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SHORT COMMUNICATION / *COMUNICAÇÃO*

Silicon and manganese on the activity of enzymes involved in rice resistance against brown spot

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ABSTRACT

This study investigated the role of silicon (Si) and manganese (Mn) rates on the activity of peroxidases (POX), polyphenoloxidases (PPO) and phenylalanine ammonia-lyases (PAL) on rice plants non-inoculated and inoculated with *Bipolaris orzyae*. Rice plants (cultivar Metica 1) were grown in nutrient solution with 0 (-Si) or 2 mmol Si L^{-1} (+Si) and with 0.5, 2.5 and 10 µmol Mn L^{-1}. Plants were inoculated with *B. oryzae* and leaf samples were collected at 6, 12, 18, 24, 36 and 48 h after inoculation (hai). Leaf samples were also collected from non-inoculated plants at these same time-points. POX activity increased for -Si plants inoculated with *B. oryzae* at both rates of 0.5 and 2.5 µmol Mn L^{-1} with one peak at 48 hai at 10 µmol Mn L^{-1}. For plants supplied with Si, POX activity also increased, but seemed to be low at the rates of 2.5 and 10 µmol Mn L^{-1}. POX activity did not show to be greatly influenced by the Mn rates in the presence of Si. However, in the absence of Si, POX activity increased. PPO activity on -Si plants increased at the rate of 0.5 µmol Mn L^{-1} and was not affected by the other two Mn rates, except a peak at 18 hai at the rate of 10 µmol Mn L^{-1}. In the presence of Si, PPO activity increased on plants supplied with 2.5 µmol Mn L^{-1} in contrast to the other two Mn rates for which the PPO activity decreased. By comparing the -Si and +Si treatments at the rate of 2.5 µmol Mn L^{-1}, PPO activity increased from 18 to 36 hai in the presence of Si than on its absence. PAL activity on -Si plants decreased regardless of the Mn rates. On plants supplied with Si and inoculated with *B. oryzae*, PAL showed low activity at the highest Mn rate and also at the rate of 0.5 µmol Mn L^{-1}, besides its activity been very low compared to the non-inoculated plants. PAL did not increase in activity in the presence of Si, but on its absence, high levels of activity were achieved. Results from this study showed that the activities of POX, PPO and PAL were not boosted by Si at any Mn rate.
Key words: *Bipolaris oryzae*, *Oryza sativa*, foliar disease, mineral nutrition.

RESUMO

Silício e manganês na atividade de enzimas envolvidas na resistência do arroz à mancha parda

O estudo investigou o efeito do silício (Si) e de doses de manganês (Mn) na atividade das enzimas peroxidases (POX), polifenoloxidases (PFO) e fenilalanina amônia-liases (FAL) em plantas não inoculadas e inoculadas com *Bipolaris oryzae*. Plantas de arroz (cultivar Metica 1) foram cultivadas em solução nutritiva com 0 (-Si) e 2 mmol Si L^{-1} (+Si) e com as doses de 0,5; 2,5 e 10 µmol Mn L^{-1}. As plantas foram inoculadas com *B. oryzae* e amostras de folhas foram coletadas às 6, 12, 18, 24, 36 e 48 h após inoculação (hai). Amostras de folhas de plantas não-inoculadas, nestes mesmos tempos, também foram coletadas. Para as doses de 0,5 e 2,5 µmol Mn L^{-1}, a atividade da POX aumentou para as plantas -Si com um pico às 48 hai na dose de 10 mmol Mn L^{-1}. Para as plantas +Si, a atividade da POX também aumentou, mas foi baixa para as doses de 2,5 e 10 mmol Mn L^{-1}. A atividade da POX não foi afetada pelas doses de Mn na presença de Si. No entanto, na ausência de Si a atividade da POX aumentou. Para a dose de 0,5 mmol Mn L^{-1}, a atividade da PFO nas plantas -Si aumentou, mas não foi afetada pelas outras duas doses de Mn, exceto um pico às 18 hai na dose de 10 mmol Mn L^{-1}. Na presença de Si, a atividade PFO aumentou nas plantas que receberam 2,5 mmol Mn L^{-1} em contraste com as outras duas doses de Mn para as quais a atividade da PFO decresceu. Ao comparar os tratamentos -Si e +Si na dose de 2,5 mmol Mn L^{-1}, atividade da PFO aumentou das 18 às 36 hai para as plantas supridas com Si. A atividade da FAL nas plantas -Si decresceu independentemente das doses de Mn. Nas plantas +Si e inoculadas com *B. oryzae*, houve menor atividade da FAL tanto na maior quanto na menor dose de Mn e também nas plantas não-inoculadas. A atividade da FAL não aumentou na presença de Si, mas na ausência desse elemento, houve aumento em atividade. Os resultados deste estudo mostram que as atividades da POX, PFO e FAL não aumentaram devido ao suprimento de Si independente da dose de Mn.
Palavras-chave: *Bipolaris oryzae*, *Oryza sativa*, mancha foliar, nutrição mineral.

