TÁSSIA BOENO OLIVEIRA

BLAST CONTROL BY SILICON FOLIAR SPRAY AND IMPAIRMENT OF PHOTOSYNTHESIS BY A SPECIFIC INHIBITOR INCREASE WHEAT SUSCEPTIBILITY TO INFECTION BY *Pyricularia oryzae*

Thesis submitted to Federal University of Viçosa, as part of the requirements for obtaining the *Doctor Scientiae* degree in Plant Physiology.

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Thesis submitted to Federal University of Viçosa, as part of the requirements for obtaining the *Doctor Scientiae* degree in Plant Physiology.

APPROVED: February 28th, 2018.

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Franklin Magnum de Oliveira Silva

Juraci Alves de Oliveira

Fabrício de Ávila Rodrigues
(Adviser)

I know You can do anything;

no purpose of yours can be thwarted.

Job 42:2

To my parents Iolanda and Getúlio,

I dedicate with love.

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BIOGRAPHY

TÁSSIA BOENO OLIVEIRA was born in Ipatinga-MG, Brazil, on January 9th, 1986. In 2010, she graduated in Biology at Federal University of São João del Rei, SJDR-MG, Brazil. In February 2014, she obtained her *Master Scientiae* degree in Plant Physiology at the Federal University of Lavras, Lavras-MG, Brazil. On March 2014, she begins her doctoral studies in the Plant Physiology Program at Federal University of Viçosa, Viçosa-MG, Brazil.

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ABSTRACT

OLIVEIRA, Tássia Boeno, D.Sc., Universidade Federal de Viçosa, February, 2018. **Blast control by silicon foliar spray and impairment of photosynthesis by a specific inhibitor increase wheat susceptibility to infection by *Pyricularia oryzae*.** Adviser: Fabrício de Ávila Rodrigues. Co-adviser: Renata Sousa Resende.

Considering the effect of blast, caused by *Pyricularia oryzae*, in reducing wheat yield this study aimed to elucidate if the potassium silicate (PS) polymerization after its foliar spray could compromise the leaf gas exchange (net CO_2 assimilation rate (A), internal CO_2 concentration (C_i), stomatal conductance to water vapor (g_s) and transpiration rate (E)) and chlorophyll *a* (maximal photosystem II quantum yield (F_v/F_m), quantum yield of non-regulated energy dissipation [Y(NO)], photochemical yield [Y(II)], electron transport rate (ETR) and quenching non-photochemical [Y(NPQ)]) parameters and, if not, it could become one strategy to reduce blast symptoms on leaf blades. Indeed, if the compromise of the photosynthetic process of wheat plants by using an inhibitor of photosynthesis could increase their susceptibility to blast. There were no significant changes in the values of A, g_s, E, C_i, F_v/F_m, Y(NO), Y(II), Y(NPQ) and ETR for plants sprayed three times (96 h interval) with PS rates of 2.5, 5.0, 7.5, 10.0 and 12.5 g L^{-1}. There was no significantly relationship between the PS rates with either absorbance, reflectance or transmittance on the leaf blades. Linear regression model best described the foliar Si concentration-PS rates relationship. Foliar Si concentration was significantly increased by 44 and 42%, respectively, for the PS and PS + fungicide treatments compared to water-sprayed plants. The area under disease progress curve was significantly lower by 64, 57 and 52%, respectively, for the treatments fungicide, PS and fungicide + PS in comparison to water-sprayed plants. No gain on disease control was achieved when PS was mixed with fungicide. The photosynthetical process, especially related to the F_v/F_m parameter, on wheat leaves was greatly impaired during the infection process of *P. oryzae*, but to a lesser extent on the leaves of PS-sprayed plants. In conclusion, the foliar spray of PS can be an environmental friendly strategy to control wheat blast without inducing any negative impact on the photosynthetic machinery. Plants from cultivar BRS 220, partially resistant to blast, were non-sprayed (control treatment) or sprayed with a solution of 10 µM of 3-(3,4-dichlorophenyl)-1,1-dimethylurea) (DCMU) at 24 h before inoculation with *P. oryzae*. The DCMU affected the functionality of the photosynthetic apparatus of wheat leaves based on the lower values of net CO_2 assimilation rate (A) coupled with increases in the internal carbon concentration. Indeed, there were dramatic reductions in the values

of g_s and E for infected plants, especially for the DCMU-sprayed ones. The lower A values obtained from leaves of DCMU-sprayed and infected plants limited the carbohydrates synthesis resulting in great starch concentration. Sucrose concentration was reduced on infected leaves mainly if they were sprayed with DCMU while fructose and glucose concentrations increased. The activities of superoxide dismutase and peroxidase were lower while catalase activity was higher for DCMU-sprayed and non-infected plants. Altogether, the results of the present study showed that the spray of DCMU increased wheat susceptibility to blast due to photosynthetic dysfunctions, impairment on sugar metabolism and a less efficient antioxidative system.

RESUMO

OLIVEIRA, Tássia Boeno, D.Sc., Universidade Federal de Viçosa, fevereiro de 2018. **Controle de brusone por aplicação foliar de silício e comprometimento da fotossíntese por um inibidor específico aumenta a suscetibilidade de trigo a infecção por *Pyricularia oryzae*.** Orientador: Fabrício de Ávila Rodrigues. Coorientadora: Renata Sousa Resende.

Considerando o efeito da brusone, causada por *Pyricularia oryzae*, na redução da produtividade do trigo este estudo objetivou elucidar se a polimeralização do silicato de potássio (SP,) após sua aplicação foliar, comprometeria os parâmetros de trocas gasosas (taxa líquida de assimilação de carbono (A), concentração interna de CO_2 (C_i), condutância estomática ao vapor de água (g_s) e taxa de transpiração (E)) e da fluorescência da clorofila *a* (rendimento quântico máximo do FSII (F_v/F_m), rendimento quântico da dissipação não regulada [Y(NO)], rendimento quântico efetivo do FSII [Y(II)], taxa de transporte de elétrons (ETR) e dissipação não fotoquímica [Y(NPQ)]) e, caso contrário, se poderia torna-se uma estratégia para reduzir os sintomas da brusone em plantas de trigo. Além disso, investigar se o comprometimento do processo fotossintético de plantas de trigo utilizando-se um inibidor da fotossíntese poderia aumentar a susceptibilidade à brusone. Não houve alterações significativas nos valores de A, g_s, E, C_i, F_v/F_m, Y(NO), Y(II), Y(NPQ) e ETR para as plantas pulverizadas com três aplicações de SP (intervalo de 96 h) com as doses de 2,5; 5,0; 7,5; 10,0 e 12,5g L^{-1}. Não houve relação significativa entre as doses de SP e a absorbância, reflectância e transmitância foliar. O modelo de regressão linear descreveu a relação entre a concentração foliar de Si e as doses de SP. A concentração foliar de Si aumentou significativamente 44 e 42%, respectivamente, para os tratamentos de SP e SP + fungicidas em comparação com as plantas pulverizadas com água. A área abaixo da curva do progresso da doença foi significativamente menor 64, 57 e 52%, respectivamente, para os tratamentos fungicida, SP e SP + fungicida em comparação com as plantas pulverizadas com água. Nenhum ganho no controle da doença ocorreu quando o SP foi misturado com fungicida. O processo fotossintético, especialmente relacionado ao parâmetro F_v/F_m, nas folhas de trigo foi negativamente afetado durante o processo infeccioso de *P. oryzae*, mas em menor grau nas folhas das plantas pulverizadas com SP. Assim, a aplicação foliar de SP pode ser uma estratégia ambientalmente sustentável para o controle da brusone do trigo sem causar nenhum impacto negativo na maquinaria fotossintética. Plantas de trigo da cultivar BRS 220, parcialmente resistente a brusone, foram pulverizadas ou não com uma solução

de 10 μM de 3-(3,4-diclorofenil)-1,1-dimetilureia) (DCMU) às 24 h antes da inoculação com *P. oryzae*. O DCMU afetou a funcionalidade do aparato fotossintético das folhas de trigo com base nos baixos valores de *A* associado com um aumento na concentração interna de carbono. Houve reduções drásticas nos valores de g_s e *E*, especialmente quando pulverizadas com DMCU. Os baixos valores de *A* nas folhas das plantas inoculadas e pulverizadas com DCMU afetou a síntese de carboidratos resultando, assim, em uma maior concentração de amido. A concentração de sacarose foi reduzida nas folhas infectadas principalmente se pulverizadas com DCMU enquanto que as concentrações de frutose e de glicose aumentaram. As atividades da superóxido dismutase e peroxidase foram menores enquanto a atividade da catalase foi maior para as plantas não infectadas e pulverizadas com DCMU. Em conclusão, os resultados do presente estudo demonstraram que o DMCU aumentou a susceptibilidade do trigo a brusone devido as disfunções fotossintéticas, comprometimento do metabolismo de açúcares e menor eficiência do sistema antioxidante.

Chapter 1

Foliar Spray of Silicon does not Limit Photosynthesis on Wheat and Contributes to Decrease Blast Symptoms on Leaves

Resumo

Considerando o efeito da brusone, causada por *Pyricularia oryzae*, na redução da produtividade do trigo e a preocupação com o fato de que a aplicação de produtos contendo silício (Si) pode afetar a fotossíntese, este estudo objetivou elucidar se a polimeralização do silicato de potássio (SP), após sua aplicação foliar, comprometiria os parâmetros de trocas gasosas (taxa líquida de assimilação de carbono (A), concentração interna de CO_2 (C_i), condutância estomática ao vapor de água (g_s) e taxa de transpiração (E)) e da fluorescência da clorofila *a* (rendimento quântico máximo do FSII (F_v/F_m), rendimento quântico da dissipação não regulada [Y(NO)], rendimento quântico efetivo do FSII [Y(II)], taxa de transporte de elétrons (ETR) e dissipação não fotoquímica [Y(NPQ)]) e, caso contrário, se poderia torna-se uma estratégia para reduzir os sintomas da brusone em plantas de trigo. Não houve alterações significativas nos valores de A, g_s, E, C_i, F_v/F_m, Y(NO), Y(II), Y(NPQ) e ETR para as plantas pulverizadas com três aplicações de SP (intervalo de 96 h) com as doses de 2,5; 5,0; 7,5; 10,0 e 12,5g L^{-1}. Não houve relação significativa entre as doses de SP e a absorbância, reflectância e transmitância foliar. O modelo de regressão linear descreveu a relação entre a concentração foliar de Si e as doses de SP. A concentração foliar de Si aumentou significativamente 44,44 e 42,22%, respectivamente, para os tratamentos de SP e SP + fungicidas em comparação com as plantas pulverizadas com água. A área abaixo da curva do progresso da doença foi significativamente menor 64,22, 57,47 e 52,4%, respectivamente, para os tratamentos fungicida, SP e SP + fungicida em comparação com as plantas pulverizadas com água. Nenhum ganho no controle da doença ocorreu quando o SP foi misturado com fungicida. O processo fotossintético, especialmente relacionado ao parâmetro F_v/F_m, nas folhas de trigo foi negativamente afetado durante o processo infeccioso de *P. oryzae*, mas em menor grau nas folhas das plantas pulverizadas com SP. Assim, a aplicação foliar de SP pode ser uma estratégia ambientalmente sustentável para o controle da brusone do trigo sem causar nenhum impacto negativo na maquinaria fotossintética.

Palavras chave: *Triticum aestivum* L., controle de doencas, doença fúngica, nutrição de plantas, parâmetros de trocas gasosas.

Abstract

Considering the effect of blast, caused by *Pyricularia oryzae*, in reducing wheat yield and the concern that the spray of silicon (Si)-containing products could affect photosynthesis, this study aimed to elucidate if the potassium silicate (PS) polymerization after its foliar spray could compromise the leaf gas exchange (net CO_2 assimilation rate (A), internal CO_2 concentration (C_i), stomatal conductance to water vapor (g_s) and transpiration rate (E)) and fluorescence of chlorophyll *a* (maximal photosystem II quantum yield (F_v/F_m), quantum yield of non-regulated energy dissipation [Y(NO)], photochemical yield [Y(II)], electron transport rate (ETR) and quenching non-photochemical [Y(NPQ)]) parameters and, if not, it could become one strategy to reduce blast symptoms on leaf blades. There were no significant changes in the values of A, g_s, E, C_i, F_v/F_m, Y(NO), Y(II), Y(NPQ) and ETR for plants sprayed three times (96 h interval) with PS rates of 2.5, 5.0, 7.5, 10.0 and 12.5 g L^{-1}. There was no significantly relationship between the PS rates with either absorbance, reflectance or transmittance on the leaf blades. Linear regression model best described the foliar Si concentration-PS rates relationship. Foliar Si concentration was significantly increased by 44.44 and 42.22%, respectively, for the PS and PS + fungicide treatments compared to water-sprayed plants. The area under disease progress curve was significantly lower by 64.22, 57.47 and 52.84%, respectively, for the treatments fungicide, PS and fungicide + PS in comparison to water-sprayed plants. No gain on disease control was achieved when PS was mixed with fungicide. The photosynthetical process, especially related to the F_v/F_m parameter, on wheat leaves was greatly impaired during the infection process of *P. oryzae*, but to a lesser extent on the leaves of PS-sprayed plants. In conclusion, the foliar spray of PS can be an environmental friendly strategy to control wheat blast without inducing any negative impact on the photosynthetic machinery.

