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RESEARCH ARTICLE

Biocontrol of sheath blight by *Trichoderma asperellum* in tropical lowland rice

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Abstract Crop damage by rice sheath blight, *Rhizoctonia solani*, can decrease rice yield by up to 45 %. The classical control method of rice sheath blight in the Amazon region is the application of fungicides. Therefore, we tested here the efficiency of a biocontrol agent, *Trichoderma asperellum*, and fungicides. Two experiments of rice cultivation were carried out with seven treatments: four isolates of *T. asperellum*, a mixture of the four isolates, the fungicide pencycuron, and the control. The first experiment involved a randomized block design, and seed and foliar spray on all plots. The second experiment involved a split-plot design with foliar spray in main plots and the 1–2 foliar sprays in subplots. Results show that all treatments reduced sheath blight progression rate. In the randomized block experiment *T. asperellum* reduced disease severity by 19 %, increased grain weight by 34 %, and increased yield by 41 %. In the split-plot design experiment, the mixture of the four *T. asperellum* isolates grain reduced disease severity by 26 %, increased grain weight by 18.5 %, and increased yield by 26 %. Our results show for the first time that a mixed isolates of *T. asperellum* was efficient in reducing disease severity and increasing yield and grain weight.

Keywords *Oryza sativa* · Biological control · Field conditions · Amazon region · *Rhizoctonia solani*

S. K. S. de França · A. F. Cardoso · G. B. da Silva (✉)
Plant Protection Laboratory, Federal Rural University of Amazon, Av Perimetral, 2501, 66077-901 Campus Belém, Pará, Brazil
e-mail: gibarata@bol.com.br

D. C. Lustosa
Federal Rural University of Western Para/IBF, Vera Paz, Sales, 68035-110 Santarém, PA, Brazil
e-mail: deniselustosa@yahoo.com.br

E. M. L. S. Ramos
Federal Rural University of Pará/Exact and Natural Science Center/ICEN, Rua Augusto Corrêa, 01, 66075-110. CP 479 Belém, Pa, Brazil
e-mail: ramos.edson@gmail.com

M. C. C. de Filippi (✉)
Plant Pathology Laboratory (Laboratório de Fitopatologia), Rice and Beans Research Center/EMBRAPA, Goiânia, Goiás, Brazil 75375-000
e-mail: cristina.filippi@embrapa.br

1 Introduction

Rice sheath blight (*Rhizoctonia solani* JG Kühn) [teleomorph *Thanatephorus cucumeris* (Frank) Donk] causes damage to rice crops in the various regions of the world where rice is grown. Crop damage from rice sheath blight can result in a loss of production of up to 45 %, depending on the plant growth stage the disease onset and under favorable conditions around the world (Kumar et al. 2009). Brazil is among the top ten world producers of rice and has environmental conditions that are favorable to the development of sheath blight (Fig. 1). Tropical environmental conditions, inadequate crop management, and the susceptibility of the cultivars grown favor the high severity of this disease (Zheng et al. 2013). Although extensive evaluation of rice germplasm has been conducted for developing rice cultivars that are genetically resistant to sheath blight, there are still no cultivars with a significant degree of resistance (Srinivasachary et al. 2013). In the Amazon region, irrigated rice is cultivated by small farmers in areas along river banks. The fields are periodically flooded under the influence of river tides, and crops are grown in the highly fertile alluvial soils without the application of fertilizers. Despite these ideal conditions, continuous cultivation of these areas results in deterioration of the natural fertility of the soil, especially decreases in phosphorous, and also results in an increase in the *R. solani* inoculum. Until now, sheath blight control strategies have relied mainly on fungicides.

