MARCELA ULI PEIXOTO ARAUJO

SILICON ALLEVIATES THE CHANGES IN THE SOURCE-SINK RELATIONSHIP ON WHEAT PLANTS INFECTED BY *Pyricularia oryzae*

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título de *Magister Scientiae*.

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APROVADA: 26 de julho de 2017.

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À minha família,
meu pai Eliomar,
minha mãe Ana Maria,
e meu irmão Vitor Tagore:
OFEREÇO E DEDICO.

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BIOGRAFIA

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ÍNDICE

ABSTRACT

ARAUJO, Marcela Uli Peixoto, M.Sc., Universidade Federal de Viçosa, July, 2017. **Silicon alleviates the changes in the source-sink relationship on wheat plants infected by *Pyricularia oryzae*.** Adviser: Fabrício de Ávila Rodrigues. Co-adviser: Jonas Alberto Rios.

Blast, caused by *Pyricularia oryzae*, has become an economically important disease on wheat in Brazil. Alternative methods for blast management are demanded by the growers and silicon (Si) stands out for its potential to decrease the intensities of important diseases in several crops. The present study aimed to investigate the effect of Si in improving the production of photoassimilates on flag leaves and their partitioning to spikes in a scenario where the blast symptoms will be lowered due to the potentiation of mechanisms of defense by this element on the source-sink interface. Wheat plants (cultivar BRS-Guamirim) were grown in hydroponic culture with either 0 or 2 mM Si and plants were inoculated with *P. oryzae* at 10 days after anthesis. The Si concentration on flag leaves and spikes of plants supplied with Si increased and contributed to decrease disease symptoms. Higher concentrations of total soluble phenolics and lignin-thioglycolic acid derivatives and greater activities of peroxidases, polyphenoloxidases, phenylalanine ammonia-lyases, *β*-1,3-glucanases and chitinases were noticed on flag leaves and spikes of plants supplied with Si and contributed to their resistance to blast. There was less concentration of pigments and an impairment of the photosynthetic performance of infected flag leaves and spikes from plants non-supplied with Si based on the values for the chlorophyll *a* fluorescence parameters maximal photosystem II quantum efficiency, fraction of energy absorbed used in photochemistry, quantum yield of non-regulated energy dissipation and quantum yield of regulated energy dissipation. On infected flag leaves and spikes from plants non-supplied with Si, the concentration of soluble sugars was lower while the hexoses-to-sucrose ratio increased on infected flag leaves. The sucrose phosphate synthase activity was lower while higher expression and activity of acid invertases was higher and lower, respectively, on flag leaves and spikes of plants non-supplied with Si in comparison to Si-supplied plants. The starch concentration on spikes of plants supplied with Si increased. All together, the results from the present study confirm the active role played by Si in increasing wheat resistance to blast, but present novel evidences of the effect of this element in

improve the source-sink relationship on infected flag leaves and spikes by preserving the alterations in assimilate production and partitioning during the grain filling process.

RESUMO

ARAUJO, Marcela Uli Peixoto, M.Sc., Universidade Federal de Viçosa, julho de 2017. **Silício alivia as mudanças na relação fonte-dreno em plantas de trigo infectadas por *Pyricularia oryzae*.** Orientador: Fabrício de Ávila Rodrigues. Coorientador: Jonas Alberto Rios.

A brusone, causada por *Pyricularia oryzae*, tornou-se uma doença no trigo economicamente importante no Brasil. Métodos alternativos para o manejo da brusone são exigidos pelos produtores e o silício (Si) se destaca por seu potencial para diminuir a intensidade de doenças importantes em várias culturas. O presente estudo teve como objetivo investigar o efeito de Si em melhorar a produção de fotoassimilados em folhas bandeiras e espigas em um cenário onde os sintomas da brusone diminuem devido à potencialização de mecanismos de defesa por este elemento na relação fonte-dreno. As plantas de trigo (cultivar BRS-Guamirim) foram cultivadas em cultura hidropônica com 0 ou 2 mM de Si e as plantas foram inoculadas com *P. oryzae* aos 10 dias após a antese. A concentração de Si em folhas bandeiras e espigas de plantas fornecidas com Si aumentou e contribuiu para diminuir os sintomas da doença. As concentrações mais elevadas de fenóis solúveis totais e derivados de ácido lignina-tioglicólico e maiores atividades de peroxidases, polifenoloxidases, fenilalanina amônia-liases, β-1,3-glucanases e quitinases foram notadas em folhas bandeiras e espigas de plantas fornecidas com Si e contribuíram para a sua resistência à brusone. Houve menos concentração de pigmentos e um comprometimento da performance fotossintética de folhas bandeiras e espigas infectadas de plantas não fornecidas com Si com base nos valores dos parâmetros da fluorescência da clorofila *a* a partir da máxima eficiência quântica do fotossistema II, fração de energia absorvida usada em fotoquímica, rendimento quântico de dissipação de energia não regulada e produção quântica de dissipação de energia regulada. Nas folhas bandeiras e espigas infectadas de plantas não fornecidas com Si, a concentração de açúcares solúveis foi menor, enquanto a relação hexose-sacarose aumentou em folhas bandeiras infectadas. A atividade da sacarose fosfato sintase foi menor, enquanto a maior expressão e atividade das invertases ácidas foi maior e menor, respectivamente, nas folhas bandeira e espigas de plantas não fornecidas com Si em comparação com plantas fornecidas por Si. A concentração de amido em espigas de plantas fornecidas com Si aumentou. Em conjunto, os resultados do presente estudo confirmam o papel ativo desempenhado pelo Si no aumento da resistência do trigo à brusone, mas apresenta novas evidências do efeito desse elemento em melhorar a relação fonte-dreno em folhas bandeiras e espigas infectadas preservando as alterações na produção de assimilados e partição durante o processo de enchimento de grãos.

Introduction

Blast, caused by the hemibiotrophic fungus *Pyricularia oryzae* Cavara (teleomorph *Magnaporthe oryzae* (T. T. Hebert) M. E. Barr) (Igarashi et al., 1986; Goulart & Paiva, 1992) has been reported to occur in some regions producing wheat in Brazil, Bolivia and Paraguay with the potential to greatly impact wheat (*Triticum aestivum* L.) yield. Frequently rainy periods, temperatures ranging from 21 to 27°C, cloudy days and high relative humidity are climate conditions that favor the occurrence of blast epidemics (Goulart et al., 2007; Kohli et al., 2011). In 2016, wheat blast reached the Asian continent and was found in about 15% of the total wheat area in Bangladesh with yield losses reaching around 90% (Malaker et al., 2016).

Disease symptoms are elliptical to round lesions on leaves and premature bleaching and death of individual or entire spikelets (Goulart et al., 2007). The occurrence of blast on the spikes cause an impedance on the translocation of photoassimilates to the developing grains which became shrivelled, deformed, small and of reduced weigh (Goulart et al. 2007). Some studies were carried out to investigate the diversity on the population of *P. oryzae*, to obtain new strategies for disease management (Maciel et al., 2014; Pagani et al., 2014; Rocha et al., 2014) and to determine the disease´s impact on wheat physiology and yield (Goulart et al., 2007; Debona et al., 2014; Rios et al., 2017). Control strategies to minimize the yield losses caused by blast include the use of resistant cultivars, when available, and fungicide application (Maciel, 2011; Castroagudín et al., 2015). Alternative methods for blast management are demanded by the growers to reduce yield losses caused by blast. Silicon (Si), although not considered an essential nutrient for plants, stands out for its potential to decrease the intensities of important diseases in several crops, especially grasses and some dicots (Datnoff et al., 2007).

The biochemical and physiological mechanisms involved in the potentiation of host defense mechanisms by Si include the high concentration of phenolics, lignin, and phytoalexins; an increase in the activities of defense enzymes such as chitinases and *β*-1,3-glucanases and the rapid transcription of genes associated with plant resistance (Rodrigues et al., 2003, 2004, 2005; Liang et al., 2006; Brunings et al., 2009). Furthermore, increased resistance of plants supplied with Si against pathogens has also been associated with a physical barrier that prevents or slows fungal penetration; this physical barrier is the result of an increase in the density of the long and short silicate cells in the leaf epidermis or from a thick silica layer below the cuticle as noted for the rice-*P. oryzae* pathosystem (Kim et al., 2002). Lower blast severity occurred on wheat plants supplied with Si (Xavier Filha et al., 2011). Aucique-Pérez et al. (2014) demonstrated that even not having a direct metabolic role in photosynthesis upon *P. oryzae* infection, the protection against blast afforded by Si could be translated into improved gas exchange performance and less dysfunction at the photochemical level of infected plants supplied with this element. These ameliorative effects of Si combined with the use of wheat cultivars with basal level of resistance to blast (Cruz et al., 2012; Debona et al., 2012) may be translated into useful tools to enhance wheat yield under conditions for the occurrence of severe blast epidemics.

Wheat yield is closely dependent on the source-sink relationship known to be a dynamic process determined by the capacity of the source tissues to produce photoassimilates through photosynthesis as well as the ability of the sink tissues to convert them into starch (Tuncel & Okita, 2013). The flag leaves and the spikes are the main photosynthetic sources that contribute to the filling of wheat grains (Tambussi et al., 2007). The starch synthesized in the chloroplasts during the day is degraded at night for a continuous supply of sugars for metabolism on leaves and to the exportation of sucrose to the sink organs (Zeeman et al., 2007). The infection process of pathogens of different life styles negatively impact the photosynthetic performance of their hosts as well as the carbohydrate composition and their partition within the infected tissues (Gammet al., 2011; Abood & Lösel, 2003).

The present study investigated the effect of Si in improving the production of photoassimilates on flag leaves and their partitioning to spikes in a scenario where the blast symptoms will be lowered due to the potentiation of mechanisms of defense.

