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Enzyme-Induced Defense Response in the Suppression of Rice Leaf Blast (*Magnaporthe Oryzae*) By Silicon Fertilization and Bioagents

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Abstract: *The effects of silicon fertilization and bioagents in suppressing leaf blast were studied in two greenhouse experiments using a susceptible upland rice cultivar, BRS Primavera. In the first experiment, the treatments consisted of four doses of SiCaMg (1,2,4 and 8 tons Si.ha^{-1}) and four bioagent (Burkholderia pyrrocinia (Bp); Pseudomonas fluorescens (Pf); Trichoderma asperellum (Ta) and a mixture of the three bioagents (Bp+Pf+Ta), in addition to untreated controls. The most effective treatment was silicon fertilization at 2tons in combination with the three bioagents, which reduced leaf blast by 96%. A second experiment was conducted to study the defense mechanism involved in disease suppression. The treatments were organized in a randomized block design, with or without Si (0 or 2 tons Si) and bioagents (non-inoculated control, Ta or Bp+Pf+Ta). The combination of silicon fertilization (2 tons SiCaMg.ha^{-1}) with T. asperellum or with a mixture of the three bioagents increased the activities of chitinase (CHI), β-1,3glucanase (GLU), peroxidase (POX) and phenylalanine ammonia-lyase (PAL) as well as the salicylic acid (SA) content in rice plants in the absence of the pathogen Magnaporthe oryzae. The mixture of the three bioagents in plants fertilized with 2 tons Si increased GLU enzyme activity and SA levels at 24 and 48 hours after infection of the plants challenged with M. oryzae. The activation of defense mechanism by combination of silicon fertilization with bioagent mixture could be an effective strategy for rice blast management.*

Keywords: *PGPR, Trichoderma, mineral nutrition, resistance, biological control.*

1. INTRODUCTION

The cost of rice production around the world is increasing due to yield declines in the main rice-growing regions of the world and the increased cost of inputs such as fertilizers and agricultural chemicals [1]. In Brazil, upland rice represents 2/3 of the total Brazilian production and has an advantage over irrigated rice because of its low negative effect on the ozone layer [2]. However, upland rice yields are low due to high disease incidence, intermittent drought periods and problems with initial plant growth, mainly in areas of successive plantings [3]. Among the biotic stresses impacting this crop, rice blast caused by the fungus *Magnaporthe oryzae* B. Couch occurs throughout the growing season [4]. Controlling rice blast is difficult due to the complex biology of the pathogen and can reduce the production potential of some improved rice cultivars by up to 100%. Consequently, growers resort to the indiscriminate use of fungicides to increase productivity and profit Chemical disease control is an efficient method in many cases; however, it

should be considered as only one component of an integrated and sustainable management regime in food production. Strategies that double food production while greatly reducing the environmental impacts of agriculture are required [5].

The potential use of bioagents such as the fungus *Trichoderma* and growth-promoting rhizobacteria (PGPR) for the biocontrol of plant diseases has been widely explored in recent years, along with silicon fertilization [3, 6]. The role of enzymes during the processes of infection by bioagents and silicon fertilization has been studied separately in different pathosystems [6-11]. However, there is little information about the combined effect of using bioagents and silicon for disease suppression in relation to enzyme-induced defense responses in rice. The objective of the present investigation was to examine enzymatic activity during the pre- and post-infection phases of leaf blast suppression using silicon fertilization in combination with bioagents.

2. MATERIALS AND METHODS

2.1. Bioagents

Three bioagents were utilized in this study: two PGPRs (*Pseudomonas fluorescens* (Pf) R-55 and *Burkholderia pyrrocinia* (Bp) R-46) from the Microorganism Culture Collection of the Embrapa Rice and Bean Research Center and a mixture composed of 4 isolates of *Trichoderma asperellum* (Ta) (T.06, T.09, T.12 and T.52) from the Fungal Culture Collection of the Plant Protection Laboratory of the Federal University of the Amazon [10, 12].

