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ORIGINAL ARTICLE

Occurrence of powdery mildew disease in wheat fertilized with increasing silicon doses: a chemometric analysis of antioxidant response

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Abstract *Blumeria graminis* (Bgt) is a pathogenic fungus that affects severally wheat plants provoking high losses in wheat production. Biochemical parameters like enzyme activity of catalase, superoxide dismutase, or peroxidases can be used to detect changes of metabolism in response to pathogen infection. We evaluated different biochemical and biometrics parameters to assess the effect of silicon, a widely recognized beneficial nutrient, in wheat infected naturally with Bgt. Integral study and interpretation of results obtained by multivariate analysis is the challenge of present work. Wheat plants growing in hydroponic solution were fertilized with increasing concentration of silicon. Responses of wheat plants to silicon treatments were assessed through the analysis of lipid peroxidation and antioxidant enzymes activity (catalase, ascorbate peroxidase and superoxide dismutase). Furthermore, biometric measures such as Pathogen Index, dry weight of shoot and roots, tiller height, spike length, spike mass, grain number and grain mass production were assessed. Pathogen Index decreased while mass and number of grain, dry weight of shoots were significantly increased, proportionally to silicon concentration increasing in culture media; biochemical parameters were also influenced by the concentration of silicon. Multivariate analysis indicated correspondence between increase of silicon treatments and decrease of antioxidant activities of APX, CAT and SOD. Relationships between the applied doses of silicon and decreasing Bgt expansion in foliar surfaces were also observed. Chemometrics proved to be an optimal tool for integrating data about metabolic status and demonstrate, in present case, that increasing concentration of silicon and the reduction of antioxidant enzyme activity are closely related to decreasing of powdery mildew disease.

Keywords Wheat · Silicon · *Blumeria graminis* · Chemometrics · PLS-DA

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Introduction

Powdery mildew is a widely spreading disease of wheat caused by fungus *Blumeria graminis* f. sp. *tritici* (Bgt) distributed in many wheat-producing countries leading to significant loss of yield (Xin et al. 2012). *Blumeria graminis*—as a biotrophic organism—needs an active plant metabolism for survival (Berger et al. 2007). The establishment of Bgt on wheat affects photosynthesis and carbon

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metabolism, increasing the invertase activity, inhibiting phloem loading in infected leaves (Sutton et al. 2007) and releasing hexoses that downregulate the expression of photosynthetic genes (Berger et al. 2007; Swarbrick et al. 2006) which suppress the transformations of sugar to starch, diminishing the transport and accumulation of assimilates in seeds (Gao et al. 2014).

The resistance to Bgt on wheat is expressed by prevention of penetration through localized cell wall strengthening and hypersensitive host cell death, both promoted by H_2O_2 signaling responses (Li et al. 2005). H_2O_2 production is associated with the formation of physical defensive barriers through mechanisms of localized papillae formation in resistant lines of wheat (Li et al. 2005; Shetty et al. 2008). On the other hand, hypersensitive response induces localized cell death to restrict pathogen growth removing nutrient supply from cell host, which is highly effective against biotrophic pathogens (Shetty et al. 2008).

It has been shown that silicon confers a general resistance status in plants under biotic and abiotic stress; however, the mechanism by which this resistance is conferred still generates many controversies. Plants supplied with silicon may develop mechanical and/or biochemical resistance by accumulation of silicates in leaves and roots and its presence in the cell wall fiber makes the cell wall tough and resistant to abiotic stress—as drought and salinity—but also to pathogens attacks, increasing its tolerance to diseases (Epstein 2009).

Wheat (*Triticum aestivum*) is considered as silicon accumulator (Guntzer et al. 2012). In the wheat–*B. graminis pv tritici* pathosystem, silicon increased the resistance to fungus infection when soluble silicon is present in the substrate (Chain et al. 2009; Fauteux et al. 2005). Histological and ultrastructural analyses on wheat–Bgt pathosystem have revealed that epidermal cells of plants treated with silicon reacted to Bgt attack with specific defense reactions including papilla formation, production of callose, fungitoxic phenolic compounds and silicon deposition at the site of infection (Bélanger et al. 2003). The compatible interaction studied by transcriptomic analysis of wheat–Bgt suggests that stress imposed by pathogen—which translates into nearly 900 regulated genes—has showed to be basically eradicated by silicon (Chain et al. 2009). In addition, biochemical response to fungus infection was reported in wheat fertilized by silicon, producing phytoalexins linked to metabolism of aconitate, which limit the diseases development (Rémus-Borel et al. 2005, 2009).

