

# Mechanisms involved in the biocontrol of rice sheath blight by *Waitea circinata*<sup>1</sup>

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## ABSTRACT

The BRS Tropical rice cultivar was developed for tropical floodplains, but is susceptible to sheath blight, making the *Waitea circinata* bioagent an important tool in managing the disease. This study aimed to assess the *W. circinata* mechanisms involved in the parasitism and resistance induction to sheath blight. The *in vitro* antagonism was assessed by optical microscopy and scanning electron microscopy, followed by quantification of the lytic enzymes involved in parasitism, such as chitinase, glucanase and protease. An effect of the *W. circinata* mycoparasite against *Rhizoctonia solani* was observed. The *W. circinata* application suppressed the sheath blight by up to 65 % and increased the chitinase, glucanase and lipoxigenase activity 72 h after the inoculation and that of the peroxidase 96 h after the inoculation. Thus, *W. circinata* showed to be efficient in suppressing sheath blight by parasitism and induced resistance via the activation of biochemical mechanisms.

KEYWORDS: *Oryza sativa*, *Rhizoctonia solani*, biological control.

## RESUMO

Mecanismos envolvidos no biocontrole da queima da bainha do arroz por *Waitea circinata*

A cultivar de arroz BRS Tropical foi desenvolvida para várzeas tropicais, mas é suscetível à queima da bainha, tornando o bioagente *Waitea circinata* uma importante ferramenta no manejo da doença. Objetivou-se avaliar os mecanismos de *W. circinata* envolvidos no parasitismo e na indução de resistência à queima da bainha. O antagonismo *in vitro* foi avaliado por microscopia óptica e microscopia eletrônica de varredura, seguida da quantificação das enzimas líticas envolvidas no parasitismo, como quitinase, glucanase e protease. Observou-se efeito do micoparasita *W. circinata* contra *Rhizoctonia solani*. A aplicação de *W. circinata* suprimiu a queima da bainha em até 65 % e aumentou a atividade da quitinase, glucanase e lipoxigenase 72 h após a inoculação e a da peroxidase 96 h após a inoculação. Assim, *W. circinata* mostrou-se eficiente em suprimir a queima da bainha por parasitismo e induziu resistência por meio da ativação de mecanismos bioquímicos.

PALAVRAS-CHAVE: *Oryza sativa*, *Rhizoctonia solani*, controle biológico.

## INTRODUCTION

Rice is one of the most widely produced and consumed cereals worldwide and a staple food of over half the global population. The estimated global production is more than 475 million metric tons, 11 million of which are produced in Brazil, which is the largest rice producer outside the Asian continent (FAO 2020). The sustainability of rice production systems is a pressing concern in maintaining food security and supporting economic growth (Devkota et al. 2020).

Sheath blight is a destructive disease caused by the necrotrophic fungus *Rhizoctonia solani* Kühn and

occurs in rice-producing areas of the world (Shu et al. 2019). Disease symptoms include damping off and plant death, causing losses of up to 50 % (Uppala & Zhou 2018).

Since there are no rice cultivars resistant to sheath blight (Bhaskar-Rao et al. 2020), the disease is controlled via foliar application of fungicides and crop management strategies (Silva et al. 2012). Pesticides are known to cause mortality in nontarget species, contaminate aquatic environments, change biodiversity and exacerbate a number of different types of cancer, neurodegenerative and autoimmune diseases (Nascimento et al. 2020).

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The number of fungicide applications may be reduced by implementing biocontrol as part of the sustainable management of this disease. The biological control of sheath blight is basically performed by a single fungal agent (*Trichoderma* spp.) (Brasil 2022), making it vital to investigate the effect of other bioagents, such as mycorrhizal fungi.

Orchid mycorrhizal fungi are bioagents little explored in the literature. The most prominent is *Waitea circinata*, which exhibits nonspecific interactions with rice and orchids (Carvalho et al. 2015, Sousa et al. 2019) and suppresses rice blast (Carvalho et al. 2021).

This is the first study to investigate the role of *W. circinata* in suppressing a disease caused by a necrotrophic pathogen. Necrotrophic pathogens are notorious for their aggressive and wide-range virulence strategies that cause the death of host cells from which they obtain nutrients for growth (Mengiste 2012).

As one of the most productive rice cultivars, BRS Tropical is still widely used, and its adaptation to tropical conditions means it may reach up to 5,145 kg ha<sup>-1</sup>; however, it is highly susceptible to sheath blight (Jordão et al. 2020). As such, the present study presents a more sustainable management strategy for sheath blight in a rice cultivar adapted to tropical conditions using *W. circinata* as a biocontrol agent.

Thus, this study aimed to assess the *W. circinata* mechanisms involved in parasitism and inducing sheath blight resistance.

## MATERIAL AND METHODS

*W. circinata* was obtained from orchid roots and described by Sousa et al. (2019) and Carvalho et al. (2022), while *Rhizoctonia solani* was obtained by collection of multifunctional microorganisms from the Embrapa Arroz e Feijão. The isolates were multiplied on potato-dextrose-agar medium (PDA; 20 g of agar, 20 g of dextrose, 1 L of water and 200 g of potato) in Petri dishes, and incubated under continuous fluorescent light for 7 days at room temperature (25 ± 2 °C). The experiments were carried out from June 2017 to July 2020, with 3 replicates.