Materials and Methods

Nutrient solution preparation

The nutrient solution used in this study was prepared based on Clark (1975) and included the following macronutrients: 1.04 M $Ca(NO_3)_2 \cdot 4H_2O$, 1 M NH_4NO_3, 0.8 M KNO_3, 0.6 M $MgSO_4 \cdot 7H_2O$, 6.9 mM KH_2PO_4 and 9.3 mM KCl; and the micronutrients: 2 mM H_3BO_3, 2 mM $ZnSO_4 \cdot 7H_2O$, 7 mM $MnCl_2 \cdot 4H_2O$, 0.5 mM $CuSO_4 \cdot 5H_2O$, 0.6 mM $ZnSO_4 \cdot 7H_2O$, 90 mM $FeSO_4 \cdot 7H_2O$ and 90 mM disodium EDTA. Silicon was supplied as monosilicic acid and was obtained by passing potassium silicate (PQ Corporation, São Paulo, Brazil) through a column with a cation exchange resin (Amberlite IR-120B, H+ form; Sigma-Aldrich, São Paulo, Brazil) (Ma et al., 2002). The Si rates used were 0 or 2 mM. The addition of monosilicic acid to the nutrient solution did not alter the pH.

Plant growth

Wheat seeds (*T. aestivum*) from cultivar BRS-Guamirim, considered to be susceptible to blast (Cruz et al., 2010), were surface sterilized in 10% (vol/vol) NaOCl for 2 min, rinsed in sterilized water for 3 min and germinated on a sand substrate at 25°C for 7 days. The germinated seedlings were transferred to plastic pots (20 cm in diameter) (Ecovaso, Jaguariúna, São Paulo, Brazil) with half-strength nutritive solution without Si for 7 days. After this period, plants were transferred to plastic pots (five plants per plastic pot) containing 5 liters of nutrient solution prepared with or without the addition of monosilicic acid. The nutrient solution was changed every 4 days and its pH was measured daily. The pH was maintained at approximately 5.8 by adding NaOH or HCl (1 M) when necessary. The plants were grown in a greenhouse with relative humidity of 70 ± 5% and temperature of 25 ± 3°C.

Plant inoculation with *P. oryzae*

A pathogenic isolate of *P. oryzae* (UFV/DFP *Po*-01) obtained from spikes of wheat plants from cultivar BR-18 was used to inoculate the plants (Debona et al., 2012). Disks of filter paper containing fungal mycelia were transferred to Petri dishes containing oat-agar medium. After fungal mycelia growth, 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 with a 24-h photoperiod for 8 days. After this period, conidia were carefully removed from the Petri dishes 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. The conidial suspension was sprayed on the adaxial surface of the leaves and spikes of plants at the growth stage 75 (Lancashire et al., 1991) with the aid of an atomizer (Paasche Airbrush Co., Chicago). After inoculation, plants were kept in a mist chamber at 25°C for 24 hai darkness. Plants were then transferred to a greenhouse (relative humidity of 80 ± 5% and temperature of 25 ± 3°C) until the end of the experiments.

Blast assessment

Blast severity on the flag leaves and spikes was assessed at 48, 72 and 96 hours after inoculation (hai). On the flag leaves, blast severity was quantified by using a diagrammatic scale (Rios et al., 2013) while on the spikes it was quantified as the mean proportion of diseased spikelets per spike (diseased spikelets/total spikelets rated × 100).

Chlorophyll *a* (Chl*a*) fluorescence imaging

Images and parameters of Chl*a* fluorescence were obtained from the flag leaves and spikes of plants at 48, 72, 96 hai using the MAXI version of the Imaging-PAM fluorometer and the Imaging Win software (Heinz Walz GmbH, Effeltrich, Germany). The Chl*a* fluorescence emission transients were captured by a CCD (charge-coupled device) camera with a resolution of 640 × 480 pixels in a visible sample area of 24 × 32 mm on each tissue. Initially, plants were dark-adapted for 60 min after which they were carefully and individually fixed in a support at a distance of 18.5 cm from the CCD camera. The flag leaves and spikes were exposed to a weak, modulated measuring beam (0.5 µmol m^{-2} s^{-1}, 100 µs, 1 Hz) to determine the initial fluorescence (F_0) when all the PSII reaction centers were "open". Next, a saturating white light pulse of 2,400 µmol m^{-2} s^{-1} (10 Hz) was applied for 0.8 s to ensure the maximum fluorescence emission (F_m) when all the PSII reaction centers were "closed". From these initial measurements, the maximum PSII photochemical efficiency of the dark-adapted flag leaves and spikes was estimated through the variable-to-maximum Chl*a* fluorescence ratio, $F_v/F_m = [(F_m - F_0)/F_m)]$. The flag leaves and spikes were subsequently exposed to actinic photon irradiance (110 µmol m^{-2} s^{-1}) for 120 s to obtain the steady-state fluorescence yield (F_s) after which a saturating white light pulse (2,400 µmol m^{-2} s^{-1}; 0.8 s) was applied to achieve the light-adapted maximum fluorescence (F_m’). Following the calculations of Kramer et al. (2004), the energy absorbed by the PSII for the following two yield components for dissipative processes were determined: the yield of photochemistry [Y(II) = (F_m’ - F_s)/F_m’)] and the yield for other non-photochemical (non-regulated) losses [Y(NO) = F_s/F_m] (Kramer et al., 2004).

Determination of photosynthetic pigments

The concentrations of chlorophyll (Chl) *a*, Chl*b* and carotenoids were determined using the dimethyl sulfoxide (DMSO) as an extractor (Santos et al., 2008). Five disks of flag leaves (1 cm in diameter) were collected at 48, 72, 96 hai, immersed in glass tubes containing 5 mL of saturated DMSO solution and calcium carbonate ($CaCO_3$)

(5 g L^{-1}) (Wellburn, 1994) and kept at room temperature during 24 h at darkness. The absorbances of the extracts were read at 480, 649 and 663 nm using a saturated solution of DMSO and $CaCO_3$ as the reference.

Determination of Si concentration on flag leaves and spikes

Flag leaves and spikes were collected from the plants used to evaluate blast severity, washed in deionized water, dried for 72 h at 65°C and then ground to pass through a 40-mesh screen with a Thomas Wiley mill (Thomas Scientific, Swedesboro, NJ). The Si concentration was determined by colorimetric analysis of 0.1 g of dried and alkali-digested tissue (Korndörfer et al., 2004).

Determination of enzymes activities

Samples of flag leaves and spikelets obtained from spikes from plants per replication of each treatment were collected at 48, 72 and 96 hai. Samples were collected at 8 a.m., kept in liquid nitrogen during sampling and then stored at -80ºC until further analysis. Enzymes were extracted according to Nunes-Nesiet et al. (2007). Activities of acid invertases (Praxedes et al., 2006) and sucrose phosphate synthase (SPS) (under saturating – V_{max} and limiting – V_{sel} substrate conditions) (Doehlert & Huber, 1983) were determined. All enzyme activities were expressed on a fresh matter (FM) basis. To obtain the extracts that were used to determine the peroxidase (POX) and polyphenol oxidase (PPO) activities, 0.3 g flag leaf tissue was macerated using a mortar and pestle with liquid nitrogen and polyvinylpyrrolidone (PVP) 2% (w/v) to obtain a fine powder. The powder was homogenized in 2 ml 100 mM potassium phosphate (pH 6.8) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and 0.1 mM ethylenediaminetetra acetic acid (EDTA). The homogenized material was centrifuged at 12 000 *g* for 15 min at 4°C and the supernatant was used for the enzymes determination. The POX activity was determined by the oxidation of pyrogallol according to the method of Kar & Mishra (1976). A mixture of 950 µl distilled water, 750 µl 100 mM potassium phosphate buffer (pH 6.8), 600 µl 100 mM pyrogallol and 600 µl 100 mM hydrogen peroxide was added to 100 µl of the extract. The absorbance was measured in a spectrophotometer at 420 nm every 10 seconds for 1 min after the addition of the extract to the mixture in a total of six readings. A molar extinction coefficient of 2.47 mM/cm was used to calculate POX activity (Chance & Maehley, 1955), which was expressed in µmol purpurogallin that was produced $min^{-1}mg^{-1}$ protein. Throughout the process, the microcentrifuge tubes were covered with aluminium foil to protect the mixture from light oxidation. The PPO activity was determined similar to that of POX, except that hydrogen peroxide was not used in the mixture. To obtain the extract that was used in the determination of the phenylalanine ammonia-lyase (PAL) activity, 0.3 g flag leaf tissue was macerated using a mortar and pestle with liquid nitrogen and PVP (2%) to obtain a fine powder. The powder was homogenized in 2 ml 0.05 M sodium borate (pH 8.3) containing 5 mM *β*-mercaptoethanol and 1 mM EDTA. The mixture was centrifuged twice at 7000 *g* for 15 min. The supernatant was used to determine PAL activity. The reaction was initiated by adding 0.5 ml of the extract to a mixture containing 2 ml sodium borate buffer 0.1 M (pH 8.8) and 1 ml 100 mM *L*-phenylalanine. The reaction mixture was incubated in a water bath at 30°C for 4 h. In the control samples, the extract was replaced with sodium borate buffer. The reaction was finalized by adding 0.1 ml 6 N HCl. The absorbance of the *trans*-cinnamic acid derivatives was measured in a spectrophotometer at 290 nm and a molar extinction coefficient of 104 mM/m (Zucker, 1965) was used to calculate the PAL activity, which was expressed in µmol/$min^{-1}mg^{-1}$ protein. To obtain the extracts that were used in the determination of the chitinase (CHI) and *β*-1,3-glucanase (GLU) activities, 0.3 g of flag leaf tissue was macerated in a mortar and pestle with liquid nitrogen and PVP (2%) to obtain a fine powder. The powder was homogenized in 2 ml buffer consisting of 50 mM sodium phosphate (pH 6.5) and 1 mM PMSF. The homogenized material was centrifuged at 20,000 *g* for 25 min at 4°C and the supernatant was used as the extract for the enzymes determination. The CHI activity was determined by the method of Roberts & Selitrennikoff (1988) as modified by Harman et al. (1993). The reaction was initiated by the addition of 20 µl aliquots of the supernatant to a mixture of 480 µl buffer, 50 mM sodium acetate (pH 5.0) and 20 µl of the substrate *p*-nitrophenyl-b-DN-N'-diacetylchitobiose (Sigma-Aldrich, Brazil, São Paulo) at a concentration of 2 mg/ml. The reaction mixture was incubated in a water bath at 37°C for 2 h. The reaction was interrupted by adding 500 µl 0.2 M sodium carbonate. In the control samples, only the sodium carbonate was used after adding the extract to the reaction mixture and the samples were incubated in a water bath at 37°C for 2 h. The absorbance of the final product that was released by the CHI was determined at 410 nm. A molar extinction coefficient of 7 x 10^4 mM/cm was used to calculate CHI activity, which was expressed in mol *p*-nitrophenyl $\text{min}^{-1}\text{mg}^{-1}$ protein. The GLU activity was determined as described by Lever (1972). The reaction was initiated by the addition of 20 µl aliquots of the supernatant to a mixture of 230 µl buffer, 100 mM sodium acetate (pH 5.0) and 250 µl of the substrate laminarin (Sigma-Aldrich, Brazil, São Paulo) at a concentration of 4 mg/ml. The reaction mixture was incubated in a water bath for 30 min at 45°C. After the incubation period, the amount of reducing sugars was determined by adding 250 µl dinitrosalicylic acid to the mixture and then incubating the resulting mixture in a water bath for 15 min at 100°C. The reaction was interrupted by cooling the samples to 30°C in an ice bath. In the control samples, the reaction mixture was the same except that the extract was added after heating the mixture at 100°C. The absorbance of the product that was released by GLU was measured at 540 nm and the GLU activity was expressed in absorbance units $\text{min}^{-1}\text{mg}^{-1}$ protein. All of the enzymes activity measurements were performed in triplicate.