Keywords: *Triticum aestivum* L., disease control, fungal disease, leaf gas exchange parameters, plant nutrition.

Introduction

Blast, caused by the hemibiotrophic fungus *Pyricularia oryzae* Sacc. (teleomorph: *Magnaphorthe grisea* (T. T. Hebert) M. E. Barr), have limited wheat production in Brazil and other South America countries (Cruz and Valent, 2017; Kohli et al., 2010). Blast was restricted to South America until its first report in Bangladesh in 2016 and more recently in India (Ceresini et al., 2018). On leaves, blast symptoms are characterized by elliptic lesions with gray center and brown margin (Goulart et al., 2007). On infected spikes, grains are small, deformed and of reduced weight (Igarashi et al., 1986). Seeds treatment with fungicides, the spray of systemic fungicides and the cultivars with high level of partial resistance have been the major control methods used by growers to avoid the negative impact of blast on grain yield (Goulart et al., 2007).

Considering that fungicides spray has been of low efficiency for blast control and the difficulty of obtaining resistant cultivars (Goulart et al., 2007; Cruz and Valent, 2017), new alternative methods for disease control need to be investigated. Several plant species, mainly grasses and some dicotyledons, exposed to both abiotic and biotic types of stresses are positively benefited by Si (Debona et al., 2017). Plants can receive Si either through soil amendment or foliar spray (Rodrigues et al., 2015). Silicon is considered immobile in the phloem and redistribution of Si in the plant is very low (Datnoff et al., 2001). Thus, some studies have shown that foliar fertilization using small amounts of Si can be a practical alternative to soil uptake and stimulate its beneficial effects (Wang and Galleta, 1998). Liquid foliar products do not increase foliar Si concentration and their polymerization on the leaf surface form a physical barrier that may affect spore germination, fungal penetration and sporulation (Liang et al., 2005; Dallagnol et al., 2012; Cacique et al., 2013). Disease control is not always satisfactory using foliar spray of Si soluble sources because several sprays are needed or by the fact that the deposition of the foliar applied Si may be easily removed by rain or irrigation water (Dallagnol et al., 2012;

Rodrigues et al., 2015, Debona et al., 2017). By contrast, Si supplied through the roots, especially for monocots, is able to reinforce the cell wall of the epidermal cells and potentiates many host defense mechanisms (Debona et al., 2017). Rice plants grown in soilless potting mix amended with Si showed more foliar Si concentration in comparison to plants Si foliar spray resulting, therefore, in lower blast severity (Cacique et al., 2013). Powdery mildew severity was reduced on leaves of melon plants that received Si through roots in contrast to the foliar spray (Dallagnol et al., 2015). For brown spot, the foliar application of Si decreased disease severity, but the level of control achieved was not as great as that obtained when Si was supplied to the roots (Rezende et al., 2009).

Wheat plants grown in soilless potting mix amended with Si showed lower blast severity and had their photosynthetic performance less impaired (Debona et al., 2014; Rios et al., 2014; Aucique-Pérez et al., 2014). Debona et al. (2014) suggested that biochemical limitation related to reduction on Rubisco activity was the main factor associated with the low *A* values during the infection process of *P. oryzae*.

This study aimed to elucidate if the potassium silicate deposition and its further polymerization on leaf blades of wheat plants could possibly decrease blast symptoms without compromise photosynthesis by examining the combination of leaf gas exchange and chlorophyll *a* fluorescence measurements.

Material and Methods

Experiment 1: Effect of potassium silicate (PS) spray on the photosynthetic performance of wheat plants

Plant growth

Wheat seeds from cultivar BRS Guamirim, susceptible to blast (Cruz et al., 2010) were surface sterilized in 10% (vol vol^{-1}) NaOCl for 2 min, rinsed in sterilized water for 3 min and sown in plastic pots filled with 1 kg of substrate (Tropstrato, Vida Verde, Mogi Mirim, SP, Brazil). A total of 1.63 g of calcium phosphate monobasic was added to each plastic pot. A total of nine seeds were sown per pot and at five days after seedlings emergence, each pot was thinned to seven seedlings. Substrate in each pot was weekly fertilized with a nutrient solution containing, in g L^{-1}, 6.4 KCl; 3.48 K_2SO_4; 5.01 $MgSO_4.7H_2O$; 2.03 $(NH_2)_2CO$; 0.009 $NH_4MO_7O_{24}.4H_2O$; 0.054 H_3BO_3; 0.222 $ZnSO_4.7H_2O$; 0.058 $CuSO_4.5H_2O$ and 0.137 $MnCl_2.4H_2O$ (Xavier Filha et al., 2011). A volume of 15 mL of nutrient solution containing 0.27 g of $FeSO_4.7H_2O$ and 0.37 g of EDTA bisodic L^{-1} was also applied after seedlings emergence. The nutrient solution was prepared using deionized water and 30 mL per pot was applied after seedlings emergence. Plants were watered as needed. Plants were grown during 40 days in a greenhouse with temperature of 25 ± 3°C, relative humidity of 70 ± 5% and natural photosynthetically active radiation of 950 ± 15 µmol photons m^{-2} s^{-1}, which was measured at midday.

Leaf gas exchange and chlorophyll *a* parameters measurements

Plants at growth stage 39 (Lancashire et al., 1991) were sprayed with PS solutions at the rates of 2.5, 5.0, 7.5, 10.0 and 12.5 g L^{-1} three times with a 96 h interval each. Plants sprayed with water served as the control treatment. At 24 h after spray, the net carbon

assimilation rate (A; μmol CO_2 m^{-2} s^{-1}), stomatal conductance to water vapor (g_s; mol H_2O m^{-2} s^{-1}), internal CO_2 concentration (C_i; μmol CO_2 mol^{-1}) and transpiration rate (E; mmol H_2O m^{-2} s^{-1}) were estimated from 09:00 to 12:00 h (solar time) on the fifth leaf, from the top to the base, of each plant per replication of each treatment using a portable open-system infrared gas analyzer (IRGA, LI-COR 6400 XT, LI-COR Biosciences Inc. Nebraska, USA). All of the measurements were conducted under artificial, saturating photon irradiance (1,000 μmol m^{-2} s^{-1}) at the leaf level at 25°C and under external CO_2 concentration of 400 μmol mol^{-1} air. The vapor pressure deficit was maintained at approximately 1.0 kPa while the amount of blue light was set to 10% of the photosynthetic photon flux density to optimize stomatal aperture.

Previously dark-adapted (30 min) leaf tissues were illuminated with weak, modulated measuring beams (0.03 μmol m^{-2} s^{-1}) to obtain the initial fluorescence (F_0). Saturating white light pulses of 8,000 μmol photons m^{-2} s^{-1} were applied for 0.8 s to ensure maximum fluorescence emissions (F_m) from which the variable-to-maximum Chl fluorescence ratio, $F_v/F_m = [(F_m - F_0)/F_m)]$ was calculated. In light-adapted leaves, the steady-state fluorescence yield (F_s) was measured following a saturating white light pulse (8,000 μmol m^{-2} s^{-1}, 0.8 s) that was applied to achieve the light adapted maximum fluorescence ($F_{m'}$). The actinic light was then turned off and far-red illumination was applied (2 μmol m^{-2} s^{-1}) to measure the light-adapted initial fluorescence (F_0'). Using these parameters, the capture efficiency of the excitation energy by the open PSII reaction centers (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 non-photochemical quenching (NPQ) was calculated as $NPQ = (F_m/F_m') - 1$. The actual quantum yield of PSII electron transport (ΦPSII) was computed as $\Phi PSII = (F_m' - F_s)/F_m'$, from which the electron transport rate (ETR) was calculated as ETR = ΦPSII × PPFD ×

$f \times \alpha$, 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 (Maxwell and Johnson, 2000).

Optical measurement

Reflectance (R) and transmittance (T) were measured at 24 h after spraying the leaves with PS rates of 0, 2.5, 5.0, 7.5, 10.0 and 12.5 g L^{-1} using an Ocean Optics model USB2000 spectrometer (Ocean Optics Inc., Dunedin, FL, USA). This spectrometer had a 2048 element detector array, 0.5 nm sampling interval and 7.3 nm spectral resolution in the 350-1000 nm range. Software was designed for signal verification, adjustment of integration time and data acquisition. The absorbance (A) values were obtained as following: A = 100 - (R + T).

Determination of foliar Si concentration

At the end of Exp. 1, leaves from plants from the replications of each treatment were collected, washed in deionized water, dried for 72 h at 65°C and ground to pass through a 40-mesh screen with a Thomas Wiley mill. The foliar Si concentration was determined by colorimetric analysis of 0.1 g of dried and alkali-digested tissue (Korndörfer et al., 2004).

Experimental design and data analysis

The experiment was arranged in a completely randomized design with six treatments (PS concentrations) and five replications. Each experimental unit corresponded to a plastic pot containing seven plants. The experiment was repeated. Data from A, g_s, C_i, E, F_v/F_m,

$F_{v'}/F_{m'}$, ETR, q_p, q_N were separately used to calculate the area under the curve (AUC). Data from AUC for each parameter was submitted to analysis of variance (ANOVA) using SAS (SAS, version 6.12; SAS Institute, Inc., Cary, NC). Polynomial regression procedures were used to determine the relationship between PS rates and foliar Si concentration as well the reflectance variables using SAS. A quadratic regression model was used to describ the effect of PS applications.

Experiment 2: Effect of PS on blast control and on the photosynthetic performance

Plants were grown as mentioned for Exp. 1. The PS rate of 12.5 g L^{-1}, which did not affect photosynthesis on plants from Exp.1, was used on Exp. 2. Plants at growth stage 39 (Lancashire et al., 1991) were sprayed with PS (12.5 g L^{-1}), fungicide (trifloxystrobin 10% + tebuconazole 20%; 0.75 L/ha) and fungicide + PS at 24 h before inoculation with *P. oryzae*. Plants sprayed with water served as the control treatment.

Plant inoculation with *P. oryzae*

An isolate of *P. oryzae* (UFV/DFP *Po*-01), obtained from spikes of wheat plants from cultivar BR-18, was used for plant inoculation. This isolate was preserved on strips of filter paper placed into glass tubes containing silica gel at 4°C. Pieces of filter paper with fungal mycelia were transferred to Petri dishes containing potato dextrose agar (PDA). After 3 days, PDA plugs containing fungal mycelia were transferred to new Petri dishes containing oat media, which were kept in a growth chamber at 25°C with a 24 h photoperiod during 10 days. After this period, conidia were carefully removed from the Petri dishes with a soft bristle brush to obtain a conidial suspension, which was calibrated with a hemacytometer to obtain a concentration of 1×10^5 conidia ml^{-1}. Thirty milliliters

of suspension was applied as a fine mist to the adaxial leaf blades of each plant until runoff with a VL Airbrush atomizer (Paasche Airbrush Co., Chicago, IL). Immediately after inoculation, plants were transferred to a growth chamber with a temperature of 25 ± 2°C and a relative humidity of 90 ± 5% and were subjected to an initial 24 h dark period. After this period, plants were transferred to a plastic mist growth chamber (temperature of 25 ± 2°C (day) to 20 ± 2°C (night) and relative humidity of 92 ± 3% by using a misting system in which nozzles (model NEB-100; KGF Company, São Paulo, Brazil) sprayed mist every 30 min above the plant canopy) for the duration of the experiment.

Blast severity assessments and determination of foliar Si concentration

The fourth and fifth leaves, from the top to the base, of each plant per replication of each treatment were marked and used to evaluate blast severity at 48, 72, 96 and 120 h after inoculation (hai) according to a diagrammatic scale proposed by Rios et al. (2013). The area under blast progress curve (AUBPC) for each leaf per plant was computed by using the trapezoidal integration of blast progress curves over time according to Shaner and Finney (1990). The foliar Si concentration was determined as described for Exp. 1 but after the samples were collected they were washed in deionized water.