A dual plate assay between *W. circinata* and *R. solani* was conducted based on the procedure described by Carvalho et al. (2015), using a completely randomized design, with 3 treatments

(*R. solani* x *W. circinata*, *R. solani* and *W. circinata*) and 3 replicates. Mycelial disks (9 mm) from the 3 treatments were placed in the Petri dishes containing PDA, one in the center and one on each end, spaced approximately 3 cm apart. The plates were incubated at 27 °C, for 15 days, in the presence of continuous light.

Assessments were performed when the *R. solani* colony reached the edge of the dish. The horizontal diameter of the pathogen colonies in both assays was measured with a digital caliper in a laminar flow chamber, at 27 °C, under continuous white light. The colony area and reductions were calculated based on the method described by Carvalho et al. (2015).

Microscopic analysis was performed at 10 days after pairing, by observing the contact region between the isolates, under an optical microscope (BelPhotonics) coupled to the BelView software. Scanning electron microscopy was performed as it follows: after *R. solani* x *W. circinata* was cultured on MN-615® filter paper immersed in PDA for 7 days, the filter paper was removed and desiccated with Neon® blue silica gel (1-4 mm) until fully dry (10 days). Five-millimeter fragments of filter paper containing the contact region between the hyphae were transferred to a stub using double-sided tape and analyzed under a scanning electron microscope (Jeol - JSM6610) equipped with EDS (Thermo Scientific NSS Spectral Imaging) (adapted from Chaibub et al. 2016).

*W. circinata* and *R. solani* mycelia grown in PDA plates were removed with a scalpel. One gram of each fungus was inoculated into Erlenmeyer flasks containing 250 mL of TLE liquid medium (KH<sub>2</sub>PO<sub>4</sub>: 2 g L<sup>-1</sup>; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>: 1.4 g L<sup>-1</sup>; MgSO<sub>4</sub>: 0.3 g L<sup>-1</sup>; CaCl<sub>2</sub>: 0.15 g L<sup>-1</sup>; FeSO<sub>4</sub>: 0.005 g L<sup>-1</sup>; MnSO<sub>4</sub>: 0.016 g L<sup>-1</sup>; ZnSO<sub>4</sub>: 0.014 g L<sup>-1</sup>). The treatments consisted of *W. circinata*, *W. circinata* + *R. solani* and *R. solani*. The flasks were incubated in a rotary shaker at 28 °C and 140 rpm, and aliquots of 1 mL were collected every 24 h up to 168 h of incubation. After this period, the cultures were filtered and used as a source of enzymes (adapted from Almeida et al. 2007 and Geraldine et al. 2013).

*In vitro* glucanase, chitinase and protease activity were determined using 1 % laminarin and colloidal chitin in sodium acetate buffer (50 mM; pH 5.0) and 0.25 % azocasein in 50 mM phosphate buffer (pH 5.0) (Almeida et al. 2007, Geraldine et al. 2013). The experiments were performed for each enzyme

and treatment, using a completely randomized design, with three treatments and 3 replicates per hour for each enzyme.

In order to assess the pathogenicity of *W. circinata* to rice plants, seeds of the BRS Tropical rice cultivar, susceptible to sheath blight, were disinfected and sown in plastic pots containing 3 kg of soil fertilized with 5 g of NPK (5-30-15), 1 g of Zn and 3 g of ammonium sulfate. Ten seeds were sown per pot and, after 20 days, 5 plants were kept in each pot. The experimental design was completely randomized, with 4 treatments and 25 replicates. The treatments consisted of *W. circinata* disks, *W. circinata* sprayed at a flow rate of 5 g L<sup>-1</sup>, *W. circinata* sprayed at a flow rate of 10 g L<sup>-1</sup> and control (sprayed with water).

A fungal disk (9 mm in diameter) was applied directly to the soil, at the base of the sheath of each plant, at 20 days after sowing (DAS). *W. circinata* mycelia grown on PDA were scraped, weighed and diluted in autoclaved distilled water to obtain 2 suspensions with concentrations of 5 and 10 g L<sup>-1</sup>. The leaves and sheaths were sprayed at 55 DAS and the plants kept under high humidity (95-100 %), with temperatures of 27-30 °C during the day and 22-25 °C at night (Prabhu et al. 2002, Mosquera-Espinosa et al. 2013). The plants were assessed every 2 days, for 10 days, observing the presence or absence of symptoms on the roots, leaves and sheaths.

Sowing and fertilization were conducted to compare *W. circinata* application methods in irrigated rice plants in three assays (A1, A2 and A3), performed as previously described for the pathogenicity assay:

A1: 4 treatments and 25 replicates, as it follows: *R. solani*; *R. solani* + *W. circinata* disks; *R. solani* + *W. circinata* sprayed at a flow rate of 5 g L<sup>-1</sup>; *R. solani* + *W. circinata* sprayed at a flow rate of 10 g L<sup>-1</sup>. At 20 DAS, a disk (9 mm in diameter) was applied directly to the soil, at the base of the sheath of each plant, followed by inoculation with *R. solani* at 55 DAS. The sheaths and leaves were sprayed with the mycelial suspension at 55 DAS, at the same time as *R. solani*. The inoculation with *R. solani* was performed based on the method described by Prabhu et al. (2002), using autoclaved wooden toothpicks containing pathogen mycelia, which were inserted between the sheath and the penultimate leaf;

A2: aimed at comparing the best treatments from A1, consisting of 3 treatments and 25 replicates: *R. solani*; *R. solani* + *W. circinata* disks; *R. solani* + *W. circinata*, sprayed at a flow rate of 5 g L<sup>-1</sup>;

A3: performed with leaf and sheath samples to quantify the activity of defense enzymes and conduct scanning electron microscopy using the best A2 treatment, consisting of 4 treatments and 10 replicates: water (absolute control); *R. solani*; *W. circinata*; *R. solani* + *W. circinata* disks.