Determination of the concentrations of carbohydrates and proteins

Samples of flag leaves and of grains obtained from inoculated plants were collected at 48, 72 and 96 hai. Samples collected from flag leaves and spikes from non-inoculated plants served as a control (0 h). Samples (250 mg of FW) were lyophilized (-48°C) and grounded in a cell disruptor using metal balls (3.2 mm in diameter) following agitation at 40 *g* for 5 min (Mini-Bead beater-96, Bio Spec Products, Bartlesville, OK, USA. Samples (15 mg) were homogenized with 600 μL 98% (v/v) ethanol and then incubated at 80ºC for 20 min following centrifugation at 14,000 *g* for 5 min. These processes were repeated after homogenizing the pellet with 80 and 50% ethanol, respectively. The concentrations of sugars (glucose, fructose and sucrose) (Gibon et al., 2004) were determined in the supernatant solutions while the concentrations of starch (Fernie et al., 2001) and proteins (Bradford, 1976) were determined in the insoluble fractions. The ratio of the sugar concentrations (RCS) was estimated as RCS = ($X_{48,\ 72\ and\ 120\text{hai}}/\overline{X}_{control}$) where $\overline{X}$ represents the mean value from non-inoculated plants (control) at 48, 72 and 120 hai.

Determination of the concentration of total soluble phenolics and lignin-thioglycolic acid (LTGA) derivatives

Samples of flag leaves and spikes from inoculated plants were collected at 48, 72 and 96 hai. Samples collected from flag leaves and spikes from non-inoculated plants served as a control (0 h). A representative sample of 0.1 g of either flag leaf or spikes from the replication of each treatment was ground into a fine powder using a pestle and mortar with liquid nitrogen. The fine powder was transferred to a microcentrifuge tube, homogenized with 1.5 mL of 80% methanol and extracted overnight on a rotary shaker (150 rpm) at room temperature. The homogenate solution was protected from light oxidation by covering the microcentrifuge tubes with aluminum foil. The dark-green methanolic extract was centrifuged at 12,000 *g* for 5 min and the supernatant was transferred to a new microcentrifuge tube and stored at -20°C. The residue was kept at -20°C until the determination of lignin and lignin-like phenolic polymers. The method developed by Zieslin & Ben-Zaken (1993) was used to determine the concentration of total soluble phenolics (TSP) with a few modifications. A volume of 150 μL of 0.25 N Folin and Ciocalteau's Phenol reagent (Sigma-Aldrich, São Paulo, Brazil) was added to 150 μL of methanolic extract and the mixture was homogenized and kept at room temperature for 5 min. Next, 150 μL of 1M Na_2CO_3 was added to the mixture, which was homogenized again and kept at room temperature for 10 min. The mixture was further homogenized with 1 mL of distilled water and kept at room temperature for 1 h. The absorbance of the developed blue color of a representative sample (500 μL) of the mixture from each replication and treatment was measured at 725 nm. The concentration of TSP was expressed as mg of phenolics (in terms of catechol) per g of FW of leaf flag or spikes. A volume of 1.5 mL of sterile distilled water was added to the residue obtained after extraction of TSP and, after homogenization, the mixture was centrifuged at 12,000 *g* for 5 min. The supernatant was discarded and the residue was left to dry at 65°C overnight. The dried alcohol insoluble residue, containing both true lignin and phenolic acids esterified to cell walls, was used for determination of lignin according to the method of Barber & Ride (1998). A volume of 1.5 mL of a 1:10 solution of thioglycolic acid (Sigma-Aldrich, São Paulo, Brazil) and 2 N HCl was added to the dried residue. The microcentrifuge tube was shaken gently to hydrate the residue and then placed in boiling water (approximately 100°C) for 4 h. The microcentrifuge tube was cooled in ice in a 4°C cold room for 10 min. The mixture was then centrifuged at 12,000 *g* for 10 min and then the supernatant was discarded. The precipitate was washed with 1.5 mL of sterile distilled water and then centrifuged at 10,000 *g* for 10 min. After centrifugation, the supernatant was discarded, the precipitate was resuspended in 1.5 mL of 0.5 N NaOH and the mixture was agitated overnight at 150 rpm in a rotary shaker at room temperature. In the next step, the mixture was centrifuged at 10,000 *g* for 10 min and the supernatant was transferred to a new microcentrifuge tube. After adding 200 μL of concentrated HCl to the supernatant, the microcentrifuge tube was transferred to a 4°C cold room for 4 h to allow the LTGA derivatives to precipitate. Following centrifugation at 10,000 *g* for 10 min, the supernatant was discarded and the orange-brown precipitate was dissolved in 2 mL of 0.5 N NaOH. The absorbance of LTGA derivatives in the supernatant was measured at 280 nm. The concentration of LTGA derivatives was expressed as mg/kg FW of leaf flag or spikes by using lignin alkali, 2-hydroxypropyl ether (Sigma-Aldrich, São Paulo, Brazil) as a standard.

Experimental design and statistical analysis

A two-by-two factorial experiment, consisting of two Si concentrations (0 and 2 mM; hereafter referred to as the -Si and +Si plants, respectively) and non-inoculated or inoculated plants, was arranged in a completely randomized design with five replications. The experiment was repeated once. Each experimental unit corresponded to a plastic pot containing five plants. A total of 100 plants were used in each experiment (25 plants per treatment at each evaluation time). The data from all the variables evaluated were subjected to an analysis of variance (ANOVA) and the means from the treatments were compared by *F* test ($P \leq 0.05$) using the SAS software (SAS Institute Inc., Cary, NC). For the Si concentration on flag leaves and spikes and the concentration of photosynthetic pigments, ANOVA was used and considered to be a two-by-two factorial experiment consisting of two Si concentrations and non-inoculated or inoculated plants. For the photosynthetic measurements, ANOVA was used and considered to be a two-by-two-by four factorial experiment consisting of two Si concentrations, non-inoculated or inoculated plants and four samplings. Pearson correlation was used to determine the relationships between Si concentration on flag leaves and spikes and blast severity for both -Si and +Si treatments in separate.

Results

Concentration of Si on flag leaves and spikes

The Si concentration on flag leaves significantly increased by 97 and 72%, respectively, for both non-inoculated and inoculated +Si plants in comparison to -Si counterparts (Fig. 1A). There were significant increases of 98 and 86%, respectively, on Si concentration on the spikes of non-inoculated and inoculated +Si plants in comparison to -Si counterparts (Fig. 1B).

Blast severity

For +Si plants, blast severity on flag leaves significantly decreased by 87 and 67%, respectively, at 48 and 96 hai in comparison to -Si plants (Fig. 2A). Blast severity on the spikes of +Si plants significantly decreased by 48 and 58% at 72 and 96 hai, respectively, in comparison to -Si plants (Fig. 2B). At 48 hai, small water-soaked lesions were noticed on flag leaves and from 72 to 96 hai they expanded, became necrotic, exhibited grayish coloration and coalesced (Fig. 3A1-A3) in contrast to the small non-coalesced lesions that developed on flag leaves of +Si plants (Fig. 3C1-C3). On the spikes of -Si plants, the gray-brown lesions caused intense tissue discoloration (Fig. 3B1-B3). By contrast, the spikes of +Si plants showed greenish coloration and elliptical lesions with light brown color in the borders and gray centers and were of small size (Fig. 3D1-D3).

Activities of defense enzymes

For flag leaves of non-inoculated plants, there was no significant difference for PAL, PPO, CHI, GLU and POX activities between -Si and +Si treatments regardless of the sampling time (Fig. 4A1, B1, C1, D1 and E1). For spikes of non-inoculated plants, there was no significant difference for PAL, CHI, GLU and POX activities between -Si and +Si treatments regardless of the sampling time (Fig. 4A2, C1, D1 and E1). At

72 hai, PPO activity significantly increased by 25% on spikes of +Si non-inoculated plants in comparison to -Si non-inoculated plants (Fig. 4B2). On flag leaves of +Si inoculated plants, there were increases on activities, respectively, of 21, 22 and 31% for PAL at 48, 72 and 96 hai, of 20% for PPO at 96 hai and of 23 and 8% for CHI at 72 and 96 hai in comparison to -Si counterparts (Fig. 4A2, B2 and C2). The GLU and POX activities significantly increased by 22 and 37%, respectively, at 96 hai on flag leaves of -Si inoculated plants in comparison to +Si counterparts (Fig. 4D2 and E2). On spikes of +Si inoculated plants, there were increases on activities, respectively, of 35% at 96 hai for PAL, of 28 and 33% at 72 and 96 hai for PPO, of 20, 14 and 8% at 48, 72 and 96 hai for CHI and of 31% at 96 hai for GLU in comparison to -Si counterparts (Fig. 4A4, B4, C4 and D4). At 96 hai, POX activity significantly decreased by 34% on spikes of +Si inoculated plants in comparison to -Si inoculated plants (Fig. 4E4).

