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Notas Científicas

Biological control of white mold by *Trichoderma harzianum* in common bean under field conditions

Daniel Diego Costa Carvalho[1], Alaerson Maia Geraldine[2], Murillo Lobo Junior[2] and Sueli Corrêa Marques de Mello[3]

[1]Universidade de Brasília, Instituto de Ciências Biológicas, Departamento de Fitopatologia, Campus Universitário Darcy Ribeiro, Bloco E, s/nº, Asa Norte, CEP 70910-700 Brasília, DF, Brazil. E-mail: daniel.carvalho@ueg.br [2]Embrapa Arroz e Feijão, Rodovia GO-462, Km 12, Zona Rural, Caixa Postal 179, CEP 75375-000 Santo Antônio de Goiás, GO, Brazil. E-mail: alaerson.geraldine@ifgoiano.edu.br, murillo.lobo@embrapa.br [3]Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Avenida W5 Norte (Final), Caixa Postal 02372, CEP 70770-917 Brasília, DF, Brazil. E-mail: sueli.mello@embrapa.br

Abstract – The objective of this work was to evaluate *Trichoderma harzianum* isolates for biological control of white mold in common bean (*Phaseolus vulgaris*). Five isolates were evaluated for biocontrol of white mold in 'Perola' common bean under field conditions, in the 2009 and 2010 crop seasons. A commercial isolate (1306) and a control treatment were included. Foliar applications at $2x10^9$ conidia mL^{-1} were performed at 42 and 52 days after sowing (DAS), in 2009, and at 52 DAS in 2010. The CEN287, CEN316, and 1306 isolates decreased the number of *Sclerotinia sclerotiorum* apothecia per square meter in comparison to the control, in both crop seasons. CEN287, CEN316, and 1306 decreased white mold severity during the experimental period, when compared to the control.

Index terms: *Phaseolus vulgaris*, *Sclerotinia sclerotiorum*, antagonists, hyperparasitism, soilborne pathogen.

Controle biológico do mofo-branco por *Trichoderma harzianum* em feijão em condições de campo

Resumo – O objetivo deste trabalho foi avaliar isolados de *Trichoderma harzianum* para o controle biológico do mofo-branco em feijão (*Phaseolus vulgaris*). Cinco isolados foram avaliados para o biocontrole do mofo-branco em feijão 'Pérola', em condições de campo, nos anos agrícolas 2009 e 2010. Um isolado comercial (1306) e um tratamento testemunha foram incluídos. Aplicações foliares a $2x10^9$ conídios mL^{-1} foram realizadas aos 42 e 52 dias após a semeadura (DAS), em 2009, e aos 52 DAS em 2010. Os isolados CEN287, CEN316 e 1306 reduziram o número de apotécios por metro quadrado de *Sclerotinia sclerotiorum*, em comparação à testemunha, nos dois anos agrícolas. CEN287, CEN316 e 1306 reduziram a severidade do mofo-branco no período experimental, quando comparados à testemunha.

Termos para indexação: *Phaseolus vulgaris*, *Sclerotinia sclerotiorum*, antagonistas, hiperparasitismo, patógenos habitantes do solo.

Fungicides have been used to manage white mold, but highly-infested areas require many applications, which greatly increase production costs. Moreover, chemical fungicides do not always provide satisfactory control and may have adverse effects on nontarget organisms (Naseby et al., 2000). In this respect, biological control is advantageous over conventional pesticides, as it provides an alternative to reduce the soil inoculum potential, without harmful effects on the environment (Harman et al., 2004).

Disease biocontrol promoted by *Trichoderma*, considering active components of natural soil, consists in a complex process that can occur through antibiosis, competition for nutrients, and mycoparasitism, among other mechanisms (Harman et al., 2004). As an additional advantage, some isolates of *Trichoderma* may also act as plant growth promoters (Carvalho et al., 2011). Different *T. harzianum* isolates have effectively reduced the incidence of white mold and other diseases in several economically important crops,

such as tomato (Abdullah et al., 2008) and common bean (Geraldine et al., 2013; Carvalho et al., 2014).