Experimental design and data analysis

The experiment was a 2 × 4 factorial and arranged in a completely randomized design with six replications. The factors studied were plants non-inoculated or inoculated with *P. oryzae* and products (plants sprayed with water, PS, fungicide and fungicide + PS). Each experimental unit corresponded to a plastic pot containing seven plants. The experiment was repeated. Data from A, g_s, C_i, E, F_v/F_m, Y(NO), Y(II), ETR and Y(NPQ) were separately used to calculate the AUC. Data from AUC for each parameter was

submitted to ANOVA and the treatment means were compared based on Tukey´s test using SAS.

Results

Experiment 1

There were no significant changes for A, g_s, E and C_i (Fig. 1) as well as for F_v/F_m, Y(NO), Y(II), Y(NPQ) and ETR (Fig. 2) regardless of the evaluation time and PS rates. The linear regression model best described the foliar Si concentration-PS rates relationship (Y = 0.80 + 0.073x, P = 0.0042, R^2 = 0.74). The foliar Si concentration significantly increased by 166% at the highest PS rate. There was no significantly relationship between the PS rates with either absorbance, reflectance or transmittance.

Experiment 2

The foliar Si concentration was significantly increased by 44.44 and 42.22%, respectively, for the PS and fungicide + PS treatments compared to the control treatment (Table 1). The AUDPC was significantly lower by 64.22, 57.47 and 52.84%, respectively, for the treatments fungicide, PS and fungicide + PS in comparison to the control treatment (Table 1).

For inoculated plants, A was significantly lower by 18.93, 31.16, 21.46 and 34.78%, respectively, for the control, fungicide, PS and fungicide + PS treatments in comparison to the non-inoculated plants (Fig. 3A). For non-inoculated plants, A was significantly higher by 24.41 and 25.92% for the control and fungicide treatments, respectively, in comparison to the fungicide + PS treatment. For inoculated plants, A was significantly lower by 35.44 and 7.37%, respectively, for the PS and fungicide + PS treatments, respectively, in comparison to the control treatment. For non-inoculated plants, C_i was significantly lower by 6.66 and 0.34% for the PS and fungicide + PS treatments, respectively, in comparison to the control treatment. For inoculated plants, C_i was

significantly lower by 8.12% for the fungicide + PS treatment in comparison to the control treatment (Fig. 3B).

For inoculated plants, F_v/F_m was significantly lower by 9.83, 5.71, 4.74 and 4.04%, respectively, for the control, fungicide, PS and fungicide + PS treatments in comparison to non-inoculated plants (Fig. 4A). The F_v/F_m was significantly lower for inoculated plants from the control treatment in comparison to the other treatments. For inoculated plants, Y(NO) was significantly higher by 8.66% for the control treatment and significantly lower by 9.13 and 19.51%, respectively, for the PS and fungicide + PS treatments in comparison to non-inoculated plants. The Y(NO) was significantly lower for non-inoculated plants from the fungicide treatment in comparison to the other treatments. For inoculated plants, Y(NO) was significantly lower by 14.01, 11.22 and 21.29%, respectively, for the fungicide, PS and fungicide + PS treatments in comparison to the control treatment (Fig. 4B). For inoculated plants, Y(II) was significantly lower by 21.62, 20.72, 40.24 and 21.00%, respectively, for the control, fungicide, PS and fungicide + PS treatments in comparison to the non-inoculated plants. For inoculated plants, Y(II) was significantly lower by 1.71 and 21.18%, respectively, for the PS and fungicide + PS treatments in comparison to the control treatment and by 2.12 and 21.51%, respectively, in comparison to the fungicide treatment (Fig. 4C). For inoculated plants, ETR was significantly lower by 22.84, 21.03, 41.04 and 21.05%, respectively, for the control, fungicide, PS and fungicide + PS treatments in comparison to the non-inoculated plants. For inoculated plants, ETR was significantly lower by 26.99, 26.96 and 23.67%, respectively, for PS treatment in comparison to the control, fungicide and fungicide + PS treatments (Fig. 4D). For inoculated plants, Y(NPQ) was significantly higher by 23.43, 30.88, 88.91 and 60.04%, respectively, for the control, fungicide, PS and fungicide + PS treatments in comparison to the non-inoculated plants (Fig. 4E). The Y(NPQ) was

significantly lower for non-inoculated plants from the PS treatment in comparison to the control and fungicide treatments. For inoculated plants, Y(NPQ) was significantly higher by 33.28 and 24.73%, respectively, for PS and fungicide + PS treatments in comparison to the control treatment (Fig. 4E).

Discussion

Considering that one concern regarding the spray of any source of soluble Si is its possible impact on photosynthesis upon polymerization over stomata on the leaf surface, the present study provides, to the best of authors´ knowledge, novel evidence that leaf gas exchange (A, C_i, g_s and E) and chlorophyll a fluorescence (F_v/F_m) parameters neither the optical properties of the wheat leaf blades were affected by PS sprays. Therefore, PS was not able to impair CO_2 influx from the environment into the carboxylation sites and any impedance to the photosynthetic process ocurred. These findings are of detrimental relevance because any impact on photosynthesis may deploy plants to mount efficient defense strategies to counteract against pathogens infection besides having their fitness changed that results in great yield losses (Rodrigues et al., 2014; Debona et al., 2017).

The possibility of using PS as a foliar spray to control blast on wheat has never been reported. In the present study, it was noticed that a higher foliar Si concentration on PS-sprayed plants contributed to decrease blast severity, but no gain on disease control was achieved when PS was mixed with fungicide. One plausible explanation for a decrease in blast symptoms on PS-sprayed leaves can be linked to the osmotic effect of its alkaline solution on conidia viability or even on their germ tube growth. Moreover, *P. oryzae* pre-penetration through an appressorium can be prevented after the PS dries over the leaf blades. On the other hand, for wheat plants having Si-supplied to the roots, blast symptoms were lowered due to the potentiation of host defense mechanisms (Rodrigues et al, 2015; Debona et al., 2017). Foliar spray of Si to wheat plants was not as effective as root-applied Si to slow powdery mildew development because Si deposition on the leaf surface was not homogeneous (Guével et al., 2007). In the melon-*Podosphaera xanthii* interaction, Si application to roots was more efficient to reduce the rate of fungal colonies expansion, fungal colonies area and conidia production in contrast to foliar applied Si

(Dallagnol et al., 2012). In rice, the efficacies of both leaf and root Si-applications were compared for blast and brown spot control. For the brown spot, the foliar-applied Si decreased disease intensity; however, the level of control achieved was not as great as that obtained when Si was supplied to the roots (Rezende et al., 2009). For the blast, the lesion size, the number of lesions per cm^2 of leaf area and the area under blast progress curve were reduced for both methods of applying Si, but Si supplied to the roots tended to be more effective in suppressing blast development than applied foliar (Cacique et al., 2013). The foliar spray of Si-containing products for powdery mildew control in cucumber, eggplant and melon plants was efficient in reducing the number of fungus colonies on the leaves (Rodrigues et al., 2015).

In the present study, the photosynthetical process on wheat leaves was greatly impaired during the infection process of *P. oryzae*, but to a lesser extent on the leaves of PS-sprayed plants. The F_v/F_m values, which represents the maximum potential quantum efficiency of PSII if all capable reaction centers were open, for inoculated plants sprayed with PS, fungicide or with their combination were less affected in comparison to non-sprayed plants. This finding indicates that the components associated with the photosynthetic machinery suffered minor damage on the leaves of these plants. It is known that an F_v/F_m value in the range of 0.79 to 0.84 is the approximate optimal value for many plant species, with lowered values indicating plant stress (Maxwell and Johnson, 2000). Some studies on the wheat-*P. oryzae* interaction reported that Si-supplied plants through the roots showed that changes in the values of F_v/F_m, F_v'/F_m', q_P and ETR were minimal indicating, therefore, that their ability to capture, explore and dissipate light energy were less affected in contrast to non Si-supplied plants (Aucique-Pérez et al., 2014, 2017). Rodrigues et al. (2015) reported no significant alterations in the values of C_i, g_s and E for common bean plants sprayed with PS, but significantly reduction on

anthracnose severity and an increase on yield were achieved. According to Ramos et al. (2013), the spray of high rates of different Si sources to control powdery mildew on pumpkin plants should be taken into account once leaf gas exchange parameters and yield can be affected. Interestingly, the combination of PS with fungicide caused a negative effect on the photosynthetic performance of wheat plants. Even though fungicides spray still be the most important control strategy to reduce blast impact on wheat yield (Rodrigues et al., 2017), growers should pay careful attention when adding fungicide and any source of soluble Si to a tank mix for wheat blast control.

In conclusion, the foliar spray of PS can be an environmental friendly strategy to control blast on wheat without causing any impact on photosynthesis.

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Table and Figures

Table 1. Foliar silicon (Si) concentration and area under blast progress curve (AUBPC) for wheat plants submitted to different treatments and inoculated with *Pyricularia oryzae*.

Treatments	Si (dag/kg)	AUBPC
Control	0.45 b	295.20 a
Fungicide	0.44 b	105.60 b
Potassium Silicate	0.65 a	125.52 b
Fungicide + Potassium Silicate	0.64 a	139.20 b
Coefficient of Variation (%)	9.99	45.98

Means within each column followed by the same letter are not significantly different ($P = 0.05$) as determined by Tukey's test. $n = 6$.

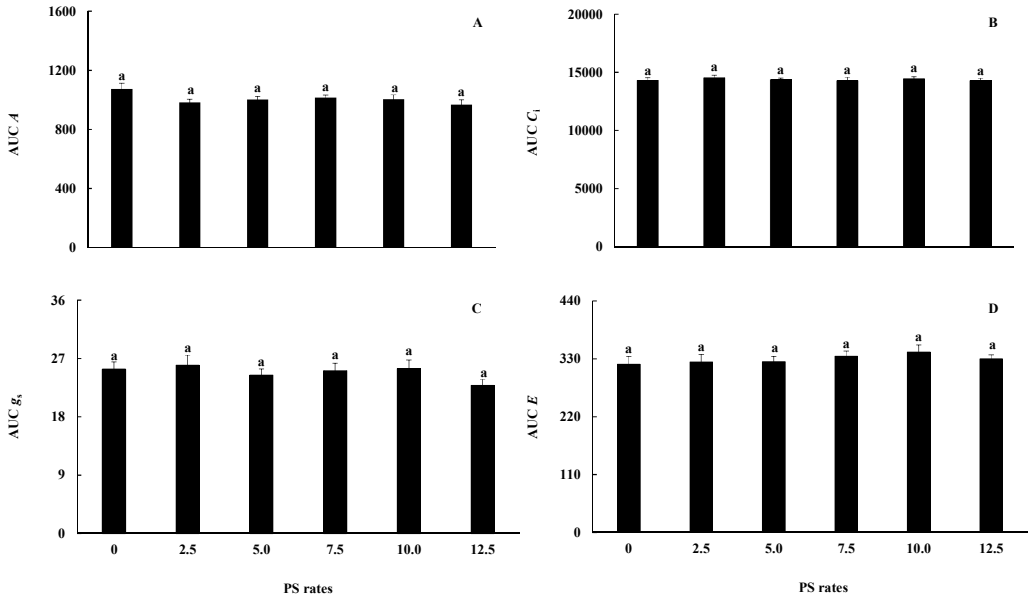

Figure 1. Area under the curve (AUC) for net CO_2 assimilation rate (A) (A), internal CO_2 concentration (C_i) (B), stomatal conductance to water vapor (g_s) and transpiration rate (E) for the leaves of wheat plants sprayed with differente potassium silicate (PS) rates. Means for each parameter followed by the same letter among the treatments are not significantly different by the Tukey´s test ($P \leq 0.05$). Bars represent the standard error of the mean. $n = 5$.

