Alterations in Gas Exchange and Oxidative Metabolism in Rice Leaves Infected by *Pyricularia oryzae* are Attenuated by Silicon

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ABSTRACT

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Rice blast, caused by *Pyricularia oryzae*, is the most important disease in rice worldwide. This study investigated the effects of silicon (Si) on the photosynthetic gas exchange parameters (net CO₂ assimilation rate [A], stomatal conductance to water vapor [g_s], internal-to-ambient CO₂ concentration ratio [C_i/C_a], and transpiration rate [E]); chlorophyll fluorescence *a* (Chl_a) parameters (maximum photochemical efficiency of photosystem II [F_v/F_m], photochemical [q_p] and nonphotochemical [NPQ] quenching coefficients, and electron transport rate [ETR]); concentrations of pigments, malondialdehyde (MDA), and hydrogen peroxide (H₂O₂); and activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and lipoxygenase (LOX) in rice leaves. Rice plants were grown in a nutrient solution containing 0 or 2 mM Si (–Si or +Si, respectively) with and without *P. oryzae* inoculation. Blast severity decreased

with higher foliar Si concentration. The values of A, g_s, and E were generally higher for the +Si plants in comparison with the –Si plants upon *P. oryzae* infection. The F_v/F_m, q_p, NPQ, and ETR were greater for the +Si plants relative to the –Si plants at 108 and 132 h after inoculation (hai). The values for q_p and ETR were significantly higher for the –Si plants in comparison with the +Si plants at 36 hai, and the NPQ was significantly higher for the –Si plants in comparison with the +Si plants at 0 and 36 hai. The concentrations of Chl_a, Chl_b, Chl_{a+b}, and carotenoids were significantly greater in the +Si plants relative to the –Si plants. For the –Si plants, the MDA and H₂O₂ concentrations were significantly higher than those in the +Si plants. The LOX activity was significantly higher in the +Si plants than in the –Si plants. The SOD and GR activities were significantly higher for the –Si plants than in the +Si plants. The CAT and APX activities were significantly higher in the +Si plants than in the –Si plants. The supply of Si contributed to a decrease in blast severity, improved the gas exchange performance, and caused less dysfunction at the photochemical level.

Additional keywords: fungal infection, *Oryza sativa*, photosynthesis.

Rice blast, caused by the hemibiotrophic fungus *Pyricularia oryzae* Cavara (teleomorph *Magnaporthe oryzae* (Catt.) B. C. Couch), is the most important disease in rice (*Oryza sativa* L.) (Ou 1980). The blast symptoms occur on leaves, leaf collars, necks, panicles, pedicels, and seed. On the leaves, lesions are initially gray-green and water soaked with a darker green border (Ou 1980). As the lesions expand, they become light tan and develop necrotic borders (Bonman 1992; Ou 1980). The blast lesions that occur on rice collars, necks, and panicles contribute to reduced rice yields due to the reduced translocation of nutrients for grain filling (Bonman 1992). Rice blast has been controlled by treating seed with fungicides, spraying systemic fungicides at different plant growth stages, and using resistant cultivars (Bonman 1992).

The most positive and consistent effects of using soluble silicon (Si) include the alleviation of abiotic and biotic stresses in a wide variety of plant species (Datnoff et al. 2007; Epstein 2009; Fauteux et al. 2005; Liang et al. 2007; Ma et al. 2001). In particular, Si has been recognized for its potential to decrease the intensities of important diseases in several crops, especially in grasses and some dicots, such as bean, cucumber, and soybean (Datnoff et al. 2007; Ma et al. 2001). For the rice–*P. oryzae* interaction, Si can potentiate biochemical mechanisms against the fungus, including increases in the concentrations of phenolics, lignin, and phytoalexins; enhanced

activities of defense enzymes such as chitinases and β-1,3-glucanases; and the rapid transcription of genes associated with defense responses (Brunings et al. 2009; Rodrigues et al. 2003, 2004, 2005). Furthermore, increased resistance of plants supplied with Si against pathogens has been associated with a physical barrier that prevents or slows fungal penetration. For example, a barrier can result from an increase in the density of the long and short silicate cells in the leaf epidermis or in the thick silica layer below the cuticle, as noted for the rice–*P. oryzae* interaction (Kim et al. 2002; Sun et al. 2010; Yoshida et al. 1962).