The presence of reactive oxygen species (ROS) is considered to act either by direct toxic effect on pathogens or as inducer signal to hypersensitive response (HR) during the early stage of pathogenesis (Liu et al. 2011). Under biotic stress conditions, inhibition of antioxidant enzymes activity accompanying ROS accumulation in the tissues induces programmed cell death (PCD) avoiding the spread of the biotic agent (Mittler 2002). In the response to biotic or abiotic agents, plants could accumulate ROS as superoxide anion, hydrogen peroxide, hydroxyl radicals, oxygen singlet and free radicals inducing oxidative stress. As a consequence, the generation of ROS is often followed by increased activity of detoxifying enzymes such as catalase (CAT), peroxidases (PX) and superoxide dismutase (SOD), (Mittler 2002; Van Breusegem et al. 2001). The detoxificant ability of antioxidant enzymes constitutes the plant response to survive to oxidative stress; therefore, quantification of enzymatic activities such as SOD, CAT and POX is useful for assessing susceptibility and resistance of plants to stress (Chaudière and Ferrari-Iliou 1999).

Multivariate calibration methods have been widely used to explain complex relationships among the data that are difficult to observe, being useful to find differences and establish groups in big data collections (Mongay Fernandez 2005). These methods have been successfully used to determine genotypical classification (Moldes et al. 2012; Pereyra-Irujoa et al. 2009), varieties of amaranth seeds (Aguilar et al. 2011), fruits (Ranalli et al. 2002), as well as propolis samples (Cantarelli et al. 2011), South American herbs (Cantarelli et al. 2010) and wines (Di Paola-Naranjo et al. 2011). In the literature, there is scarce information about the use of multivariate analysis applied to antioxidant enzyme activities in biological systems. However, chemometrics appears as appropriate statistical tool, considering the complex nature of biochemical responses to stress and their interrelationships (Sinha et al. 2009). In a previous work, chemometrics has been applied to biological systems to analyze antioxidant enzyme activities (catalase, ascorbate peroxidase and guaiacol peroxidases) in cotton plants, showing that inhibition of such enzymes was associated to increasing doses of silicon in non-stressed plants (Moldes et al. 2013). Furthermore, the antioxidant and fermentative variables were analyzed in pears by chemometrics, whose results showed that core browning of pears (*Pirus communis*) involves different metabolic pathways (Larrigaudière et al. 2004). Partial least squares regression (PLSR) using antioxidant enzymes activity as biochemical variables of stored broccoli (*Brassica oleracea*) revealed correlation of enzymatic activities with the release of volatile compounds, which is useful to obtain indicators for changes in processed and stored vegetables (Raseetha et al. 2013). In the cases mentioned above, chemometric models—based on antioxidant enzymatic activities—constituted an important tool to reveal an integral antioxidant response in biological systems. For the exposed, the aim of this work was to assess the effect of silicon on the responses of antioxidant enzymes in wheat plants under natural exposure to pathogenic fungus *B. graminis* sp. tritici (Bgt). Chemometric models as principal

components analysis (PCA) and partial least square discriminant analysis (PLS-DA) were obtained for the interpretation of biochemical behavior of antioxidant enzymes on wheat plants fertilized by silicon.

