

Nutritional status of rice plants supplied with silicon in response to *Pyricularia oryzae* infection

Gisele Pereira Domiciano¹, Leonardo Araujo², Henrique Silva Silveira Duarte³, Cecilia Chagas Freitas¹, Andersom Milech Einhardt¹, Fabrício Ávila Rodrigues^{1*}

1.Universidade Federal de Viçosa - Departamento de Fitopatologia - Viçosa (MG), Brazil.

2.Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina, Fitopatologia - Florianópolis (SC), Brazil.

3.Universidade Federal do Paraná - Departamento de Fitotecnia e Fitossanitarismo - Curitiba (PR), Brazil.

ABSTRACT: Considering the importance of silicon (Si) to increase rice resistance to blast, this study investigated the nutritional status of rice plants supplied with this element in response to *Pyricularia oryzae* infection. Plants were grown in nutrient solutions containing 0 and 2 mmol·L⁻¹ of Si (referred to -Si and +Si plants, respectively). The area under blast progress curve decreased for +Si plants due to a significant increase of 714% on the foliar Si concentration in contrast to -Si plants. For +Si plants, the foliar N, P, K, Ca, Mg, Cu, and B concentrations were significantly lower in comparison to -Si plants. On infected plants, the foliar N, K, and Cu concentrations were significantly lower in comparison to noninfected ones. The foliar Zn, Fe, and Mn concentrations were significantly lower for -Si inoculated plants in comparison to -Si noninoculated ones.

The foliar Mn and S concentrations were significantly lower for +Si inoculated plants in comparison to +Si noninoculated ones. The foliar Fe concentration was significantly lower for +Si inoculated plants in comparison to +Si noninoculated ones. Foliar Zn and Mn concentrations were significantly lower for +Si plants in comparison to -Si plants regardless of their inoculation with *P. oryzae*. Foliar Fe and S concentrations were significantly lower for +Si plants in comparison to -Si plants, respectively, for noninoculated and inoculated plants. Taken together, Si stood for itself in reducing blast symptoms without the need of high foliar concentrations of either macro or micronutrients that could affect plant growth.

Key words: foliar disease, host resistance, plant nutrition.

*Corresponding author: fabricio@ufv.br

Received: Sept. 26, 2018 – Accepted: Apr. 29, 2019



Rice (*Oryza sativa* L.) is one of the most important food crops worldwide, and blast, caused by the hemibiotrophic fungus *Magnaporthe oryzae* (T. T. Hebert) Yaegashi & Udagawa Barr [anamorph *Pyricularia oryzae* (Cooke) Sacc.], is its major disease, causing yield losses (Ou 1985). The use of resistant cultivars and fungicides spray have been used for rice blast management (Gao et al. 2011). However, the emergence of new races of *P. oryzae* and fungal isolates resistant to some fungicides (e.g., strobilurins) (Kim et al. 2002) drive our attention to find new strategies for blast management. The beneficial role of Si in enhancing host resistance to various biotic (e.g., pest attack and pathogens infection) and abiotic stress (e.g., drought, salinity, and metal ion toxicities) has been reported in many studies (Debona et al. 2017). Silicon (Si) stands out for its potential to decrease the intensities of important diseases in several crops. The potentiation of host defense mechanisms by Si against pathogens infection include the high concentrations of phenolic acids, flavonoids, lignin, and some phytoalexins, an increase in the activities of chitinases and β -1,3-glucanases, and the rapid transcription of some defense genes (Debona et al. 2017). A physical barrier formed by Si polymerization below the cuticle may also prevent or slow fungus penetration as reported for the rice-*P. oryzae* interaction (Kim et al. 2002; Debona et al. 2017). Rice can uptake large amounts of Si that are several folds higher compared to the macronutrients. Plants take up Si from the soil solution in the form of monosilicic acid, which is mainly deposited in the cell lumen and cell wall in the form of amorphous silica gel (Debona et al. 2017). A few studies have associated the infection process of pathogens with the nutritional profile of their hosts in the presence of Si (Gao et al. 2011; Cruz et al. 2014; Araujo et al. 2016). Gao et al. (2011) reported that Si enhanced the resistance of rice plants to blast, which showed lower foliar concentrations of K, Na, Ca, Mg, and Fe. By contrast, Tamai and Ma (2008) showed that even though plants from a rice mutant [low Si (*lsi1*)] deficient in an active Si uptake accumulated much less Si compared to plants from the wild-type (cultivar 'Oochikara'); there was no difference between the *lsi1* and wild-type plants regarding the foliar content of N, P, and K.

The present study aimed to provide new insights into the nutritional status of rice plants supplied with Si in response to *P. oryzae* infection considering the scarce information in the literature regarding this subject, especially under controlled conditions. Rice plants (cultivar 'Metica-1') were grown in nutrient solution as described by Domiciano et al. (2015).