Brown spot, caused by *Bipolaris oryzae* (Breda de Haan) Shoemaker, negatively affects rice yield and grain quality (Ou, 1985). Brown spot has been controlled primarily with the spray of fungicides (Lee, 1992). Adding silicon (Si) to the soil has provided a viable method to reduce brown spot severity in rice (Datnoff et al., 2007).

The mode of Si action in reducing the intensities of diseases on several crops has been investigated by many researchers (Datnoff et al., 2007). Particularly in rice, the high density of silicified buliform in the leaf epidermis due to Si application reduced the symptoms of leaf blast (Kim et al., 2002). Rice plants supplied with Si showed a differential

accumulation of *β*-1,3-glucanases, peroxidases, and PR-1 transcripts besides a higher concentration of phenolics compounds and lignin (Rodrigues et al., 2005). Brunings et al. (2009) reported that the involvement of chitinases genes in rice defense response against *Pyricularia grisea* and also those phenylalanine ammonia-lyases and peroxidases genes were up-regulated after fungal infection in the absence of Si. Dallagnol et al. (2009) showed that in the presence of Si, brown spot intensity was less reduced in the rice mutant (*lsi1*, low silicon 1) plants than on its wild-type counterpart (cv. Oochikara) showing the importance of the active Si uptake by rice to achieve a desirable level of resistance against this disease.

Many biochemical reactions in plants are affected by manganese (Mn) (Thompson & Huber, 2007). Peroxidases, related to phenolic compounds and flavonoids production, are dependent on Mn (Hamond-Kosak & Jones, 2000). Some diseases such as root rot, take-all, powdery mildew, leaf and stem rust on cereals; damping-off and wilt on cotton; late blight and scab on potato; leaf spots on soybean, and many others diseases in different crops had their intensities decreased by Mn (Thompson & Huber, 2007). Brown spot severity is negatively correlated with the content of Mn on rice leaf tissues (Kaur et al., 1979). However, the deficiency or toxicity of this micronutrient can increase or decrease plant resistance against pathogens infection (El-Jaoual & Cox, 1998). Rice plants supplied with Si showed reduction on brown spot severity regardless of the presence of either low or high Mn rates in the nutrient solution (Zanão Júnior et al., 2009). It is also known that plants tolerance to Mn toxicity can be increased in the presence of Si (Horiguchi, 1988).

This study was undertaken to investigate the effects of Si and Mn on the activity of enzymes commonly involved in host defense against pathogens infection to support the findings of Zanao Júnior et al. (2009) that previously demonstrated that on the presence of Si, regardless of low and high Mn rates, brown spot severity was greatly reduced.

The nutrient solution used in this study was prepared according to Zanão Júnior et al. (2009). The Mn rates used were 0.5, 2.5 and 10 µmol L^{-1}. Silicon was supplied as monosilicic acid, which was prepared by passing potassium silicate through a cation-exchange resin (Amberlite IR-120B, H^+ form; Sigma-Aldrich, São Paulo) (Ma et al., 2001). The Si rates used were 0 and 2 mM. Rice seeds (cultivar Metica-1) were germinated on distilled water-soaked germitest paper (Fisher Scientific Inc., Pittsburgh, PA, USA) in a germination chamber at 25ºC for five days. Germinated seedlings were transferred to plastic containers (25-cm in diameter) with one-half-strength of the nutrient solution without the addition of Si or Mn for three days. After this period, plants were transferred to new plastic containers with nutrient solution prepared using the Si and Mn rates mentioned above. The nutrient solution, without aeration, was changed every four days. The pH was checked daily and kept around 5.5 by using NaOH or HCl (1 M) when needed.