For all 3 assays, the plants were kept in a greenhouse under high humidity (95-100 %), daytime temperatures of 27-30 °C and 22-25 °C at night (Prabhu et al. 2002, Mosquera-Espinosa et al. 2013).

The presence and size of lesions were monitored every 2 days post-inoculation (DPI) (2, 4, 6, 8 and 10 DPI). Severity was calculated based on the previous evaluation (Prabhu et al. 2002), whereby the size of the lesion relative to the tiller was equal to its size divided by the total tiller length. The area under the disease progress curve (AUDPC) was calculated in accordance with Silva et al. (2012).

Scanning electron microscopy was carried out for all four treatments in the third assay (A3), using the collected plant fragments, which were fixed, dehydrated and attached to stubs for visualization (Chaibub et al. 2020, Sousa et al. 2019).

Samples of individual sheaths and leaves (biological replicates) were collected for the four A3 treatments, resulting in 3 replicates per treatment at 0, 24, 48, 72 and 96 h after inoculation (HAI) with the pathogen. Next, protein extraction and quantification were performed for each sample (Bradford 1976). The activity (U mg<sup>-1</sup>) of chitinase, glucanase, lipoxygenase, peroxidase and phenylalanine ammonium lyase were quantified *in vivo* (Chaibub et al. 2016).

Univariate analysis was performed for severity, and analysis of variance (Anova) with the Tukey test ( $p < 0.05$ ) for AUDPC and enzymatic activity, using the package Agricolae of the R<sup>®</sup> software, version 3.4.3. The Box-Cox transformation was applied to meet the assumptions of the Anova. Multivariate analysis was applied to determine the variables severity and enzymes in the third assay. For the multivariate analyses, the data set was standardized, so that each variable was maintained with null mean and unit variance. The variables were organized in a matrix for principal component analysis (PCA), using the Past<sup>®</sup> software, version 2.16.

## RESULTS AND DISCUSSION

When paired with *W. circinata*, the *R. solani* pathogenic isolate colony formed fewer smaller

sclerotia (Figure 1C) than the control (Figure 1A), but *W. circinata* did not significantly inhibit the *R. solani* colony growth (Figure 1C).

When paired with *R. solani*, the *W. circinata* colony exhibited a mycelial mass with a more vivid yellow color than the control (Figures 1B and 1C). Under an optical microscope, the *W. circinata* hyphae were thinner and coiled around the hyphae of the pathogen, which were much thicker (Figure 1E). The same was detected by scanning electron microscopy (Figure 1F). A dense hyphal coiling of *W. circinata* around *R. solani* hyphae was observed, demonstrating a mycoparasite effect.

In this form of parasitism, the fungus uses pathogen structures as a source of nutrients, thus preventing host growth, reducing sclerotium production and causing cell wall degradation (Asad et al. 2014). In other study, *W. circinata* inhibited other rice pathogens with *Magnaporthe oryzae*, *Cochliobolus miyabeanus*, *Monographella albescens* and *Sarocladium oryzae* (Carvalho et al. 2022).

*W. circinata* and *R. solani* secreted glucanase, chitinase and protease, when cultured in TLE during the study period (Figure 2). For glucanase, *R. solani* showed the highest activity (6.86, 5.66 and 9.60 U mg<sup>-1</sup> at 24, 72 and 96 h, respectively)