The lesions caused by hemibiotrophic and necrotrophic pathogens can affect plant physiology by negatively affecting leaf gas exchange due to losses in healthy leaf area or by lowering the efficiency of the photosynthetic process, even in asymptomatic leaf tissues (Alves et al. 2011; Gao et al. 2011; Padhi et al. 1978; Resende et al. 2012; Shtienberg 1992; Swiech et al. 2001; Yun et al. 2000). For different host–pathogen interactions, reductions in the pigment concentrations, structural damage to the chloroplasts, impairments in energy dissipation (which can be determined via chlorophyll *a* [Chl_a] fluorescence kinetics), and increases in leaf temperature are the most notable negative effects that result from pathogen infection (Baker 2008; Bastiaans 1991; Bastiaans and Roumen 1993; Krause and Weis 1991; Lichtenthaler and Miehe 1997; Meyer et al. 2001). In addition, pathogens can cause leaf damage at the cuticular and stomatal levels, which can lead to changes in transpiration, the plant water balance, and the plant canopy temperature (Padhi et al. 1978; Resende et al. 2012). Because photosynthesis is the major physiological process that fuels biomass production, any alterations in photosynthesis affect

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crop growth and performance. Therefore, a proper assessment of the photosynthetic apparatus upon pathogen infection (e.g., via measurements of the leaf gas exchange and Chl_a fluorescence kinetics) is very important (Balachandran and Osmond 1994).

Stressful conditions induced by biotic factors can impair photosynthetic performance (Bastiaans 1991; Bastiaans and Roumen 1993; Berger et al. 2007), increase the excitation energy to exceed the amount required for photosynthetic metabolism, and contribute to the generation of reactive oxygen species (ROS) (Resende et al. 2012). The ROS, especially hydrogen peroxide (H_2O_2), have been recorded to result from the infection process of several pathogens (Shetty et al. 2007; Unger et al. 2005). Broadly, any type of stress occurring on ROS-producing organelles during pathogen infection may contribute to ROS production (Apel and Hirt 2004). An antioxidant system normally maintains the ROS balance within plant cells due to the contribution of the antioxidative enzymes involved in the ROS detoxification, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR). Other antioxidants, including ascorbate and reduced glutathione, also play a pivotal role in maintaining the ROS pools at controlled levels (Havir and McHale 1989; Mittler 2002; Moller 2001; Nakano and Asada 1981).

Resende et al. (2012) demonstrated that Si can be beneficial for sorghum plants that are infected with *Colletotrichum sublineolum*, mainly due to greater SOD, CAT, APX, and GR activities. The authors found that Si was beneficial for the sorghum plants by maintaining carbon fixation and enhancing the antioxidant system, resulting in increased ROS scavenging and, ultimately, reducing cell membrane damage. Mohaghegh et al. (2011) observed greater CAT and APX activities on cucumber roots supplied with Si and infected with *Phytophthora melonis*. Sun et al. (2010) observed that rice plants supplied with Si and infected by *Pyricularia oryzae* had increased CAT and lipoxygenase activities and lower malondialdehyde concentrations.

Given the facts described above and considering that Si could help maintain the photosynthetic rates in plants infected with foliar pathogens, it was hypothesized that the supply of Si could mitigate the deleterious effects of *P. oryzae* infection in rice leaves and maintain the functionality of the photosynthetic apparatus. To test this hypothesis, the combined gas exchange and Chl_a fluorescence measurements, along with an analysis of the antioxidant system and pigments pools, were used to examine the photosynthetic performance of rice plants supplied with Si during the infection process of *P. oryzae*.

MATERIALS AND METHODS

Nutrient solution preparation. The nutrient solution was prepared based on Hoagland and Arnon (1950), with some modifications,

and included the following macronutrients: 1.0 mM KNO_3 , 0.25 mM $\text{NH}_4\text{H}_2\text{PO}_4$, 0.1 mM NH_4Cl , 0.5 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 1.0 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$. In addition, the nutrient solution included the following micronutrients: 0.30 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.33 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 11.5 μM H_3BO_3 , 3.5 μM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.1 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 25 μM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and 25 μM EDTA disodium. Si was supplied as monosilicic acid, which was prepared by passing potassium silicate through a cation-exchange resin (Amberlite IR-120B, H^+ form; Sigma-Aldrich, São Paulo, Brazil) (Ma and Yamaji 2006). The Si was applied at 2 mM in the treatments and no Si was applied (0 mM) to the control. The addition of monosilicic acid to the nutrient solution did not alter the pH.

Plant growth. Rice seed from 'Metica-1' were surface-sterilized in 10% (vol/vol) NaOCl for 3 min, rinsed in sterilized water for 3 min, and germinated on distilled-water-soaked germitest paper (20 by 20 cm, cellulose at 120 g/m²; Germilab; BioGenética Ltd., São Paulo, Brazil) in a germination chamber at 25°C for 6 days. The germinated seedlings were transferred to plastic pots containing one-half strength nutrient solution without Si for 5 days. Subsequently, five plants were transferred to new plastic pots containing 5 liters of nutrient solution prepared with or without Si. The nutrient solution was changed every 4 days, and the electrical conductivity and pH of the nutrient solutions were measured daily. The pH was maintained at approximately 5.5 by adding NaOH or HCl (1 M) as necessary. The plants were grown in a greenhouse with a relative humidity of $65 \pm 5\%$, a temperature of $30 \pm 5^\circ\text{C}$, and a natural photon irradiance of $900 \pm 15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (measured at midday).