Plants were inoculated with *B. oryzae* after 39 days in hydroponic culture with the treatments. A pathogenic isolate of *B. oryzae* (CNPAF-HO 82) obtained from symptomatic rice plants and provided by Dr. Anne Sitarama Prabhu (Embrapa - National Center for Rice and Beans Research) was used to inoculate the plants. This isolate was preserved on filter paper at -80ºC. Pieces of filter paper containing fungal mycelia were transferred to Petri dishes with potato dextrose agar (PDA). After three days, PDA plugs containing fungal mycelia were transferred to Petri dishes with PDA medium. Petri dishes were kept in a growth chamber at 25ºC with a 12 h photoperiod for 10 days. A conidial suspension of *B. oryzae* (10^4 conidia mL^{-1}) was applied as a fine mist to the adaxial leaf blades of leaves per each plant until runoff with sprayer (D. Vilbiss no. 15). Gelatin (1%, w/v) was added to the sterile water to aid conidial adhesion to the leaf blades. Immediately after inoculation, plants were transferred to a mist chamber at 25 ± 2ºC with an initial 24 h dark period. After this period, plants were incubated using a 12 h photoperiod of ≈ 162 µE m^{-2} s^{-1} provided by cool-white fluorescent lamps. The relative humidity inside the mist chamber was 90 ± 5% throughout the experiments. Plants were kept inside the mist chamber for the duration of the experiments.

For determination of peroxidases (POX, EC 1.11.1.7), polyphenoloxidases (PPO, EC 1.10.3.1), and phenylalanine ammonia-lyases (PAL, EC 4.3.1.5) activities, leaf samples from plants of each replication for each treatment were collected at 6, 12, 18, 24, 36 and 48 hai with *B. oryzae*. Leaf samples were also collected from non-inoculated plants at the same time-points above. Leaf samples were kept in liquid nitrogen (N_2) and stored at -80ºC. For enzyme extracts of POX, 0.6 g of leaf tissues were ground into a fine powder in a pestle and mortar with liquid N_2 and the fine power was homogenized in an ice bath in 20 mL 100 mM potassium phosphate buffer (pH 6.8) containing 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.1 mM ethylenediaminetetraacetic acid (EDTA), and polyvynilpolypyrrolidone (PVPP) (1%, w/v). For enzyme extracts of PPO, a total of 1 g of leaf tissues were ground into a fine powder in a pestle and mortar with liquid N_2 and the fine powder was homogenized in an ice bath in 3 mL 0.2 M sodium phosphate buffer (pH 6.0) containing 1 mM PMSF, 0.1 mM EDTA, and PVPP (1%, w/v). The POX and PPO activities were determined as described by Dallagnol et al. (2011). For enzyme extracts of PAL, a total of 0.1 g of leaf tissues was ground into a fine powder using a pestle and mortar with liquid nitrogen. The fine powder was homogenized in an ice bath in 15 mL 0.1 M sodium borate buffer (pH 8.8) containing PVPP (1%, w/v). The PAL activity was determined as described by Pereira et al. (2009). Four separate extractions were performed for each treatment for assay of the activities of the three enzymes. The protein contents of the extracts were measured according to Pereira et al. (2009).

A 2 × 3 × 2 factorial experiment, consisting of two Si rates (referred thereafter as -Si and +Si treatments), three Mn rates, and plants inoculated or non-inoculated with *B. oryzae* was arranged in a completely randomized design with four replications. Each experimental unit consisted of one plastic container with 4 L of nutrient solution and four rice plants. Data were analyzed by analysis of variance (ANOVA) and means comparisons between non-inoculated and inoculated plants at each sampling time for each Mn rate by Student's test ($P \leq 0.05$) using the software SAEG 5.0 (Viçosa Federal University).

For non-inoculated plants, POX activity increased from 6 to 18 hai and increased thereafter for the -Si 0.5 µmol Mn L^{-1} treatment (Figure 1A). For the -Si 2.5 µmol Mn L^{-1} treatment, POX activity increased from 6 to 18 hai (Figure 1C). POX activity was kept constant from 6 to 24 hai, and increased thereafter for the -Si 10 µmol Mn L^{-1} treatment (Figure 1E). For the treatment +Si 0.5 µmol Mn L^{-1} treatment, POX activity peaked at 24 hai (Figure 1B). POX activity increased from 6 to 18 hai for the +Si 2.5 µmol Mn L^{-1} treatment (Figure 1D). For the +Si 10 µmol Mn L^{-1} treatment, POX activity attained constant values from 6 to 48 hai (Figure 1F). Upon inoculation of -Si plants with *B. oryzae*, POX activity was high at 6 hai and increased thereafter at the rate of 0.5 µmol Mn L^{-1} (Figure 1A). At the rate of 2.5 µmol Mn L^{-1}, POX activity increased from 6 to 18 hai and peaked at 36 hai (Figure 1C). POX activity peaked at both 24 and 48 hai at the rate of 10 µmol Mn L^{-1}