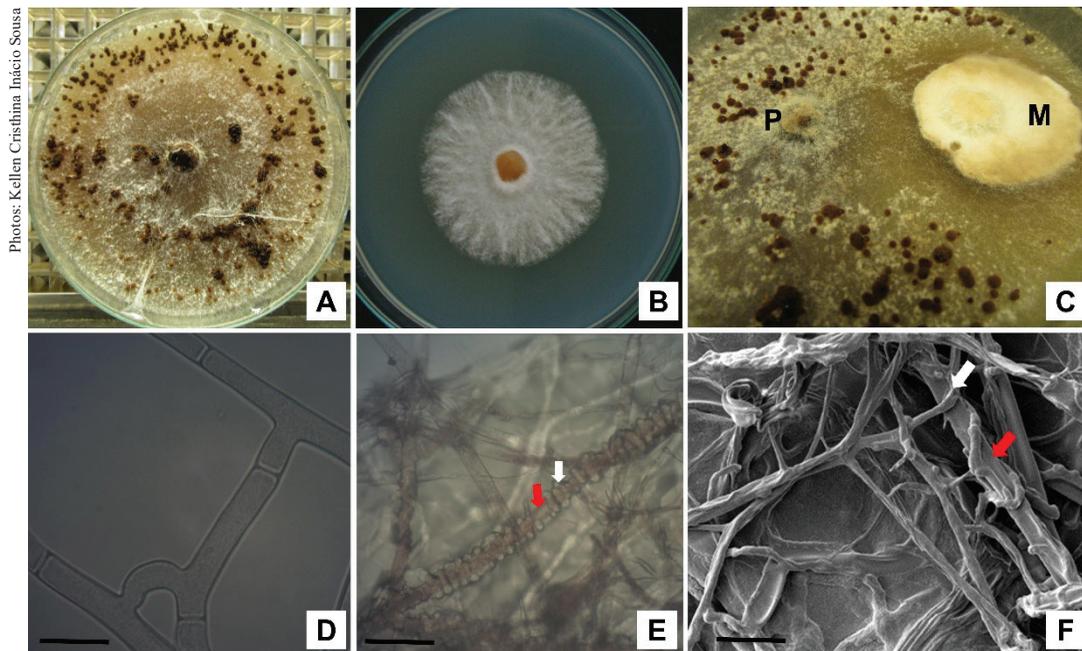


Figure 1. *In vitro* interaction between *Waitea circinata* and *Rhizoctonia solani*: A) *R. solani* in PDA culture medium; B) *W. circinata* in PDA culture medium; C) *R. solani* pathogen (P) and *W. circinata* mycorrhizal fungus (M) paired in Petri dishes; D) *R. solani* hyphae; E) *W. circinata* hyphae (white arrow) coiled around *R. solani* hyphae (red arrow) under an optical microscope; and F) under a scanning electron microscope. Bars: 10 µm.

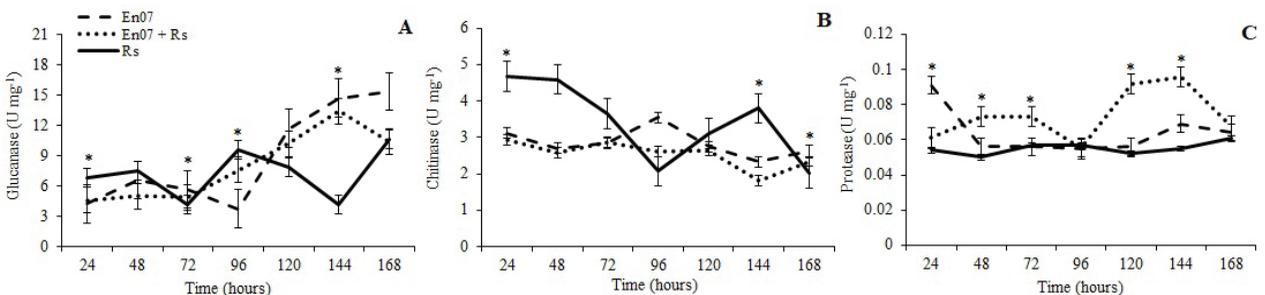


Figure 2. *In vitro* enzymatic activity of *Waitea circinata* in co-culture with *Rhizoctonia solani*: A) glucanase; B) chitinase; C) protease. \* Means significantly different, according to the Tukey test ( $p < 0.05$ ). Bars indicate the mean standard error.

and *W. circinata* at 72 and 144 h, respectively with 5.66 and 14.7. When *W. circinata* + *R. solani* were cultivated together, the greatest activity occurred at 96 and 144 h (7.63 and 13.42 U mg<sup>-1</sup>) (Figure 2A). For chitinase, *R. solani* exhibited the highest activity at 24 and 144 h (4.67 and 3.80 U mg<sup>-1</sup>) and *W. circinata* at 168 h (2.63 U mg<sup>-1</sup>). There was no difference between *W. circinata* + *R. solani* and *W. circinata* and *R. solani* alone (Figure 2B). For protease, *R. solani* alone did not differ from *W. circinata* and *W. circinata* + *R. solani*. The highest activity for *W. circinata* was observed at 24 h (0.091 U mg<sup>-1</sup>), and for *W. circinata* + *R. solani* at 48, 72, 120 and 144 h (0.073, 0.073, 0.092 and 0.096 U mg<sup>-1</sup>) (Figure 2C).

The high lytic enzyme production observed during the bioagent-pathogen interaction demonstrates the bioagent's parasitism of the pathogen as a source of nourishment, as previously reported for bioagents such as *Cladosporium cladosporioides* and *Trichoderma* spp. (Geraldine et al. 2013, Chaibub et al. 2020). However, this is the first such report for *Waitea circinata*.

In the pathogenicity assay, no spots or lesions were observed on sprayed or disk-inoculated rice plants (Figures 3B and 3C) or controls sprayed only with water (Figure 3A). In addition, *W. circinata* mycelia were observed in the soil (Figure 3D), in close proximity to the sheaths and roots, albeit without causing damage.

The present study showed that the two *W. circinata* application methods (via spraying or mycelial disks applied directly to the soil) did not damage the BRS Tropical rice plants at 20 and 55 DAS. Carvalho et al. (2015) inoculated BRS Primavera rice plants with *W. circinata* and also found

that the fungus is not pathogenic to rice, confirming our results of no pathogenicity during the vegetative or reproductive stages and corroborating Mosquera-Espinosa et al. (2013).