Fig. 1 Irrigated rice field experiment under tropical environmental conditions, favorable to sheath blight development (**a**). *Rhizoctonia solani* damage on untreated rice plants (*left*) or treated with *Trichoderma asperellum*, under field conditions (**b**)

The genus *Trichoderma* (Hypocreales, Ascomycota) is known for its antagonistic activity against several plant pathogens, including *R. solani* (Harman 2006). There are many studies reporting that biological control with *Trichoderma* may be effective in minimizing the incidence of sheath blight in rice (Das and Hazarika 2000; Tewari and Singh 2005; Naeimi et al. 2010). However, most of them were performed in vitro and in the greenhouse (Naeimi et al. 2010) and only few reported efficiency under field conditions considering method of application in the plant (foliar spray or treatment of seed) and formulation. The main objective of this study was to develop an effective method of applying *Trichoderma* spp. for controlling sheath blight in rice under flooded conditions.

2 Materials and methods

Two experiments were conducted on the Rio Guamá flood plain, in Belem, Pará State, Brazil. Each experiment used a distinct planting system. Experiment 1 used a sowing system with two distinct and consecutive planting times and was conducted during the November 2010 crop season. Experiment 2 used a transplanting system with two distinct and consecutive planting times and was conducted during the December 2011 crop season.

2.1 The pathogen (*R. solani*) and the biological agent (*Trichoderma asperellum*)

The pathogen inoculum consisted of 2-cm long toothpick segments that were sterilized for 20 min at 120 °C and placed in Petri plates containing a potato dextrose agar (PDA) medium colonized by *R. solani* (4F1, AG1 IA anastomosis group, Embrapa Rice and Beans microorganism collection).

The biological agents used were isolates of *T. asperellum* (T.06, T.09, T.12, and T.52), which were isolated from rhizospheric soils of reforested and native forest areas in the Amazon. Prior to the experiment, these strains were assessed and identified in vitro and through greenhouse studies by the Federal Rural University of Amazonia (UFRA) Plant Protection Laboratory. The *T. asperellum* isolates were grown in Petri dishes containing BDA for 5 days and bioformulated as described by Silva et al. (2012).

2.2 Sowing system experiment (experiment 1)

The experiment occupied a field area of 287.55 m^2 and consisted of two trials planted consecutively during the 2011/2012 season. The minimum temperature was 23.2 °C and the maximum temperature was 33.7 °C. Relative humidity ranged from 69 to 100 %, and rainfall levels ranged from 136.2 to 520.3 mm (INMET 2012) during the season. The field was prepared by mechanically incorporating crop debris. The experimental design was a randomized block with four replications, consisting of two trials with consecutive planting dates performed during a single season. Each plot had seven 2.5-m long rows spaced 0.2-m apart, with 80 seeds planted per meter. Prior to planting, seeds of the rice cultivar BRS Tropical were sterilized in alcohol (70 %) and hypochlorite (2 %) and rinsed in water.

2.2.1 Experiment 1 treatments

The treatments included five seed treatments (ST) with the biological agent *T. asperellum* isolate (T1=T.06, T2=T.09, T3=T.12, T4=T.52, and T5=mix of four isolates), a fungicide treatment (T6=pencycuron), and a control (T7=water). Following the seed treatment, the plants were treated twice by foliar spraying with the same isolate or product used in seed treatment. The first spray, at 57 days after sowing (DAS), was considered preventive, and the second spray, at 66 DAS, was considered curative. The biocontrol seed treatments were

performed at concentrations of 10 g of powdered *T. asperellum* per 1 kg of seed and 250 g ai kg^{-1} of pencycuron per 1 kg of seed. For all treatments, the foliar sprays were performed with an SS 5 L backpack sprayer at a pressure of 7–12.6 $kgfcm^{-2}$ and a spray volume of 500 L ha^{-1}. The concentration of the biological solutions was 10^8 conidia per milliliter and the chemical fungicide concentration was 250 g ai L^{-1}. The control treatment group was sprayed with water only.

2.2.2 Experiment 1 inoculation procedure

The toothpick segments colonized with *R. solani* were inserted between the flag leaf sheath and plant culm of the marked main tillers located along the central line of each plot. The plants were inoculated at 62 days after sowing (DAS), which was 5 days after the first foliar spray (57 DAS) and 5 days before the second foliar spray (66 DAS).