Concentration of TSP

For non-inoculated plants, there was no significant difference between -Si and +Si plants regardless of the sampling time (Fig. 5A and C). For flag leaves of inoculated plants, the concentration of TSP significantly increased by 12, 24 and 22%, respectively, at 48, 72 and 96 hai for +Si plants in comparison to -Si plants (Fig. 5B). The concentration of TSP significantly decreased by 15% at 48 hai and significantly increased by 16 and 17%, respectively, at 72 and 96 for +Si plants in comparison to -Si plants (Fig. 5D).

Concentration of LTGA derivatives

For flag leaves of non-inoculated plants, there was no significant difference between -Si and +Si treatments regardless of the sampling time for the concentration of LTGA derivatives (Fig. 6A). The concentration of LTGA derivatives significantly increased by 42% at 96 hai for spikes of +Si plants in comparison to -Si plants (Fig. 6C). For flag leaves of inoculated plants, the concentration of LTGA derivatives significantly increased by 31 and 42%, respectively, at 72 and 96 hai for +Si plants in comparison to -Si plants (Fig. 6B). The concentration of LGTA derivatives significantly increased by 47% at 96 hai for spikes of +Si plants in comparison to -Si plants (Fig. 6D).

Imaging of Chl*a* fluorescence parameters

The first visual changes in the images of the parameters F_v/F_m, F_m, Y(II), Y(NPQ) and Y(NO) were noticed at 48 hai on flag leaves and spikes of -Si and +Si plants (Fig. 7). As the lesions expanded on flag leaves and spikes of -Si plants, especially at 96 hai, there were alterations in the images of these five parameters (Fig. 7). By contrast, on flag leaves and spikes of +Si plants, alterations in the images of the parameters F_v/F_m, F_m, Y(II), Y(NPQ) and Y(NO) were less evident (Fig. 7) and this pattern was likely associated with lower disease severity (Figs. 2 and 3). The changes of the images of the five parameters in the area surrounding the lesions on flag leaves and spikes of -Si plants were more pronounced in comparison to those in the flag leaves and spikes of +Si plants (Fig. 7). Based on the semi-quantitative analysis of these Chl*a* fluorescence parameters, there was no significant difference for F_m and Y(II) between flag leaves from -Si and +Si non-inoculated plants regardless of the sampling time (Fig. 8B1 and C1). For flag leaves of non-inoculated plants, Y(NPQ) significantly increased by 11% at 72 hai and decreased by 32% at 96 hai while Y(NO) significantly increased by 18% at 96 hai for +Si plants in comparison to -Si plants (Fig. 8D1 and E1). On flag leaves of +Si inoculated plants, there were increases, respectively, of 13, 63 and 28% at 48, 72 and 96 hai for F_v/F_m, of 44 and 39% at 72 and 96 hai for F_m, of 28, 24 and 66% at 48, 72 and 96 hai for Y(II) and of

35 and 30% at 48 and 72 hai for Y(NPQ) in comparison to -Si plants (Fig. 8A2, B2, C2 and D2). At 96 hai, Y(NPQ) and Y(NO) significantly decreased by 23 and 42% on flag leaves of +Si inoculated plants in comparison to -Si counterparts (Fig. 8D2 and E2). For spikes of non-inoculated plants, there was no significant difference between -Si and +Si treatments for none of the parameters of Chl*a* evaluated regardless of the sampling time (Fig. 8A3, B3, C3, D3 and E3). On spikes of +Si inoculated plants, there were increases, respectively, of 4, 15 and 28% at 48, 72 and 96 hai for F_v/F_m, of 72% at 96 hai for F_m, of 29 and 49% at 72 and 96 hai for Y(II) and of 30% at 96 hai for Y(NPQ) in comparison to -Si plants (Fig. 8A2, B2, C2 and D2). Y(NO) significantly decreased by 19 and 47%, respectively, at 72 and 96 hai on spikes of +Si inoculated plants in comparison to -Si counterparts (Fig. 8D4 and E4).

Ratio of sucrose, fructose and glucose concentrations

On flag leaves of +Si inoculated plants, there were significant increases of 28 and 44%, respectively, at 72 and 96 hai for the ratio of sucrose concentration and significant decreases of 49 and 50% for the ratio of fructose concentration and of 40 and 25% for the ratio of glucose concentration, respectively, at 72 and 96 hai in comparison to -Si counterparts (Fig. 9A, C and E). On spikes of +Si inoculated plants, there were significant increases of 19, 42 and 60%, respectively, at 48, 72 and 96 hai for the ratio of sucrose concentration and significant decreases of 13% for the ratio of fructose concentration at 48 hai and of 43 and 34% for the ratio of glucose concentration, respectively, at 72 and 96 hai in comparison to -Si counterparts (Fig. 9B, D and F).

Concentration of starch

The starch concentration was significantly increased by 23% at 96 hai on spikes of +Si non-inoculated plants in comparison to -Si plants (Fig. 10A). On spikes of +Si inoculated plants, the concentration of starch was significantly increased by 26 and 31%, respectively, at 72 and 96 hai in comparison to -Si plants (Fig. 10B).

Concentration of pigments

The concentration of Chl*a*+*b* and carotenoids were significantly increased by 13 and 12%, respectively, at 48 and 72 hai and by 20% at 72 hai on flag leaves of +Si non-inoculated plants in comparison to -Si counterparts (Fig. 11A and C). On flag leaves of +Si inoculated plants, the concentration of Chl*a*+*b* significantly increased by 18 and 40%, respectively, at 72 and 96 hai and carotenoids by 27% at 96 hai in comparison to -Si counterparts (Fig. 11B and D).

Activities of sucrose phosphate synthase and invertases acid

For flag leaves and spikes of non-inoculated plants, there was no significant difference for the activities of sucrose phosphate synthase and invertases acid between -Si and +Si treatments regardless of the sampling time (Fig. 12A1, A3, B1 and B3). On flag leaves of +Si inoculated plants, there were increases of 39 and 32% at 48 and 96 hai, respectively, for sucrose phosphate synthase activity and of 30 and 21% at 72 and 96 hai, respectively, for invertases acid activity in comparison to -Si plants (Fig. 12A2 and B2). On spikes of +Si inoculated plants, there were increases of 29 and 28% at 72 and 96 hai, respectively, for sucrose phosphate synthase activity and of 27 and 43% at 72 and 96 hai, respectively, for invertases acid activity in comparison to -Si plants (Fig. 12B2 and B4).

Discussion

The present study provides, to the best of authors' knowledge, the effect of Si on enhancing wheat resistance to blast at both physiological and biochemical levels considering the source-sink interface.

The greater Si concentration on both flag leaves and spikes resulted in lower disease severity on these organs. It is plausible that Si deposition and polymerization below the cuticle of the flag leaves and on spikes' tissue prevented or delayed *P. oryzae* penetration. Rice resistance to *P. oryzae* infection was attributed to Si deposition below the cuticle (Kim et al., 2002). Wheat plants supplied with Si showed great deposition of osmiophilic material around the haustoria of *Blumeria graminis* f.sp. *tritici* that resulted in lower disease severity (Bélanger et al., 2003). It can be postulated that wheat plants supplied with Si responded quickly and more efficiently against *P. oryzae* infection because in areas of heavy Si deposition, a delay in fungal ingress and a slow tissue colonization by fungal hyphae provided the wheat plants with enough time to activate mechanisms of defense. The high CHI, GLU, PAL, POX and PPO activities on flag leaves, mainly on spikes, of plants supplied with Si helped them to counteract against the deleterious effect of *P. oryzae* infection. It has been reported in the literature that the resistance of several plant species against pathogens of different life styles can be potentiated by Si (Cruz et al., 2015; Datnoff et al., 2007; Resende et al., 2009; Rodrigues et al., 2009; Debona et al., 2017). The CHI and GLU are key enzymes involved in host defense response against pathogens attack because they catalyze the hydrolysis of the carbohydrates chitin and β-1,3-glucan, respectively, found in their cell wall (Keen & Yoshikawa, 1983; Mauch et al., 1988). Xavier Filha et al. (2011) reported a high CHI activity on the leaves of wheat plants supplied with Si in response to *P. grisea* infection. PAL activity was of great importance on flag leaves than on spikes of plants supplied with Si. This enzyme catalyzes the deamination of *L*-phenylalanine for the synthesis of phenolics and some phytoalexins with the lignin being the final product (Campbell & Sederoff, 1996). Therefore, the high concentrations of TSP and LTGA derivatives on the flag leaves and spikes of wheat plants was of detrimental importance for their resistance against blast. Cruz et al. (2015) reported that the colonization of *P. oryzae* in the leaf tissue of wheat plants supplied with Si was limited due to the intense deposition of phenolics. It is known that phenolics are associated with an increase in fungal membrane permeability, leakage of cell contents, and cytoplasm aggregation (Southerton & Deverall, 1990). The reinforcement of the cell walls in both flag leaves and spikes as the result of a high concentration of LTGA derivatives protected them from the deleterious action of both hydrolytic enzymes and the non-host selective toxins produced by *P. oryzae*. The polymerization of phenolics that led to an increase in tissue lignification involves the participation of both PPO and POX (Grisebach, 1981; Vidhyasekaran, 1988). In the present study, PPO and POX activities were greater on flag leaves and spikes of plants supplied with Si. High POX and PPO activities on rice and wheat plants supplied with Si increased their resistance against the infections caused, respectively, by *Monographella albescens* and *P. oryzae* (Cruz et al., 2014; Tatagiba et al., 2014).