2.2. Silicon Fertilization and Planting

Two greenhouse experiments were conducted utilizing the cultivar BRS Primavera, which is susceptible to rice blast. Calcium and magnesium silicate (SiCaMg), in the registered form of Agrosilício®, containing 10.5% silicon, 27% calcium and 6% magnesium, was utilized as the silicon source. Soil was collected from virgin, non-cultivated cerrado, which was identified as an oxisol (darkred latosol according to Brazilian soil classification) with the following chemical characteristics before the application of fertilizers: pH H_2O 5.4; clay 589.0 $g.kg^{-1}$; silt 66.0 $g.kg^{-1}$; sand 144.0 $g.kg^{-1}$; K^+ 63.0 $mg.dm^{-3}$; P 4.0 $mg.dm^{-3}$; Ca^{+2}: 0.4 $mg.dm^{-3}$; Mg^{+2}: 0.2 $Cmolc.dm^{-3}$; Al^{+3}: 0.1 $Cmolc.dm^{-3}$; Si: 3.0 $mg.kg^{-1}$. The soil was corrected with lime before fertilization with the various doses of silicon and was incubated for 30 days in plastic trays (20x40x20 cm) containing 3 kg of soil. The soil in all trays was fertilized with NPK (5-30-15) + Zn and FTE at sowing. Nitrogen was applied in the form of ammonium sulfate ($(NH_4)_2SO_4$ + Fe and Bo) as top dressing 19 days after planting the cultivar BRS Primavera. Seeds were sown in each tray in eight 5.0 cm-long rows and then thinned after germination to maintain 10 to 12 plants per row.

2.3. Experiment 1

The first experiment used a randomized block design with eight repetitions. A total of 25 treatments were performed, consisting of 5 doses of silicon (0, 1, 2, 4, and 8 $SiCaMg.ha^{-1}$) and 5 bioagents (bioagent-free control; seed treatment following soil drenching with Bp; spraying plants with Pf 48 hours before challenge with the pathogen *M. oryzae*; seed treatment followed by soil drenching with the Ta isolate; and a mixture of the three bioagents Bp, Pf and Ta). The method of applying the mixture of bioagents consisted of treating seeds with the Bp and Ta isolates, followed by soil drenching with Bp and Ta and spraying with the Pf isolate 48 hours before challenge with the pathogen *M. oryzae* (10; 12).

2.4. Experiment 2

A second greenhouse experiment was conducted utilizing the best combinations of the silicon dosage and the bioagents (*T. asperellum* alone and the mixture of all three bioagents) from the first experiment. The experiment was organized in a randomized block design with four repetitions. The 12 total treatments consisted of 6 non-inoculated treatments and 6 in which plants were inoculated with the pathogen *M. oryzae*: 1) Control (without silicon fertilization or bioagents), 2) *T. asperellum*, 3) the mixture of the three bioagents, 4) Si fertilization, 5) Si+ *T. asperellum* and 6) Si+ the mixture of three bioagents. Silicon fertilization of soil previously corrected with calcium dolomite was conducted with 2 tons $SiCaMg.ha^{-1}$. The *T. asperellum* treatment consisted of seed treatment followed by soil drenching 48 hours before inoculation with the pathogen. The method of applying the mixture of bioagents was the same as in experiment 1.

2.5. Inoculation and Evaluation

A single conidial isolate of *M. oryzae*, Py-461, compatible with cv. BRS Primavera, maintained on sterilized filter paper discs in the culture collection of the Embrapa Rice and Bean Research Center, was utilized for the inoculation of plants in these two experiments. The fungus multiplication and sporulation and plant inoculation procedures were performed [13]. Twenty-two-day old plants were inoculated by spraying a spore suspension ($3x10^5$ conidia/mL) on the leaves until run-off, using an atomizer connected to air compressor. The inoculated plants were incubated in a humid plastic chamber in the dark for 24 hours and then transferred to greenhouse benches. The disease reaction was assessed eight days after inoculation using a 10–grade visual rating scale (0%, 0.5%, 1%, 2%, 4%, 8%, 16%, 32%, 64%, 82%) based on the percentage of the area infected, as described by [14].In the second experiment, the disease was assessed four times (1, 2, 4 and 8 days after the appearance of symptoms) to calculate the area under the disease progress curve (AUDPC).

2.6. Enzymatic Activity

2.6.1. Collection of samples

Leaf samples were collected 24 and 48 hours after inoculation with *M. oryzae*. Twenty leaves per treatment, including the non-inoculated control, were collected and stored in a freezer.

Protein extraction: A sample of five leaves was macerated in liquid nitrogen with a pestle until it became a powder. The buffer solution was composed of Tris-HCl 10 mM; NaCl [150 mM]; EDTA [2 mM]; DTT [2 mM]; PMSF [1 mM]; leupeptin [10 μg mL^{-1}] and aprotinin [10 μg mL^{-1}]. Protein measurement was performed according to the methodology of Bradford [15].