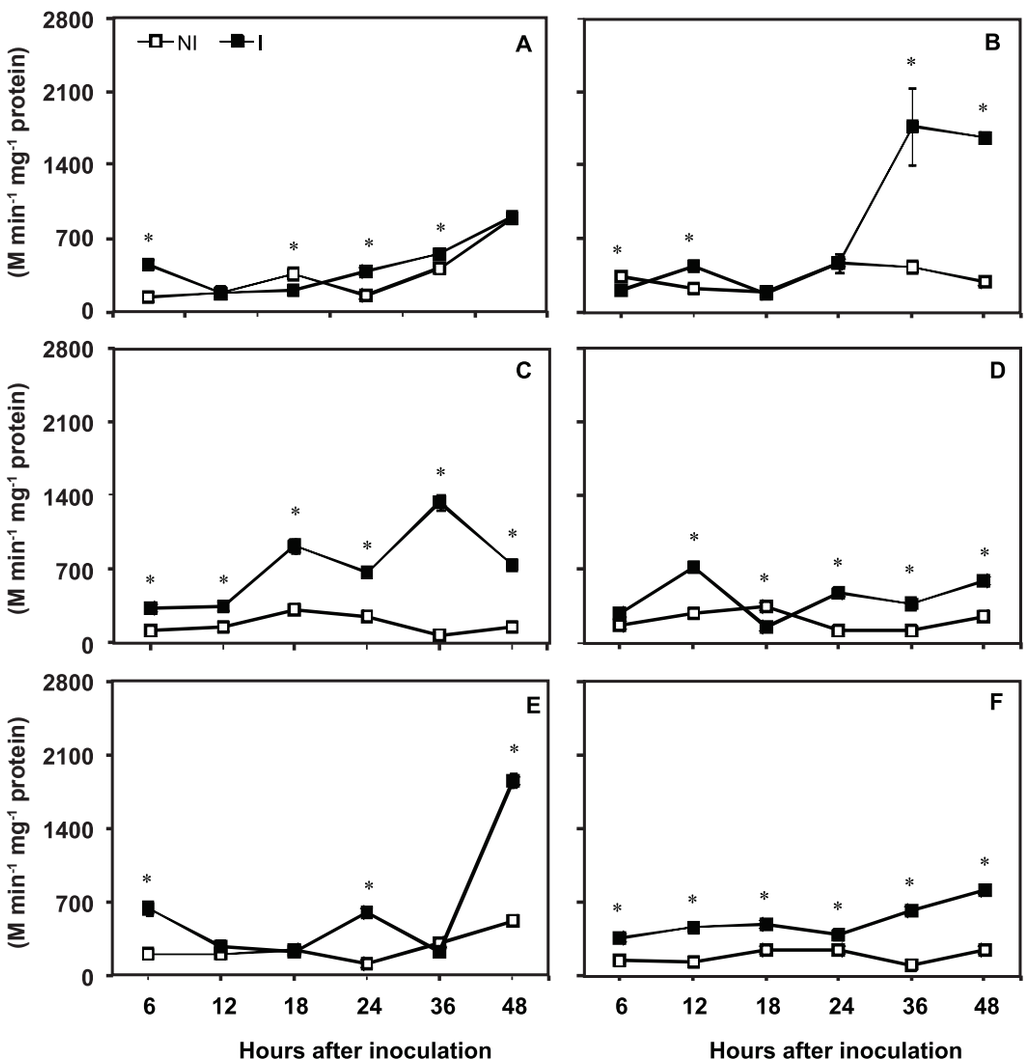

FIGURE 1 - Peroxidase (POX) activity in leaf tissue of rice plants grown in hydroponic culture in the absence (**A, C,** and **E**) or presence (B, D, and F) of silicon (Si) at the rates of 0.5 (**A** and **B**), 2.5 (**C** and **D**) and 10 µmol Mn L^{-1} (**E** and **F**) and non-inoculated (NI) or inoculated (I) with *Bipolaris oryzae*. Each point represents the mean of four replications. Error bars represent standard deviation of the means. Means from NI and I treatments followed by an asterisk (*) are different ($P \leq 0.05$) by *t*-test.