In A1, the disease severity in all the treatments was lower than that of the control (6.77 cm), particularly for simultaneous spraying (5 g L<sup>-1</sup>) + pathogen (1 cm), early application of disks + pathogen (2.23 cm) and simultaneous spraying (10 g L<sup>-1</sup>) (2.2 cm) (Figure 4A). The AUDPCs for the treatments with disk inoculation or simultaneous spraying with 5 g L<sup>-1</sup> or 10 g L<sup>-1</sup> were 4.02, 11.32 and 12.89, respectively, and 25.70 for the controls (Figure 4B).

In A2, the treatments with disk inoculation and simultaneous spraying (5 g L<sup>-1</sup>) showed disease severity of 1.52 and 1.87 cm, respectively, and 5.35 cm for the controls (Figure 4C). The AUDPCs for mycelial disks and simultaneous spraying (5 g L<sup>-1</sup>) were 3.8 and 5.19, respectively, and 14.25 for the control treatment (Figure 4D).

There was a difference between disk inoculation (5.4 cm) and control (14.3 cm) in A3 (Figure 4E). The AUPDC was also smaller in the disk treatment (18.1) than in the controls (53.75) (Figure 4F). The lesions in the mycorrhized and inoculated plants were light brown and did not coalesce. Plants inoculated with the pathogen exhibited dark brown lesions and necrosis at 10 DAS (Figures 5C and 5D), forming sclerotia near the inoculum (Figure 5D).

Scanning electron microscopy images showed closed stomata and normal glandular trichomes (Figure 5A) on the leaves of plants sprayed only with water, while in mycorrhized plants the glandular trichomes in the guard cells closed the stomata (Figures 5A and 5B). Leaves submitted to

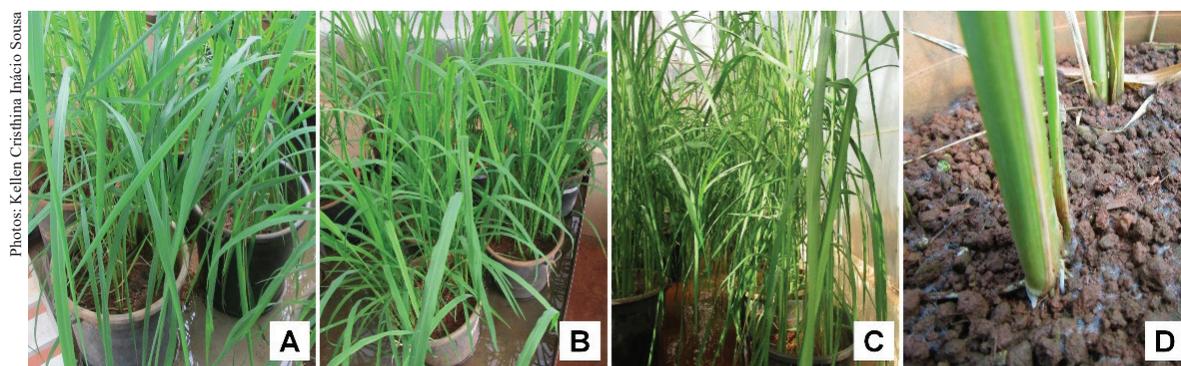


Figure 3. Evaluation of *Waitea circinata* pathogenicity in rice plants: A) plants sprayed only with water; B) plants sprayed with 5 g L<sup>-1</sup>; C) plants sprayed with 10 g L<sup>-1</sup>; D) plants inoculated with 9-mm disks of *W. circinata* applied to the soil.

