origins and on many substrata were analyzed. "Genealogical Concordance Phylogenetic Species Recognition" based on sequences of four loci (i.e. act, cal, ITS nrDNA, tef1) and other species delimitation approaches, indicate the presence of at least ten species in addition to the apparently uncommon *T. harzianum* sensu stricto, which is restricted to Europe and North America. Although morphology remains of little diagnostic value, some of the recognized cryptic species correlate to geographical distribution, ecology (e.g. endophyte, mycoparasite, or saprophyte), and known teleomorphs. Teleomorphs in *T. harzianum* are apparently rare, found only in a few clades. In addition, one of the endophytic species shows possible host preference. *Trichoderma harzianum* may be an example of the ‘end of morphology' in taxonomy where there are too few visible characters to account for the rapid speciation and genetic isolation which generally precedes the divergence of morphological character states.

S040

Controle de doenças de plantas usando isolados brasileiros de *Trichoderma harzianum*.
Mello SCM, Carvalho DDC, Junior ML. 1Embrapa Genetic Resources and Biotechnology,, Brasília, DF. smello@cenargen.embrapa.br. [Biological control of plant diseases using Brazilian isolates of *Trichoderma harzianum*]

Knowledge about potential use of fungi in agriculture has been followed with increasing interest, especially in terms of environmental protection and development of biotechnological processes and products. These biological resources (not only fungi, but also other microbial agents), if well characterized and evaluated under rigorous scientific approach, may uncover important biocontrol agents and generate clean technologies to combat plant-health problems that threaten the country's yields.

The availability of selected biocontrol agents is essential, given that use of agricultural pesticides together with intensive application of chemical fertilizers has been identified as the main cause of environmental imbalance, that endanger the sustainability of agro-ecosystems. The most visible consequence of these imbalances is the exacerbated proliferation of several agricultural pests, constantly threatening crops and demanding ever more pesticides. The Society's growing awareness about the indiscriminate use of pesticides and the need to preserve both environment and human health lead us to expect a worldwide agricultural scenario where biological inputs will be a key tool on plant disease management. Because Brazil is a mega-diverse country, it provides excellent conditions for carrying out bio-prospection studies, which will be of vital importance to the development of national bio-products, using lineages from its different eco-systems, with high biocontrol efficiency.

So far, many fungal agents for the biocontrol of plant pathogens evaluated for their ability to reduce plant disease belong to the genus *Trichoderma* (De Marco et al., 2000; Silvestri, 2005). First described at about 200 years ago (Persoon, 1794), this genus gathers anamorphic species that can be found in a wide variety of habitats. When isolated from soil samples and from host structures (sclerotia, for example), competitive isolates can be selected in the laboratory and afterwards applied in the field.

Considering the importance of this group of fungi, the genus Trichoderma has been prominent in research carried out at Embrapa Genetic Resources and Biotechnology in partnership with Embrapa Rice and Beans and the University of Brasilia, focusing on the exploration of microbial biodiversity conserved in culture collections. The objective of this research is to characterize and discover lineages with higher parasitic activity with good adaptation to the different environments where these agents will be used. Research results generated from studies on the potential for using these organisms points several possibilities of use of this biological diversity and its metabolites.

To address this issue, we report below the search for isolates for control of two main diseases that threaten common bean (*Phaseolus vulgaris* L.), according to Paula Júnior et al. (2008): white mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary and fusarium yellows wilt caused by *Fusarium oxysporum* Schlecht. f. sp. *phaseoli* Kendrick & Snyder. These two pathogens survive in crop debris and in the soil, by means of their resistance structures, thus making it difficult to control them physically or chemically in regions where the common bean crop is constantly grown (Cavalcanti et al., 2002; Abdullah et al., 2008).

Starting from 40 isolates belonging to the Collection of Fungi for Biological Control of Plant Pathogens at Embrapa Genetic Resources and Biotechnology (Brasília, DF), five *T. harzianum* isolates were selected (CEN287, CEN 288, CEN289, CEN290 and CEN316) based on in vitro assays in Petri dishes using dual culture technique. Besides, aiming to evaluate the interaction between pathogens and antagonists, further examinations were also performed on samples taken from areas of confrontation between the two species, using the scanning electron microscopy technique. Subsequently, the following tests were performed with these five best isolates, at Embrapa Rice and Beans (Santo Antônio de Goiás, GO).