Enzymatic activities were assessed in triplicate. The specific activity (U mg^{-1}) was calculated as the ratio between the enzymatic activity in previously defined units (U) and the protein content quantified in each sample (mg).

β-1,3-glucanase activity (EC 3.2.1.6): The activity of β-1,3-glucanase (GLU) in rice leaf extracts from the different treatments was assayed by measuring the rate of reduction of sugar production using laminarin (Sigma) as the substrate [16]. DNS (dinitro salicylic acid) reagent was used as the colorimetric agent.

Chitinase activity (CHI) (EC 3.2.1.14): Chitinase (CHI) activity in rice leaf extracts from different treatments was assayed using a modified form of the method of [16]. The rate of N-acetylglucosamine production was measured using colloidal chitin as the substrate. DNS reagent was used as the colorimetric agent.

Peroxidase activity (EC 1.11.1.7): Peroxidase (POX) activity was assayed by measuring the rate of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) oxidation, using its colorimetric property. One unit was defined as the enzymatic activity that catalyzed the formation of 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) to increase the absorbance by 1 unit of abs per hour [17].

Phenylalanine ammonia-lyase (EC 4.3.1.5): The determination of Phenylalanine ammonia-lyase (PAL) enzyme activity was performed utilizing 2 mL of 10 mM phenylalanine solution in 0.1 M borate buffer, pH 9.0, and 50 μL of a plant extract sample. The mixture was homogenized and subjected to quantification of the product in a spectrophotometer (Fento 600 Plus) at 290 nm (ultraviolet).

Lipoxygenase (EC 1.13.11.12): Lipoxygenase (LOX) activity was assayed utilizing linoleic acid at 10 mmol L^{-1} as a substrate. There action mixture consisted of 1000 μL of sodium phosphate buffer, pH 6.0, 20 μL of substrate and 10 μL of sample. The molar extinction coefficient (ε) from hydroperoxides of linoleic acid was 25.000$mol.L^{-1}$ cm^{-1}. One unit of specific LOX activity was determined with a spectrophotometer (234 nm) and was defined as one micromole of hydroperoxides produced from linoleic acid per hour, per milligram of protein [18].

Salicylic acid (SA): SA extraction was performed by adding to 200 mg of macerated plant tissue to 1 mL of methanol (90%), followed by centrifugation. The supernatant was transferred to a new

tube, to which 2 mL of trichloro acetic acid (5%) and 2 mL of an ethyl acetate, cyclopentane and isopropanol solution (50:50:1) were added. After centrifugation, the supernatant was freeze-dried. The samples were recovered in 200 µL of 23% methanolin an acetate buffer solution (20 µmol, pH 5.0), followed by filtration (0.45-µm filter). Salicylic acid determination was performed through high performance liquid chromatography (HPLC) on a5 µm x 250 mm x 2.1 mm C18 column, at a temperature of 30°C, using isocratic methanol: acetate buffer solution (20 µmol, pH 5.0) (23:77) at 0.2 mL.min^{-1} as the mobile phase. A sample volume of 20 µL was applied. The retention time was determined to be 4.8 minutes [8].

2.7. Data Analysis

The data were analyzed using analysis of variance and polynomial regression with SPSS, version 19.0. The means were compared using the Duncan test at 5% probability.

3. Results and Discussion

3.1. Experiment 1

Statistically significant differences in the suppression of leaf blast were observed between the treatments involving different silicon doses and bioagents. The soil containing 2 tons SiCaMg.ha^{-1} combined with the mixture of three bioagents (*B. pyrrocinia*, *P. fluorescens* and *T. asperellum*) reduced the leaf area by 0.56, corresponding to a decrease of 96% in relation to the non-treated control (Table 1). Among the tested silicon doses, 8 tons.ha^{-1} of SiCaMg, reduced the leaf blast severity from 14.02 in the control to 1.3, corresponding to a decrease of 90.73%, whereas among the bioagents, the rhizobacterium *P. fluorescens* reduced the severity to 4.16, corresponding to a decrease of 70.33%, when sprayed over the rice plant leaves 48 hours before inoculation with the pathogen *M. oryzae.*

Regression analysis showed that the relationship between the silicon dose and leaf blast severity with and without bioagents was quadratic. While the dose of SiCaMg showing the maximum efficiency in reducing blast severity without a bioagent was 5.66ton.ha^{-1}, the addition of bioagents using the mixture reduced the required dose to 5.2 tons.ha^{-1} (Fig.1).