Inoculation procedure. A pathogenic isolate of *P. oryzae* (CNPAF-1048) was used to inoculate the plants. Disks of filter paper containing fungal mycelia were transferred to Petri dishes (90 by 15 mm) containing oat-agar medium. After growing the disks containing mycelia, the media with the fungus were transferred to new Petri dishes containing the same medium. The dishes were incubated in a growth chamber at 25°C under continuous light for 10 days. After this period, conidia were carefully removed from the media with a soft bristle brush using water-containing gelatin (1% wt/vol). The conidial suspension was calibrated with a hemacytometer to obtain a concentration of 1×10^5 conidia mL^{-1} . The conidial suspension was sprayed with an atomizer (Paasche Airbrush Co., Chicago) on the adaxial surface of the plant leaves 45 days after emergence. After inoculation, the plants were kept in the dark in a mist chamber at 25°C for 24 h. Next, the plants were transferred to a greenhouse with a relative humidity of $80 \pm 5\%$ and a temperature of $25 \pm 3^\circ\text{C}$.

Evaluation of blast severity. The second and third leaves (from the base to the top) of each plant per replication for each treatment were marked and used to evaluate the blast severity at

Fig. 1. A, Blast severity (Sev) and **B,** area under blast progress curves (AUBPC) for rice plants grown in hydroponic culture containing 0 mM (–Si) or 2 mM (+Si) silicon. For blast development, the means for the –Si and +Si treatments followed by an asterisk (*) at each evaluation time are significantly different according to a Student's *t* test ($P \leq 0.05$). Error bars represent the standard error of the mean.

36, 60, 84, 108, and 132 h after inoculation (hai) according to the scale proposed by IRRI (1996). The area under the blast progress curve (AUBPC) for each leaf was computed using the trapezoidal integration of the blast progress curve over time (Shaner and Finney 1977).

Photosynthetic measurements. The leaf gas exchange parameters were simultaneously determined by measuring the chlorophyll *a* (Chl_a) fluorescence with a portable open-flow gas exchange

system (LI-6400XT; LI-COR, Lincoln, NE) equipped with an integrated fluorescence chamber head (LI-6400-40; LI-COR Inc.). The net CO₂ assimilation rate (*A*), stomatal conductance to water vapor (*g_s*), internal-to-ambient CO₂ concentration ratio (*C_i/C_a*), and transpiration rate (*E*) were measured on the attached leaves (fourth leaf from the top of the plant for each replication of each treatment) at approximately 08:00 h (0, 36, 60, 84, 108, and 132 hai) and 12:00 h (solar time) (0, 42, 66, 90, and 114 hai) with a saturating

Fig. 2. A and B, Net carbon assimilation rate (*A*); C and D, stomatal conductance to water vapor (*g_s*); E and F, transpiration rate (*E*); and G and H, the internal-to-ambient CO₂ concentration ratio (*C_i/C_a*) determined at 08:00 h (A, C, E, and G) and at 12:00 h (B, D, F, and H) in the leaves of rice plants grown in hydroponic culture containing 0 mM (–Si) or 2 mM (+Si) silicon and inoculated with *Pyricularia oryzae*. Bars represent the standard error and asterisks indicate the means that differed significantly at each evaluation time (*t* test, *P* ≤ 0.05).

photon irradiance of approximately $1,200 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the leaf level under the naturally fluctuating ambient CO_2 concentrations. All of the measurements were performed at 25°C and the vapor pressure deficit was maintained at approximately 1.0 kPa. The amount of blue light was set to 10% of the photon irradiance to optimize the stomatal aperture.

Previously dark-adapted (30 min) leaf tissues were illuminated with weak modulated measuring beams ($0.03 \mu\text{mol m}^{-2} \text{s}^{-1}$) to obtain the initial fluorescence (F_0). Saturating white light pulses of $8,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ were applied for 0.8 s to ensure maximum fluorescence emissions (F_m). The variable-to-maximum Chl fluorescence ratio $F_v/F_m = [(F_m - F_0)/F_m]$ was calculated from

Fig. 3. A and B, Maximum photochemical efficiency of photosystem II (PSII) (F_v/F_m); C and D, photochemical (q_p) and E and F, nonphotochemical (NPQ) quenching coefficients; and G and H, the electron transport rate (ETR) determined at 08:00 h (A, C, E, and G) and at 12:00 h (B, D, F, and H) in the leaves of rice plants grown in hydroponic culture containing 0 mM (–Si) or 2 mM (+Si) silicon and inoculated with *Pyricularia oryzae*. Bars represent the standard error and asterisks indicate the means that differed significantly for each evaluation point (t test, $P \leq 0.05$).