In laboratory trials, seeds of common bean ‘Jalo Precoce' and ‘BRS Valente' artificially infected by *S. sclerotiorum* and *F. oxysporum*, respectively, were treated with 2 mL 100 g-1 seeds from antagonist suspensions (2.5 x 108 conidia mL-1), sown in a paper roll (4 repetitions of 50 seeds) and incubated at 25 and 20° C. The incidence percentages, diseased and normal plantlets were evaluated at 7 and 9 days, respectively. These experiments were repeated twice. In both of them, an absolute control (seeds without infestation) and a commercial *T. harzianum* isolate were included.

To assess the effects of the isolates on the control of white mold in the field from July-September, in 2009, the antagonists were applied at 42 and 52 days after seeding (DAS) the common bean ‘Pérola'. Each plot of 6.25 m2 was sprayed with 1500 mL of suspension (106 conidia mL-1). Each treatment was composed of four plots, following a randomized block design.

The inoculum density of S. sclerotiorum was determined at 62 DAS in terms of apothecia/m2. White mold severity evaluation took place at 69 DAS, with the help of a scale adapted from Napoleão et al. (2005): 1: all plants healthy; 2: 1 to 5% of the area covered with symptoms - acs; 3: 6 to 20% acs; 4: 21 to 50% acs; 5: 51 to 70% acs; 6: 71 to 90% acs; 7: 91 to 100% acs and dead plants. Harvest took place at 97 DAS.

Field experiments were also carried out to assess the biological control of fusarium yellows wilt. In 2009/2010 (October-January) and 2010 (April-July), plots of 1 m2 (1 x 1 m) were infested with the isolate FOP 46 of F. oxysporum f.sp. phaseoli. Next, cv. BRS Valente seeds were sown in furrows sprayed with T. harzianum (1.2 x 1012 conidia ha-1). Each treatment was composed of four plots, following a randomized block design. As well as the selected isolates, a commercial isolate of T. harzianum, an absolute control and a positive control (plots infested with the pathogen and without application of the antagonists) were used. At 68 DAS, the severity of wilt was evaluated in accordance with the scale developed by Abawi & Pastor-Corrales (1990): 1 - symptomless; 3 - approximately 10% of leaves showing wilt and chlorosis; 5 - approx. 25%; 7 - approx. 50%; 9 - approx. 75%, plants atrophied and dead. The harvest took place at 85 DAS. All results from laboratory and field were submitted to ANOVA and mean separation according to Scott-Knot's test (5%).

CEN287 and CEN316 were the most effective in the control of S. sclerotiorum and F. oxysporum in seeds (reduction of 90 and 92%; and 40 and 31% of incidence, respectively). The five Trichoderma isolates did not affect the percentage of normal plantlets, which ranged from 84.5 to 94%. In the field experiment with white mold, the average number of apothecia/m2 was lower in treatments with CEN287, CEN290 and CEN316 (6.75, 8.5 and 5.25, respectively), in comparison to the control (12.5). For these three isolates, the average severity grades (between 2.1 and 2.6) were lower than the control (grade 3.5). There was no significant difference for productivity, where values varied between 1820 and 2162 kg ha-1. As regards the study with F. oxysporum, severity grades of 1.89 and 2.23 for CEN287 and 1.84 and 1.88 for CEN316 were obtained based on the fusarium wilt symptoms in the 2009/2010 and 2010 trials, respectively. These two treatments were statistically lower than the others in the two field trials, including the positive control (grades 3.36 and 3.64, respectively), which exhibited the highest wilt severity. There was no significant difference for productivity, in the same agricultural year, which varied between 2878 and 3664 kg ha-1 (2009/2010) and 3336 and 3948 kg ha-1 (2010).

These findings sum up with other research results obtained previously in recent years with other pathosystems, and show that relevant biocontrol agents can be effectively obtained from a large biodiversity. It's clear that we know only a small part of the Brazilian *Trichoderma* diversity, and there's still a lot to be done, in terms of germplasm characterization and selection of other superior isolates for pathogen control. In spite of that, the current results show that important diseases can be managed with the help of antagonists. Therefore, biological control using *Trichoderma* will be effectively incorporated into crop management programs, taking over where other techniques are inefficient or undesirable.

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