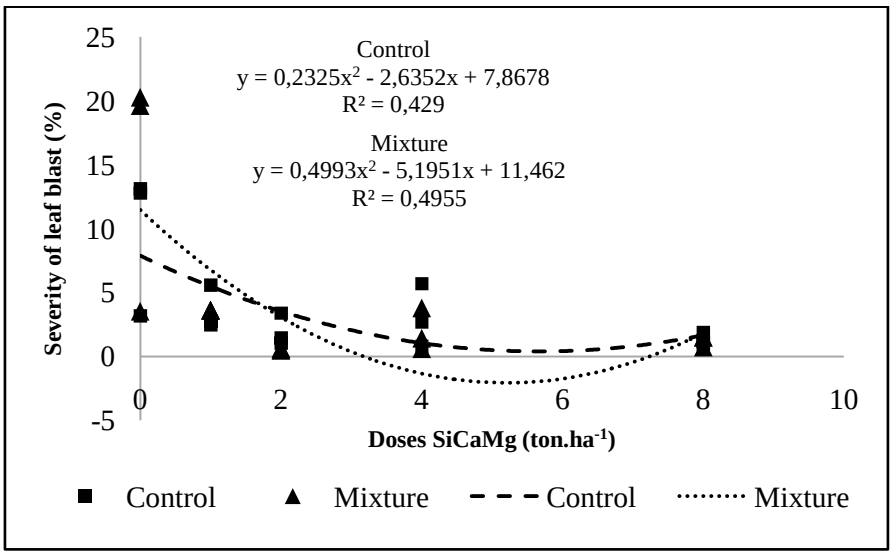

Fig1. *Relationship between silicon (SiCaMg) doses in combination with a mixture of three bioagents (Burkholderia pyrrocinia+Pseudomonas fluorescens+Trichoderma asperellum) and leaf blast severity (%).*

Table1. *The effect of silicon fertilization and the bioagents, both individually and in combination, on leaf blast severity (%) of Experiment 1.*

Doses of silicon (ton.ha^{-1})	Bioagents				
	Control	*T. asperellum*	*P. fluorescens*	*B. pyrrocinia*	**Mixture**
0	14.02 Dd	9.60 cBC	4.16 bA	8.06 dB	9.63 dC
1	3.61 cB	2.46 bB	1.14 aA	3.87 cB	3.63 cB
2	1.95 abC	1.24 aB	1.41 aB	1.01 aB	0.56 aA
4	3.05 bcC	0.87 aA	1.27 aB	1.82 bB	1.94 bB
8	1.30 aA	1.60 bB	4.12 bC	2.31 bB	1.25 abA

Means followed by lowercase letters in a vertical column and capital letters in a line do not differ significantly according to the Duncan test (P≤0.05).

3.2. Experiment 2

The differences among the various treatments were statistically significant in relation to the leaf blast severity as well as AUDPC. The differences between the responses to the treatments with *T. asperellum* and the mixture of bioagents combined with 2 tons of silicon fertilization were not significant despite reducing leaf blast compared with the control. However, the reduction of leaf blast was significantly greater for the condition with bioagent mixture treatment consisting of seed treatment with *B. pyrrocinia* and *T. asperellum* isolates, followed by soil drenching with the same bioagents and spraying plants with *P. fluorescens*, combined with 2 tons of silicon fertilization. The diseased leaf area was reduced from 14.24% in the control to 3.96% with the bioagent mixture, and the AUDPC decreased from 33.8 to 13.17, respectively (Fig.2A and 2B).

The treatments also resulted in differences in lesion type. In the treatment involving only fertilization with 2 tons SiCaMg.ha^{-1}, the blast lesions were elliptical with a brown margin and grey center. These lesions were sporulating and often coalesced, covering a larger portion of the green leaf area (Figure 3a). In contrast, the lesions were brown, round to oval and non-sporulating on the leaves of plants fertilized with 2 tons SiCaMg.ha^{-1} and seed treated with the bioagent *T. asperellum* (Ta) (Figure 3b). In plants fertilized with 2 tons of SiCaMg.ha^{-1} and treated with the mixture of bioagents, the lesions were pinhead-sized and non-sporulating (Figure 3c).