F_m . In the light-adapted leaves, the steady-state fluorescence yield (F_s) was measured following the application of a saturating white light pulse ($8,000 \mu\text{mol m}^{-2} \text{s}^{-1}$; 0.8 s) to achieve the maximum light-adapted fluorescence (F_m'). Next, the actinic light was turned off and far-red illumination was applied ($2 \mu\text{mol m}^{-2} \text{s}^{-1}$) to measure the light-adapted initial fluorescence (F_0'). Using these parameters, the efficiency of the PSII reaction centers for capturing excitation energy (F_v'/F_m') was estimated as $F_v'/F_m' = (F_m' - F_0')/F_m'$. The coefficient for photochemical quenching (q_p) was calculated as $q_p = (F_m' - F_s)/(F_m' - F_0')$, while that for nonphotochemical quenching (NPQ) was calculated as $\text{NPQ} = (F_m'/F_m') - 1$. The actual quantum yield of PSII electron transport (Φ_{PSII}) was computed as $\Phi_{\text{PSII}} = (F_m' - F_s)/F_m'$, from which the apparent electron transport rate (ETR) was calculated as $\text{ETR} = \Phi_{\text{PSII}} \times \text{PPFD} \times f \times \alpha$, where f is a factor that accounts for the partitioning of energy between PSII and PSI and is assumed to be 0.5, which indicates that the excitation energy is distributed equally between the two photosystems, and α is the leaf absorbance by the photosynthetic tissues and is assumed to be 0.84 (Baker 2008).

Determination of leaf pigments. Leaf tissue (0.5 g) was ground into a fine powder using a mortar and pestle with liquid nitrogen and 1 mg of calcium carbonate. Next, the fine powder was homogenized in 2 ml of aqueous acetone (80%, vol/vol) for 1 min in a room with reduced light intensity ($12 \mu\text{mol m}^{-2} \text{s}^{-1}$). The suspension was filtered through a Whatman number 1 filter paper and the residue was washed four times with 80% acetone. The volume was adjusted to 25 ml using the same solvent in a volumetric flask. The absorbance of the samples was recorded at 470, 646.8, and 663.2 nm, and the concentrations of photosynthetic pigments (Chl_a , Chl_b , and total carotenoids) were estimated according to Lichtenthaler (1987).

Biochemical assays. Samples from the fourth and fifth leaves (from the top to the base) of each plant in each replication and treatment were collected at 36, 60, 84, 108, and 132 h after inoculation at approximately 12:00 h. The leaf samples collected from noninoculated plants served as a control (0 h). The leaf samples were kept in liquid nitrogen during sampling and were stored at -80°C until analysis.

To determine the SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), APX (EC 1.11.1.11), GR (EC 1.8.1.7), and lipoxygenase (LOX) (EC 1.13.11.12) activities, 200 mg of leaf tissue was ground into a fine

powder in a mortar and pestle with liquid nitrogen. The fine powder was homogenized in an ice bath with 60 mg of polyvinylpyrrolidone and the following components: for SOD, 1 ml of 100 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, and 0.1% Triton X-100 (vol/vol); for CAT, 1 ml of 100 mM potassium phosphate buffer (pH 7.0) and 0.1 mM EDTA; for APX, 1 ml of 50 mM potassium phosphate buffer (pH 7.0) and 1 mM ascorbate; for GR, 1 ml of 100 mM Tris-HCl (pH 7.5), 50 mM EDTA, 10 mM isoascorbate, 9 mM 2-mercaptoethanol, and 0.1% Triton X-100 (vol/vol); and, for LOX, 2 ml of 50 mM potassium phosphate buffer (pH 7.0) and 1 mM hydroxylamine. For SOD, CAT, APX, and GR, the homogenates were centrifuged at $15,000 \times g$ for 15 min at 4°C and the supernatants were used as crude enzyme extracts. For LOX, the homogenate was centrifuged at $16,000 \times g$ for 20 min at 4°C , and the supernatants were used as crude enzyme extracts. The SOD activity was determined by measuring its ability to photochemically reduce *p*-nitrotriazol blue (NTB) (Giannopolitis and Ries 1977). The reaction was started by adding 5 μl of the crude enzyme extract to 3 ml of a mixture containing 50 mM potassium phosphate buffer (pH 7.8), 14 mM methionine, 75 μM NTB, 0.1 μM EDTA, and 2 μM riboflavin. The production of formazan blue, resulting from the photoreduction of NTB, was monitored as the increase in absorbance at 560 nm (Giannopolitis and Ries 1977). One unit of SOD was defined as the amount of enzyme necessary to inhibit NTB photoreduction by 50%. The CAT activity was estimated from the rate of H_2O_2 decomposition at 240 nm (Havir and McHale 1989). The reaction was initiated after adding 20 μl of the crude enzyme extract to 3 ml of a mixture containing 50 mM potassium phosphate buffer (pH 7.8) and 12.5 mM H_2O_2 . For APX, the reaction was started after adding 20 μl of the crude enzyme extract to 3 ml of a mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, and 0.1 mM H_2O_2 . The APX activity was determined from the rate of ascorbate oxidation at 290 nm (Nakano and Asada 1981), and the GR activity was determined from the rate of NADPH oxidation at 340 nm. The reaction was initiated after adding 20 μl of the crude enzyme extract to a mixture containing 50 mM Tris-HCl (pH 7.5), 10 mM reduced glutathione, 3 mM MgCl_2 , and 0.15 mM NADPH. For each enzyme, six separate extractions were performed using samples from each treatment and