2.3 Transplanting system experiment (experiment 2)

The experiment occupied 296.84 m^2 and consisted of two trials planted consecutively during the 2011/2012 season.

2.3.1 Seedling production and transplantation

Seeds of rice cultivar BRS Tropical were sown on 1.5×20 m plots. When the seedlings were 30 days old and 15 cm in height, two to three seedlings were transplanted to the experimental plots, which were fertilized with 20 g of NPK (11-23-20+S) per crop row. Minimum temperatures ranged from 23.5 to 32 °C, relative humidity ranged from 67 to 100 %, and rainfall ranged from 139.1 to 440 mm (INMET 2012). A randomized block design was used with three replications and seven treatments in a split-plot design. The plots consisted of six 5-m long rows with 2.5-long subplots, with the rows and furrows spaced at 0.2 m.

2.3.2 Experiment 2 treatments

The treatments consisted of one or two foliar spray treatments with the biological agent *T. asperellum* isolate (T1=T.06, T2=T.09, T3=T.12, T4=T.52, T5=mix of four), a fungicide (T6=pencycuron), or a control (T7=water). Each subplot was treated with either one or two foliar sprays. The first spray treatment was applied the first 20 days after transplanting (50 DAT) and was considered a preventive application. The second foliar spray was applied 30 days after transplanting (60 DAT) and was considered a curative application.

2.3.3 Experiment 2 inoculation procedure

R. solani was inoculated 25 days after transplanting (DAT) using the same methods described for the experiment 1 inoculation.

2.4 Experiments 1 and 2 sheath blight severity and yield assessment

For both experiments (experiments 1 and 2) disease severity, disease progression (r) and a number of plant productivity parameters were assessed. In experiment 1, disease severity was assessed at 64, 66, 67, 69, and 74 days after sowing (DAS). At 64 DAS, the plants had been treated with a preventive spray and inoculated with *R. solani*. At 66 DAS and the other evaluation dates thereafter, the plants had been treated with both sprays (preventive and curative) and inoculated with *R. solani*. In experiment 2, disease severity was assessed at 57, 59, 62, 64, and 66 days after sowing (DAS). At 57 and 59 DAS, the plants had been treated with the preventive spray and inoculated with *R. solani*. On the remaining evaluation dates, the plants had been treated with both sprays (preventive and curative) and inoculated with *R. solani*. Severity of sheath blight was based on vertical lesion length, which was measured on 20 main tillers per repetition and evaluated for 5 days at intervals of 1 to 5 days. Yield parameters included panicle length (cm) and mass (g), grain weight (g) and weight of 100 grains (g), and yield (kg ha^{-1}) measured along the central row of each plot.

Area under disease progress curve (AUDPC) was calculated using the disease severity data according to Shanner and Finney (1977). Disease progression rate (r) was determined by linear regression. The parameter b of the model equation was obtained from the model that was the best fit for the data. The empirical models tested included: logistic $Y = 1/1 + \exp(-(\beta + r \times t))$; monomolecular $Y = 1-(1-y_0 \times \exp(-r \times t)$; and Gompertz $Y = \exp(-(-\ln(y_0)) \times \exp(-r \times t))$ according to Campbell and Madden (1990). For each experiment, the best model was selected based on a high R^2 value, a low mean square value, and a plot of standardized residuals ($y_{obs} - y_{exp}$) that did not show trends or values close to the x-axis (data not shown).

3 Results and discussion

3.1 Epidemic sheath blight

The statistical analysis showed that there was no difference between the two trials in either in experiment 1 or 2, indicating that the treatments had the same effect regardless of planting date. In both trials of experiments 1 and 2, all treatments