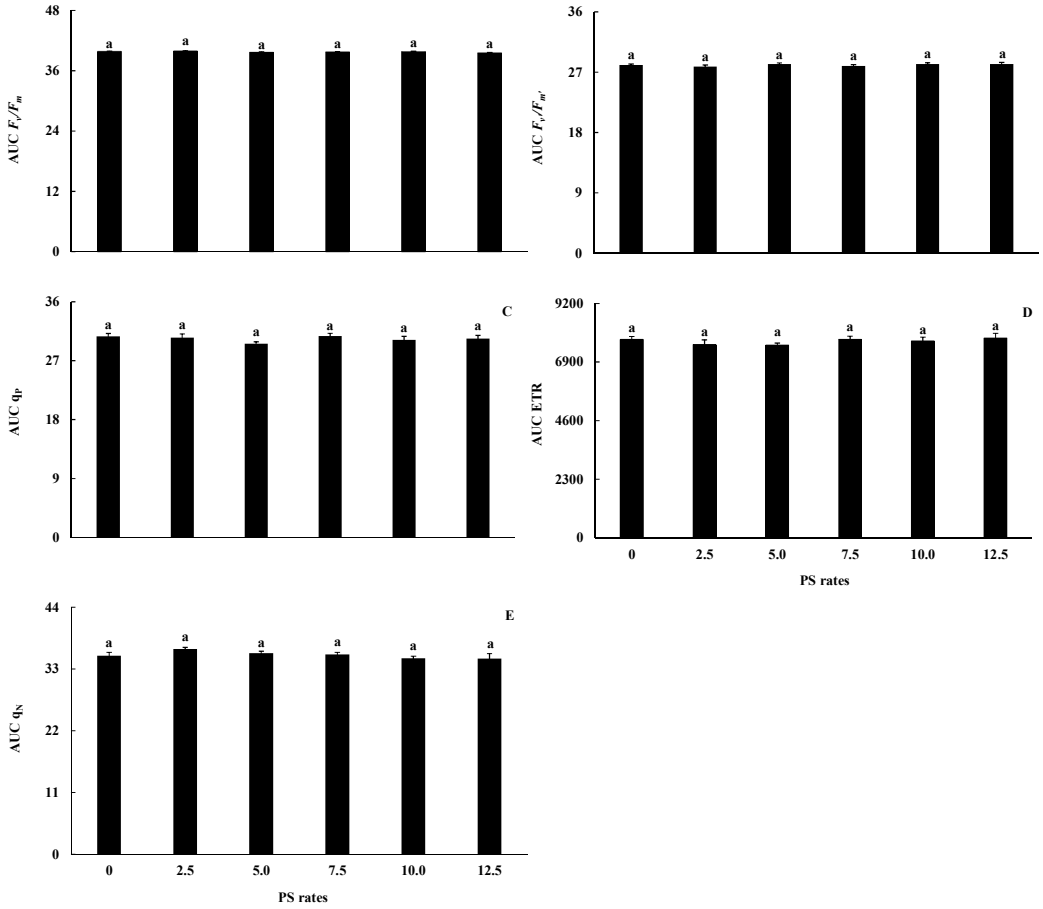

Figure 2. Area under the curve (AUC) for maximal photosystem II quantum yield (F_v/F_m) (A), capture efficiency of excitation energy by the open PSII reaction centers (F_v'/F_m') (B), coefficient for photochemical quenching (q_P) (C), electron transport rate (ETR) (D) and the non-photochemical quenching of variable chlorophyll fluorescence (q_N) (E) for the leaves of wheat plants sprayed with differente potassium silicate (PS) rates. Means for each parameter followed by the same letter among the treatments are not significantly different by the Tukey´s test ($P \leq 0.05$). Bars represent the standard error of the mean. $n = 5$.

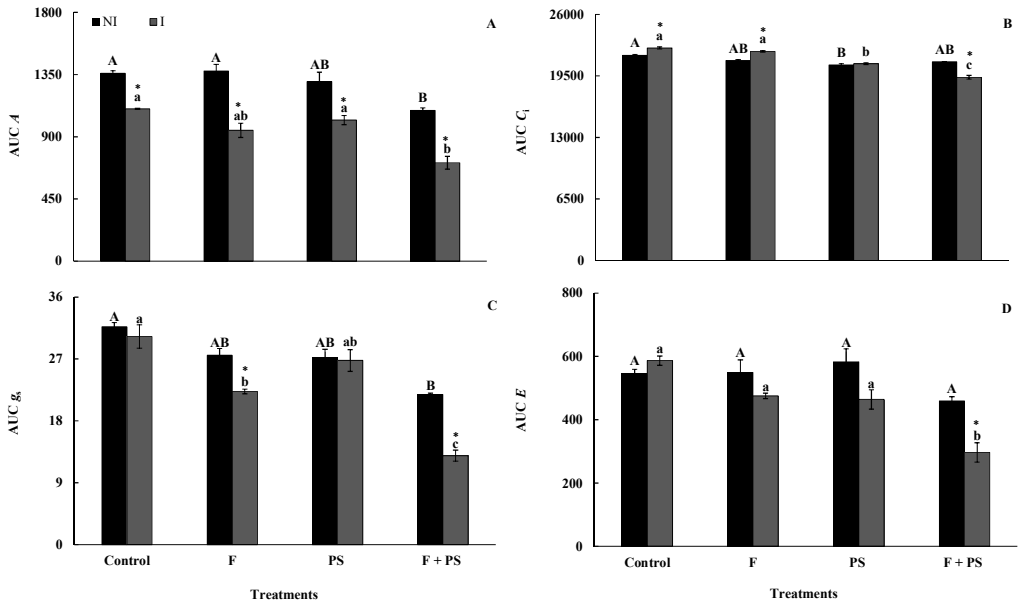

Figure 3. Area under the curve (AUC) for net CO_2 assimilation rate (A) (A), internal CO_2 concentration (C_i) (B), stomatal conductance to water vapor (g_s) and transpiration rate (E) for the leaves of wheat plants sprayed with water (control), fungicide (F), potassium silicate (PS) and fungicide + potassium silicate (F+PS) and non-inoculated (NI) or inoculated (I) with *Pyricularia oryzae*. Means for the NI and I treatments followed by an asterisk (*) for control, F, PS and F + PS treatments, are significantly different by F test ($P \leq 0.05$). For the NI or I treatments, means followed by the same uppercase or lowercase letters, respectively, are not significantly different according to Tukey′s test ($P \leq 0.05$). Bars represent the standard error of the mean. $n = 5$.

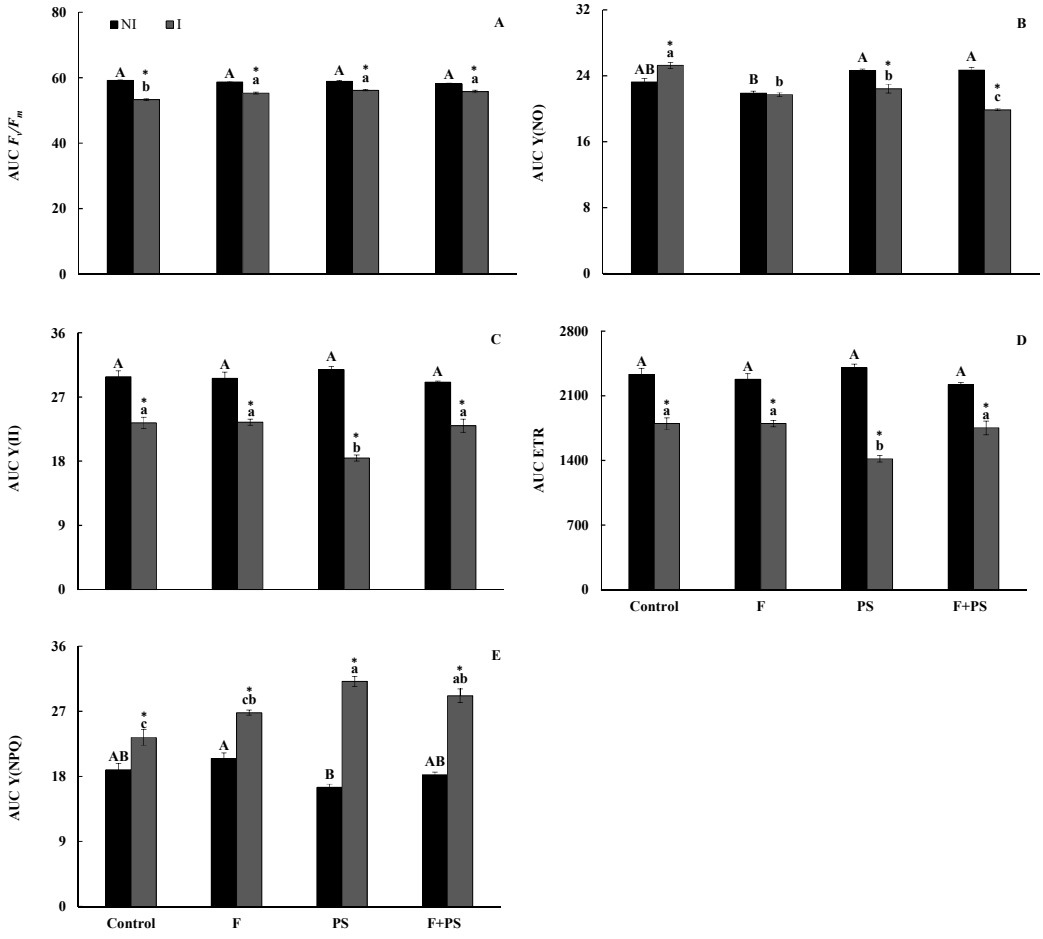

Figure 4. Area under the curve (AUC) for maximal photosystem II quantum yield (F_v/F_m) (A), quantum yield of non-regulated energy dissipation [Y(NO)] (B), photochemical yield [Y(II)] (C), electron transport rate (ETR) (D) and quenching non-photochemical [Y(NPQ)] (E) for the leaves of wheat plants sprayed with water (control), fungicide (F), potassium silicate (PS) and fungicide + potassium silicate (F+PS) and non-inoculated or inoculated with *Pyricularia oryzae*. Means for the NI and I treatments followed by an asterisk (*) for control, F, PS and F + PS treatments, are significantly different by *F* test ($P \leq 0.05$). For the NI or I treatments, means followed by the same uppercase or lowercase letters, respectively, are not significantly different according to Tukey´s test ($P \leq 0.05$). Bars represent the standard error of the mean. $n = 5$.

Chapter 2

Impairment of Photosynthesis by a Specific Inhibitor Increase Wheat Susceptibility to Blast

Resumo

Considerando a brusone, causada por *Pyricularia oryzae*, como seria ameaça à produção de trigo e a importância da fotossíntese como uma fonte de energia a ser utilizada pelas plantas para à ativação de mecanismos de defesa em resposta a infecção por patógenos, este estudo objetivou investigar se o comprometimento do processo fotossintético de plantas de trigo utilizando-se um inibidor da fotossíntese poderia aumentar a susceptibilidade à brusone. Plantas de trigo da cultivar BRS 220, parcialmente resistente a brusone, foram pulverizadas ou não com uma solução de 10 μM de 3-(3,4-diclorofenil)-1,1-dimetilureia) (DCMU) às 24 h antes da inoculação com *P. oryzae*. O DCMU afetou a funcionalidade do aparato fotossintético das folhas de trigo com base nos baixos valores da taxa de assimilação liquida de CO_2 (*A*) associado com um aumento na concentração interna de carbono. Houve reduções drásticas na condutância estomática ao vapor de água e na taxa de transpiração nas plantas infectadas, especialmente quando pulverizadas com DMCU. Os baixos valores de *A* nas folhas das plantas inoculadas e pulverizadas com DCMU afetou a síntese de carboidratos resultando, assim, em uma maior concentração de amido. A concentração de sacarose foi reduzida nas folhas infectadas principalmente se pulverizadas com DCMU enquanto que as concentrações de frutose e de glicose aumentaram. As atividades da superóxido dismutase e peroxidase foram menores enquanto a atividade da catalase foi maior para as plantas não infectadas e pulverizadas com DCMU. Em conclusão, os resultados do presente estudo demonstraram que o DMCU aumentou a susceptibilidade do trigo a brusone devido as disfunções fotossintéticas, comprometimento do metabolismo de açúcares e menor eficiência do sistema antioxidante.

Palavras chave: *Triticum aestivum* L., brusone, doença fúngica, metabolismo antioxidativo, parâmetros de trocas gasosas.

Abstract

Considering blast, caused by *Pyricularia oryzae*, as a key threat to impact wheat yield and the importance of photosynthesis as the source of energy to be used by plants to activate their mechanisms of defense to counteract pathogens infection, this study aimed to investigate if the compromise of the photosynthetic process of wheat plants by using an inhibitor of photosynthesis could increase their susceptibility to blast. Plants from cultivar BRS 220, partially resistant to blast, were non-sprayed (control treatment) or sprayed with a solution of 10 μM of 3-(3,4-dichlorophenyl)-1,1-dimethylurea) (DCMU) at 24 h before inoculation with *P. oryzae*. The DCMU affected the functionality of the photosynthetic apparatus of wheat leaves based on the lower values of net CO_2 assimilation rate (*A*) coupled with increases in the internal carbon concentration. Indeed, there were dramatic reductions in the values of stomatal conductance to water vapor and transpiration rate for infected plants, especially for the DCMU-sprayed ones. The lower *A* values obtained from leaves of DCMU-sprayed and infected plants limited the carbohydrates synthesis resulting in great starch concentration. Sucrose concentration was reduced on infected leaves mainly if they were sprayed with DCMU while fructose and glucose concentrations increased. The activities of superoxide dismutase and peroxidase were lower while catalase activity was higher for DCMU-sprayed and non-infected plants. Altogether, the results of the present study showed that the spray of DCMU increased wheat susceptibility to blast due to photosynthetic dysfunctions, impairment on sugar metabolism and a less efficient antioxidative system.