Fig. 4. Concentrations of **A**, chlorophyll *a* (Chl_a); **B**, chlorophyll *b* (Chl_b); **C**, total chlorophyll (Chl_{a+b}); and **D**, carotenoids in the leaves of rice plants grown in hydroponic culture containing 0 mM (–Si) or 2 mM (+Si) silicon and inoculated with *Pyricularia oryzae*. Bars represent the standard error and asterisks indicate the means that differed significantly at each evaluation time (*t* test, $P \leq 0.05$). FW = fresh weight.

each reaction was performed three times. The LOX activity was determined after adding 50 μ l of the supernatant to a mixture containing 50 mM sodium phosphate buffer (pH 6.5) and 50 μ M sodium linoleate (pH 6.0) to obtain a total volume of 1 ml. The absorbance of the reaction mixture was recorded every 30 s over 120 s at 234 nm (Axelrod et al. 1981). The soluble protein concentrations on the extracts were measured using the method described by Bradford (1976).

The H_2O_2 concentration was determined by grinding 200 mg of leaf tissue into a fine powder in a mortar and pestle with liquid nitrogen. The fine powder was homogenized in an ice bath in 2 ml of 50 mM potassium phosphate buffer (pH 6.5) containing 1 mM hydroxylamine. The homogenate was centrifuged at $10,000 \times g$ for 15 min at 4°C (Kuo and Kao 2003) and the supernatant was used to determine the H_2O_2 concentration. The reaction was initiated after adding 50 μ l of the supernatant to a mixture containing 25 mM sulfuric acid, 250 μ M ammonium ferrous sulfate, 250 μ M xylenol orange, and 100 mM sorbitol to obtain a total volume of 2 ml (Gay and Gebicki 2000). The samples were stored in the dark for 30 min and the absorbance was recorded at 560 nm. Control samples (blank) for the color of the reagents and for the sample extracts were prepared concurrently with the test samples and subtracted from the absorbance of each sample.

The oxidative damage to the lipids was estimated as the concentration of the total 2-thiobarbituric acid (TBA)-reactive substances and expressed as equivalents of malondialdehyde (MDA) according to Cakmak and Horst (1991), with few modifications. Briefly, 200 mg of leaf tissue was homogenized in 2 ml of 0.1% (wt/vol) trichloroacetic acid (TCA) solution at 4°C . After centrifugation at $10,000 \times g$ for 15 min, 0.5 ml of the supernatant was reacted with 1.5 ml of TBA (0.5% in 20% TCA) for 20 min in a boiling water bath. Next, the reaction was stopped by immersion in an ice bath. The samples were centrifuged at $13,000 \times g$ for 4 min and the absorbance of the supernatant was recorded at 532 nm. The concentrations of MDA formed in each sample were calculated by using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

Determination of the foliar Si concentration. At the end of the blast severity experiment, the leaves were collected from the plants of each replication and each treatment, washed in deionized water, dried for 72 h at 65°C , and ground to pass through a 40-mesh screen using a Thomas Wiley mill (Thomas Scientific, Swedesboro, NJ). The foliar Si concentration was determined by colorimetric analysis using 0.1 g of dried and alkali-digested tissue (Korndörfer et al. 2004).