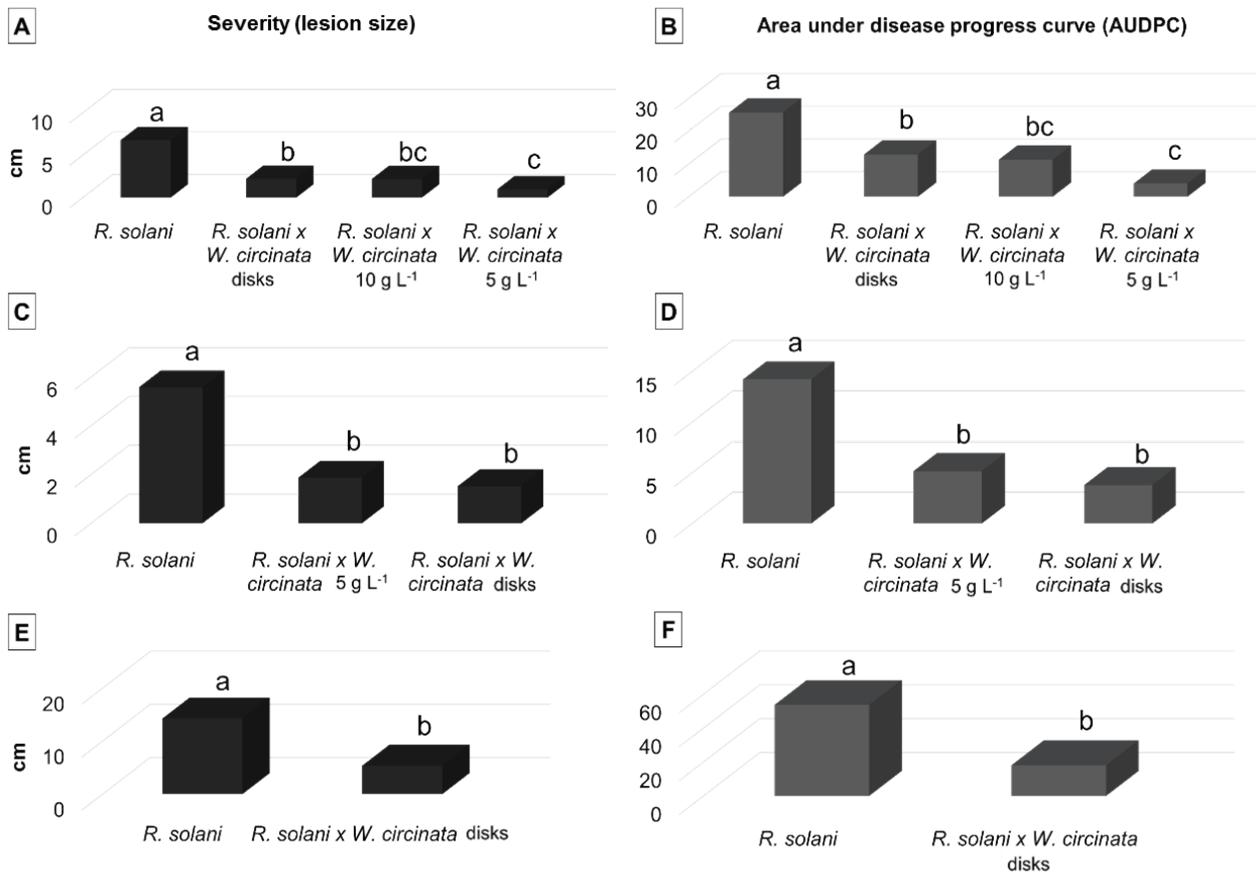


Figure 4. Sheath blight severity (A, C and E) and area under the disease progress curve - AUDPC (B, D and F) for the greenhouse assays (A1: A-B; A2: C-D; A3: E-F) with *Waitea circinata* applied beforehand (disks) or simultaneously (sprayed with 5 and 10 g L<sup>-1</sup>).

mycorrhization and inoculation with the pathogen exhibited a waxy layer (Figure 5E) and the presence of crystals on the abaxial surface (Figure 5F), whereas in control plants the leaves displayed several pathogenic hyphae (Figure 5C) and the sheaths were necrotic with mycelial growth of the pathogen (Figure 5D).

In the present study, there was a 73 % reduction in the size of the sheath blight lesions and a 77 % decrease in the AUDPC, when *W. circinata* was sprayed on rice plants simultaneously to *R. solani* inoculation by segment deposition. Plants previously inoculated with *W. circinata* disks and subsequently with the pathogen showed lesion size and AUDPC reductions of 62 and 65 %, respectively. Similarly, Mosquera-Espinosa et al. (2013) found that rice plants submitted to mycorrhization with *Ceratobasidium* spp. exhibited smaller lesions than those on plants inoculated with *R. solani* alone. Carvalho et al. (2022) demonstrated rice blast suppression in plants sprayed

with 10 g L<sup>-1</sup> of *W. circinata* by up to 84 %. Sprays of spore suspensions on leaves and seed treatment with other fungi with *Trichoderma* sp. reduced rice sheath blight in field conditions (Tewari & Singh 2012, França et al. 2015). There was an increase in the chitinase activity at 72 HAI in plants with early application of *W. circinata* and later challenged with the pathogen (*W. circinata* x *R. solani*), when compared to plants treated only with *W. circinata* or the pathogen (Figure 6A). A significant increase in glucanase activity was also observed at 72 HAI in plants treated with *W. circinata* x *R. solani*, in relation to the other treatments (Figure 6B).

The lipoxygenase activity increased significantly at 72 HAI in plants treated with *W. circinata* x *R. solani* (Figure 6C), differing significantly from those that received the other treatments. There was no significant difference between treatments for phenylalanine ammonia-lyase activity at any of the assessed times (Figure 6D). However, the peroxidase