Brazilian soils contain a rich diversity of beneficial microorganisms. Among them, *Trichoderma* species have been targets for collection and screening, aiming at the discovery of efficient isolates for biological control (Carvalho et al., 2014). Five isolates (CEN287, CEN288, CEN289, CEN290, and CEN316) were previously selected in vitro against major common bean pathogens (Carvalho et al., 2011). Their effectiveness, previously observed in controlled-environment and field studies (Carvalho et al., 2015), motivated further tests, also designed to determine their biocontrol amplitude in agroecosystems.

The objective of this work was to evaluate *Trichoderma harzianum* isolates for biological control of white mold in common bean (*Phaseolus vulgaris*).

The five isolates of *T. harzianum* used in the present study belong to the fungi collection for biological control of plant pathogens and weeds of Embrapa Recursos Genéticos e Biotecnologia, located in the municipality of Brasília, in Distrito Federal, Brazil. All *Trichoderma* isolates were originally obtained from the Cerrado biome. Cultures were stored in liquid nitrogen and recovered in potato dextrose agar medium. In addition, a commercial isolate of *T. harzianum*, 1306 Trichodermil, (Itaforte Bioprodutos, Itapetininga, SP, Brazil), recommended for the biocontrol of soilborne pathogens and for plant growth promotion, was used.

Two field experiments were conducted in the same area, at Fazenda Palmital (16º26'04"S, 49º24'07"W, at an altitude of 735 m), within Embrapa Arroz e Feijão facilities, located in the municipality of Goianira, in the state of Goiás, Brazil, in the 2009 and 2010 crop seasons (July-October), when the average air temperature was 21.2 and 21.5°C, respectively. The soil of the experimental area is classified as a Latossolo Vermelho ácrico (Rhodic Acrustox) (Santos et al., 2006) with clay texture, and presented the following characteristics: 6.6 pH; 3.24, 1.25, 0, and 3.35 $cmol_c$ dm^{-3} Ca, Mg, Al, and H+Al, respectively; 11.6, 111, 2.9, 3.6, 110, and 50 mg dm^{-3} P, K, Cu, Zn, Fe, and Mn, respectively; and 20 g dm^{-3} organic matter. The experimental area had been previously cropped for pasture and had no record of previous annual crop. A total of 2.5 L ha^{-1} glyphosate was applied, and the distribution of sclerotia in the experimental area was carried out soon after crop sowing, with an average of 145 sclerotia per square meter in 2009 and 2010, in alignment with Huang et al. (2000). The experimental area was fertilized with N-P_2O_5-K_2O (5-25-15 at 400 kg ha^{-1}) and sown with 'Pérola' common bean (24 seeds per square meter).

Plots composed by five 2.5-m planting rows were arranged in a randomized complete block design, with four replicates. The spacing between rows was 0.5 m, whereas plots were spaced at 1.0 m. Outer guard rows (2.5 m) were sown with the same crop to protect the total experimental area (400 m^2) and to support a disease-conducive microclimate. The experiments were sprinkled irrigated, favoring proper common bean growth and apothecia development. Other cultural practices followed the recommendations of Barbosa & Gonzaga (2012).

In order to produce *T. harzianum* inoculum, 5.0-mm mycelial plugs of each *T. harzianum* isolate were transferred to 250-mL flasks (six plugs per flask), containing 15 g parboiled rice, previously moistened (60% w/v) and autoclaved (121ºC for 40 min). Flasks were kept at 25ºC, under a 12-hour photoperiod. After 7 days, spores were harvested with distilled water and filtered through sterile gauze, and their concentration was adjusted to 1×10^6 conidia mL^{-1} with a Neubauer chamber.

