP, i.e., during the vegetative phase (Schneider & Kelly, 2000; Chaudhary et al., 2006; Miranda et al., 2007; Bilgi et al., 2008). In field, our results indicate that the FRR assessment at R5 might provide results more consistent than at R7 stage (beginning of pod formation). Assessment at the earliest reproductive stage implies time and money savings in the screening of genotypes for resistance to FRR.

We conclude that there are advanced lines from the common bean breeding program of Minas Gerais with the same levels of FRR resistance of those in the line A-300. Additionally, our results indicate that FRR assessment at R5 stage is more effective than either at V3 or R7 stage for both greenhouse and field experiments.

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