(Figure 1E). For the +Si 0.5 µmol Mn L^{-1} treatment, POX activity increased from 6 to 12 hai and from 24 to 36 hai (Figure 1B). POX activity increased from 6 to 12 hai for the +Si 2.5 µmol Mn L^{-1} treatment (Figure 1D). For the +Si 10 µmol Mn L^{-1} treatment, POX activity increased after 24 hai (Figure 1F). For the -Si treatment, significant differences between non-inoculated and inoculated plants for POX activity occurred at 6, 18, 24 and 36 hai at the rate of 0.5 µmol Mn L^{-1} (Figure 1A), from 6 to 48 hai at the rate of 2.5 µmol Mn L^{-1} (Figure 1C), and at 6, 24, and 48 hai at the rate of 10 µmol MnL^{-1} (Figure 1E). For the +Si treatment, POX activity was significantly different between non-inoculated and inoculated plants at 6, 12, 36 and 48 hai at the rate of 0.5 µmol Mn L^{-1} (Figure 1B), from 12 to 48 hai at rate 2.5 µmol Mn L^{-1} (Figure 1D), and from 6 to 48 hai at rate of 10 µmol Mn/L (Figure 1F).

For non-inoculated plants, PPO activity increased from 6 to 12 hai for the -Si 0.5 µmol Mn L^{-1} treatment (Figure 2A). For the -Si 2.5 µmol Mn L^{-1} treatment, PPO activity peaked at 18 hai and increased after 24 hai (Figure 2C). PPO activity peaked at 18 and 36 hai for the -Si 10 µmol Mn L^{-1} treatment (Figure 2E). For the +Si 0.5 µmol Mn L^{-1} treatment, PPO activity peaked at 18 hai (Figure 2B). PPO activity decreased from 6 to 48 hai for the +Si 2.5 µmol Mn L^{-1} treatment (Figure 2D). For the +Si 10 µmol Mn L^{-1} treatment, PPO activity peaked at 24 hai (Figure 2F). Upon inoculation of -Si plants with *B. oryzae*, PPO activity increased after 18 hai at the rate of 0.5 µmol Mn L^{-1} (Figure 2A). At the rate of 2.5 µmol Mn L^{-1}, PPO activity increased from 6 to 12 hai (Figure 2C). PPO activity peaked at 18 and 36 hai at the rate of 10 µmol Mn L^{-1} (Figure 2E). For the +Si 0.5 µmol Mn L^{-1} treatment, PPO activity peaked at 18 hai (Figure 2B). PPO activity increased from 6 to 36 hai for the +Si 2.5 µmol Mn L^{-1} treatment (Figure 2D). For the +Si 10 µmol Mn L^{-1} treatment, PPO activity increased from 6 to 12 hai (Figure 2F). For the -Si treatment, significant differences between non-inoculated and inoculated plants for PPO activity occurred at 6, 18, 36 and 48 hai at the rate of 0.5 µmol Mn L^{-1} (Figure 2A), from 6 to 48 hai at the rate of 2.5 µmol Mn L^{-1} (Figure 2C), and at 6, 18, 24, and 48 hai at the rate of 10 µmol Mn L^{-1} (Figure 2E). For the +Si treatment, PPO activity was significantly different between non-inoculated and inoculated plants at 24, 36 and 48 hai at the rate of 0.5 µmol Mn L (Figure 2B), at 6, 18, 24, and 36 hai at the rate of 2.5 µmol Mn L^{-1} (Figure 2D), and at 6, 12, 24 and 36 hai at the rate of 10 µmol Mn L^{-1} (Figure 2F).

For non-inoculated plants, PAL activity peaked at 12 and 24 hai for the -Si 0.5 µmol Mn L^{-1} treatment (Figure 3A). For the -Si 2.5 µmol Mn L^{-1} treatment, PAL activity peaked at 12 hai (Figure 3C). PAL activity peaked at 6 hai for the -Si 10 µmol Mn L^{-1} treatment (Figure. 3E). For the +Si 0.5 µmol Mn L^{-1} treatment, PAL activity increased from 6 to 18 hai (Figure 3B). PAL activity increased after 12 hai for the +Si 2.5 µmol Mn L^{-1} treatment (Figure 3D). For the +Si 10 µmol Mn L^{-1} treatment, PAL activity peaked at 36 hai (Figure 3F). Upon inoculation of -Si plants with *B. oryzae*, PAL activity peaked at 36 hai at the rate of 0.5 µmol Mn L^{-1} (Figure 3A). At the rate of 2.5 µmol Mn L^{-1}, PAL activity increased from 6 to 12 hai and peaked at 36 hai (Figure 3C). PAL activity peaked at 18 hai at the rate of 10 µmol Mn L^{-1} (Figure 3E). For the +Si 0.5 µmol Mn L^{-1} treatment, PAL activity peaked at 24 hai (Figure 3B). PAL activity slightly increased from 12 to 48 hai for the +Si 2.5 µmol Mn/L treatment (Figure 3D). For the +Si 10 µmol Mn L^{-1} treatment, PAL activity increased after 36 hai (Figure 3F). For the -Si treatment, significant differences between non-inoculated and inoculated plants for PAL activity occurred at 12, 18, 24, 36 and 48 hai at the rate of 0.5 µmol Mn L^{-1} (Figure 3A) and at 6, 18, 36 and 48 hai for both 2.5 and 10 µmol Mn L^{-1} (Figures 3C and E). For the +Si treatment, PAL activity was significantly different between non-inoculated and inoculated plants at 12, 18 and 24 hai at the rate of 0.5 µmol Mn L^{-1} (Figure 3B), at 6, 36, and 48 hai at the rate of 2.5 µmol Mn/L (Figure 1D), and at 24, 36, and 48 hai at the rate of 10 µmol Mn L^{-1} (Figure 3F).