Keywords: *Triticum aestivum* L., antioxidative metabolism, blast, fungal disease, leaf gas exchange parameters.

Introduction

Wheat (*Triticum aestivum* L. subsp. *aestivum*), an important staple food crop cultivated in South America, especially in Bolivia, Brazil and Paraguay (Goulart and Paiva, 1992; Kohli et al., 2011), can have its yield greatly impacted due to blast epidemics, caused by the hemibiotrophic fungus *Pyricularia oryzae* Cavara (teleomorph *Magnaporthe oryzae* (T. T. Hebert) M. E. Barr). Blast was reported on wheat fields in Bangladesh in 2016 and it was disseminated into India warned the Asian governments to this devastating disease (Callaway, 2016; Islam et al., 2016). On leaves, blast symptoms begin as gray-green, water-soaked lesions with dark green borders that become necrotic after they have expanded completely besides causing premature bleaching and death of individual or entire spikelets (Igarashi et al., 1986; Goulart et al., 2007; Debona et al., 2012). Blast epidemics are more prone to occur in growing regions under wet rainy seasons and temperature greater than 20°C (Goulart et al., 2007; Kohli et al., 2011). Fungicides sprayed during flowering stage and the use of cultivars with high level of partial resistance are key control strategies to reduce yield losses caused by blast (Rodrigues et al., 2017).

The infection by *P. oryzae* affects indirect and or directly several physiological processes on wheat leaves (Debona et al., 2014; Rios et al., 2017). When blast severity is higher than 40%, there is a compromise of the photosynthetic efficiency of the infected plants with drastic reductions in the values of the leaf gas exchange parameters net carbon assimilation rate (A), stomatal conductance to water vapour (g_s) and transpiration rate (E) as well as on the concentration of photosynthetic pigments (Aucique-Pérez et al., 2014; Debona et al., 2014). The attenuation of the cellular damage caused by *P. oryzae* infection on plants from cultivar BR 18, with a high level of partial resistance to blast, was attributed to a more efficient antioxidant system in the removal of the reactive oxygen

species (ROS) generated during fungal infection (Debona et al., 2012). Rios et al. (2017) reported that wheat plants infected by *P. oryzae* showed a reduction in the efficiency of the PSII that negatively impacted their photosynthetic capacity. The advance in knowledge of the relationship between photosynthesis and the different metabolic pathways that result in the production of defense compounds highlights the pivotal role played by the chloroplasts in plant resistance against pathogens infection more specifically regarding the ROS production, changes in the cellular redox potential as well as on ions flux (Trotta et al., 2014; Torres-Zabala et al., 2015; Cheng et al., 2016). For several host-pathogen interactions, the use of specific inhibitors has become a strategy in an attempt to correlate the functional operations of the PSI and PSII with the host defense responses originated from the primary metabolism (Barth et al., 2001; Torres-Zabala et al., 2015). The 3-(3,4-dichlorophenyl)-1,1-dimethylurea) (DCMU), an algicide and herbicide of the phenylurea class that inhibits photosynthesis, binds specifically to the Q_B site on the D1 protein of the PSII reaction center preventing, therefore, the electron transfer from Q_A to Q_B (Bowyer et al., 1991). During light, the binding of DCMU to the PSII reaction center results in the formation of the radical pair P^{+}_{680} pheophytin^{-}, whose recombination produces the triplet state of P680 that reacts with oxygen to form singlet molecular oxygen ($^1O_2$) (Asada, 1996). Under such conditions, the probability for the formation of the triplet states of PSII antennae chlorophylls increases and these react with oxygen to increase $^1O_2$ production while hydrogen peroxide production in the chloroplasts is lowered (Marshall et al., 2002; Flors et al., 2005). Indeed, the fluorescence of chlorophyll *a* increases to its maximum and then is kept at physiological levels (Petrášek et al., 2005; Baker, 2008). Finally, the production of NADPH and ATP is blocked and the interruption on carbon fixation results in the inactivation of carbohydrates and the occurrence of oxidative stress (Yu et al., 2010). In several plant species, the foliar

spray of DCMU causes chlorosis, necrosis and leaf wilt; alters the epicuticular wax layer and causes morpho-anatomical changes on leaves; damage to the photosynthetic process; reduction in plant growth and decrease in grain yield (Goltsev et al., 2001; Bell and Duke, 2005; Moskova et al., 2011; Oswalt et al., 2014; El-Nahhal and Hamdona, 2015; Sadler et al., 2016).

Considering blast as a key threat to impact wheat yield and the importance of photosynthesis as the major source of energy to be used for plants to activate their mechanisms of defense to counteract pathogens infection, this study aimed to investigate if the compromise of the photosynthetic process of wheat plants by using the DCMU could increase their susceptibility to blast.

Material and methods

Plant growth

Wheat seeds from cultivar BRS 220 that is partially resistant to blast (Cruz et al., 2010) were surface sterilized in 10% (vol vol^{-1}) NaOCl for 2 min, rinsed in sterilized water for 3 min and sown in plastic pots filled with 1 kg of substrate (Tropstrato, Vida Verde, Mogi Mirim, SP, Brazil). A total of 1.63 g of calcium phosphate monobasic was added to each plastic pot. A total of nine seeds were sown per pot and at five days after seedlings emergence, each pot was thinned to seven seedlings. Substrate in each pot was weekly fertilized with a nutrient solution containing, in g $liter^{-1}$, 6.4 KCl; 3.48 K_2SO_4; 5.01 $MgSO_4.7H_2O$; 2.03 $(NH_2)_2CO$; 0.009 $NH_4MO_7O_{24}.4H_2O$; 0.054 H_3BO_3; 0.222 $ZnSO_4.7H_2O$; 0.058 $CuSO_4.5H_2O$ and 0.137 $MnCl_2.4H_2O$ (Xavier Filha et al., 2011). A volume of 15 mL of nutrient solution containing 0.27 g of $FeSO_4.7H_2O$ and 0.37 g of ethylenediamine tetraacetic acid (EDTA) bisodic L^{-1} was also applied after seedlings emergence. The nutrient solution was prepared using deionized water and 30 mL per pot was applied after seedlings emergence. Plants were watered as needed. Plants were grown during 40 days in a greenhouse with temperature of 25 ± 3°C, relative humidity of 70 ± 5% and natural photosynthetically active radiation of 900 ± 15 μmol photons m^{-2} s^{-1}, which was measured at midday.

Inoculum production, DCMU spray and plant inoculation with *P. oryzae*

An isolate of *P. oryzae* (UFV/DFP *Po*-01), obtained from spikes of wheat plants from cultivar BR 18, was used for plant inoculation. This isolate was preserved on strips of filter paper placed into glass tubes containing silica gel at 4°C. Pieces of filter paper with fungal mycelia were transferred to Petri dishes containing potato dextrose agar (PDA).

After 3 days, PDA plugs containing fungal mycelia were transferred to new Petri dishes containing oat media, which were kept in a growth chamber at 25°C with a 24 h photoperiod during 10 days. After this period, conidia were carefully removed from the Petri dishes with a soft bristle brush to obtain a conidial suspension, which was calibrated with a hemacytometer to obtain a concentration of 1×10^5 conidia ml^{-1}. At 24 h before inoculation, plants were sprayed with a solution of 10 μM of DCMU (30 ml per plant). This concentration was selected based on the preliminary experiment aimed to determine the effect of different DCMU concentrations on the photosynthetic performance of wheat plants. The adaxial leaf blades of each plant was sprayed with 30 mL of suspension as a fine mist until runoff using a VL Airbrush atomizer (Paasche Airbrush Co., Chicago, IL). Immediately after inoculation, plants were transferred to a growth chamber with a temperature of 25 ± 5°C and a relative humidity of 85 ± 5% and were subjected to an initial 24 h dark period. After this period, plants were transferred to a plastic mist growth chamber (temperature of 25 ± 2°C (day) to 20 ± 2°C (night) and relative humidity of 92 ± 3% by using a misting system in which nozzles (model NEB-100; KGF Company, São Paulo, Brazil) sprayed mist every 30 min above the plant canopy) for the duration of the experiments.

Blast severity assessments

Blast severity was assessed at 48, 72 and 96 hours after inoculation (hai) on the fourth and fifth leaves, from the top to the base, of each plant per replication of each treatment according to a diagrammatic scale proposed by Rios et al. (2013).

Leaf gas exchange parameters measurements

The leaf gas exchange parameters net CO_2 assimilation rate (*A*), stomatal conductance to water vapor (g_s), internal CO_2 concentration (C_i) and transpiration rate (*E*) were measured by using a portable open-flow gas exchange system (LI-6400XT; Li-Cor Inc., Lincoln, NE). Measurements were made at the attached fifth leaf, from the top to the base, of each plant per replication of each treatment from 09:00 to 12:00 h (solar time) when *A* was at its maximum at 48 and 72 hai. These parameters were also measured on the leaves of non-inoculated plants, at these same evaluation times, to serve as controls. The experimental conditions during measurements were: artificial photosynthetically active radiation (PAR) of 1000 µmol photons m^{-2} s^{-1} at the leaf level, 400 µmol atmospheric CO_2 mol^{-1} air; 25°C, vapor pressure deficit at approximately 1.0 kPa and 10% of blue light (relative to total PAR) to optimize the stomatal aperture.

Histochemical localization of hydrogen peroxide (H_2O_2) superoxide and anion radical ($O_2^{\bullet-}$) on wheat leaves

Five fragments of the fourth and fifth leaves (≈ 10 cm of length), from the top to the base, of each plant per replication of each treatment were collected at 48, 72 and 96 hai. The leaves were also collected from non-inoculated plants, at these same sampling times, to serve as controls. For the detection of H_2O_2, fragments were placed in glass vials containing a solution of 3,3′-diaminobenzidine tetrahydrochloride (1 mg ml^{-1}) (Sigma-Aldrich, São Paulo, Brazil) and kept in the dark at 25°C for 12 h. After this period, leaf fragments were cleared in boiling 80% ethanol (80%) for 60 min and then stored in glycerol solution (70%). For detection of $O_2^{\bullet-}$, leaf fragments were placed in glass vials, infiltrated with a solution of 0.1% nitro blue tetrazolium (Sigma-Aldrich, São Paulo,

Brazil) in 10 mM potassium phosphate buffer (pH 6.8) for 40 min, cleared in boiling 80% ethanol for 60 min and then stored in glycerol solution (70%) until be analyzed.

Biochemical assays

For all biochemical assays, the fourth and fifth leaves, from the base to the top, of each plant per replication of each treatment were collected at 48, 72 and 96 hai. Leaves were also collected from non-inoculated plants, at these same sampling times, to serve as controls. Leaf samples were kept in liquid nitrogen during sampling and then stored at -80ºC until further analysis.

Determination of malondialdehyde (MDA) concentration

The concentration of total 2-thiobarbituric acid (TBA) reactive substances, expressed as equivalents of MDA (Cakmak and Horst, 1991), was determined as an indicator of oxidative damage in the leaf cells. A total of 200 mg of leaf tissue was ground into a fine powder using a mortar and pestle with liquid nitrogen. The fine powder was homogenized in 2 ml of 0.1% (wt vol^{-1}) trichloracetic acid (TCA) solution in an ice bath. The homogenate was centrifuged at 12000 *g* for 15 min at 4°C. After centrifugation, a total of 0.5 ml of the supernatant was reacted with 1.5 ml of TBA solution (0.5% in 20% TCA) for 30 min in a boiling water bath at 95°C. After this period, the reaction was stopped in an ice bath. The samples were centrifuged at 9000 *g* for 10 min and the specific absorbance was determined at 532 nm. The nonspecific absorbance was estimated at 600 nm and subtracted from the specific absorbance value. The extinction coefficient of 155 mM^{-1} cm^{-1} (Heath and Packer, 1968) was used to calculate the MDA concentration, which was expressed as μmol kg^{-1} of fresh weight.