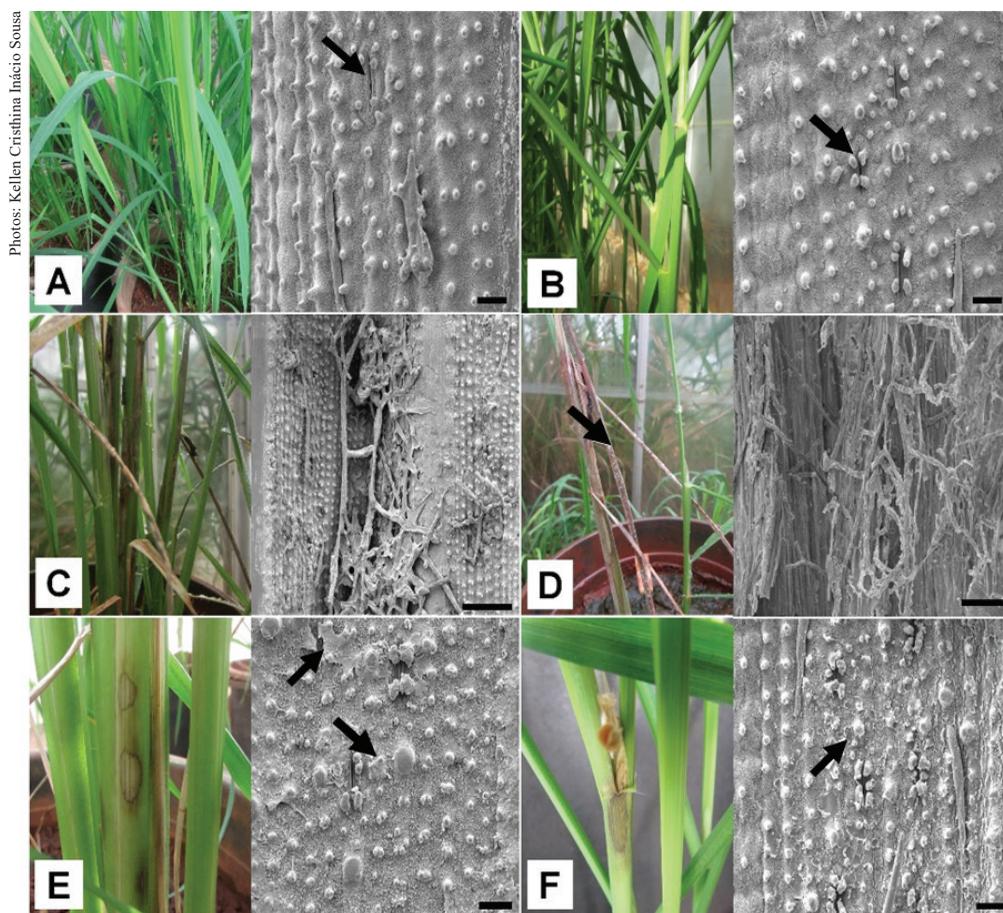


Figure 5. *In vivo* interaction between *Waitea circinata* and *Rhizoctonia solani*, with rice plants in a greenhouse (left) and respective scanning electron microscopy (right): A) plants sprayed only with autoclaved distilled water; B) plants treated with *W. circinata* soil disks (20 days after sowing - DAS); C-D) plants inoculated only with *R. solani* at 55 DAS, large number of hyphae on leaves (C), sheath degraded by the pathogen (D); E-F) plants treated with *W. circinata* disks at 20 DAS and subsequently inoculated with *R. solani* at 55 DAS. Arrows: A-B) glandular trichomes in the guard cells closed the stomata; D) necrotic sheaths with mycelial growth of the pathogen; E) waxy layer; F) crystals. Bars: 10  $\mu\text{m}$  (A, B, E, F) and 50  $\mu\text{m}$  (C, D).

activity increased significantly at 96 HAI in plants treated with the pathogen alone (Figure 6E).

The chitinase activity at 72 HAI was positively correlated (0.9997) with sheath blight severity (Figure 6A). A positive correlation was also observed between the chitinase activity at 48 HAI and phenylalanine ammonia-lyase activity at 96 HAI (0.99964), and between the lipoxygenase activity at 48 HAI and glucanase activity at 96 HAI (0.99799). On the other hand, there was a negative correlation between the peroxidase activity at 48 HAI and chitinase activity at 96 HAI (-0.99789) and between the phenylalanine ammonia-lyase and peroxidase activity at 96 HAI (-0.9974) (Figure 7A).

The PCA showed that the first two components accounted for 89.77 (component 1) and 11.58 %

(component 2) of the total variance (Figure 7B). As expected, there was no correlation between any factors for plants treated with *W. circinata* and the variable severity, contrary to the results obtained for plants inoculated with the pathogen (Figure 7B). Based on the analysis of variance (Figure 6), the PCA indicated a correlation between the chitinase activity at 72 HAI and the presence of the pathogen, in addition to increased glucanase activity at 72 h in the *W. circinata* x *R. solani* treatment.

In plants treated with *W. circinata* disks, the chitinase and glucanase activity increased significantly at 72 and 48 HAI, respectively, indicating that *W. circinata* can trigger primary defense mechanisms in the plant. A greater chitinase and glucanase activity in rice and bean plants treated

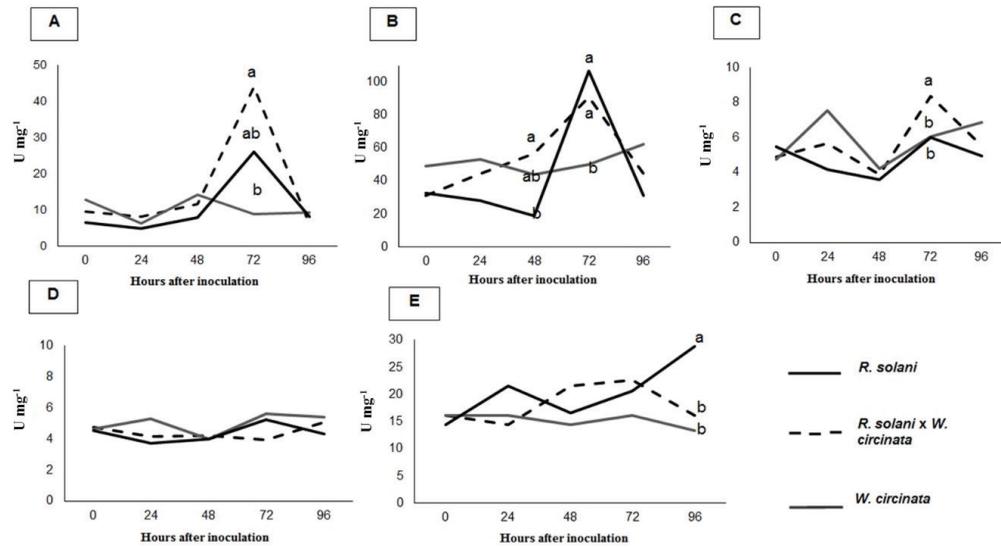


Figure 6. *In vivo* enzymatic activity [A) chitinase; B) glucanase; C) lipoxygenase; D) phenylalanine ammonia-lyase; E) peroxidase] in rice plants treated with *Waitea circinata* and subsequently inoculated with the pathogen *Rhizoctonia solani* (*R. solani* x *W. circinata*), treated with *W. circinata* alone and inoculated only with the pathogen (*R. solani*).