In 2009, two *T. harzianum* applications of 1.5 L of the conidial suspension were performed per plot (6.25 m^2, equivalent to 2.4×10^{12} conidia ha^{-1}): the first at 5% bean flowering, at 42 days after sowing (DAS), and the second, 10 days after the first one (Huang et al., 2000). In 2010, *T. harzianum* was spread only once at 42 DAS, at the same dose as in 2009. In both experiments, a pre-compression sprayer, model 417-02 (Guarany Indústria e Comércio Ltda., Itu, SP, Brazil), with real tank volume of 3.8 L was used to spray the conidial suspensions. After the antagonist was applied, the experiments were irrigated to facilitate the spread of conidia in the soil.

The number of apothecia present on the soil surface was estimated at the full-bloom and first-pod formation stages (62 DAS), in a 1.0-m^2 area in the center of each plot. White mold severity was evaluated at 72 DAS, at the pod-filling stage. For this evaluation, two 1.0-m^2 areas were randomly chosen within each plot. For severity evaluation, the rating scale described by Napoleão et al. (2005) was used. For statistical analysis, the mean point of each assigned grade was

considered. Manual harvesting was performed at 97 DAS in the two central rows of each plot at 1.5-m length. Besides grain yield (kg ha^{-1}), the number of pods per plant, grains per pod, and mass of 100 grains (g) were determined. The results were subjected to the analysis of variance and to the Scott-Knott test, at 5% probability, using the software Sisvar, version 5.3 (Ufla, Lavras, MG, Brazil).

Soil infestation was successful, because it allowed the development of white mold in all plots, in both experiments, in 2009 and 2010. Differences were found among treatments. The CEN287, CEN316, and 1306 isolates reduced the pathogen inoculum density in both crop seasons, when compared to the control treatment not inoculated with *T. harzianum* (Table 1). The reduction in the average number of apothecia per square meter observed in the plots treated with these effective isolates was 46, 58, and 62% in 2009, and 73, 61, and 52% in 2010, respectively. CEN290 also reduced the number of apothecia in 2009, but did not present the same effect the following year, under higher inoculum pressure.

Furthermore, only the CEN287, CEN316, and 1306 isolates proved to be effective biocontrol agents of white mold under field conditions in the two crop seasons, reducing disease severity by 77, 74, and 76% in 2009, and by 96, 80, and 84% in 2010, respectively, in comparison to the untreated control. Even with the increase in the general mean of white mold severity between 2009 and 2010, these three isolates maintained their efficiency in controlling the disease, as shown by the grouped means obtained by the Scott-Knot test in both crop seasons.

There were no differences between the isolates for yield and its components (Table 2). However, in the first experiment, in 2009, the mass of 100 grains of CEN287 was higher than that of the other isolates. This was the only significant difference observed in the means of yield and its components (Table 2). In addition, an increase in the general grain yield was verified between 2009 and 2010.

Reductions in the number of apothecia per square meter and in the severity of white mold did not positively

Table 1. Effect of *Trichoderma harzianum* on the number of apothecia per square meter and on white mold severity in 'Pérola' common bean (*Phaseolus vulgaris*) under field conditions, in the 2009 and 2010 crop seasons[1].

Trichoderam harzianum isolate	Apothecia per square meter		Severity[2] (%)	
	2009	2010	2009	2010
CEN287	6.7aA	28.2aB	6.7aA	2.3aA
CEN288	10.5bA	114.2bB	11.6aA	33.7bB
CEN289	17.5cA	50.7aB	27.5bA	32.2bA
CEN290	8.5aA	85.7bB	6.8aA	32.2bB
CEN316	5.2aA	40.5aB	7.5aA	11.7aA
1306	4.7aA	50.2aB	6.7aA	9.2aA
Control[3]	12.5bA	105.5bB	28.5bA	58.7cB
Mean	9.39A	67.89B	13.62A	25.76B
CV (%)	21.61	27.06	25.36	29.60

[1]Means followed by equal letters, lowercase in the columns and uppercase in the rows, do not differ by the Scott-Knott test, at 5% probability. [2]Severity was evaluated according to the rating scale described by Napoleão et al. (2005). [3]Without application of *Trichoderma harzianum.*

Table 2. Effect of *Trichoderma harzianum* application, for biocontrol of white mold, on grain yield of 'Pérola' common bean (*Phaseolus vulgaris*) and its components, under field conditions, in the 2009 and 2010 crop seasons[1].