By decreasing blast severity, Si helped the wheat plants to preserve the functionality of the photosynthetic apparatus of their flag leaves and spikes during the infection process of *P. oryzae*. It was demonstrated that wheat plants supplied with Si had the functionality of their photosynthetic apparatus and gas exchange capacity increased upon infection by *P. oryzae* when compared to plants non-supplied with this element (Aucique-Pérez et al., 2014). In the present study, the values for F_v/F_m decreased to below 0.80 on flag leaves and spikes of wheat plants infected with *P. oryzae* and non-supplied with Si suggesting, therefore, the occurrence of chronic photoinhibition of photosynthesis. Indeed, the values of Y(II) were lower and coupled with a progressive loss of chloroplastidic pigments indicating that the apparent electron transport activity on infected flag leaves and spikes of plants non-supplied with Si was negatively impaired. These alterations were associated with lower and high Y(NPQ) and Y(NO) values, respectively, which reflected the inability of plants non-supplied with Si to regulate their mechanisms of photoprotection on flag leaves and spikes. These alterations reflected a photooxidative damage on the infected host tissue that could have resulted in a lower provision of ATP and a reduced power for CO_2 assimilation (Rolfe & Scholes, 2010). The reduction of photosynthesis on leaves of wheat plants infected by *P. oryzae*, based on gas exchange analyses, was due to chiefly to biochemical constraints linked to presumably lower RuBisCO activity (Debona et al., 2014).

Wheat plants supplied with Si showed great production of photoassimilates on flag leaves and their efficient partitioning to spikes due to reduced disease symptoms on these organs. Given that photosynthesis was drastically constrained on infected flag leaves of plants non-supplied with Si, a lower availability of photoassimilates to be exported towards the sink tissues was expected. Indeed, the concentration of sucrose, the main sugar form transported in plants, was lower on infected flag leaves and grains obtained from infected spikes of plants non-supplied with Si. The reduction on sucrose concentration was linked to lower SPS activity and, mainly, to an increase on invertase activity on infected flag leaves of plants non-supplied with Si contributing, therefore, to depress the sucrose pools by increasing its breakdown to produce hexoses. Notably, the concentrations of fructose and glucose also increased as disease developed on flag leaves of plants non-supplied with Si in contrast to plants supplied with this element. Consequently, the hexoses-to-sucrose ratio increased as disease developed correlating with an induction in cell wall invertases. The results from the present study are in agreement with those of Berger et al. (2004) who observed an increase on hexoses-to-sucrose ratio on tomato leaves infected with *Pseudomonas syringae* and *Botrytis cinerea*. Considering the information reported for other host-pathogen interactions (Maust et al., 2003; Gamm et al., 2011) and for the wheat-*P. oryzae* interaction in particular, the association of reduced photosynthesis with an increase in invertase activity for plants non-supplied with Si coupled with an increase on the hexoses-to-sucrose ratio suggests, therefore, a sink status in the infected tissues. Additionally, the increase on sucrose uptake and accumulation of hexoses may be an indicative of additional sinks in plants to favor pathogen infection (Fotopoulos et al., 2003). Conversely, the invertase activity was lower in the infected spikelets of plants non-supplied with Si and can be linked to an decrease in the concentration of sucrose on grains obtained from spikes of plants non-supplied with Si. In the present study, the starch pools dramatically decreased on grains obtained from infected spikes of plants non-supplied with Si in comparison to plants supplied with this element. According to Rios et al. (2017), this effect may be associated with an impairment of AGPase activity (lower capacity of starch production) and higher expression of the *α*- and *β-amylase* genes (higher breakdown of starch in the infected grains). Taken together, these alterations should remarkably impair the endosperm filling process that ultimately resulted in small and shrunken grains on the infected spikes obtained from plants non-supplied with Si.

All together, the results from the present study confirm the active role played by Si in increasing wheat resistance to blast, but present novel evidences of the effect of this element in improve the source-sink relationship on infected flag leaves and spikes by preserving the alterations in assimilate production and partitioning during the grain filling process. Therefore, the beneficial effect of Si on this scenario may contribute for great grain biomass and, consequently, high wheat yield.

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Figures

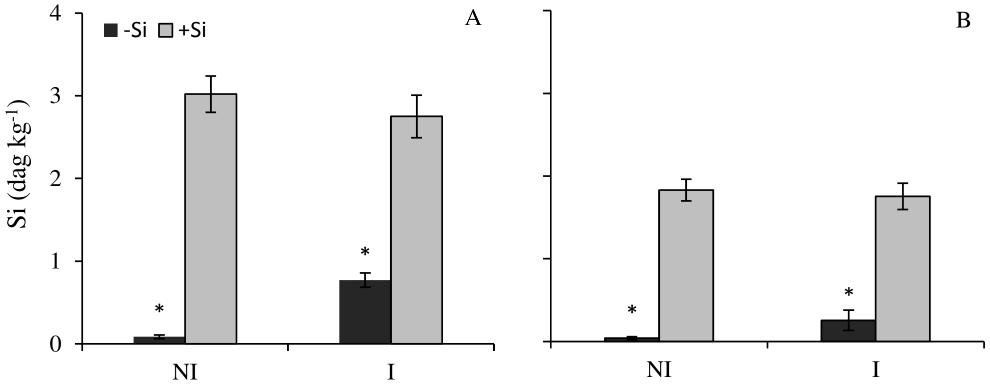

Figure 1. Concentration of silicon (Si) on flag leaves (A) and spikes (B) of wheat plants supplied with 0 (-Si) or 2 mM (+Si) silicon and non-inoculated (NI) or inoculated (I) with *Pyricularia oryzae*. Means from -Si and +Si treatments followed by an asterisk (*), for NI and I treatments, are significantly different ($P \leq 0.05$) by the F test. Bars represent the standard deviations of the means. $n = 5$.

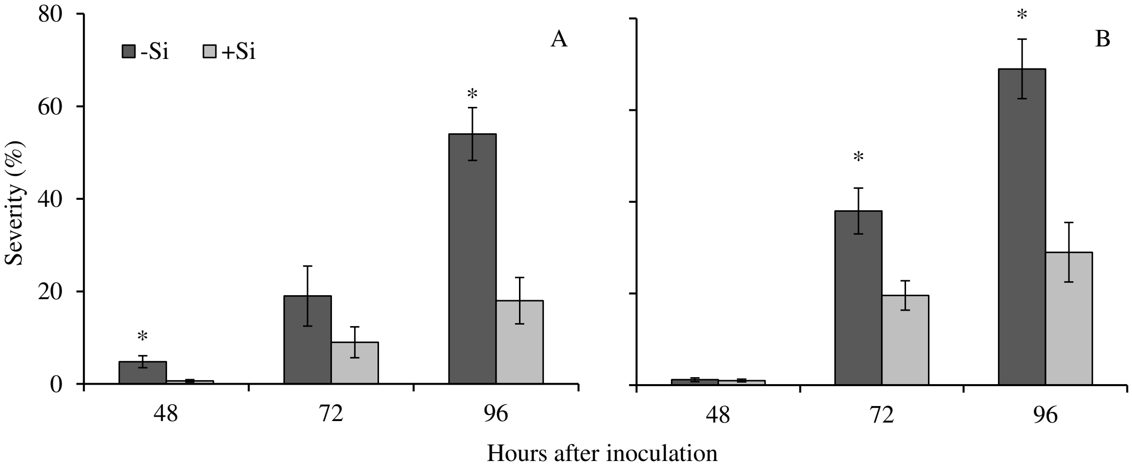

Figure 2. Severity of blast on flag leaves (A) and spikes (B) of wheat plants supplied with 0 (-Si) or 2 mM (+Si) silicon (Si) and inoculated with *Pyricularia oryzae*. Means from -Si and +Si treatments followed by an asterisk (*), at each evaluation time, are significantly different ($P \leq 0.05$) by the F test. Bars represent the standard deviations of the means. $n = 5$.

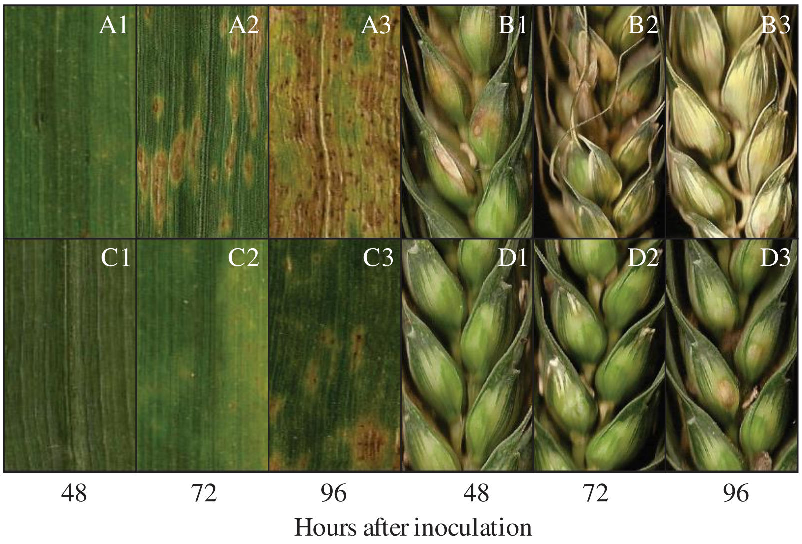

Figure 3. Symptoms of blast on flag leaves (A1-A3 and C1-C3) and spikes (B1-B3 and D1-D3) of wheat plants supplied with 0 (-Si) (A1-A3 and B1-B3) or 2 mM (+Si) silicon (Si) (C1-C3 and D1-D3).