Materials and methods

Plant materials, treatments and experimental design

Wheat BR40 cultivar was used as a variety susceptible to Bgt. Wheat seeds were superficially sterilized with bleach solution (2 %), rinsed with sterile water and sown in mini spots with sand–vermiculite (2:1) mixture. After 7–10 days of germination, seedlings were transferred to 2-L plastic pots containing hydroponic solution. Hydroponic culture was chosen to minimize effects of substrate over availability of silicon. The seedlings were adapted to liquid nutrient media in pots containing fivefold diluted Johnson nutrient solution at pH 6 (Johnson et al. 1957). After 7 days, diluted nutrient medium was replaced by full nutrient solution with addition of soluble sodium metasilicate (Vetec, Brasil) to stablish the following treatments: 0, 10, 25, 50 and 100 mg Si L^{-1}. Plants were placed at 20 cm each other. Treatment solutions were adjusted at pH 6 and replaced weekly. Seedlings were grown in a greenhouse at 25 °C and 45 % humidity. Experiment was carried out in a complete randomized design with six replications for each silicon treatment. Greenhouse assay was synchronized with the period of crop establishment (March–July) to grow the plants under an optimal regime of light intensity and photoperiod. At 13 days after emergency, natural infection of fungus was developed in plants, from propagules already present in the greenhouse from previous assays inoculated with Bgt. The natural infection was preferred to imitate field conditions, involving a close coordination with the previous experiment. A reduced space between plants and a randomized design experiment, contributed to a homogeneous pathogen exposition. Identification of *B. graminis* was performed visually by typical morphology of colonies in foliar surface. Samples were collected at 60 days after emergency (approximately at the middle of culture cycle) from each of replicates. Samples consisted of leaves from top–middle–bottom of plant, pooled, frozen in liquid nitrogen and stored in ultrafreezer at −80 °C until analysis of antioxidant enzymes activity, lipid peroxidation and silicon content. Finally, entire plants were collected at 130 days post-emergency and the following parameters were determined: spike length (cm), tiller height (cm), spike mass (g), grain mass $plant^{-1}$ (kg), grain number $plant^{-1}$, individual grain mass (g) and dry weight of shoots and roots (g) by drying samples at 60 °C to constant weight.

Pathogen index

Sixty days after emergence, a visual evaluation of the second leaf was made. Two independent evaluators scored the degree of infection of second leaf determining the percentage of leaf area affected by powdery mildew following the James's scale (James 1971). Both evaluations were averaged for final score.

Silicon quantification

Silicon extraction from wheat leaves was made by the autoclave-induced digestion method and its content quantified through colorimetric determination (Elliott and Snyder 1991). Briefly, samples were dried at 60 °C and grounded in a stainless steel mill to pass through a 0.84-mm mesh screen. A subsample of 100 mg was placed in a polyethylene tube (100 mL) with 2 mL H_2O_2 50 % and 3 mL NaOH 50 % and then boiled in water bath for 1 h. After this treatment, 45 mL of distilled water was added and the obtained suspension autoclaved for 1 h. Aliquots of 1 mL were added with 19 mL distilled water (final volume: 20 mL) and color development was accomplished by adding 1 mL HCl 50 % and 2 mL ammonium molybdate 10 %. After color development (about 10 min), 2 mL of oxalic acid 7.5 % was added and absorbance measured at 410 nm. Three measurements were made per sample for silicon content and it was expressed as % Si (g Si 100 g^{-1} dry weight of leaves).

Lipid peroxidation

Malondialdehyde (MDA) content in leaves was determined by weighting 250 mg of fresh leaves, which were then frozen, crushed and homogenized in a mortar with 1.3 mL of 0.1 % trichloroacetic acid (TCA) solution and 0.2 mg of insoluble polyvinylpyrrolidone (PVPP). The homogenate was centrifuged at 10,000 rpm for 5 min and 250 μL of the supernatant was added to 1 mL of 0.5 % 2-thiobarbituric acid (TBA), 20 % TCA solution, and incubated in a water bath at 95 °C for 20 min. Measures were made in three replicates by sample and the concentration of MDA was calculated from the absorbance at 532 nm using the absorbance coefficient 155 mM^{-1} cm^{-1}, followed by a correction for unspecific turbidity determined at 600 nm (Moldes et al. 2008).

Enzymes extraction and enzymatic activity measurements

Crude enzyme extracts were obtained from 1 g of wheat leaves crushed and homogenized in a mortar with 3 mL of extraction buffer containing 100 mmol L^{-1} potassium

phosphate buffer (pH 7.5), 1 mmol L^{-1} ethylene diamine tetraacetic acid (EDTA), 3 mmol L^{-1} DL-dithiothreitol and 5 % (w/v) insoluble PVPP. The homogenate was centrifuged at 10,000 rpm for 30 min and the supernatant was stored in separate aliquots at −80 °C for all enzyme activity determinations (Gomes-Junior et al. 2006). Measures of enzymes activity was made by triplicate for each sample.

Catalase (CAT-EC 1.11.1.6) activity was assessed at 25 °C in Eppendorf tube containing 1 mL of reaction buffer [100 mmol L^{-1} potassium phosphate buffer (pH 7.5) and 7.5×10^{-3} % H_2O_2 solution]. The reaction was initiated by the addition of 20 µL crude enzyme extract and determined by monitoring H_2O_2 degradation in spectrophotometer at 240 nm over 1 min. CAT activity was calculated using an extinction coefficient of 39.4 mM^{-1} cm^{-1} for H_2O_2 and expressed in nmol H_2O_2 min^{-1} mg $protein^{-1}$ (Moldes et al. 2008).