Antioxidant enzymes assays

A total of 300 mg of leaf tissue was ground into a fine powder in a mortar and pestle with liquid nitrogen to be used to determine the activities of ascorbate peroxidase (APX, EC 1.11.1.11), catalase (CAT, EC 1.11.1.6), peroxidase (POX, EC 1.11.1.7) and superoxide dismutase (SOD, EC 1.15.1.1). The fine powder was homogenized in an ice bath in 2 mL of a solution containing 50 mM potassium phosphate buffer (pH 6.8), 0.1 mM EDTA, 1 mM phenylmethylsulphonyl fluoride (PMSF) and 2% (w/v) polyvinylpolypyrrolidone (PVPP). The homogenate was centrifuged at 12,000 *g* for 15 min at 4°C and the supernatant was used as the crude enzyme extract. To determine the glutathione reductase (GR) activity, a total of 300 mg of leaf tissue was ground as described above and the fine powder was homogenized in an ice bath in 2 mL of a solution containing 100 mM potassium phosphate buffer (pH 7.5), 0.1 mM EDTA, 1 mM DL-dithiothreitol, 1 mM PMSF and 2% (w/v) PVPP. The homogenate was centrifuged as previously described. For APX activity, the reaction was started after the addition of 50 µM of the crude enzyme extract to 1.95 mL of the reaction mixture containing 50 mM potassium phosphate buffer (pH 6.8), 1 mM H_2O_2 and 0.8 mM ascorbate (Nakano and Asada, 1981). The APX activity was measured based on the rate of ascorbate oxidation at 290 nm for 1 min at 25°C. The extinction coefficient of 2.8 mM^{-1} cm^{-1} was used to calculate the APX activity (Nakano and Asada, 1981). The CAT activity was determined based on the rate of H_2O_2 decomposition at 240 nm for 1 min at 25°C (Havir and McHale, 1987). The reaction was initiated after the addition of 50 µM of the crude enzyme extract to 1.95 mL of the reaction mixture containing 50 mM potassium phosphate buffer (pH 6.8) and 20 mM H_2O_2. An extinction coefficient of 36 M^{-1} cm^{-1} was used to calculate CAT activity (Anderson et al., 1995). The POX activity was assayed following the colorimetric determination of pyrogallol oxidation (Kar and Mishra, 1976) after adding of 15 µM of the crude enzyme

extract to 1.98 mL of the substrate mixture containing 25 mM potassium phosphate (pH 6.8), 20 mM pyrogallol and 20 mM H_2O_2. The POX activity was determined based on the absorbance of coloured purpurogallin recorded at 420 nm for 1 min at 25°C. The extinction coefficient of 2.47 mM^{-1} cm^{-1} was used to calculate the POX activity (Chance and Maehly, 1955). The SOD activity was measured based on the ability of this enzyme to photochemically reduce the nitroblue tetrazolium (NBT) (Del Longo et al., 1993). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 0.1 mM EDTA and 2 µM riboflavin. The reaction was started after the addition of 60 µM of the crude enzyme extract to 1.94 mL of the reaction mixture. The reaction was allowed to proceed at 25°C under a 15 W lamp. After 10 min of light exposure, the light was blocked and the production of formazan blue, which resulted from the photoreduction of NBT, was monitored spectrophotometrically to determine the increase in absorbance at 560 nm (Giannopolitis and Ries, 1977). The control reaction mixtures were kept in darkness for 10 min and the absorbance was measured at 560 nm. The control values obtained were subtracted from the experimental values obtained. One unit of SOD was defined as the amount of enzyme necessary to inhibit NBT photoreduction by 50% (Beauchamp and Fridovich, 1971). The reaction mixture used to determine GR activity contained 100 mM potassium phosphate (pH 7.5), 1 mM EDTA, 1 mM oxidized glutathione (GSSG) and 0.1 mM NADPH prepared in 0.5 mM Tris-HCl buffer (pH 7.5) (Carlberg and Mannervik, 1985). The reaction was started after the addition of 100 µM of the crude enzyme extract to 1.9 mL of the substrate mixture. The decrease in absorbance at 340 nm was determined for 1 min at 30°C. The extinction coefficient of 6.22 mM^{-1} cm^{-1} was used to calculate GR activity (Foyer and Halliwell, 1976). Four separate extractions were performed on leaf samples from plants of each treatment to determine the activity of each enzyme. Each extraction was read

three times. The concentration of soluble protein of the extracts was measured by the method of Bradford (1976) using bovine serum albumin as the standard protein.

Determination of carbohydrates, amino acids and proteins concentrations

Leaf sample was lyophilized (-48°C) and ground 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). Leaf samples (15 mg) were homogenized with 500 µL of 100% ethanol, 500 µL of 80% ethanol and 500 µL of 50% ethanol and then incubated at 80°C for 30 min following centrifugation at 13000 *g* for 5 min. This process was repeated after homogenizing the pellet with 80 and 50% ethanol. The concentrations of sugars (fructose, glucose and sucrose) and amino acids (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.

Experimental design and data analysis

A 2 × 2 factorial experiment with six replications, consisting of non-inoculated and inoculated plants as well as plants non-sprayed or sprayed with DCMU arranged in a completely randomized design, was carried out to evaluate blast severity, determine the leaf gas exchange parameters and to obtain the leaf samples for the biochemical analysis. The experiment was repeated once. Each experimental unit corresponded to a plastic pot containing seven plants. Data from all leaf gas exchange parameters and biochemical variables were analyzed by analysis of variance and means from the treatments were compared using *F* test ($P \leq 0.05$) (SAS, version 6.12; SAS Institute, Inc., Cary, NC).

Results

Blast severity

The AUC for severity was significantly higher by 32.27% for DCMU-sprayed plants in comparison to non-sprayed ones (Fig. 1).

MDA concentration

There were significant increases of 38.63 and 59.21%, respectively, at 48 and 96 hai for inoculated and DCMU non-sprayed plants and of 48.69, 46.42 and 196.62%, respectively, at 48, 72 and 96 hai for inoculated and DCMU sprayed plants relative to their control counterparts (Fig. 2A). At 48 hai, MDA concentration was significantly reduced by 30.02 and 25.15%, respectively, for non-inoculated and inoculated plants sprayed with DCMU relative to their control counterparts. At 72 and 96 hai, there were significantly increases of 30.98 and 68.53%, respectively, for inoculated DCMU-sprayed plants in comparison to inoculated DCMU non-sprayed ones.

Histochemical localization of H_2O_2 and $O_2^{\bullet-}$

The H_2O_2 production, as indicated by the brown color, increased from 48 to 96 hai in the leaves of plants non-sprayed with DCMU in comparison to non-inoculated ones (control). By contrast, on the leaves of plants sprayed with DCMU, the $O_2^{\bullet-}$ production was more remarkable from 48 to 96 hai in comparison to H_2O_2 production. The DCMU spray resulted in more $O_2^{\bullet-}$ production and non-detectable H_2O_2 production in the leaves of non-inoculated plants (Fig. 3A-B).

Leaf gas exchange parameters

For inoculated DCMU non-sprayed plants, there were significantly decreases of 100 and 100%, respectively, at 48 and 72 hai for *A* and of 90.42 and 89.02%, respectively, for g_s and *E* at 48 hai relative to their control counterparts. The g_s, C_i and *E* values significantly increased by 229.00, 62.18 and 69.23%, respectively, at 72 hai for inoculated DCMU non-sprayed plants in comparison to their control counterparts. There were significantly decreases of 92.85% at 48 hai for g_s and of 92.11 and 55.95%, respectively, for *E* at 48 and 72 hai for inoculated DCMU-sprayed plants in comparison to their control counterparts. The C_i values were significantly higher by 45.67 and 20.63%, respectively, at 48 and 72 hai for inoculated DCMU-sprayed plants in comparison to their control counterparts. For non-inoculated DCMU-sprayed plants, there were significantly decreases of 97.99 and 97.26%, respectively, at 48 and 72 hai for *A*, of 26.31 and 42.86%, respectively, at 48 and 72 hai for g_s and of 12.5 and 45%, respectively, at 48 and 72 hai for *E* relative to their control counterparts. C_i values significantly increased by 89.42 and 83.65%, respectively, at 48 and 72 hai for non-inoculated DCMU-sprayed plants and by 163.05 and 36.49%, respectively, at 48 and 72 hai for inoculated DCMU-sprayed plants relative to their control counterparts. For inoculated DCMU-sprayed plants, there were significantly decreases of 100.00 and 100.00%, respectively, at 48 and 72 hai for *A*, of 90 and 91.30%, respectively, at 48 and 72 hai for g_s and of 88.66 and 86.15%, respectively, at 48 and 72 hai for *E* relative to their control counterparts.

Activities of antioxidant enzymes

For inoculated DCMU non-sprayed plants, there were significantly increases of 79.11% at 48 hai for SOD activity, of 142.56, 80.00 and 39.02%, respectively, at 48, 72 and 96 hai for CAT activity, of 159.77 and 92.75%, respectively, at 48 and 96 hai for POX

activity and of 49.56, 75.80 and 50.83%, respectively, at 48, 72 and 96 hai for APX activity relative to their control counterparts. SOD activity significantly decreased by 30.21% at 96 hai for inoculated and DCMU non-sprayed plants in comparison to non-inoculated and DCMU non-sprayed ones. For inoculated DCMU-sprayed plants, there were significantly increases of 66.61 and 83.78%, respectively, at 48 and 96 hai for SOD activity, of 115.63 and 128.77% at 48 hai for CAT and APX activities and of 114.28% at 96 hai for GR activity relative to their control counterparts. Significantly decreases of 39.67% at 72 hai POX activity and of 65.88 and 55.60%, respectively, at 72 and 96 hai for APX occurred for inoculated DCMU sprayed plants in comparison to non-inoculated and DCMU-sprayed ones. For non-inoculated and DCMU-sprayed plants, there were significantly decreases of 25.23 and 41.15%, respectively, at 72 and 96 for SOD activity relative to their control counterparts. For non-inoculated and DCMU-sprayed plants, there were significantly increases of 63.41% at 96 hai for CAT activity and of 106.69 and 154.15%, respectively, at 48 and 96 hai for POX activity relative to their control counterparts. For inoculated DCMU-sprayed plants, there were significantly decreases of 38.55 and 52.68%, respectively, for SOD and CAT activities at 72 hai, of 24.44 and 32.40%, respectively, at 48 and 72 hai for POX activity and of 80.97 and 64.33%, respectively, at 72 and 96 for APX activity relative to their control counterparts. There were significantly increases of 55.98% at 96 hai for SOD activity, of 35.49% at 48 hai for APX activity and of 328.57% at 96 hai for GR activity for inoculated DCMU-sprayed plants relative to their control counterparts.

Concentrations of glucose, fructose, sucrose and starch

For inoculated DCMU non-sprayed plants, there were significantly decreases of 25.79% at 96 hai for glucose concentration, of 81.75 and 65.46%, respectively, at 48 and 72 hai

for sucrose concentration, of 44.91% at 48 hai for starch concentration and of 41.48% at 72 hai for malate concentration relative to their control counterparts. There were significantly increases of 113.81% at 72 hai and of 91.92% at 48 hai, respectively, for starch and malate concentrations for inoculated DCMU non-sprayed plants relative to their control counterparts. There were significantly decreases of 30.54% at 96 hai for glucose concentration, of 70.98% at 96 hai for fructose concentration, of 85.49% at 96 hai for starch concentration and of 63.00% at 48 hai for malate concentration for inoculated DCMU-sprayed plants in comparison to their control counterparts. Significantly increases of 147.22% at 96 hai for fructose concentration and of 147.27% at 48 hai for malate concentration occurred for non-inoculated DCMU-sprayed plants in comparison to their counterparts. For non-inoculated DCMU-sprayed plants, there were significantly decreases of 67.80 and 40.96% at 48 hai, respectively, for sucrose and starch concentrations and of 35.01% at 72 hai for malate concentration relative to their control counterparts. The concentrations of fructose significantly increased by 444.57% at 48 hai and of starch significantly decreased by 74.46% at 72 hai for inoculated DCMU-sprayed plants relative to their control counterparts.

Concentrations of amino acids and proteins

For amino acids concentration, there were significantly decreases of 34.41% at 96 hai and of 48.97% at 48 hai, respectively, for inoculated DCMU non-sprayed and inoculated DCMU-sprayed plants in comparison to their control counterparts. At 96 hai, there was significantly increase of 106.86% for inoculated DCMU-sprayed plants in comparison to non-inoculated DCMU-sprayed ones. There were significant increases of 104.77% at 48 hai and of 52.63% at 96 hai, respectively, non-inoculated DCMU-sprayed plants and non-inoculated DCMU non-sprayed ones relative to their control counterparts. At 96 hai, there

was significant increase of 64.22% for inoculated DCMU-sprayed plants in comparison to inoculated non-sprayed ones (Fig. 8A). For proteins concentration, there was a significantly increase of 63.79% for inoculated DCMU-sprayed plants in comparison to non-inoculated DMCU-sprayed ones at 96 hai. There was a significantly decrease of 37.24% at 96 hai for DCMU-sprayed and non-inoculated plants in comparison to non-DCMU sprayed and non-inoculated plants (Fig. 8B).