reduced sheath blight severity, AUDPC and the sheath blight progression rate (r) compared to those of the control (Fig. 2). Among the *T. asperellum* isolates, T.06 was associated with the lowest AUDPC and differed statistically only from the T.09 isolate (Fig. 2). The *T. asperellum* and fungicide treatments reduced the disease progression rate compared to those of the control. However, there were no differences in disease progression rate among the *T. asperellum* isolates or the fungicide, with r ranging from 0.62 to 0.76 in experiment 1 and from 0.54 to 0.61 in experiment 2. The disease progression rate was best explained by the logistic model $Y = 1/1 + \exp(-(\beta + r \times t))$, where Y is the ratio of disease severity expressed as a percentage, β the integration constant, r is the disease progression rate, and t is the time in days (Campbell and Madden 1990). For both experiments, the selection of this model was based on a higher R^2 value, a lower mean square value and a plot of the standardized residuals ($y_{obs} - y_{exp}$) that did show any trends or values close to the x-axis (data not shown).

There was no significant difference between the fungicide and the isolates of *T. asperellum* concerning sheath blight severity, in all dates assessed, in experiment 2 trials. Additionally, no significant difference was observed among fungicide, T.52 and mixed-isolate for AUDPC; however, for the same parameter, the fungicide pencycuron statistically differed from T.09 and T.06.

3.2 Yield

Rice yield parameters differed among the treatments in experiment 1, except for number of grains per panicle (Table 1). Except for T.52, all treatments were associated with a panicle length that was higher than that of the control. The mixed-isolate treatment showed relatively higher values than the other treatments for grain mass/panicle, 100-grain weight, and productivity. The correlations between AUDPC and the yield parameters were negative (Table 2) for all evaluated parameters. The only statistically significant correlations with AUDPC were for panicle length (r=−0.298), grain weight (r=−0.317), and yield (r=−0.317). In experiment 2, all treatments improved yield and yield parameters (Table 1). Panicle weight and length and grain weight/panicle were statistically higher for all treatments compared to those of the control. The fungicide treatment was associated with the highest 100-grain mass, followed by the T.09, T.52, and mixed-isolate treatments. In all treatments, 100-grain mass was statistically