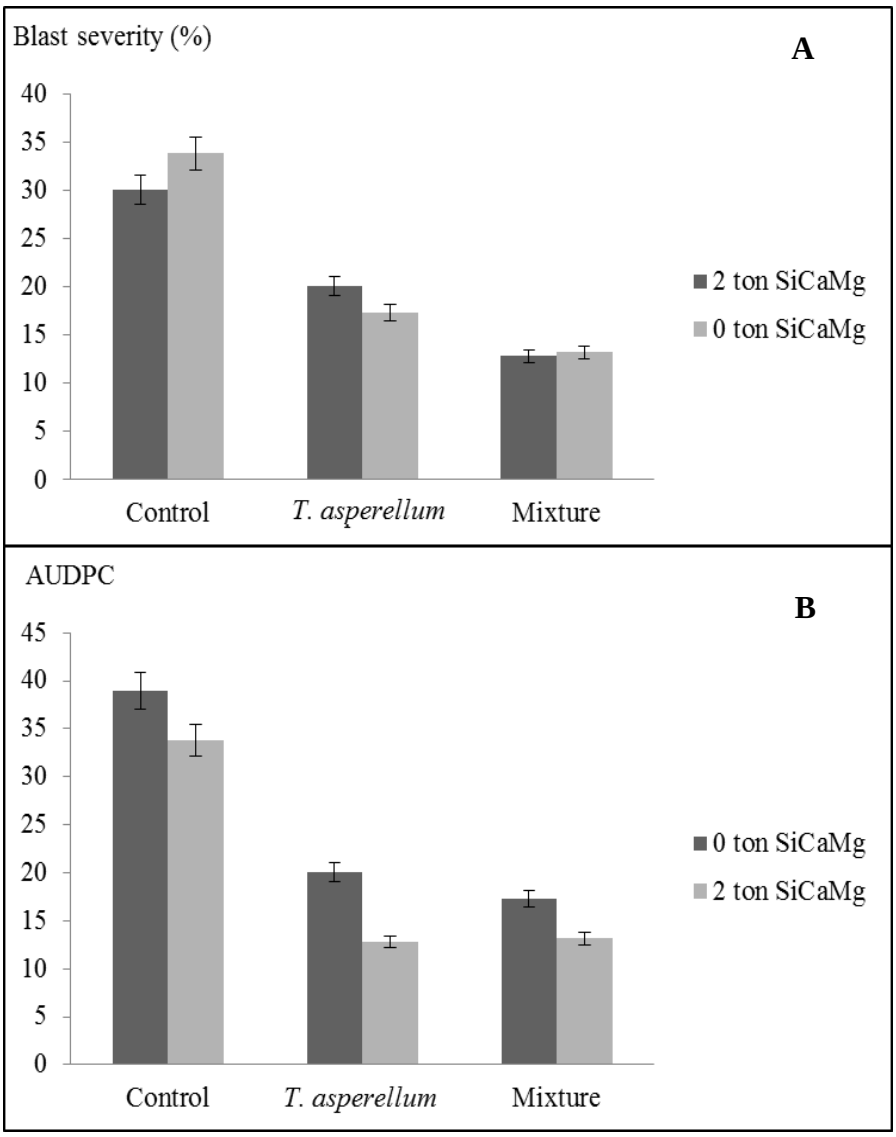

Fig2. *The effect of silicon fertilization and the bioagents, both individually and in combination, on leaf blast severity (%) of Experiment 2* ***(A)****, and Area under Disease Progress Curve (AUDPC) of Experiment 2* ***(B)****. Duncan test (P≤0.05).*

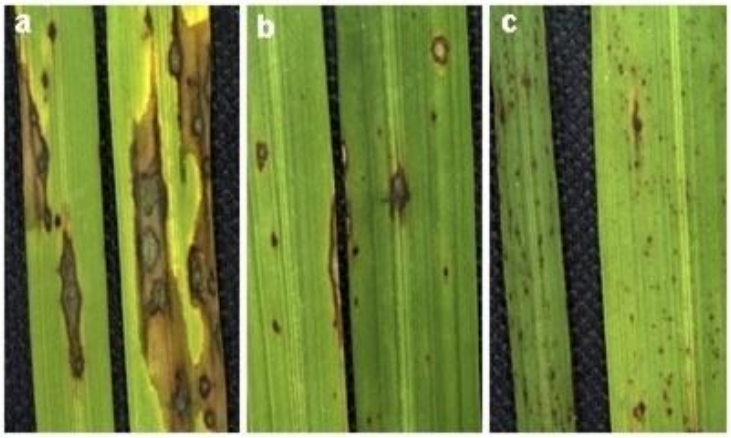

Fig3. *Symptoms of leaf blast in response to inoculation with a conidial suspension of M. oryzae (3x10^{5} con.mL^{-1}) in plants fertilized with 2 tons.ha^{-1} of SiCaMg.* ***a*** *= Control (not treated with the bioagent mixture);* ***b*** *= T. asperellum;* ***c*** *= the mixture of three bioagents (Burkholderia pyrrocinia + Pseudomonas fluorescens + Trichoderma asperellum).*