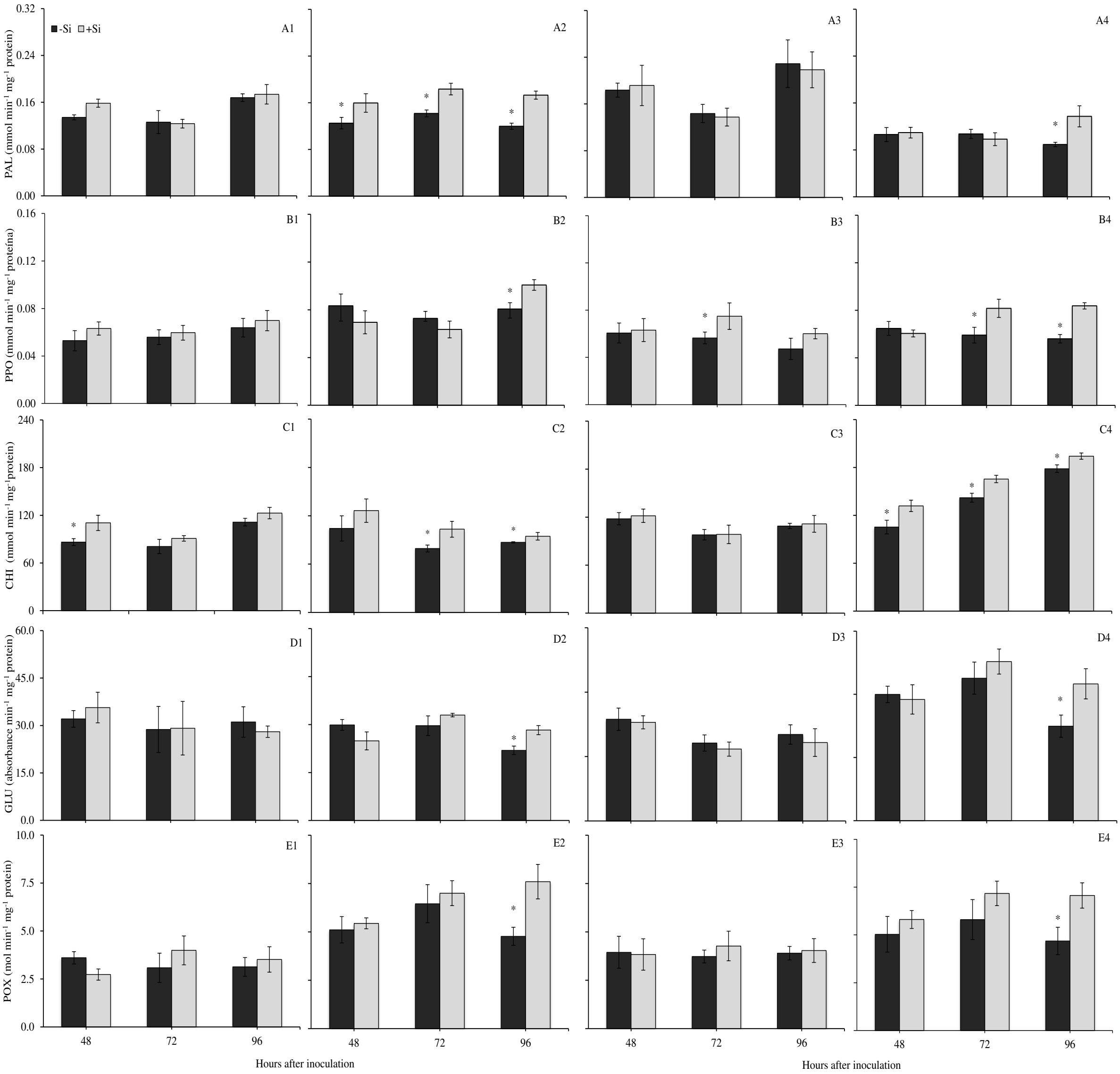

Figure 4. Activities of phenylalanine ammonia-lyases (PAL) (A1-A4), polyphenoloxidases (PPO) (B1-B4), chitinases (CHI) (C1-C4), *β*-1,3-glucanases (GLU) (D1-D4) and peroxidases (POX) (E1-E4) on flag leaves (A1-A2, B1-B2, C1-C2, D1-D2 and E1-E2) and spikes (A3-A4, B3-B4, C3-C4, D3-D4 and E3-E4) of wheat plants supplied with 0 (-Si) or 2 mM (+Si) silicon (Si) and non-inoculated (A1, A3, B1, B3, C1, C3, D1, D3, E1 and E3) or inoculated (A2, A4, B2, B4, C2, C4, D2, D4, E2 and E4) with *Pyricularia oryzae*. Means from -Si and +Si treatments followed by an asterisk (*), at each sampling time, are significantly different ($P \leq 0.05$) by the *F* test. Bars represent the standard deviations of the means. $n = 5$.

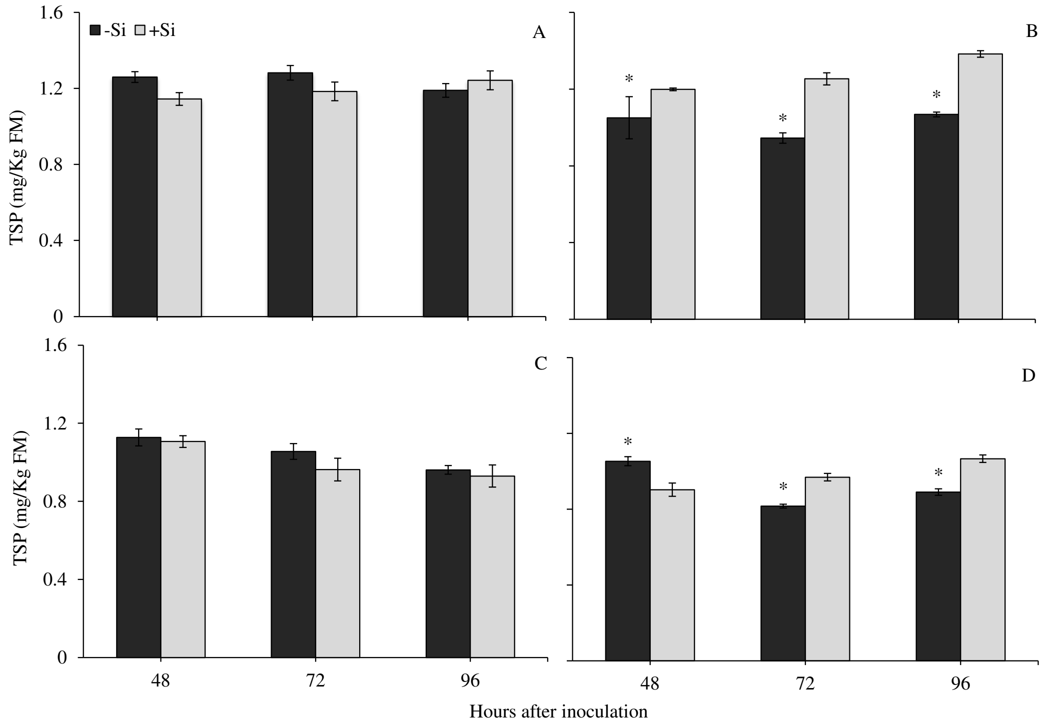

Figure 5. Concentration of total soluble phenolics (TSP) on flag leaves (A and B) and spikes (C and D) of wheat plants supplied with 0 (-Si) or 2 mM (+Si) silicon (Si) and non inoculated (A and C) or inoculated (B and D) with *Pyricularia oryzae*. Means from -Si and +Si treatments followed by an asterisk (*), at each sampling time, are significantly different ($P \leq 0.05$) by the F test. Bars represent the standard deviations of the means. FM = fresh matter. $n = 5$.

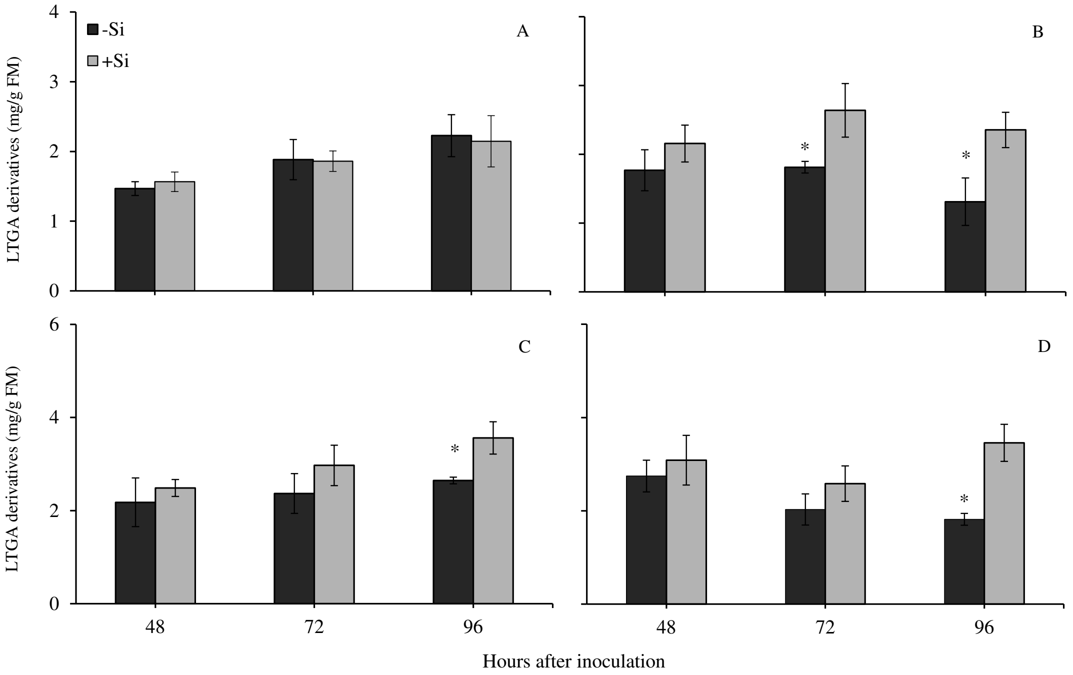

Figure 6. Concentrations of lignin-thioglycolic acid (LTGA) derivatives on flag leaves (A and B) and spikes (C and D) of wheat plants supplied with 0 (-Si) or 2 mM (+Si) silicon (Si) and non inoculated (A and C) or inoculated (B and D) with *Pyricularia oryzae*. Means from -Si and +Si treatments followed by an asterisk (*), at each sampling time, are significantly different ($P \leq 0.05$) by the *F* test. Bars represent the standard deviations of the means. FM = fresh matter. $n = 5$.