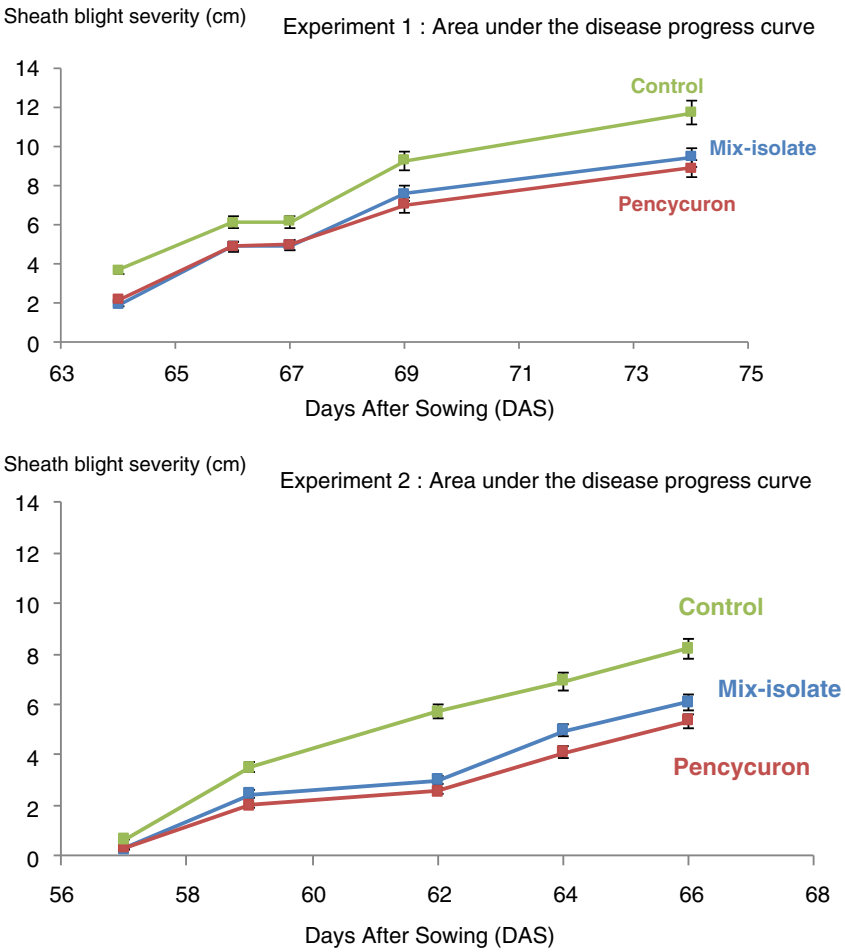

Fig. 2 Area under the disease progress curve (AUDPC) for rice plants treated with the biocontrol agent *Trichoderma asperellum* and the fungicide pencycuron in a tropical lowland environment, Belém, PA, November 2010 (experiment 1). Treatments consisted of seed treatment + one preventative spray + one curative spray (experiment 2). Treatments consisted of one preventative spray + one curative spray days after sowing

Table 1 Yield parameters from the sowing system experiment (experiment 1: November 2010) and transplanting system experiment (experiment 2: December 2011) for treatments with isolates of *Trichoderma asperellum* and the fungicide pencycuron for the control of sheath blight in rice in tropical lowland environments

Treatment[a]	Panicle			100-grain mass (g)	Yield (kg ha^{-1})
	Length (cm)	Grain number	Grain mass (g)		
Sowing system[b] (E1)					
T.06	21.58±0.24bc[c]	102.07±3.35a	2.37±0.17bc	2.30±0.12b	3.238.63ab
T.09	22.60±0.62abc	95.30±7.43a	2.23±0.15bc	2.37±0.12b	3.428.44ab
T.12	22.81±0.63abc	108.11±6.85a	2.24±0.17bc	2.06±0.06b	3.498.68ab
T.52	21.20±0.36c	93.48±7.51a	2.16±0.13bc	2.35±0.15b	3.045.64b
Mix	24.07±1.23a	101.10±9.51a	2.97±0.17a	3.12±0.34a	4.377.51a
Pencycuron	23.52±0.33ab	107.83±10.60a	2.55±0.18ab	2.49±0.25b	3.026.32b
Control	21.36±0.34c	98.38±5.12a	2.03±0.13c	2.05±0.08b	2.565.61c
CV (%)	8.72	20.65	21.68	24.81	38.64
Transplanting system[d] (E2)					
T.06	23.38±0.21a	4.27±0.14a	3.54±0.08a	2.61±0.06b	2.794.05a
T.09	23.58±0.22a	4.35±0.19a	3.58±0.14a	2.67±0.05ab	2.858.09a
T.12	23.70±0.32a	4.33±0.21a	3.46±0.11a	2.58±0.06b	2.748.40a
T.52	23.73±0.25a	4.41±0.16a	3.63±0.12a	2.63±0.08ab	2.818.35a
Mix	23.93±0.15a	4.63±0.18a	3.88±0.11a	2.65±0.07ab	2.882.87a
Pencycuron	23.58±0.26a	4.69±0.26a	4.01±0.20a	2.92±0.21a	3.092.18a
Control	21.48±0.27b	3.35±0.22b	2.78±0.19b	2.16±0.04c	2.135.08b
CV (%)	4.84	18.09	16.69	14.81	16.82

CV coefficient of variation

[a] T.06, T.09, T.12, and T.52 = *T. asperellum* isolates; mix = combination of four *T. asperellum* isolates; pencycuron (fungicide); control = water

[b] Sowing system experiment (experiment 1): seed treatment + one preventative spray + one curative spray (n = 8)

[c] Data [mean±standard error] followed by the same letter in the column do not differ by a Duncan test (P<0.05)

[d] Transplanting system experiment (experiment 2): one preventative spray+one curative spray (n = 12)

Table 2 Correlation coefficients for sheath blight and yield parameters in a sowing system experiment (experiment 1) and transplanting system experiment (experiment 2) with rice treated with isolates of *Trichoderma asperellum* in a tropical lowland environment