3.3. Enzymatic activity

3.3.1. Chitinase

In non-inoculated plants, the treatments involving the bioagent mixture, both with and without Si fertilization, resulted in significantly greater chitinase activity in leaves collected after 24 hours (1.67 and 1.35 U.mg^{-1}) and 48 hours (0.69 and 0.77 U.mg^{-1}) compared with control (Table 2). After challenge with the pathogen, the same treatments resulted in lower enzymatic activities of 0.67 and 0.69 U.mg^{-1}, respectively, after 24 hours, and a significantly increase was observed after 48 hours compared with the control. In contrast, *T. asperellum*+Si led to greater activity compared with the control after 48 hours for both non-inoculated and inoculated plants. Chitinase activity was significantly increased, by 1.49 and 1.45 U.mg^{-1}, respectively (Table 2).

3.3.2. β-1, 3-glucanase

In non-inoculated plants, the bioagent mixture without Si resulted in a significantly higher level of glucanase activity (2.1 U.mg^{-1}, Table 2) in leaf samples collected after either 24 or 48 hours; however, when the bioagent mixture was combined with Si, higher activity than the control was only observed after 48 hours. Treatment with the mixture, either with or without Si, led to greater enzymatic activity at both 24 and 48 hours after challenge with the pathogen. Treatment with the bioagent *T. asperellum* combined with Si resulted in significantly higher activity (3.08 U.mg^{-1}) than the other treatments in samples collected after 48 hours in both non-inoculated and inoculated plants (Table 2).

3.3.3. Peroxidase

The bioagent mixture, either with or without silicon, did not alter the peroxidase activity in inoculated plants compared with non-inoculated plants. The activity of this enzyme was significantly higher (0.594 U.mg^{-1}) in plants treated with the bioagent mixture without Si in non-inoculated plants (Table 2).

3.3.4. Phenylalanine Ammonia-Lyase

Treatment with the bioagent mixture together with Si resulted in the highest phenylalanine ammonia-lyase activity (20.5x10^{-5}) observed in samples collected after 24 hours in non-inoculated plants. Treatment with the bioagent mixture without silicon did not exhibit any effect on PAL activity. Treatment with *T. asperellum*, both with and without silicon, significantly increased PAL activity, by 4.76 and 12.6 x10^{-5} U.mg^{-1} after 48 hours in non-inoculated and inoculated plants, respectively (Table 2).

3.3.5. Lipoxygenase

The LOX activity level for this treatment was significantly greater (4.17 x 10^{-3} U.mg^{-1}) than for any of the other conditions and was also greater in plants treated with *T. asperellum* than in non-treated plants at 48 hours after inoculation (Table 2).

3.3.6. Salicylic Acid

In the control treatment without silicon, the salicylic acid content was high (911.33 $ng.g^{-1}$) in non-inoculated plants. At 24 hours after challenge with *M. oryzae,* the plants treated with the mixture of the three bioagents showed an increase in contents to 1096.07 $ng.g^{-1}$. In plants challenged with *M. oryzae,* Si alone increased salicylic acid content to 1007.00 $ng.g^{-1}$ compared with the control without silicon. The treatment with *T. asperellum* combined with Si led to the highest content (1045.30 $ng.g^{-1}$), followed by the bioagent mixture with or without Si, compared with control in non- inoculated plants. At 48 hours after challenge with the pathogen, there were no significant differences between the treatment with Si alone and those with Si and the mixture of bioagents or *T. asperellum* (Table 2).

The results presented herein show, for the first time, that the use of silicon fertilization in combination with the use of bioagents is more efficient at suppressing rice leaf blast than silicon fertilization alone. The mixture of the three bioagents combined with silicon fertilization at low concentration (2 tons $SiCaMg.ha^{-1}$) suppressed leaf blast.

The treatment differences in experiment 2 were not statistically significant. However, a greater efficiency of the mixture of three bioagents in combination with silicon fertilization was obtained compared with application of *T. asperellum* considering AUDPC and the lesion type. The lesions produced in response to treatment with the three bioagents combined with silicon fertilization were also non-sporulating, contrary to the few small sporulating lesions dispersed on the leaf surfaces of plants treated only with the mixture of the *T. asperellum* isolates (Figures 3b and 3c).