Ascorbate peroxidase activity (APX, EC 1.11.1.11) was determined by monitoring the rate of ascorbate oxidation at 290 nm at 30 °C. The reaction was initiated by the addition of 40 µL crude enzyme to 1 mL of reaction medium containing 50 mmol L^{-1} potassium phosphate buffer (pH 7.0), 0.5 mmol L^{-1} ascorbate, 0.1 mmol L^{-1} EDTA and 0.1 mmol L^{-1} H_2O_2. APX activity, expressed as nmol ascorbate min^{-1} mg^{-1} protein, was calculated using the extinction coefficient of 2.8 $mmol^{-1}$ L cm^{-1} for ascorbate (Moldes et al. 2008).

SOD activity was performed according to Paoletti et al. (1986) using a spectrophotometric method based on the inhibition of oxidation of NADH by superoxide radicals. The reaction mixture contains 100 mmol L^{-1} triethanolamine–diethanolamine (pH 7.4), 7.5 mmol L^{-1} NADH, EDTA/$MnCl_2$ (100 mmol L^{-1}/50 mmol L^{-1}, pH 7.4) and 10 mmol L^{-1} 2-mercaptoethanol. The reaction started by the addition of 1 mL of buffer reaction to 20 µL crude enzyme extract and changes in absorbance were monitored at 340 nm for 10 min. One unit of SOD was defined as the amount of SOD capable of inhibiting by 50 % the rate of NADH oxidation observed in a control standard.

SOD staining gel electrophoresis

Activity staining for SOD was carried out in non-denaturing polyacrylamide electrophoresis (SDS was excluded) as described by Gomes-Junior et al. (2006) with minor modifications. Then, a volume of crude extract containing 60 µg of protein was used for each treatment. Electrophoresis was carried out at constant current of 15 mA for approximately 3 h. The protein concentration was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as standard.

Statistical analysis

Univariate analysis was carried out by ANOVA, Tukey's multiple range and linear regression test using the SAS statistical program (SAS Institute Inc., NC, USA). Multivariate analysis was performed by principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) using the Unscrumbler X software package, version 10.3 (CAMO AS, Norway).

Results

Effects of silicon treatments over biometric parameters

Biometric parameters summarized in Table 1 show the effect of silicon treatment on productive parameters of wheat. Significant increases in dry weight of leaves, tiller height, spike and grain mass at 100 mg Si L^{-1} silicon treatment were observed. However, spike length, tiller height, and grain number were not different as silicon concentration increased.

On the other hand, the increase of silicon content in plant showed inverse proportionality to Pathogen Index indicating an effective action of silicon against Bgt infection in the foliar surface (Fig. 1). Therefore, silicon decreased the detrimental effect of Bgt infection over growth and productivity of wheat. Pathogen Index established a close relationship between the antioxidant response, the silicon content and the severity of disease under actual assay conditions.

Response of lipid peroxidation and antioxidant enzymes to silicon treatments

The increasing of silicon in treatments presented an inverse relationship to the lipid peroxidation level (MDA), showing significant decrease ($P < 0.05$) at 50 and 100 mg Si L^{-1} treatments (Table 2). Furthermore, APX and CAT activities had not significant differences between treatments (Table 2). However, linear regression analysis confirmed results of Tukey test for lipid peroxidation (MDA) and, in addition, CAT significantly decreased ($P < 0.05$) in response to increase of silicon content (Table 3). Total SOD activity, determined by spectrophotometrics, showed a high SOD activity at 10 mg Si L^{-1} treatment (Table 2). Similar behavior could be observed in several SOD isoenzymes in non-denaturant gel staining (Fig. 2) which showed a differential intensity of bands, suggesting the highest SOD activity at 0, 10 and 25 mg Si L^{-1} treatment for Isozymes II, III and IV.