Experimental design and data analyzes. An experiment consisting of two Si concentrations (0 or 2 mM, hereafter referred to as the -Si and +Si plants, respectively) arranged in a completely randomized design with 10 replications was used to evaluate the blast severity and the foliar Si concentration. The photosynthetic measurements were obtained from an experiment consisting of two Si concentrations arranged in a completely randomized design with 10 replications. In addition, leaf samples were obtained from these experiments for the biochemical assays. The analysis of variance for these experiments was used to test two $\times$ six factors consisting of two Si concentrations and six evaluation times (photosynthetic measurements) and six sampling times (biochemical assays). Each experimental unit consisted of a plastic pot with five plants, and all experiments were repeated once. Data from the AUBPC and foliar Si concentration were pooled based on Bartlett's test, which was used to determine whether the replications from the two experiments were from populations with equal variances. The mean comparisons of the treatments were obtained by conducting a Student's t test ($P \leq 0.05$) using SAS (SAS Institute, Inc., Cary, NC). The blast severity and the photosynthetic measurements were correlated using Pearson's linear correlation analysis.

RESULTS

Foliar Si concentration. The foliar Si concentration significantly increased by 683% for the +Si plants relative to the -Si plants (47 and 6 g kg^{-1} , respectively).

Blast severity and AUBPC. The blast severity was significantly higher on the leaves of the -Si plants relative to the leaves of the +Si plants from 60 to 132 hai (Fig. 1A). The AUBPC was reduced by 89% for the +Si plants in comparison with the -Si plants (Fig. 1B).

Leaf gas exchange parameters. Overall, the values of A , g_s , and E were greater for the +Si plants than for the -Si plants upon *P. oryzae* infection at 08:00 and 12:00 h (Fig. 2). In the early morning (08:00 h), when the gas exchange performance is less affected by the prevailing environmental conditions and the applied treatments are more accurately affected, the A and g_s values were significantly higher at 84, 108, and 132 hai (Fig. 2A and C) and the E value was significantly higher at 0, 84, 108, and 132 hai (Fig. 2E) for the +Si plants relative to the -Si plants. At 12:00 h, A was significantly higher at 66 and 144 hai (Fig. 2B), while g_s and E were higher at 42, 66, and 114 hai (Fig. 2D and F) for the +Si plants relative to the -Si plants. There was no changes in the C_i/C_a ratio for the -Si and +Si plants at 08:00 or 12:00 h (Fig. 2G and H).

Photochemical parameters. At 08:00 h, the F_v/F_m , q_p , NPQ, and ETR values were higher for the +Si plants than for the -Si plants

Fig. 5. Concentration of **A**, malondialdehyde (MDA); **B**, concentration of hydrogen peroxide (H_2O_2); and **C**, lipoxygenase (LOX) activity in the leaves of rice plants grown in hydroponic culture containing 0 mM (-Si) or 2 mM (+Si) silicon and inoculated with *Pyricularia oryzae*. Means for the -Si and +Si treatments followed by an asterisk (*) for each evaluation time are significantly different according to a Student's t test ($P \leq 0.05$). Error bars represent the standard error of the mean.

at 108 and 132 hai (Fig. 3A, C, E, and G). The q_p and ETR values were significantly higher for the –Si plants than for the +Si plants at 36 hai (Fig. 3C and G) and at 0 and 36 hai for NPQ (Fig. 3C). At 12:00 h, the q_p , NPQ, and ETR values were significantly lower for the +Si plants in comparison with the –Si plants at 42, 90, 114 (Fig. 3D), 66 (Fig. 3F), 90, and 114 hai (Fig. 3H), respectively. In comparison with the +Si plants, the NPQ for the –Si plants was significantly higher at 42, 90, and 114 hai (Fig. 3F).

Photosynthetic pigments. The concentrations of the carotenoids and Chl_a, Chl_b, and Chl_{a+b} were significantly greater for the +Si plants than the –Si plants at 108 and 132 hai (Fig. 4A–D). For the –Si plants, the carotenoid concentration was significantly higher at 36 hai in comparison with the +Si plants (Fig. 4D).

MDA and H₂O₂ concentrations. The MDA concentration was significantly higher for the –Si plants than for the +Si plants at 0, 36, 60, 108, and 132 hai (Fig. 5A). For the –Si plants, the H₂O₂ concentration was significantly higher at 36, 108, and 132 hai in comparison with the +Si plants, and was significantly lower for the +Si plants at 60 and 84 hai (Fig. 5B).

Enzyme activities. The LOX activity was significantly higher at 108 and 132 hai for the +Si plants compared with the –Si plants (Fig. 5C). The SOD and GR activities were significantly higher for the –Si plants than for the +Si plants at 36 and 84 hai (Fig. 6A) and at 84 hai (Fig. 6D), respectively. The CAT and APX activities were significantly higher for the +Si plants than for the –Si plants at 84 and 108 hai (Fig. 6B) and at 108 hai (Fig. 6C), respectively.

Pearson correlations. At 08:00 h, the E was positively correlated with the A and g_s . In addition, C_i/C_a was negatively correlated with A and with E and positively correlated with g_s , and the blast severity was negatively correlated with A and positively

correlated with C_i/C_a (Table 1). At 12:00 h, the blast severity was negatively correlated with E . In addition, g_s was positively correlated with E and C_i/C_a . Furthermore, A was positively correlated with E and g_s (Table 1).