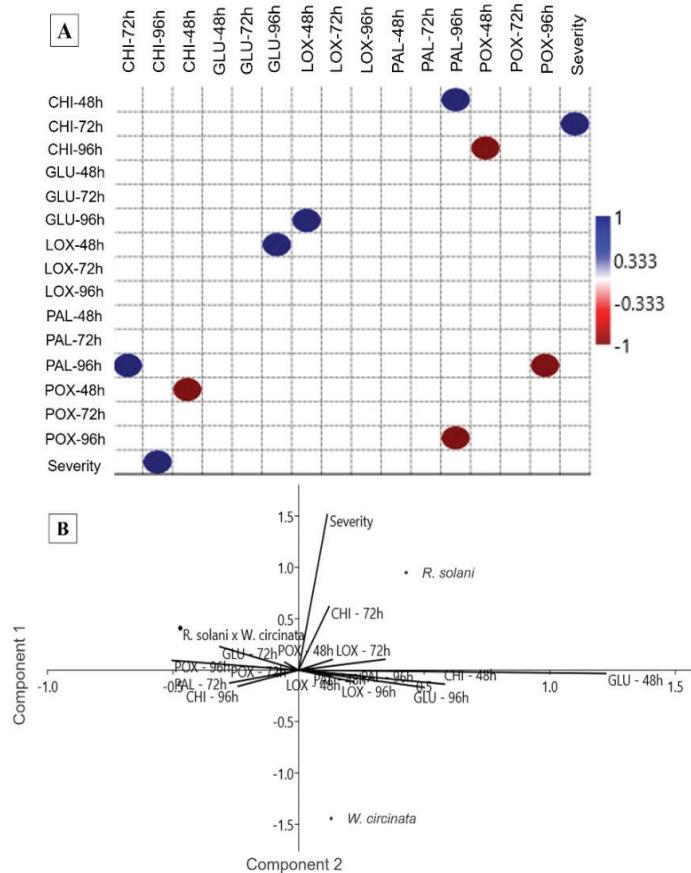


Figure 7. Multivariate analysis of enzymatic activity and disease severity for plants treated only with *Waitea circinata*, inoculated with *Rhizoctonia solani* alone or treated with *W. circinata* and inoculated with *R. solani*: A) Pearson's linear correlation, with Bonferroni correction, between chitinase (CHI), glucanase (GLU), lipoxygenase (LOX), phenylalanine ammonia-lyase (PAL) and peroxidase (POX) activity and severity; B) principal component analysis of the studied variables (enzymes: CHI, GLU, LOX, PAL and POX and severity).

with other fungal biological control agents, such as *Trichoderma* sp., *Cladosporium* sp. and *Epicoccum* sp., has also been reported (Silva et al. 2012, Sena et al. 2013, Mayo et al. 2015, Chaibub et al. 2016). The lipoxygenase activity doubled at 72 HAI in rice plants treated with *W. circinata* and the pathogen. The peroxidase activity increased at 96 HAI in plants inoculated with the pathogen alone. This indicates a possible programmed response to the phytotoxins released by *R. solani*, since the pathogen causes necrosis a few days after infection, what did not occur in plants treated with *W. circinata*.

Studies have suggested that these enzymes are directly involved in defense pathways mediated by salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). LOX is related to both the JA and POX pathways, as well as SA synthesis pathways, apoptosis and hypersensitive reaction (HR) (Mengiste 2012, Nair et al. 2015, Mayo et al. 2015). The activity of antioxidants such as superoxide dismutase (SOD) and guaiacol peroxidase (GPX) was also observed in rice plants inoculated with the endophytic fungus *Piriformospora indica* against *R. solani* (Nassimi & Taheri 2017).

The correlation between sheath blight severity and chitinase activity at 72 HAI explains the rice defense response to *R. solani*. Plants treated with *W. circinata* and subsequently inoculated with the pathogen showed a greater enzyme activity (chitinase, glucanase and lipoxygenase) than those inoculated only with the pathogen (peroxidase) or *W. circinata*. These results, along with the shape of the glandular trichomes near the stomata and wax deposition on the leaves evident in scanning electron microscopy, indicate the activation of preestablished mechanisms in plants treated with *W. circinata*.

Our results suggest that *W. circinata* was efficient at suppressing sheath blight by parasitism and induced resistance via the activation of biochemical mechanisms, and may, therefore, be included in sheath blight management strategies for the studied rice cultivar.

## CONCLUSION

The parasitism exerted by *Waitea circinata*, in addition to its *in vivo* effect in suppressing sheath blight and activating the defense system, was proven by the activity of enzymes in the plant, showing its ability to act in different ways.

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