Table 1 Determination of biometrics parameters

Silicon dose (mg Si L^{-1})	DW leaves (g)	DW root (g)	Spike length (cm)	Tiller height (cm)
0	3.91 ± 0.40b	0.59 ± 0.18a	8.60 ± 0.64a	56.32 ± 3.25c
10	4.08 ± 1.02ab	0.61 ± 0.18a	8.81 ± 0.35a	66.33 ± 3.27ab
25	4.14 ± 0.75ab	0.70 ± 0.10a	8.96 ± 0.51a	63.41 ± 2.74b
50	4.80 ± 0.66ab	0.83 ± 0.10a	9.13 ± 0.54a	68.08 ± 2.79a
100	5.15 ± 0.59a	0.82 ± 0.22a	8.76 ± 0.45a	65.95 ± 4.43ab

Silicon dose (mg Si L^{-1})	Spike mass (g)	Grain mass per spike (g)	Grain number per spike	Individual grain mass (mg)
0	3.45 ± 0.97c	1.91 ± 0.61c	136 ± 31.8a	13.89 ± 2.33b
10	3.81 ± 0.67bc	2.20 ± 0.44bc	143 ± 23.0a	15.31 ± 0.72b
25	4.65 ± 0.51abc	2.62 ± 0.55bc	177 ± 13.4a	14.75 ± 2.07b
50	5.42 ± 0.33ab	3.18 ± 0.36ab	171 ± 2.0a	18.55 ± 2.03ab
100	6.00 ± 0.16a	3.83 ± 0.07a	178 ± 24.0a	22.40 ± 4.26a

Means of $n = 6 \pm$ standard deviation

Letters indicate significant differences by Tukey means test ($P < 0.05$)

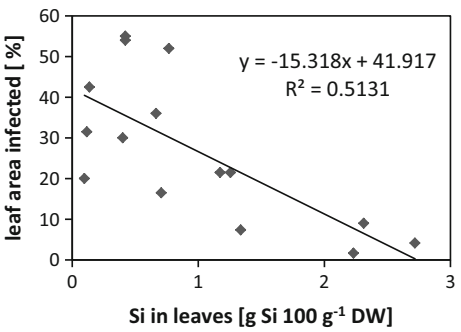

Fig. 1 Correlation between infected foliar area and Si content in leaves

Chemometric analysis by PCA and PLS-DA

Multivariate analysis was introduced as a further study of classical statistics. Principal component analysis (PCA) was done using the following variables: MDA, APX, CAT, SOD, and silicon content (Si) (Fig. 3a) using a model with three PCs—48.6 % for PC1, 29.7 % for PC2 and for PC3 18.3 % of explained variance (total 96.6 %). Loading plot indicates APX and SOD as influencing variables for grouping. Scores plot showed that treatment with 100 mg Si L^{-1} generated a well-differentiated group from the rest of the treatment (Fig. 3b). Three-dimensional PLS-DA was performed with the same variables other than PCA with explained variances of 48.2 % for PLS1, 28.6 % for PLS2 and for PLS3 22.0 % of explained variance (total 98.8 %). Score plot of PLS-DA (Fig. 4) shows grouping of 100 mg Si L^{-1} treatment similar to behavior showed by PCA. Both score plots of PCA and PLS-DA showed changes from 0 to 100 mg Si L^{-1}. Loading plot shows that CAT, SOD, and APX are the three most influent variables (Fig. 5). Interpretation of chemometric analysis indicates that enzymatic activity of CAT, APX and SOD decreased, showing alteration in basal level of antioxidant enzymes when the silicon concentration increased in the nutrient media.

Discussion

Results demonstrate that silicon had significant effects in plant performance improving several production parameters of wheat exposed to Bgt. Significant changes were evident in biometrics and biochemical variables due increase of silicon in wheat under influence of Bgt. In addition, multivariate analysis allowed the assessing of the biochemical variables' behavior in wheat plants grown under increasing doses of silicon in presence of Bgt infection.

Glucose is the preferred carbon source absorbed by the powdery mildew haustoria (Sutton et al. 2007). Because *B. graminis* demands hexoses, it induced the redirection of carbon metabolites (by the down-regulation photosynthesis) increasing the invertase activity and very often inducing a transition of source tissues into sink tissues (Berger et al. 2007). In addition, powdery mildew appears to influence the expression of starch synthesis-related enzymes, repressing transformation of sugars to starch and inhibiting the starch synthesis, which leads to yield losses (Gao et al. 2014). Biometric measures showed that there were not alterations in those constitutive parameters of plant development as spike length or grain number per spike. Nevertheless, filling of seeds had significant changes on wheat under silicon treatment increasing significantly