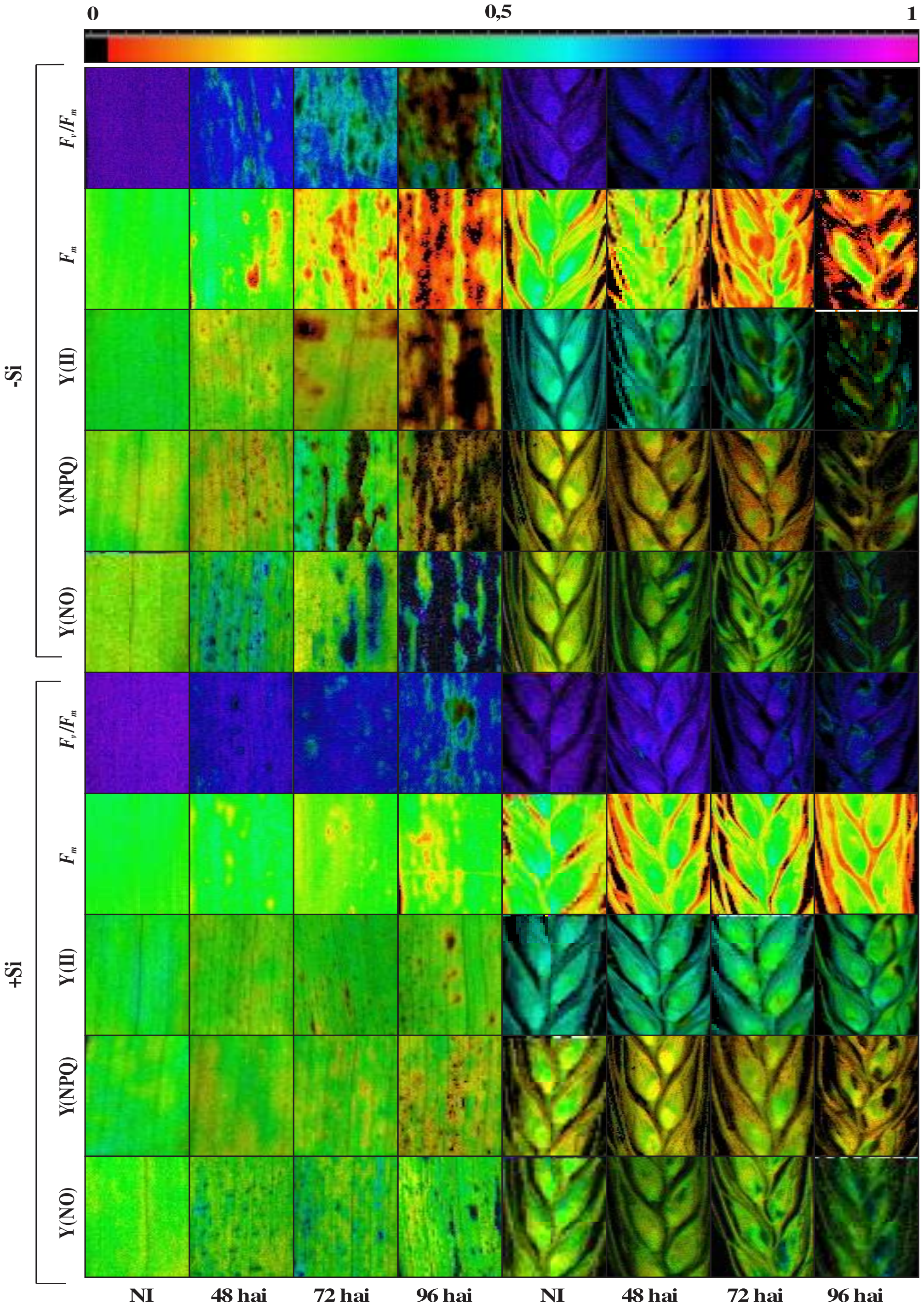

Figure 7. Maximal photosystem II quantum yield (F_v/F_m), maximal fluorescence (F_m), fraction of energy absorbed used in photochemistry (Y(II)), quantum yield of regulated energy dissipation (Y(NPQ)) and quantum yield of non-regulated energy dissipation (Y(NO)) determined on flag leaves and spikes of wheat plants supplied with 0 (-Si) or 2 mM (+Si) silicon (Si) and non-inoculated (NI) or at 48, 72 and 96 hours after inoculation (hai) with *Pyricularia oryzae*.

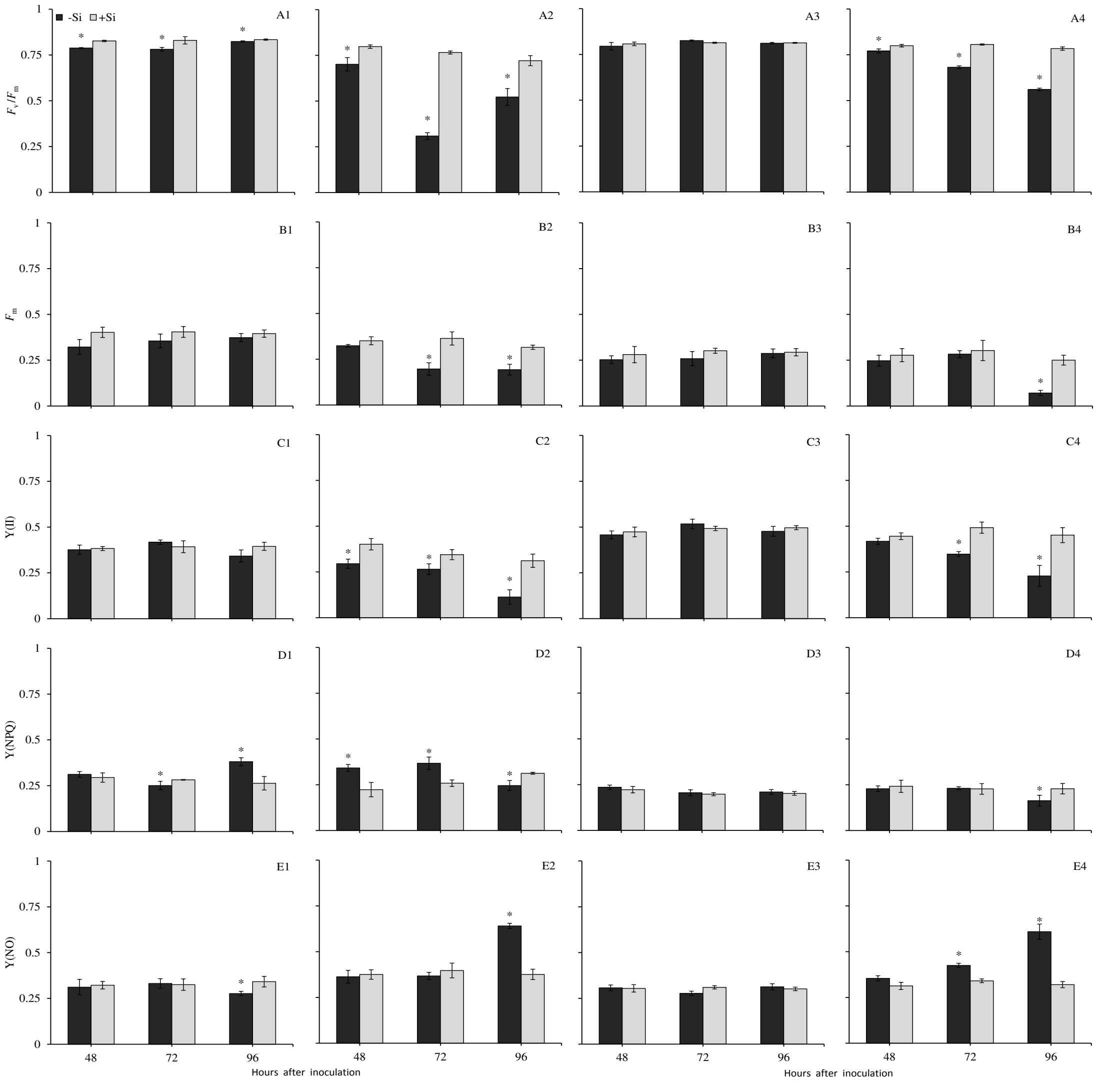

Figure 8. Parameters of chlorophyll *a* fluorescence: maximal photosystem II quantum efficiency (F_v/F_m), maximal fluorescence (F_m), fraction of energy absorbed used in photochemistry (Y(II)), quantum yield of regulated energy dissipation (Y(NPQ)) and quantum yield of non-regulated energy dissipation (Y(NO)) determined on flag leaves (A1-A2, B1-B2, C1-C2, D1-D2 and E1-E2) and spikes (A3-A4, B3-B4, C3-C4, D3-D4 and E3-E4) of wheat plants supplied with 0 (-Si) or 2 mM (+Si) silicon (Si) and non-inoculated (A1, A3, B1, B3, C1, C3, D1, D3, E1 and E3) or inoculated (A2, A4, B2, B4, C2, C4, D2, D4, E2 and E4) with *Pyricularia oryzae*. Means from -Si and +Si treatments followed by an asterisk (*), at each sampling time, are significantly different ($P \leq 0.05$) by the *F* test. Bars represent the standard deviations of the means. $n = 5$.