Parameter	Length	Number	Mass	100-grain mass	Yield
Sowing system (E1)					
AUDPC	−0.298*	−0.141	−0.317*	−0.214	−0.297*
Panicle length (cm)	–	0.205	0.488**	0.358**	0.274*
Number (grain number/panicle)		–	0.504**	−0.430**	−0.114
Grain mass (g)			–	0.550**	0.194
100-grain mass (g)				–	0.305*
Yield (kg ha^{-1})					–
Transplanting system (E2)					
AUDPC	−0.288**	−0.475**	−0.381**	−0.541**	−0.371**
Panicle length (cm)	–	0.379**	0.547**	0.185	0.501**
Number (grain number/panicle)		–	0.841**	0.584**	0.305**
Grain mass (g)			–	0.568**	0.448**
100-grain mass (g)				–	0.229*
Yield (kg ha^{-1})					–

AUDPC area under disease progress curve

*P<0.05; ** P<0.01; n=84

higher than in the control. AUDPC was statistically correlated with all yield parameters (Table 2).

R. solani is a plant pathogen with strong saprophytic abilities that forms sclerotia in the soil, and the availability of pesticides that control this pathogen is very low (Prabhu et al. 2002; Naeimi et al. 2010). Successful biological control of sheath blight by the bioagent *Trichoderma* has been recorded by several authors, mostly in greenhouse studies, with few studies conducted under field conditions (Krishnamurthy et al. 1999; Mathivanan et al. 2005; Silva et al. 2012). Our results showed that *T. asperellum* isolates suppressed sheath blight, promoted planted growth, and increased plant yields under tropical lowland field conditions. Seed treatment with *T. asperellum* followed by a single foliar spraying reduced sheath blight severity by 36 % (measured at 64 DAS), and seed treatment with *T. asperellum* followed by two foliar sprays reduced sheath blight severity by 21 % at the end of the epidemic (scored at 74 DAS). In experiment 2, sheath blight was reduced by 27 % at 66 DAS in transplants that were sprayed only once with *T. asperellum*. However, there were no significant differences in disease severity if plants were sprayed once or twice in experiment 2 (data not shown). In both experiments, treatments where fungicide was sprayed, sheath blight severity was lower by 25 and 35 %, respectively, when compared to that of the control. Silva et al. (2012) reported antibiosis by mycoparasitism and toxic compounds production for the same four *T. asperellum* isolates (T.06, T.09, T.12, T.52) used in this study on *R. solani*. We attribute the success of *T. asperellum* in suppressing sheath blight severity under field conditions to the same mechanisms described by Silva et al. (2012). Abdel-Fattah et al. (2007) found that applications of *Trichoderma harzianum* sprayed at 15-day intervals reduced the severity of brown spot on rice leaves grown in a field. The author noted that *Trichoderma* spp. can induce systemic and localized resistance by the plant and form an antagonistic relationship with the pathogen by directly attacking the pathogen or inhibiting pathogen growth and colonization of the plant. Even though we observed that seed treatment (experiments 1 and 2) reduced sheath blight severity and increased plant yields, it was not possible to confirm mechanisms of local or systemic resistance. However, we will seek evidence of this mechanism in future experiments. Although sheath blight is caused by a resident soil pathogen, it showed polycyclic behavior, evidenced by rapid disease development within the same host cycle.

Seven days after pathogen inoculation, sheath blight severity increased 44 and 76 % in experiments 1 and 2, respectively. Based on the control treatment, it was observed that the daily growth rate of disease severity was 0.8 cm, in both experiments. We believe that the rapid development of sheath blight was due to the aggressiveness of the pathogen and the favorable tropical lowland environmental conditions, such as high temperature and humidity. Based on the epidemiological parameters we measured, AUDPC and *r*, a logistic model best explained sheath blight development (based on the high R^2 value, low mean square value, and plot of standardized residuals in both experiments). We found decreases in AUCPD of 22 and 34 % for plants sprayed with *T. asperellum* in experiments 1 and 2, respectively. Based on the values for disease progression rate of 28 and 17 % in experiment 1 and 2, respectively, *T. asperellum* reduced the increment of daily progression of the disease.