Table 2 Determination of biochemical parameters and silicon content of leaves

Silicon dose (mg Si L^{-1})	Lipid peroxidation (nmol MDA g FW^{-1})	APX activity (nmol ascorbate min^{-1} mg $prot^{-1}$)	CAT activity (nmol H_2O_2 min^{-1} mg $prot^{-1}$)	SOD activity (unit mg $prot^{-1}$)	Silicon content (g Si 100 g^{-1} DW)
0	9.47 ± 0.65a	33.91 ± 9.03a	31.83 ± 4.02a	36.95 ± 7.22ab	0.12 ± 0.02d
10	10.17 ± 2.10a	30.88 ± 6.16a	31.16 ± 3.41a	41.36 ± 3.57a	0.41 ± 0.01dc
25	9.86 ± 0.89a	27.15 ± 4.22a	29.68 ± 7.64a	35.24 ± 6.31ab	0.71 ± 0.05c
50	6.67 ± 0.99b	25.62 ± 5.29a	25.44 ± 6.00a	34.98 ± 5.29ab	1.26 ± 0.08b
100	5.86 ± 0.58b	22.94 ± 3.88a	21.35 ± 4.20a	33.15 ± 5.59b	2.42 ± 0.26a

Means of $n = 6 \pm$ standard deviation

Letters indicate significant differences by Tukey means test ($P < 0.05$)

Table 3 Results of linear regression analysis ($y = A + Bx$; R^2 regression coefficient; P calculated level of significance)

Parameters	A	B	R^2	P^a
MDA	10.36	−0.04734	0.5947	**0.0008**
APX activity	31.09	−0.09929	0.2369	0.0658
CAT activity	31.71	−0.10592	0.5823	**0.0009**
SOD activity	37.38	−0.04243	0.0747	0.3243
Silicon content	0.15	0.02266	0.9832	**<0.0001**
DW shoot	3.63	0.01698	0.4690	**0.0048**
DW root	0.62	0.00244	0.2755	**0.0446**
Spike length	8.82	0.00116	0.0072	0.7633
Tiller height	62.41	0.06691	0.2310	0.0698
Spike mass	3.51	0.02654	0.5556	**0.0014**
Grain mass $plant^{-1}$	1.89	0.02028	0.6437	**0.0003**
Grain number	139.31	0.35016	0.1222	0.2014
Grain mass	13.37	0.10743	0.5286	**0.0021**

MDA lipid peroxidation, *CAT* catalase, *APX* ascorbate peroxidase, *SOD* superoxide dismutase

[a] P values <0.05 (in bold) indicate B value statistically different from 0

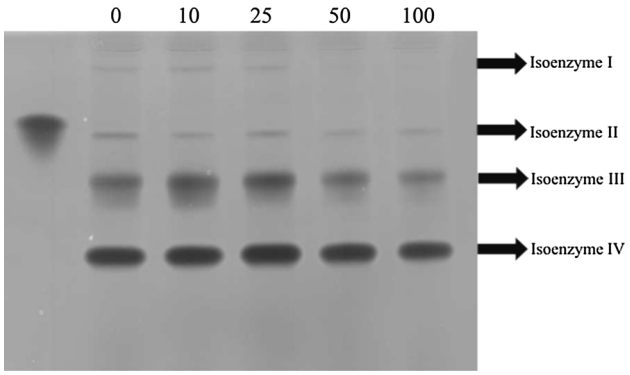

Fig. 2 SOD isoenzyme electrophoresis of wheat leaf extracts for treatments 0, 10, 25, 50 and 100 mg Si L^{-1}

the weight of spikes and grains. Inverse correlation between silicon content and percentage of infected leaf area (Fig. 1) indicates that reduction of Bgt in leaves could affect carbon metabolism from photosynthesis to the transport of assimilates, reducing the consume of hexoses by Bgt and channeling photoassimilates to storage sinks as seeds in favor of plant development: silicon played a role as resistance generator in susceptible BR18 wheat. It is widely known that silicon contributes to formation of a physical barrier by deposition of phytoliths in epidermal tissues especially in several monocots denominated as silicon accumulators (Epstein 2009). Silicon is successful against both abiotic (Ma et al. 2016; Rizwan et al. 2012; Tale Ahmad and Haddad 2011; Liang et al. 2008) and biotic (Van Bockhaven et al. 2015; Debona et al. 2014;