DISCUSSION

This study supports previous findings that Si can increase the resistance of several monocots against foliar pathogens (Datnoff et al. 2007; Domiciano et al. 2010; Fauteux et al. 2005; Resende et al. 2012), including the resistance of rice to blast (Rodrigues et al. 2003, 2004, 2005; Seebold et al. 2001). To the best of our knowledge, this study is the first to describe the physiological features associated with increases in rice resistance against *P. oryzae* infection due to the presence of Si. Very few studies have evaluated the effects of Si on the physiological processes of plants under pathogen infections (Gao et al. 2011; Mohaghegh et al. 2011; Resende et al. 2012). However, because the infection process of a certain pathogen is a dynamic event, data obtained at a single time point may not properly reflect what happens to the host physiology upon pathogen infection.

The gas exchange performance of the –Si plants was dramatically impaired over the course of *P. oryzae* infection. However, in the presence of Si (the +Si counterparts), these impairments were reduced. Overall, Si per se provoked negligible if any effect on the physiological parameters measured in this study, as noted for the +Si plants at 0 hai. Many studies have reported that fungal infections negatively affect A due to the physical limitations of CO₂ influx (Berger et al. 2007; Chou et al. 2000; Resende et al. 2012). Regarding blast, the disintegration of cells in the necrotic tissue of the lesions due to nonhost selective toxins can reduce the transport of water and photoassimilates (Yoshii 1937) and potentially trigger

Fig. 6. Activities of **A**, superoxide dismutase (SOD); **B**, catalase (CAT); **C**, ascorbate peroxidase (APX); and **D**, glutathione reductase (GR) in the leaves of rice plants grown in hydroponic culture containing 0 mM (–Si) or 2 mM (+Si) silicon and inoculated with *Pyricularia oryzae*. Means for the –Si and +Si treatments followed by an asterisk (*) for each evaluation time are significantly different according to a Student's t test ($P \leq 0.05$). Error bars represent the standard error of the mean.

stomatal closure, decreasing the CO₂ influx into the leaf. Nevertheless, stomatal limitations to photosynthesis should be dismissed in this study. In the early morning (08:00 h), when the magnitude of the gas exchange is less affected by environmental conditions, changes in the *A* did not correspond with the changes in the *g_s*. Instead, the *A* was negatively correlated with the *C_i/C_a* ratio. Together, these results indicate that the decreases in *A* over the course of blast development were not strongly associated with diffusive (stomatal) limitations to photosynthesis. Furthermore, the *g_s* values were generally greater than 0.5 mol m⁻² s⁻¹, which was more than sufficient for supporting the *A* values (Detmann et al. 2012). Thus, the changes in *A* were likely not driven by the changes in *g_s*. Although the *A* was positively correlated with *g_s* even at midday, no significant correlation was observed between the *A* and *C_i/C_a* ratio, indicating that stomatal limitations did not significantly affect the photosynthetic performance of the rice leaves.

It is possible that the diffusional limitations at the mesophyll level had little impact on the magnitude of the *A*, given that the *C_i/C_a* ratio increased with time (particularly at 08:00 h). Therefore, biochemical dysfunctions at the chloroplast level likely played a primary role in constraining the *A* upon infection by *P. oryzae*. In addition, similar results were reported by other authors when measuring the gas exchange parameters for the interactions of hemibiotrophic *Mycosphaerella* spp.–*Eucalyptus globulus* and wheat–*P. oryzae* (Aucique-Perez et al. 2014; Debona et al. 2014; Pinkard and Mohammed 2006) and biotrophic *Puccinia psidii*–*E. urophylla* (Alves et al. 2011).

Pyricularia oryzae can release lytic enzymes and nonhost selective toxins into the leaf tissues that could impair carbon fixation reactions or reduce the concentrations of photosynthetic pigments (Aver'yanov et al. 2007), as noted in the present study. These dysfunctions could be mitigated in the presence of Si given that the physical barrier formed on the rice leaf blades following Si deposition (Yoshida et al. 1962) should contribute to the preservation and functionality of the photosynthetic apparatus in the +Si plants. In addition, because the impairments in the *A* of rice leaf tissues infected by *P. oryzae* may occur in asymptomatic tissues (Bastiaans 1991), the higher *A* values likely resulted from the maintenance of adequate carbon fixation rates in the asymptomatic tissues which made up a greater proportion of leaf tissue.