Disease severity and AUCPD were higher in experiment 1 compared to those in 2, in which was combined seed treatment and foliar spray with *Trichoderma*. This result is similar to those reported by Tewari and Singh (2005) and Naeimi et al. (2010) that a spore suspension sprayed on the leaves significantly reduced sheath blight severity and was more effective than soil treatment or seedling root dip. However, these studies were conducted in greenhouse conditions, with no seed treatment combined with spore suspension sprayed on the leaves and yield was not evaluate.

In the presented study, sheath blight negatively affected all yield parameters (Table 2). Although the plants treated with *T. asperellum* presented an increase in yield in both experiments, the best treatment for reducing disease severity has not provided the greatest productivity gain. In experiment 1, plants treated with the mixed-isolate (combination of all four *T. asperellum* isolates) presented increases of 34 % in 100-grain weight, 41 % in yield, and 19 % in disease severity reduction compared to those in the control. In contrast, in experiment 2, the mixed-isolate treatment was associated with an increase of only 18.5 % in 100-grain mass and of 26 % in yield and 26 % in disease severity reduction. This result indicates that first of all, the combination of four *T. asperellum* isolates had a synergistic effect and the application method, seed treatment followed by foliar spray completed each other, allowing that the biological agent *T. asperellum* acted not only as a disease antagonist but also as a growth promoter.

Based on greenhouse and laboratory studies by Silva et al. (2012), it demonstrated that the application of the same four isolates of *Trichoderma* to rice plants, grown under greenhouse conditions, resulted in increased biomass, root length, and plant size, and reduced the severity of sheath blight. Among the known mechanisms involved in achieving these results was the production of phytohormones such as indoleacetic acid (IAA), the production of biomolecules involved in metabolic pathways that cause walling off of the *Trichoderma* thallus, phosphate solubilization, and induced systemic resistance.

The fungicide treatment also increased 100-grain weight and yield by 20 and 15 %, respectively, compared to that of the control, in experiment 1. However, it presented 30 % less in productivity when compared to the treatment composed of *T. asperellum* mixed-isolate. In contrast, in experiment 2, although these two treatments were similar, fungicide

treatment increased productivity in 30 % when compared to that of the control (Table 2). Fungicide seed treatments provide protection only during the residual period. In this way, fungicides are different from biological agents, which provide protection for longer period during the process of germination and seedling growth because the agents colonize the host.

The benefits of *Trichoderma* spp. for plant growth and yield have been observed in greenhouse and field experiments, especially when certain isolates are used in combination (Harman et al. 2004; Hoyos-Carvajal et al. 2009). Reduced sheath blight and increased yields have been observed in rice (Mathivanan et al. 2005) and other crops (Raj et al. 2005; Saber et al. 2009; Tchameni et al. 2011), although the mechanisms involved mechanisms are not yet fully understood. There are many variables at play in the complex interactions between host-pathogen-antagonists under field conditions. Therefore, additional studies are needed to elucidate all the modes of action by which *Trichoderma* can reduce sheath blight and promote growth (Howell 2003; Harman 2006; Vinale et al. 2008).

4 Conclusion

We demonstrated that mixed isolates of *T. asperellum* combined on seed treatment and foliar spray was efficient on reducing disease severity and increasing yield and grain weight, in cultivated rice under flooded conditions. Considering all of the parameters evaluated in this study, the treatments that included the bioagent *T. asperellum* showed a level of efficiency that was similar to that of the fungicide pencycuron in both experiments. Trichoderma as a plant growth promoter could play an important role in maintaining sustainable rice production in the Amazon, by promoting increases in yield and reducing the contamination of the rivers, which sustain the floodplains used for household and large-scale production. To insert *Trichoderma* in the sheath blight integrated management, some studies will be necessary regarding the benefit cost, the establishment of a process for large-scale production, and official reports to enforcement of the law. Despite the long distance between scientific research and market, the demand for sustainable production systems and food quality by society will continues to push this kind of investigations.

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