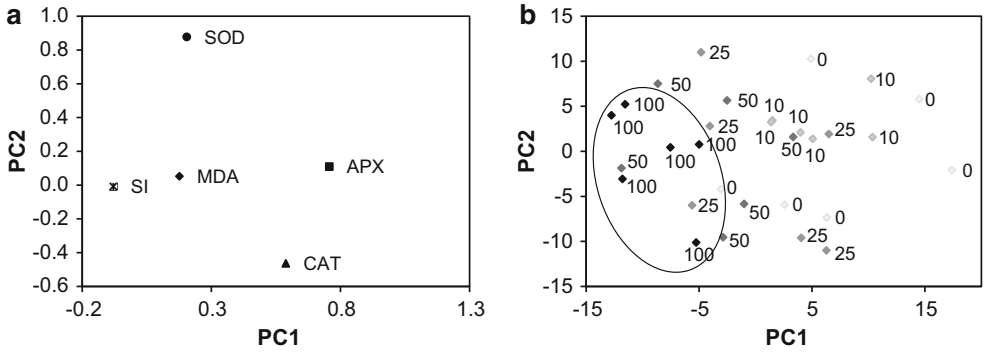

Fig. 3 Loading plot (**a**) shows the influence of variables in the PCA model. Score plot (**b**) shows the discrimination of Si treatments