In the biological control of plant diseases, the tested bioagents and silicon induce pathogen resistance via different pathways. The PGPRs and *Trichoderma* activate mechanisms that are responsible for nutrient competition and antibiosis of plant pathogens via the production of antimicrobial substances such as phytoalexins and pathogenesis-related proteins (PRPs) [19, 20], resulting in the induction of systemic resistance (ISR) [21].

Silicon has been shown to be efficient in controlling many plant diseases, including rice diseases [22]. The results obtained to date indicate that this protective effect is mainly due to the absorption of monosilicic acid by roots and the deposition of amorphous silicon in the cell wall between the cuticle and epidermis in rice plants [23]. Some authors have demonstrated that in addition to fortifying the cell wall, silicon activates the host plant's defense system [6, 24, 25]. In the present study, the treatment with *T. asperellum* + Si (plants treated with *T. asperellum* + 2 tons $SiCaMg.ha^{-1}$) and the treatmentwith the mixture of three bioagents + fertilization with 2 tons of $SiCaMg.ha^{-1}$ resulted in increases in CHI, GLU, POX and PAL activity and SA levels, even in the absence of *M. oryzae* (Table 2).These results are in accord with those obtained by Cruz and collaborators [6], who observed increased activity of PAL, CHI, GLU and polyphenol oxidase in soybean plants subjected to calcium nitrate supplementation in the absence of *Phakopsora pachyrhizi.*

A positive effect of silicon was observed after challenging with *M. oryzae* when plants were treated with the mixture of three bioagents in increasing the activity of CHI and GLU after 48 hours. A similar effect of silicon was not observed in activating PAL, the precursor enzyme of SA. However, the treatments employing silicon either individually or in combination with the three bioagents led to a significant increase in SA contents at 24 and 48 hours after challenging with the pathogen. GLU and CHI are enzymes related to the pathogenesis associated with the PR-2 and PR-3families. They exhibit hydrolytic functions, disrupting the structures of polymers such as β-1,3-glucanase and chitinase, which are present in the cell wall. PAL is a secondary metabolic enzyme related to the phenyl propanoid pathway that catalyzes the formation of *trans*-cinnamic acid, the precursor of diverse compounds involved in plant defense such as phytoalexins and salicylic acid [26]. SA is an important signaling molecule involved in the defense against plant pathogens that induces the expression of proteins related to systemic acquired resistance (SAR).The enzyme POX is responsible for fortifying the host plant cell wall via the oxidation of phenols as well as suberization and lignification during the plant defense against pathogen attack (Rodrigues and Datnoff 2005). The function of LOX is to impair the growth of the pathogen by inducing the production of phytoalexins, aldehydes and jasmonic acid, and it also participates in signal transduction [27].

Table2. *CHI, GLU, POX, PAL and LOX activities* ***(U.mg^{-1})*** *and SA concentration* ***(ng.g^{-1})*** *in rice leaves collected after 24 and 48 hours from non-inoculated plants and plants inoculated with Magnaporthe oryzae.*

Treatments	CHI		GLU		POX		PAL		LOX	SA	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	48 h	24 h	48 h
Non- inoculated											
Control-Si[1]	0.59 bc[3]	0.44 ef	1.36 cde	1.23 fg	0.31 cde	0.36 ab	1.90 b[3]	3.10 f	4.07 a	911.33 b	477.87 g
T. asperellum-**Si**	0.32 c	0.58 def	0.97 ef	1.37 f	0.20 de	0.49 ab	5.60 b	4.76 c	2.92 b	877.07 b	895.97 cde
Mixture-Si	1.67 a	0.77 c	2.08 b	2.10 c	0.59 ab	0.40 ab	6.66 b	3.46 de	1.81 d	203.33 f	1036.57 ab
Si	0.65 bc	0.72 cd	1.15 def	1.83 cd	0.25 de	0.40 ab	2.36 b	3.36 de	1.10 fg	493.43 de	902.07 bcde
T. asperellum+**Si**[2]	0.67 bc	1.49 a	0.77 f	3.08 a	0.44 bc	0.98 a	1.60 b	0.80 h	1.21 efg	625.60 c	1045.30 a
Mixture+Si	1.35 a	0.68 cd	1.50 cd	1.71 de	0.23 de	0.18 b	20.5 a	1.86 g	1.71 de	881.17 b	963.70 abc
Inoculated											
Control-Si	1.33 a	0.56 def	2.81 a	1.47 ef	0.38 cd	0.46 ab	3.33 b	5.93 b	1.20 efg	451.53 e	810.13 e
T. asperellum-**Si**	1.18 ab	0.40 f	1.56 cd	1.08 h	0.38 cd	0.91 a	2.23 b	12.6 a	2.38 c	917.93 b	822.70 de
Mixture-Si	0.69 bc	1.14 b	1.66 bc	1.79 d	0.37 cd	0.58 ab	2.20 b	3.26 ef	1.22 efg	1096.07 a	603.90 f
Si	0.53 c	0.61 cde	1.28 cde	1.99 cd	0.75 a	0.67 ab	2.93 b	3.53 d	1.55 def	883.57 b	1007.00 abc
T. asperellum+**Si**	0.65 bc	1.44 a	1.16 def	1.94 cd	0.26 cde	0.37 ab	2.60 b	2.00 g	0.91 g	585.57 cd	949.70 abcd
Mixture+Si	0.67 bc	1.27 b	1.52 cd	2.39 b	0.15 e	0.13 b	1.03 b	2.03 g	0.67 g	499.62 de	919.75 abcde