Overall, changes in photosynthesis photochemistry were more prominent at the advanced stages of fungal infection, which were only evident for the –Si plants. These results involved the following changes: (i) decreases in the *q_P*, suggesting that a lower fraction of the absorbed light was dissipated photochemically or that an increased proportion of oxidized Q_A was present (which represents the fraction of the PSII centers that are prone to suffer photo-inhibitory damage) (Lima et al. 2002); (ii) decreases in the NPQ, which corresponds with lower thermal dissipation of excess energy (Logan et al. 2007); and (iii) dramatic decreases in the ETR during

the advanced stages of fungal infection, which can be attributed to the lytic enzymes and nonhost selective toxins produced by *P. oryzae* in the rice leaf tissue that can disrupt the electron transport chain in the thylakoid membranes (Chen et al. 2007, 2008). The observed values of ETR are more than sufficient for supporting the photochemical requirements of the actual *A* values (Baker 2008; Lichtenthaler and Miehe 1997). Therefore, the decreases in the *A* upon fungal infection could not be related to photochemical dysfunctions. It can be hypothesized that these dysfunctions might contribute to blast development by potentiating the occurrence of oxidative stress.

Due to the chronic overexcitation state on the leaves of –Si plants during the time course of *P. oryzae* infection, it can be hypothesized that these plants were subjected to an oxidative stress condition because the excess reducing power could not be fully dissipated. This hypothesis was supported by the higher H₂O₂ and MDA concentrations which reflect lipid peroxidation and the greater LOX activity for the –Si plants. The increased LOX activity with the progression of blast severity could be linked to the MDA production. Notably, the +Si plants generally accumulated H₂O₂ at the intermediate stages of fungal infection, which could be important for triggering some unknown mechanism of rice resistance to blast. In contrast to the results obtained from this study, the concentrations of H₂O₂ and MDA were maintained on the leaves of rice plants supplied with Si and infected by *P. oryzae* in a study by Sun et al. (2010). It was speculated that the high H₂O₂ concentrations for the +Si plants infected with *P. oryzae* can be readily controlled by the CAT activity because its activity was enhanced for the +Si plants at 84 hai, immediately after the peak H₂O₂ concentration. Therefore, it is plausible that the +Si plants could not only accumulate H₂O₂ but also temporally regulate its concentration through changes in the CAT activity. In the –Si plants, greater H₂O₂ concentrations upon *P. oryzae* infection could result in increases in the SOD activities, which catalyze the dismutation of O₂⁻ to H₂O₂ when coupled with higher LOX activities that were not compensated for by increases in the concentrations of enzyme associated with H₂O₂ removal, such as CAT and APX. Indeed, the activities of these enzymes varied at different time periods, during *P. oryzae* infection and were accompanied only by inconsistent alterations in the GR activity. At 132 hai, no significant variations in the SOD, CAT, APX, and GR activities were observed in the presence of Si despite the enhanced excitation pressure (lower *q_P* coupled with lower NPQ) for these plants. This result implies that these plants were unable to induce further antioxidant defenses for coping with overexcitation, which ultimately helped to exacerbate the occurrence of oxidative stress and blast severity.

Overall, adequate *g_s* and *A* values were maintained in the leaves of the rice plants supplied with Si. The supply of Si helped protect the photosynthetic apparatus against chronic photoinhibition during *P. oryzae* infection and indirectly protected the photosystems against damage, which often results from a state of chronic hyperexcitation. Therefore, the rice plants supplied with Si and infected with *P. oryzae* are less prone to this chronic state of hyperexcitation than in the absence of Si. Moreover, the results of the present study show that the oxidative stress in rice plants infected by *P. oryzae* was alleviated in the presence of Si due to regulations in the activities of the enzymes involved in ROS scavenging, mainly the SOD, CAT, and APX.

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TABLE 1. Pearson's correlation coefficients among the net carbon assimilation rate (*A*), stomatal conductance to water vapor (*g_s*), transpiration rate (*E*), internal-to-ambient CO₂ concentration ratio (*C_i/C_a*), and blast severity (Sev) determined in the leaves of rice plants grown in hydroponic culture containing 0 mM (–Si) or 2 mM (+Si) silicon^a

Variables	<i>A</i>	<i>g_s</i>	<i>E</i>	<i>C_i/C_a</i>	Sev
<i>A</i>	...	0.16 ^{ns}	0.74*	–0.55*	–0.59*
<i>g_s</i>	0.76*	...	0.55*	0.58*	0.29 ^{ns}
<i>E</i>	0.81*	0.88*	...	–0.14*	–0.17 ^{ns}
<i>C_i/C_a</i>	–0.20 ^{ns}	0.41*	0.17 ^{ns}	...	0.68*
Sev	–0.35 ^{ns}	–0.47*	–0.38*	–0.24 ^{ns}	...

^a Values above and below the diagonal are the measurements made at 08:00 and 12:00 h, respectively; * = significant at 1% of probability according to the Student's *t* test and ns = not significant.

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