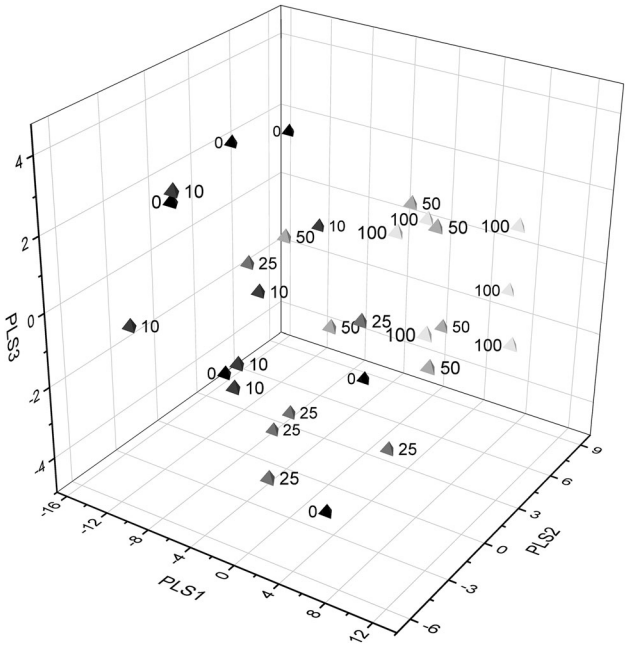

Fig. 4 Scores plot shows the discrimination of Si treatments by PLS-DA

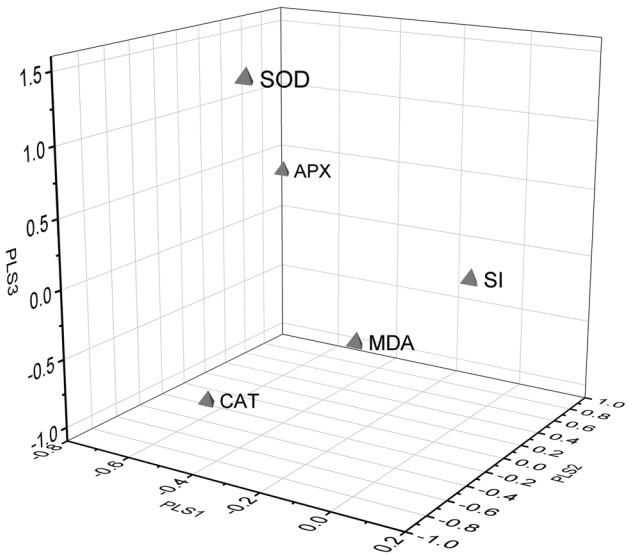

Fig. 5 Loading plot shows the influence of variables on the PLS-DA model

Xavier Filha et al. 2011; Silva et al. 2010) stresses. Nevertheless, mechanisms of resistance conferred by silicon are discussed even in silicon accumulators as to purely physical mechanism, induced mechanism of biochemical responses, or combination of both (Epstein 2009; Van Bockhaven et al. 2013). Lipid peroxidation (measured as MDA produced in thiobarbituric acid–lipoperoxides reaction) is usually an indicator of ROS proliferation measuring the level of damage in membranes by peroxidation of insaturations in lipophilic tale of phospholipids (Farmer and Mueller 2013). This indirect indicator is not a measure of ROS proliferation in localized tissues or specific sites in cell, although it is an indicator of how it was affected by the plant stressor. In compatible interaction, as wheat–Bgt in present study, increasing lipid peroxidations showed ROS proliferation but it had not a role in plant defense. The persistence of Bgt in foliar surface induced an increase of basal content of ROS producing oxidative stress and, as consequence, an increase of basal antioxidant activity of CAT, SOD and APX. This suggests that ROS production was not effective to activate mechanism reinforcing in the cell wall through papillae formation or to establish an efficient hypersensitive response in low silicon doses 0, 10 and 25 mg Si L^{-1} possibly due to a dispersion of ROS signal. Nevertheless, we observed that silicon confers resistance characteristics to susceptible BR18 wheat at 50 and 100 mg Si L^{-1} treatments. In this context, we determined low values of lipid peroxidation at high doses of silicon indicating diminished oxidative stress. The decreasing of antioxidant enzymes activity in wheat leaves supplied by silicon indicates that ROS generation was limited by silicon (Debona et al. 2014). The comparison of wheat plants infected by Bgt with and without silicon treatment (Si+Bgt+ versus Si−Bgt+) showed that 699 genes were regulated, from which 685 of them were

"down-regulated" (including 25 stress response genes) (Chain et al. 2009). Univariate analysis showed that only SOD activity is modified as silicon increases. Nevertheless, consideration of all parameters in one integrator multivariate analysis (PCA or PLS-DA) showed that activity of SOD, CAT and APX decreases as silicon increases in treatments. Decreasing of antioxidant activity is consistent with a "down-regulation" of antioxidant defense genes at transcriptional level (Chain et al. 2009). SOD profile of isoenzymes indicate that Isozyme III and IV are Cu/Zn-SOD present in all cellular compartment, Isozyme II is a Mn SOD present in mitochondria, and Isozyme I is a Fe SOD localized in chloroplasts (Huseynova et al. 2014). Activity of SODs isoforms in all compartments was similar showing evident decreasing of SOD activity at 50 and 100 mg Si L^{-1} treatments. Reduction of SOD activity is established in all cellular compartments (cytosol, chloroplast and mitochondria) indicating that ROS modulation could be affected inducing an ROS signal dispersion when the activity of antioxidant enzymes is high at low dose of silicon in presence of Bgt. Decreasing the activity of antioxidant enzymes could be beneficial for a potential defense response because papillae formation and the hypersensitive response require modulation of ROS signaling: even minimal changes in basal activity of antioxidant enzymes could affect such modulation. In addition, under low antioxidant activity, a more effective regulation of ROS content in cell compartment is inducible. In a previous work, we ask the question whether silicon intervenes in the antioxidant metabolism to adjust the plant fitness for a potential defense response against pathogen, founding that presence of silicon changed the basal activities of antioxidant enzymes of cotton leaves (dicotyledonous non-silicon accumulator) as CAT (down-regulated) and APX (up regulated) (Moldes et al. 2013). In addition, we suggested that decreasing of ROS detoxification induced by silicon, established conditions on leaves for more efficient mechanisms regulated by ROS signaling, (as papillae formation and/or hypersensitive response) in the case of pathogen attack. However, more studies are necessary for answering the question of why antioxidant enzymes activity should be decreased and its relationship with resistance mechanism.

According to the results, it can be concluded that there exists a relationship between the applied doses of silicon and decreasing Bgt infection in foliar surfaces and such correspondence is closely related to the reduction of basal antioxidant enzyme activity and ROS. Because ROS intervenes in precisely modulated signaling process, the decrease of antioxidant enzymes influenced by silicon could generate an ROS status for more efficient responses of resistance to Bgt. Those changes in antioxidant parameters could be observed using chemometric tools such as PCA and PLS-DA, demonstrating that even though results statistically "not significant" are obtained by univariate statistical analysis, those can contain useful information to determine the behavior of wheat under studied conditions. These results could contribute to understand how metabolism of wheat plants responds to silicon under biotic stress affecting the antioxidant response and modulation of ROS by introducing chemometric tools for analysis of biochemistry parameters. On the other hand, it was demonstrated that silicon fertilization improved the wheat culture productivity when a deleterious biotic factor such as Bgt is influencing the culture, suggesting that applications of silicon at field scale could decrease economic losses provoked by powdery mildew disease.

Author contribution statement OFSF and SMT designed the study. OFSF performed the experiment. JMC introduced new analytic computational tools for data analyses. CAM, OFSF and JMC analyzed the data. CAM, LJM and JMC wrote the paper.

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