Discussion

There are many studies reporting the use of inhibitors of photosynthesis, including the DCMU, in order to gain further insights of the importance of this physiological process on plants exposed to many types of stresses, including the infection by pathogens of different lifestyles (Abdollahi et al., 2015; Torres-Zabala et al., 2015; Cheng et al., 2016; Arase et al., 2017). However, for the wheat-*P. oryzae* interaction, the present study provides, to the best of authors´ knowledge, novel evidence of the deleterious effect of DCMU to disturb photosynthesis on wheat leaves and increasing their susceptibility to blast based on the high severity and great cell damage as reflected by higher MDA concentration. Moreover, the functionality of the photosynthetic apparatus on wheat leaves, based on the lower A values coupled with increases in the C_i values, was dramatically compromised by DCMU. The DCMU is a potent inhibitor of the electron transport from the primary quinone electron acceptor to the secondary quinone electron acceptor in the PSII (Marshall et al., 2002).

In the present study, there were reductions in A, g_s and E values for wheat plants infected by *P. oryzae*, especially if they were sprayed with DCMU. Some studies also related the effect of *P. oryzae* infection in the photosynthetic process of wheat plants mainly in the photochemical phase associated with lower concentration of photosynthetic pigments and a lower biochemical capacity for carbon fixation due to a decrease on RuBisCO activity (Aucique-Pérez et al., 2014; Debona et al., 2014; Rios et al., 2017). Torres-Zabala et al. (2015) reported reduction on photosynthesis of *Arabidopsis thaliana* plants infected by *Pseudomonas syringae* indicating, therefore, the importance of chloroplasts for host resistance against bacterium infection. Chloroplasts are involved in the biosynthetic pathways of hormones that participate in the cell signaling pathways mediating plant defense responses against pathogens infection, in redox homeostasis as

well as on carbon fixation (Maxwell et al., 2002; Stael et al., 2015; Cheng et al., 2016). Thus, the manipulation of chloroplasts by effector proteins released by the pathogens on the infected tissues is an efficient mechanism to inhibit any defense response by the plant´ side (Torres-Zabala et al., 2015).

It is important to point out that any reduction in the photosynthetic capacity of plants can affect their response to counteract pathogens infection either by influencing their overall energy status, the reducing power of cells (*e.g.* ATP, NADPH and carbon skeletons) or the production of ROS in the chloroplasts (Foryer and Noctor, 2000; Gechev et al., 2006; Kangasjärvi et al., 2012). The lower *A* values on leaves sprayed with DCMU and infected by *P. oryzae* limited carbohydrates synthesis resulting in great starch concentration. On maize leaves sprayed with DCMU, there was an induction on starch degradation pathway mediated by light through nitrate reduction (Basra et al., 2002). The sucrose concentration, form by which sugar is transported in plant tissue, was reduced on wheat leaves infected by *P. oryzae* mainly if they were sprayed with DCMU due to lower availability of substrate while fructose and glucose concentrations increased. According to Rios et al. (2017), invertase and sucrose-phosphate synthase activities increased and decreased, respectively, in flag leaves of wheat plants infected by *P. oryzae* contributing, therefore, to reduce sucrose concentration which was used for hexoses production. In rice transgenic plants expressing the Pathogenesis-Related gene originated from maize, a higher sucrose concentration acted as an endogenous signal for the activation of host defense mechanisms in response to infection by *Bipolaris oryzae*, *Pyricularia oryzae* and *Fusarium verticillioides* (Gómez-Ariza et al., 2007). In addition, increased sucrose uptake and hexose accumulation may indicate additional consumption by plants as a result of pathogens infection (Gómez-Ariza et al., 2007).

Although the production of ROS is considered an important defense strategy mounted by plants in response to pathogens infection (Magbanua et al., 2007), their accumulation due to an imbalance between production and an efficient removal system may damage the host tissue furthermore (Lima et al., 2002). In the present study, there were lower SOD and POX activities and higher CAT activity for plants non-infected by *P. oryzae* and sprayed with DCMU. According to Debona et al. (2012), APX, CAT, POX and SOD activities increased on wheat leaves infected by *P. oryzae*. The DCMU is able to inhibit hydrogen peroxide production by oxygen photoreduction in the PSI besides increasing the production of singlet oxygen through the PSII (Flors et al., 2006; Mubarakshina et al., 2010). The PSII plays an important role in plant resistance in response to pathogens infection through the production of ROS that not only have a direct effect against them and to the components of the photosynthetic electron transfer chain on plant tissue, but also acts as important retrograde signaling molecules (Rodríguez-Herva et al., 2012; Torres-Zabala et al., 2015; Serrano et al., 2016). The oxidative damage may be linked to the antioxidative system that lower ROS concentration on plant tissue (Sharma et al., 2012; Rais et al., 2017). Therefore, the activities of some antioxidative enzymes do not need to increase when the ROS concentration is kept high (Sharma et al., 2012; Rais et al., 2017). Although it is believed that the ROS is deleterious to plants infected by pathogens, their concentration does not reach a certain level capable of efficiently preventing their colonization on plant tissues, but may act as signaling molecules for the activation of host defense mechanisms (Samalova et al., 2014; Marroquin-Guzman et al., 2017; Segal and Wilson, 2017).

Altogether, the results of the present study showed that the spray of DCMU increased wheat susceptibility to blast due to photosynthetic dysfunctions, impairment on sugar metabolism and a less efficient antioxidative system.

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Figures

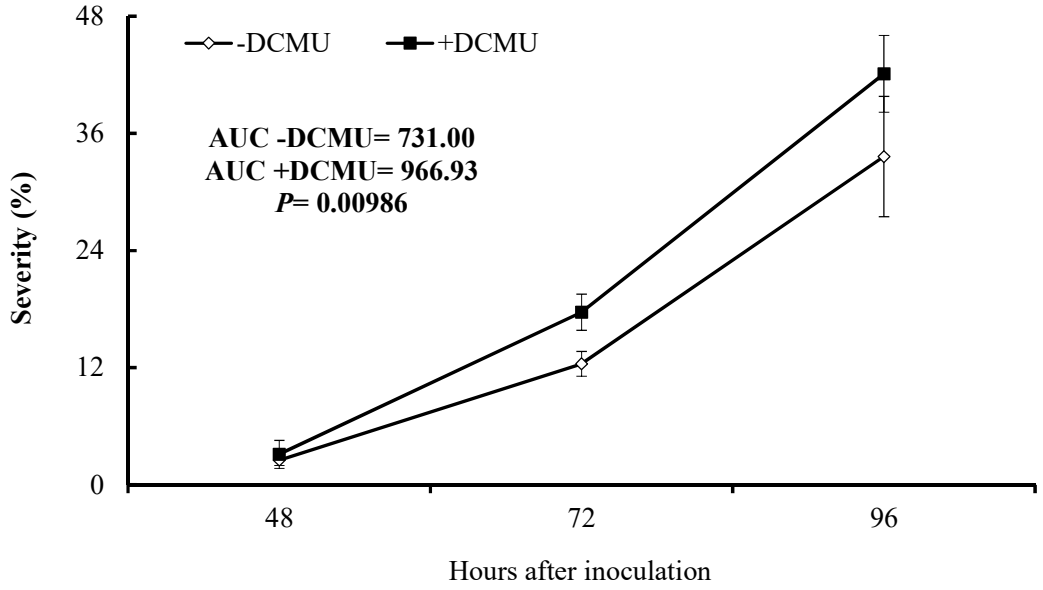

Figure 1. Severity of blast in the leaves of wheat plants non-sprayed (-DCMU) or sprayed (+DCMU) with the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) at different times after inoculation with *Pyricularia oryzae*. Data from severity for the -DCMU and +DCMU treatments were used to calculate the area under the curve (AUC) for severity. Error bars represent the standard deviations of means. $n = 6$.

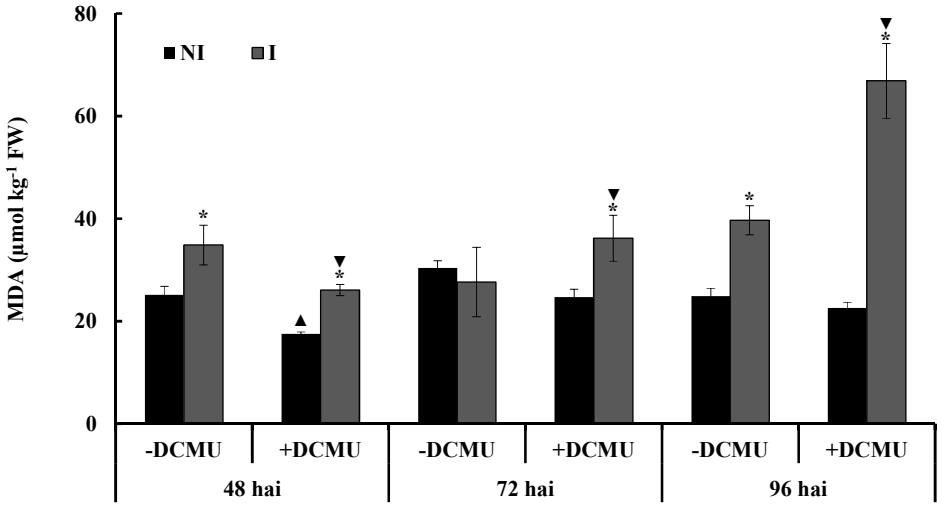

Figure 2. Concentration of malondialdehyde (MDA) in the leaves of wheat plants non-sprayed (-DCMU) or sprayed (+DCMU) with the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and non-inoculated (NI) at 48, 72 and 96 hours after inoculation (hai) with *Pyricularia oryzae* (I). Means for the NI and I treatments followed by an asterisk (*) at -DCMU and +DCMU treatments and evaluation time are significantly different according to *F* test ($P \leq 0.05$). Symbols filled triangle and filled inverted triangle indicate differences between -DCMU and +DCMU treatments, respectively, for NI and I treatments, at each evaluation time, according to *F* test ($P \leq 0.05$). Bars represent the standard error of the means. $n = 6$. FW = fresh weight.

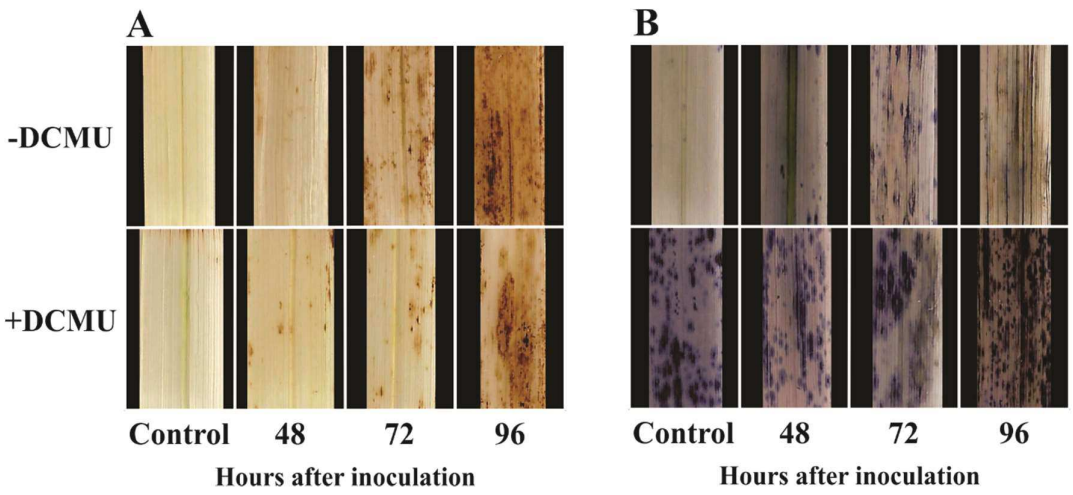

Figure 3. Histochemical detection of hydrogen peroxide (A) and superoxide anion radical (B) in the leaves of wheat plants non-sprayed (-DCMU) or sprayed (+DCMU) with the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and non-inoculated (control) or at 48, 72 and 96 hours after inoculation with *Pyricularia oryzae*.