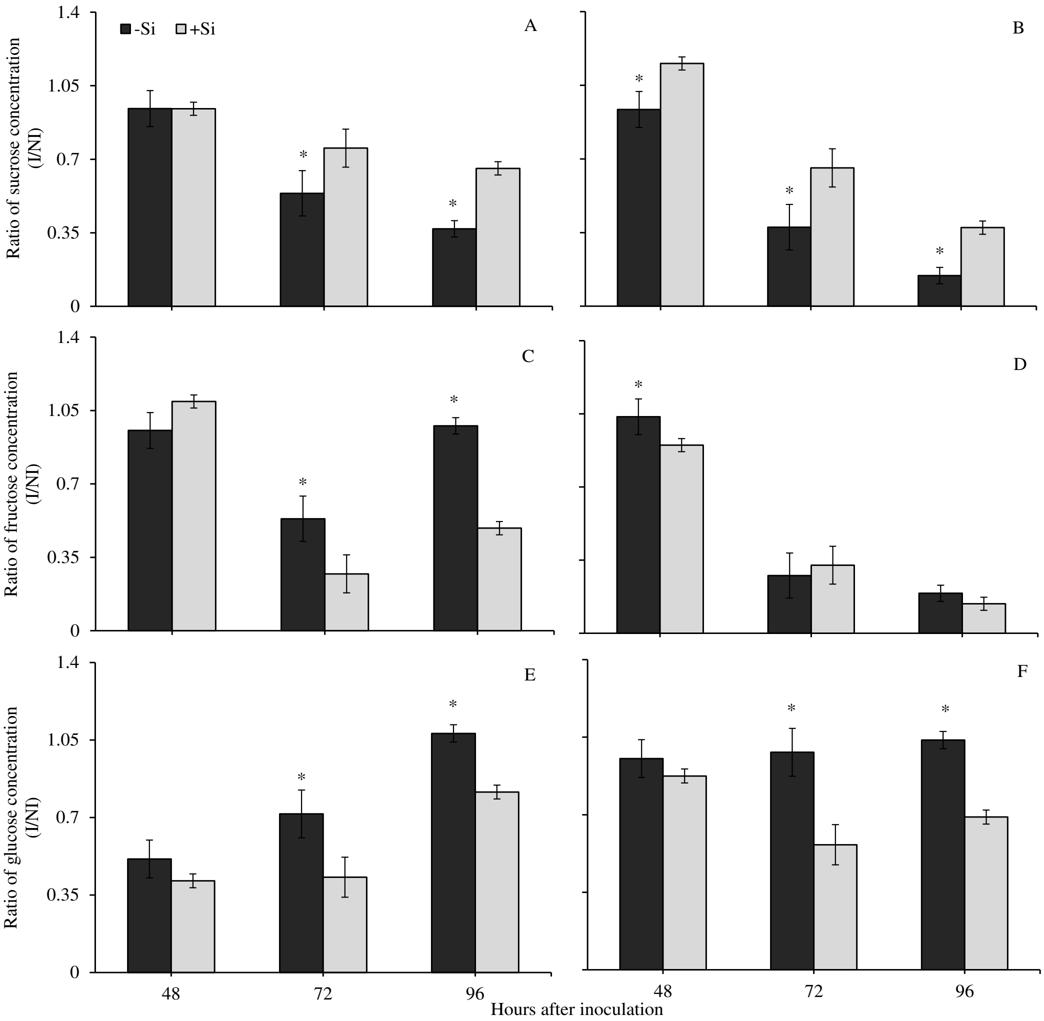

Figure 9. Ratio of sucrose (A and B), fructose (C and D) and glucose (E and F) concentrations on flag leaves (A, C and E) and spikes (B, D and F) of wheat plants supplied with 0 (-Si) or 2 mM (+Si) silicon (Si) and inoculated with *Pyricularia oryzae*. Means from -Si and +Si treatments followed by an asterisk (*), at each sampling time, are significantly different ($P \leq 0.05$) by the *F* test. Bars represent the standard deviations of the means. $n = 5$.

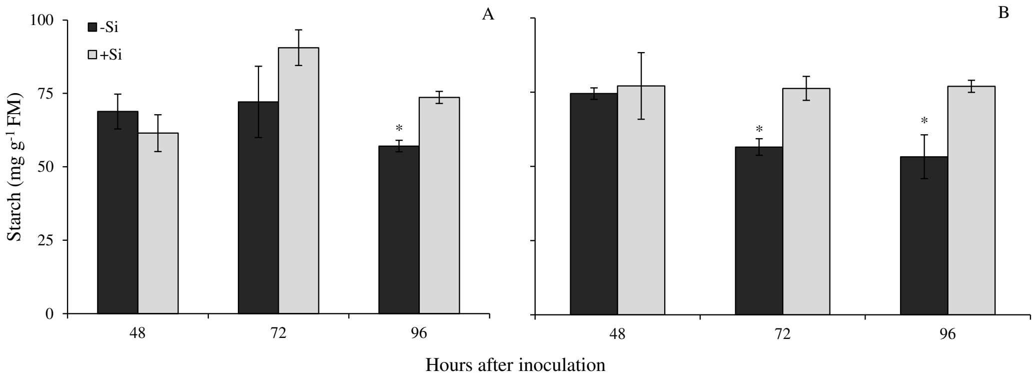

Figure 10. Starch concentration on spikes of wheat plants supplied with 0 (-Si) or 2 mM (+Si) silicon (Si) and non-inoculated (A) or inoculated (B) with *Pyricularia oryzae*. Means from -Si and +Si treatments followed by an asterisk (*), at each sampling time, are significantly different ($P \leq 0.05$) by the *F* test. Bars represent the standard deviations of the means. FM = fresh matter. $n = 5$.

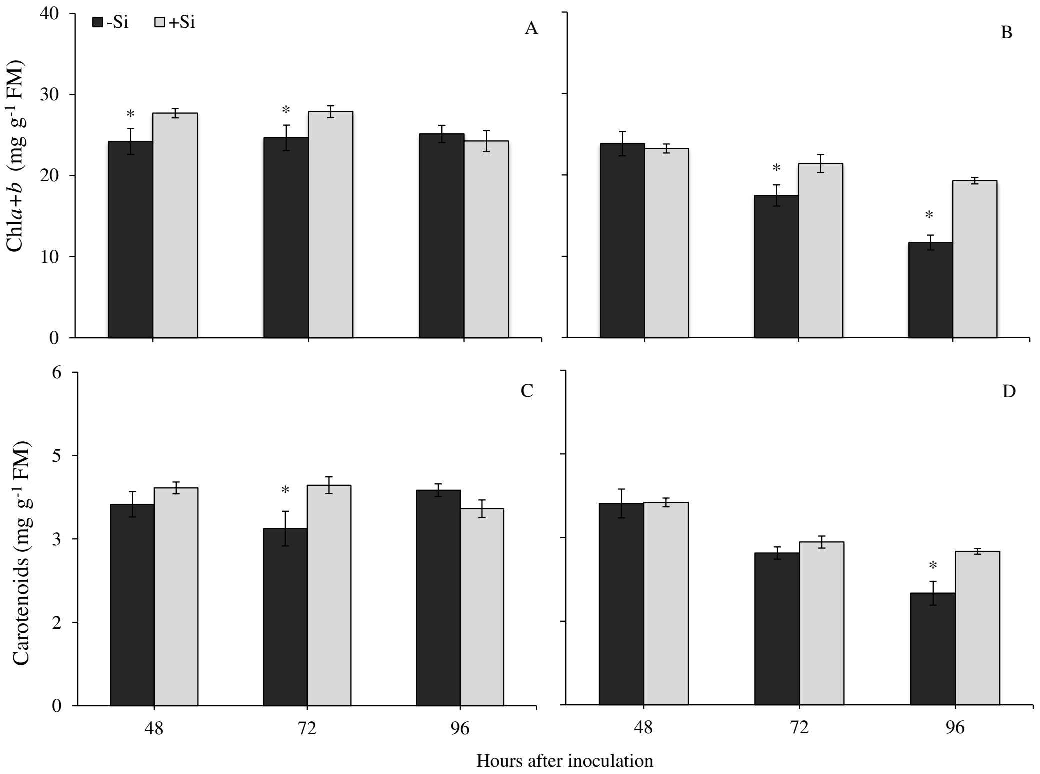

Figure 11. Concentrations of total chlorophylls (Chl*a*+*b*) (A and B) and carotenoids (C and D) on flag leaves of wheat plants supplied with 0 (-Si) or 2 mM (+Si) silicon (Si) and non-inoculated (A and C) or inoculated (B and D) with *Pyricularia oryzae*. Means from -Si and +Si treatments followed by an asterisk (*), at each sampling time, are significantly different ($P \leq 0.05$) by the *F* test. Bars represent the standard deviations of the means. FM = fresh matter. $n = 5$.

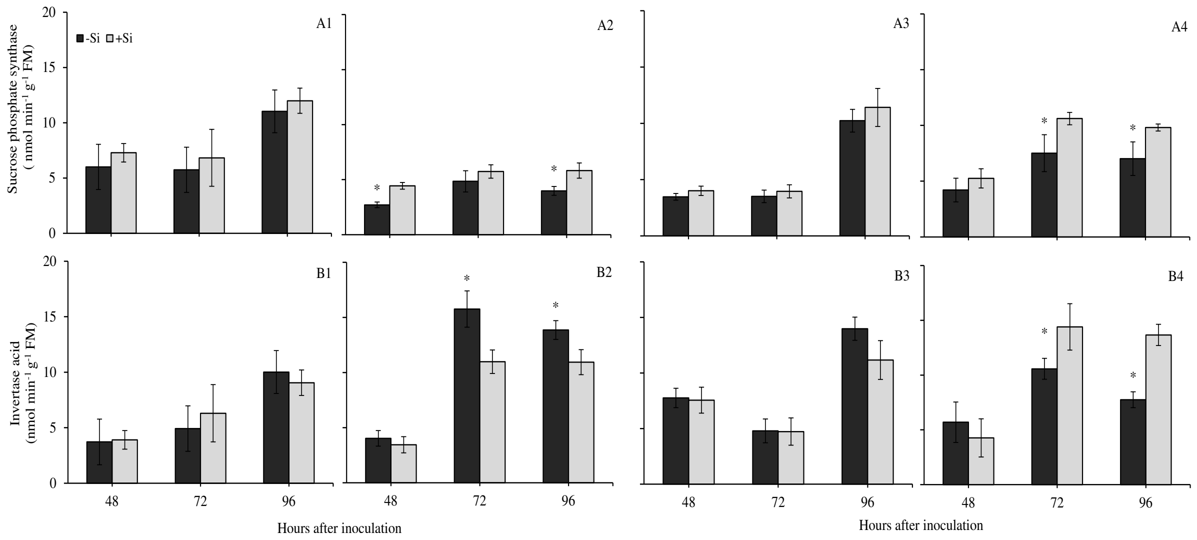

Figure 12. Activities of sucrose phosphate synthase (A1-A4) and invertases acid (B1-B4) on flag leaves (A1-A2 and B1-B2) and spikes (A3-A4 and B3-B4) of wheat plants supplied with 0 (-Si) or 2 mM (+Si) silicon (Si) and non-inoculated (A1, A3, B1 and B3) or inoculated (A2, A4, B2 and B4) with *Pyricularia oryzae*. Means from -Si and +Si treatments followed by an asterisk (*), at each sampling time, are significantly different ($P \leq 0.05$) by the F test. Bars represent the standard deviations of the means. FM = fresh matter. $n = 5$.