[1]*-Si = soil not fertilized with silicon.*

[2]*+Si = soil fertilized with silicon.*

[3] *Means followed by the same letters do not differ significantly according to the Duncan test (P≤0.05)*

The roles of silicon and bioagents in biochemical plant defense mechanisms have been investigated in various pathosystems, including rice [7, 9, 11]. Rodrigues and collaborators [28] observed that plants susceptible to *M. oryzae* subjected to treatment with calcium silicate showed greater expression of GLU at 36 hours after inoculation, accumulation of POX at 12 hours after inoculation, and PR-1 accumulation at 60 and 96 hours after inoculation. In the same pathosystem, Datnoff [11] observed increased expression of CHI in plants fertilized with silicon at 24 hours after inoculation with *M. oryzae*. Filippi and collaborators [20] tested the use of *B. pyrrocinia* (soil drenching 15 days before challenge with *M. oryzae*) and *P. fluorescens* (sprayed 2 days before challenge with *M. oryzae*) in rice and observed increased activities of CHI, GLU and POX at 72 hours after inoculation with *M. oryzae*.

Some authors have obtained similar results in other pathosystems. Cruz and collaborators [6] demonstrated that fertilization of soybean with calcium silicate, with or without challenge with *Phakopsora pachyrhizi*, increased the activities of CHI and PAL at 141 hours after inoculation and that of GLU at 72 hours after inoculation. Chérif and collaborators [29] reported that cucurbit plants fertilized with potassium silicate and challenged with *Pythium aphanidermatum* displayed high GLU expression at 6 days after inoculation. Oliveira [13] tested cotton plants cultivated in soil containing calcium silicate and challenged with *Xanthomonas citri*s sp. *malvacearum* and showed that even in non-accumulator plants, silicon treatment increased the activity of POX, PAL and GLU at 24 hours after inoculation, in addition to reducing disease severity by 55%.

Silva and collaborators [10] observed that *Trichoderma* spp. Isolates increased the activity of POX in cucumber plants when challenged with *Colletotrichum lagenarium*, reducing the disease severity by88%. Saikia and collaborators [8] tested the ability of *Pseudomonas aeruginosa* isolates to induce resistance to *R. solani* in rice grown under greenhouse conditions and showed that the bacteria increased the SA content at 24 hours after inoculation and reduced the disease severity by 33%.

However, there are no available reports addressing the combined effects of silicon and bioagents on enzymatic activity. The results of the present study indicated that during the combined application of silicon fertilization, using 2 tons. ha^{-1} SiCaMg, and the mixture of three bioagents for leaf blast suppression, the enzymes CHI, GLU, POX, LOX and the hormone SA were all

activated before and at 24 and 48 hours after challenging with *M. oryzae*. Furthermore, a reduction in the number and size of blast lesions on the leaves was observed only in plants treated with silicon in combination with the bioagent mixture.

4. CONCLUSION

The interaction between bioagents and silicon produced promising results in inducing resistance to rice leaf blast. The combination of *T. asperellum* or the mixture of bioagents with soil fertilization using 2 tons of $SiCaMg.ha^{-1}$ may be considered an efficient strategy for use in sustainable management of blast disease. This should permit the reduced use of silicon fertilizer and agricultural chemicals and increase disease resistance.

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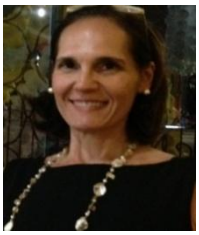

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