One of the mechanisms involved in Si-mediated host resistance, especially in the model pathosystem rice-*P. grisea*, has been attributed to the deposition of this element below the cuticle (Kim et al., 2002). However, probing more deeply into how Si affects brown spot development in rice, it was found that plants supplied with this element responded more promptly to *B. oryzae* infection by increasing the production of phenolic compounds, lignin and the activity of POX and chitinases (Dallagnol et al., 2011). However, to the best of our knowledge, no information can be found in the literature about the biochemical mechanisms of rice resistance against *B. oryzae* potentiated by Si in interaction with different rates of the micronutrient Mn. Zanão Júnior et al. (2009) demonstrated that the area under brown spot progress curve was more affected by Si than by different Mn rates in the rice-*B. oryzae* interaction. On the other hand, Baba & Takahashi (1957) found that the number of lesions of brown spot and their size were reduced on leaves of rice plants grown in different soils containing high levels of Mn. Zanão Júnior et al. (2009) also reported that in the absence of Si, brown spot progress on rice leaves grown in nutrient solution was negatively affected when Mn concentration on leaf tissues was high, which was achieved at the rate of 10 µmol Mn L^{-1}.

Peroxidases are glycoproteins capable of catalyzing numerous reactions in the plant, such as those related to lignin and suberin production on roots, which in turn helps to decrease the host tissue colonization by some pathogens (Goodman et al., 1986). In the present study, POX activity on -Si plants increased as disease progressed at both rates of 0.5 and 2.5 µmol Mn L^{-1} and with one major peak occurring at 48 hai at the rate of 10 µmol Mn L^{-1}. For plants supplied with Si, POX activity also increased, but seemed to be very low at the rates of 2.5 and 10 µmol Mn L^{-1} (activity reaching values below 700 M min^{-1} mg^{-1} of protein), except with the two major peaks that occurred at 36 and 48 hai at the rate of 0.5 µmol Mn L^{-1}. In general, POX activity did not show