Trichoderma harzianum isolate	Pods (number per plant)		Grains (number per pod)		Mass of 100 grains (g)		Grain yield (kg ha^{-1})	
	2009	2010	2009	2010	2009	2010	2009	2010
CEN287	10.7aA	10.5aA	5.4aA	5.0aA	29.7aA	27.7aA	2,162aA	3,217aB
CEN288	9.8aA	12.2aA	5.1aA	5.1aA	26.5bA	25.7aA	1,820aA	2,285aA
CEN289	8.7aA	11.3aA	5.1aA	5.0aA	26.0bA	27.5aA	1,950aA	2,700aA
CEN290	9.1aA	8.5aA	5.2aA	5.1aA	26.0bA	27.1aA	2,056aA	2,744aA
CEN316	12.0aA	11.3aA	5.2aA	5.1aA	25.5bA	26.5aA	1,944aA	2,426aA
1306	11.9aA	11.3aA	5.5aA	5.0aA	26.6bA	26.6aA	1,929aA	3,471aB
Control[2]	8.4aA	9.4aA	5.2aA	5.3aA	26.9bA	25.1aA	1,990aA	2,555aA
Mean	10.1A	10.7A	5.2A	5.1A	26.8A	26.6A	1,979A	2,771B
CV (%)	19.33	29.36	7.00	10.11	5.12	6.29	23.59	22.07

[1]Means followed by equal letters, lowercase in the columns and uppercase in the rows, do not differ by the Scott-Knott test, at 5% probability. [2]Without application of *Trichoderma harzianum*.

affect grain yield. This can be explained by the fact that 'Pérola' common bean, a known susceptible cultivar to white mold (Napoleão et al., 2005; Geraldine et al., 2013), shows indeterminate growth linked to yield compensation after biotic or abiotic stress (Kelly et al., 1998). In this case, an extended flowering period may originate a new set of pods, out of reach of decayed apothecia, and compensate at least partial yield losses from the disease. Although severity of white mold was not enough to cause changes in grain yield, disease biocontrol with *Trichoderma* species can inhibit the formation of new sclerotia in the area (Abdullah et al., 2008).

Pathogen inoculation in 2009, together with new sclerotia formed in the first experiment, and a new artificial infestation in 2010 resulted in increased inoculum density, from 9.4 to 67.9 apothecia per square meter in the experimental area, from one crop season to the other (Table 1). However, the antagonist did not survive for 2 years and had to be inoculated again. The increasing soil infestation with sclerotia is in alignment with reports on buildup of *S. sclerotiorum* inocula over time (Duncan et al., 2006); despite the increasing disease pressure, the effective antagonists CEN287, CEN316, and 1306 were still able to reduce the pathogen inoculum density in both experiments, showing their advantages for biocontrol (Table 1).

The grouping of CEN287, CEN288, CEN290, CEN316, and 1306 regarding white mold severity in 2009 was considered a consequence of the lower number of apothecia per square meter in the first crop season. CEN288 was statistically similar to these isolates in terms of white mold severity in 2009, but presented a statistically higher average number of apothecia per square meter at 62 DAS, except when compared to CEN289 and the control. In the following experiment, only the CEN287 and CEN316 isolates were effective in showing stable results in both crop seasons, with their biocontrol capacity unchanged in 2010 with just one application and under increased disease pressure. This result is in agreement with Zeng et al. (2012), who highlighted that bioagents were more effective when disease pressure was high.

CEN287 and CEN316 were sprayed as conidial suspension in water, devoid of the technological formulations of 1306, i.e., emulsifiable concentrate suspension. However, all three isolates proved to be effective for management of *Fusarium* wilt of common bean in field conditions (Carvalho et al., 2014, 2015; Guimarães et al., 2014). Therefore, also due to their high capacity for spore production, these isolates are considered a promising tool for biocontrol of white mold in common bean.

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