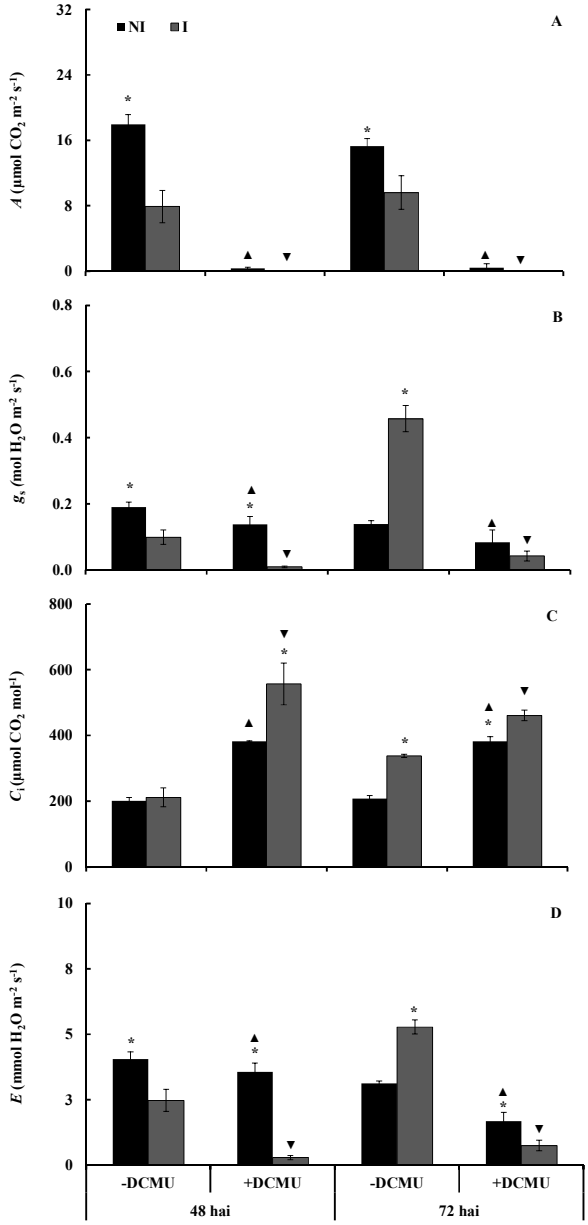

Figure 4. Net CO_2 assimilation rate (A) (A), stomatal conductance to water vapor (g_s) (B), internal CO_2 concentration (C_i) (C) and transpiration rate (E) (D) determined in the leaves of wheat plants non-sprayed (-DCMU) or sprayed (+DCMU) with the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and non-inoculated (NI) or at 48 and 72 hours after inoculation (hai) with *Pyricularia oryzae* (I). Means for the NI and I treatments followed by an asterisk (*) at -DCMU and +DCMU treatments and evaluation time are significantly different according to F test ($P \leq 0.05$). Symbols filled triangle and filled inverted triangle indicate differences between -DCMU and +DCMU treatments, respectively, for NI and I treatments, at each evaluation time, according to F test ($P \leq 0.05$). Bars represent the standard error of the means. $n = 6$. FW = fresh weight.

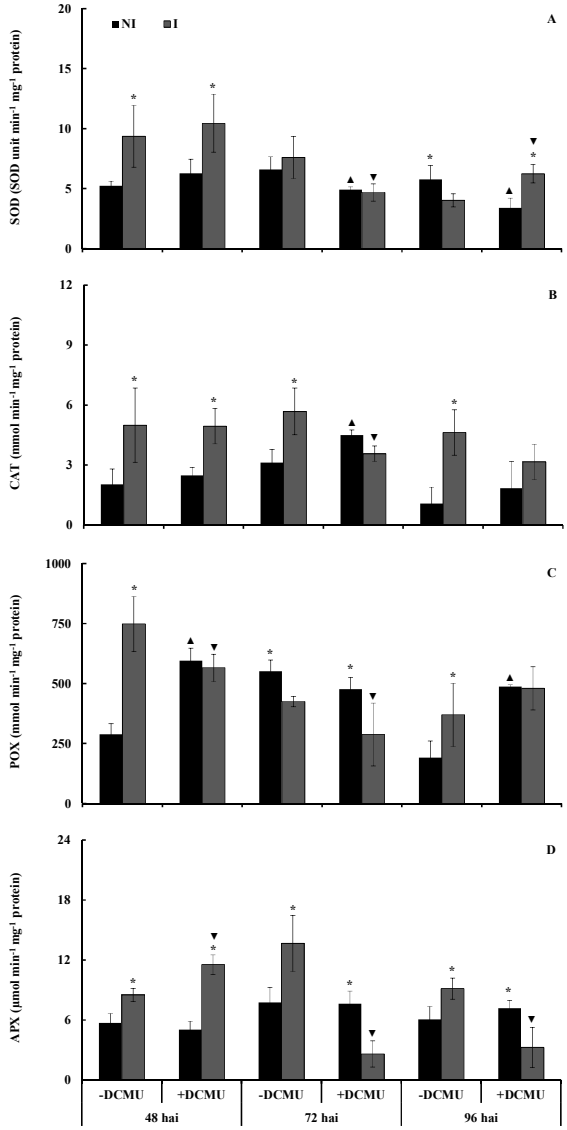

Figure 5. Activities of superoxide dismutases (SOD) (A), catalases (CAT) (B), peroxidases (POX) (C), ascorbate peroxidases (APX) (D) and glutathione reductases (GR) (E) determined in the leaves of wheat plants non-sprayed (-DCMU) or sprayed (+DCMU) with the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and non-inoculated (NI) or at 48, 72 and 96 hours after inoculation (hai) with *Pyricularia oryzae* (I). Means for the NI and I treatments followed by an asterisk (*) at -DCMU and +DCMU treatments and evaluation time are significantly different according to *F* test ($P \leq 0.05$). Symbols filled triangle and filled inverted triangle indicate differences between -DCMU and +DCMU treatments, respectively, for NI and I treatments, at each evaluation time, according to *F* test ($P \leq 0.05$). Bars represent the standard error of the means. n = 6.

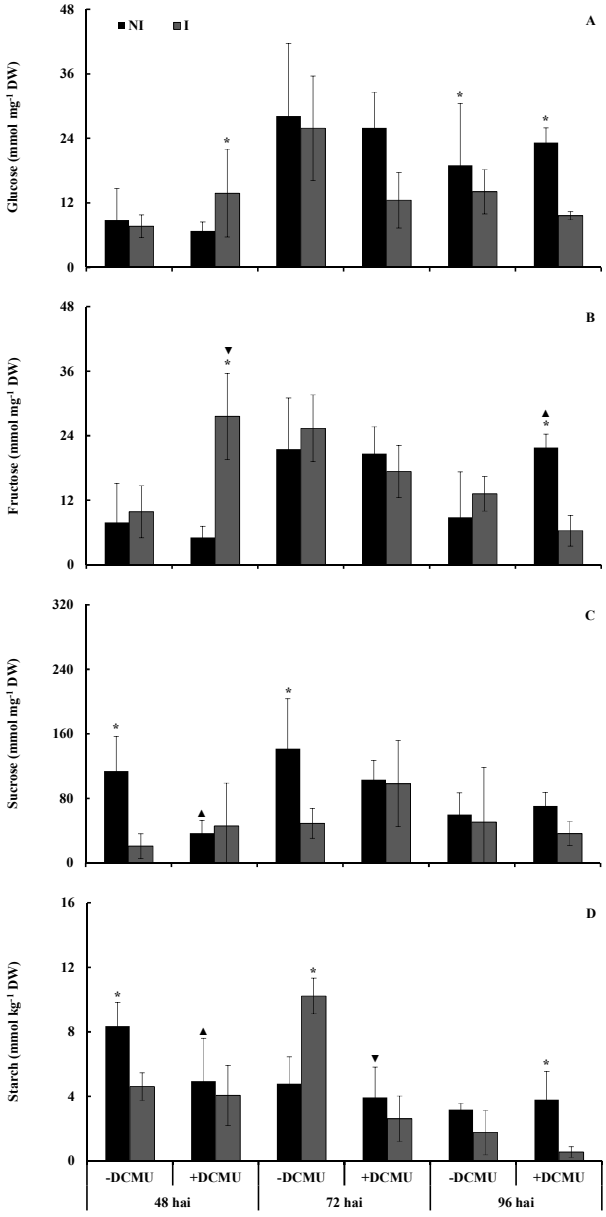

Figure 6. Concentrations of glucose (A), fructose (B), sucrose (C) and starch (D) determined in the leaves of wheat plants non-sprayed (-DCMU) or sprayed (+DCMU) with the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and non-inoculated (NI) or at 48, 72 and 96 hours after inoculation (hai) with *Pyricularia oryzae* (I). Means for the NI and I treatments followed by an asterisk (*) at -DCMU and +DCMU treatments and evaluation time are significantly different according to *F* test ($P \leq 0.05$). Symbols filled triangle and filled inverted triangle indicate differences between -DCMU and +DCMU treatments, respectively, for NI and I treatments, at each evaluation time, according to *F* test ($P \leq 0.05$). Bars represent the standard error of the means. $n = 6$. FW = fresh weight.

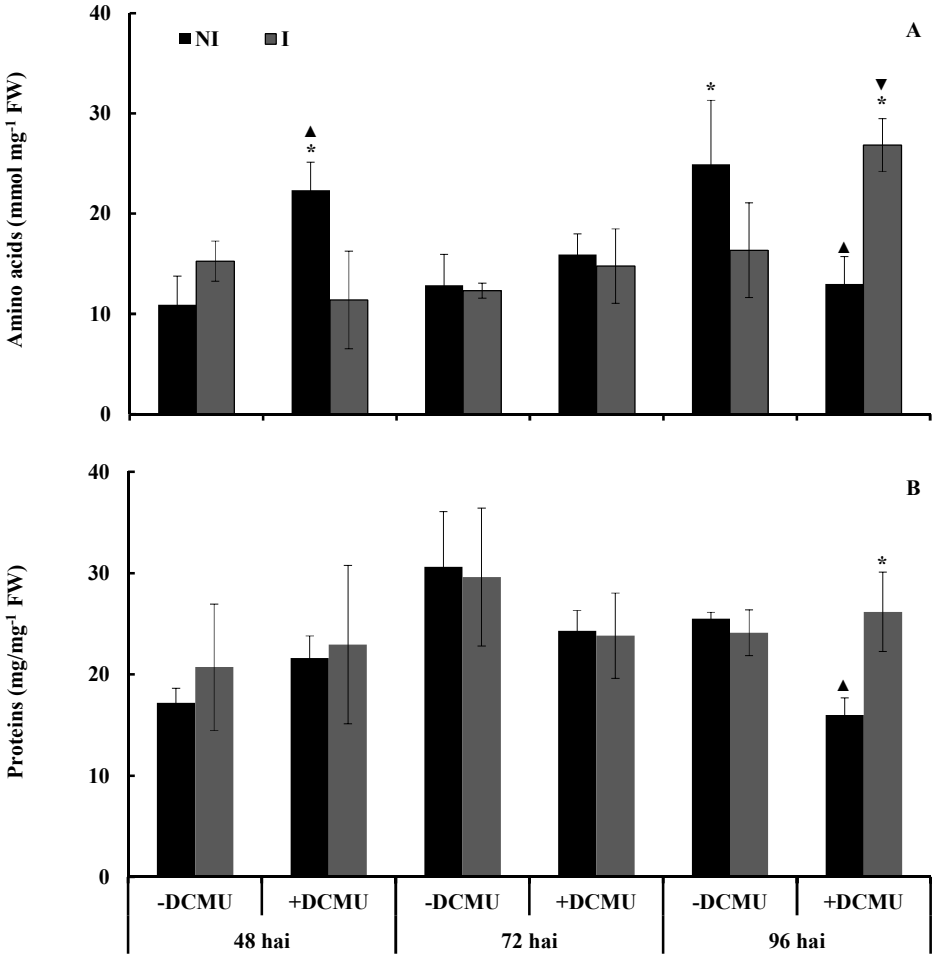

Figure 7. Concentrations of amino acids (A) and proteins (B) determined in the leaves of wheat plants non-sprayed (-DCMU) or sprayed (+DCMU) with the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and non-inoculated (NI) or at 48, 72 and 96 hours after inoculation (hai) with *Pyricularia oryzae* (I). Means for the NI and I treatments followed by an asterisk (*) at -DCMU and +DCMU treatments and evaluation time are significantly different according to *F* test ($P \leq 0.05$). Symbols filled triangle and filled inverted triangle indicate differences between -DCMU and +DCMU treatments, respectively, for NI and I treatments, at each evaluation time, according to *F* test ($P \leq 0.05$). Bars represent the standard error of the means. $n = 6$. FW = fresh weight.