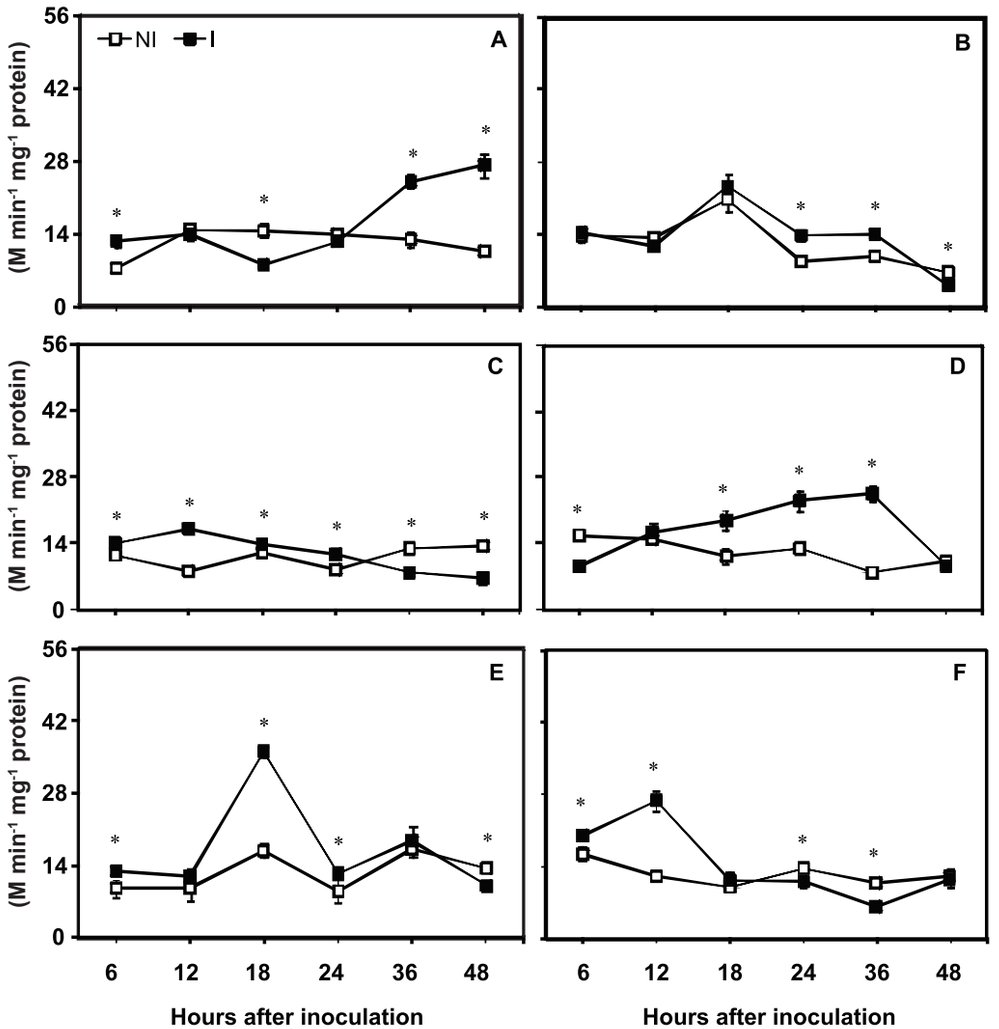

FIGURE 2 - Polyphenoloxidase (PPO) activity in leaf tissue of rice plants grown in hydroponic culture in the absence (**A, C,** and **E**) or presence (**B, D,** and **F**) of silicon (Si) at the rates of 0.5 (**A** and **B**), 2.5 (**C** and **D**) and 10 µmol Mn L^{-1} (**E** and **F**) and non-inoculated (NI) or inoculated (I) with *Bipolaris oryzae*. Each point represents the mean of four replications. Error bars represent standard deviation of the means. Means from NI and I treatments followed by an asterisk (*) are different ($P \leq 0.05$) by *t*-test.

to be greatly influenced by the Mn rates in the presence of Si. However, in the absence of Si, POX activity seemed to increase for the inoculated plants upon 18 dai. Rodrigues et al. (2005) showed that an accumulation of POX transcripts was associated with an increase in rice resistance to blast, presumably due to the participation of POX in the biosynthesis of lignin. Cucumber plants showed an increase in the activity of the enzymes POX, PPO and PAL when supplied with Si and inoculated with the powdery mildew fungus *Podosphaera xanthii* as compared to plants not supplied with this element (Liang et al., 2005).

The PPO participates in the oxidation of many types of phenolic compounds leading to the production of quinones, which are extremely toxic to several pathogens (Campbell & Sederoff, 1996). According to Dallagnol et al. (2011), PPO activity on rice plants supplied with this element did not increase in activity in contrast to what was observed for POX. Regarding the present study, PPO activity on -Si plants increased at the rate of 0.5 µmol Mn L^{-1} and was not affected by the other two Mn rates, except a peak that occurred at 18 hai at the rate of 10 µmol Mn L^{-1}. In the presence of Si, PPO activity increased on plants supplied with µmol Mn L^{-1} in contrast to the other two Mn rates for which the PPO activity decreased. By comparing the -Si and +Si treatments at the rate of µmol Mn L^{-1}, PPO activity increased from 18 to 36 hai in the presence of Si than on its absence.

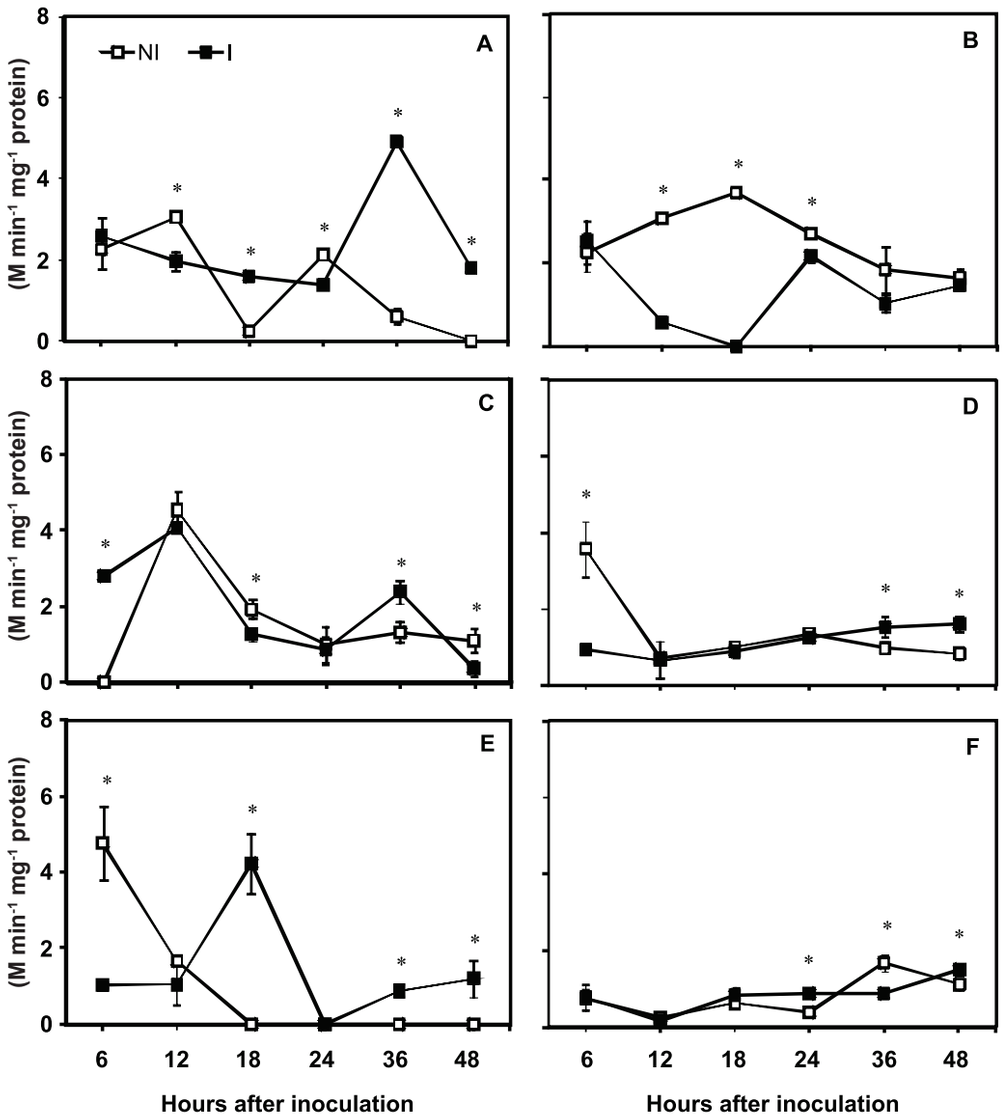

FIGURE 3 - Phenylalanine ammonia-lyase (PAL) activity in leaf tissue of rice plants grown in hydroponic culture in the absence (**A, C,** and **E**) or presence (**B, D,** and **F**) of silicon (Si) at the rates of 0.5 (**A** and **B**), 2.5 (**C** and **D**) and 10 µmol Mn L^{-1} (**E** and **F**) and non-inoculated (NI) or inoculated (I) with *Bipolaris oryzae*. Each point represents the mean of four replications. Error bars represent standard deviation of the means. Means from NI and I treatments followed by an asterisk (*) are different ($P \leq 0.05$) by *t*-test.

The PAL catalyzes the desamination of the aminoacid *L*-phenylalanine with the formation of trans-cinnamic acid, which is the precursor of several types of phenolics in the phenylpropanoid pathway, with lignin being the final product (Campbell & Sederoff, 1996). In the current study, PAL activity on -Si plants decreased regardless of the Mn rates, except for the major peaks occurring at 36 and 18 hai, respectively, for 0.5 and 10 µmol Mn L^{-1}. On plants supplied with Si and inoculated with *B. oryzae*, PAL showed low activity at the highest Mn rate and also at the rate of 0.5 µmol Mn L^{-1}, besides its activity been very low compared to non-inoculated plants. In general, PAL did not increase in activity in the presence of Si, but on its absence, high levels of activity were achieved. It is plausible to conclude that the passive role played by Si on plants supplied with this element, regardless of Mn, was detrimental for rice resistance against brown spot as previously confirmed by Zanão Júnior et al. (2009) than enhancing the activity of the three enzymes evaluated as demonstrated in the present study.

Results of this study clearly suggest that the involvement of the enzymes POX, PPO and PAL in rice resistance against brown spot was minimum regardless of the presence of Si or the rate of Mn in the nutrient solution.

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