Plants were supplied with monosilicic acid obtained by passing potassium silicate through a cation-exchange resin (Amberlite IR-120B, H⁺ form; Sigma-Aldrich, São Paulo, Brazil) (Ma and Yamaji 2006). The Si doses used were 0 (-Si) and 2 mmol·L⁻¹ (+Si). The isolate UFV-DFP Po-22 of *P. oryzae* was used to inoculate the plants. Inoculum production was obtained following the procedures described by Domiciano et al. (2015). At 45 days after emergence, plants were sprayed with the conidial suspension (1×10^5 conidia·mL⁻¹) of *P. oryzae* using an atomizer (Paasche Airbrush Co., Chicago, IL, USA). After that, they were kept in a plastic mist growth chamber [temperature of 25 ± 2 °C (day) to 22 ± 2 °C (night) and relative humidity of 90 ± 5 %]. The maximum natural photon flux density at plants canopies was $950 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Blast severity was evaluated at 36, 60, 84, 108, and 132 hours after inoculation (hai) on the second and third leaves, from base to top, of each plant per replication of each treatment according to the scale proposed by IRRI (1996). The area under blast progress curve (AUBPC) for each leaf was computed using the trapezoidal integration of the blast progress curves over time (Araujo et al. 2016). At the end of the experiments, the leaves of each plant per replication of each treatment were collected, washed in deionized water, placed into paper bags, and dried for 72 h at 65 °C. Shoot and roots dry weights of plants per replication of each treatment were determined. After that, plant material was ground to pass through a 40-mesh screen to obtain the leaf tissue for chemical analysis. The foliar concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), zinc (Zn), iron (Fe), copper (Cu), and manganese (Mn) were determined by using atomic spectrum absorption (Malavolta et al. 1997). The foliar boron (B) concentration was determined colorimetrically (Battaglia and Raij 1990). The foliar Si concentration was determined according to Araujo et al. (2016). A 2×2 factorial experiment, consisting of two Si doses (0 and 2 mmol·L⁻¹ Si, referred to -Si and +Si plants) and plant inoculation (noninoculated and inoculated plants), was arranged in a completely randomized design with six replications. Each experimental unit consisted of a plastic pot containing five plants. The experiment was repeated once. Data from AUBPC and the foliar concentrations of Si and the macro and micronutrients from the two experiments were analyzed using the MIXED procedure of SAS software (Release 8.02 Level 02 M0 for Windows, SAS Institute) to determine if data from these two experiments could be combined (Moore and Dixon 2015) and then submitted to analysis of

→

variance (ANOVA). Treatments means were compared by Tukey's test using SAS (SAS Institute, Inc., 1989, Cary, NC). Principal components analysis (PCA) technique was used to determine the relationship between the variables evaluated. Data were analyzed using the Minitab software v.18.

The AUBPC was significantly reduced by 86% for +Si plants (125) in comparison to -Si plants (856). Based on the ANOVA, the factor Si doses was significant only for the foliar concentrations of N, P, K, Ca, Mg, and S ($p \leq 0.05$). The factor plant inoculation was not significant only for the foliar concentrations of Ca, Mg, and S ($p \leq 0.05$). The interaction Si doses \times plant inoculation was not significant only for the foliar S concentration ($p \leq 0.05$). The factor Si doses were significant only for the foliar concentrations of Zn, Fe, Mn, Cu, B, and Si. The factor plant inoculation was not significant only for the foliar concentrations of B and Si. The interaction Si doses \times plant inoculation was significant only for the foliar concentrations of Zn, Fe, and Mn. The foliar concentrations of N, P, K, Ca, Mg, Cu, and B was significantly higher for -Si plants in comparison to +Si ones (Table 1). The foliar Si concentration significantly increased by 714% for +Si plants in contrast to -Si ones (Table 1). For +Si plants, the foliar N, P, K, Ca, Mg, Cu, and B concentrations were significantly lower in comparison to -Si plants. On infected plants, the

foliar N, K, and Cu concentrations were significantly lower in comparison to noninfected ones (Table 1). The foliar Zn, Fe, and Mn concentrations were significantly lower for -Si inoculated plants in comparison to -Si noninoculated ones. The foliar Mn and S concentrations were significantly lower for +Si inoculated plants in comparison to +Si noninoculated ones (Table 2). The foliar Fe concentration was significantly lower for +Si inoculated plants in comparison to +Si noninoculated ones. Foliar Zn and Mn concentrations were significantly lower for +Si plants in comparison to -Si plants regardless of their inoculation with *P. oryzae*. Foliar Fe and S concentrations were significantly lower for +Si plants in comparison to -Si plants, respectively, for noninoculated and inoculated plants (Table 2). Shoot and roots dry matter significantly increased by 70 and 56%, respectively, for +Si plants (7.7 and 13 g per plant, respectively) in comparison to -Si plants (2.1 and 3.3 g per plant, respectively). The first two PCs covered most of the variation of the dataset; PC 1 and PC 2 explained 62 and 13% of the total variance, respectively (Fig. 1). The PCA revealed that the treatments -Si, +Si, noninoculated plants, and inoculated plants did not cluster together indicating their impact on the foliar Si and nutrients concentrations (Fig. 1a). The PC1 showed positive scores for AUBPC and the foliar concentrations of N, Cu, Mn, K, P, Fe, Zn, Ca, Mg, S, and

Table 1. Foliar concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), boron (B), and silicon (Si) in rice plants grown in nutrient solution containing 0 (-Si) or 2 mmol.L⁻¹ (+Si) Si and non-inoculated or inoculated with *Pyricularia oryzae*. $n = 12$.

Treatments	Foliar Concentrations (dag·kg ⁻¹)							
	N	P	K	Ca	Mg	Cu	B	Si
-Si	3.59a	0.57a	3.50a	0.58a	0.57a	14.36a	16.35a	0.14b
+Si	3.01b	0.53b	2.52b	0.48b	0.48b	10.25b	9.49b	3.51a
Noninoculated plants	3.67a	0.56a	3.29a	0.54a	0.54a	13.60a	13.00a	1.90a
Inoculated plants	2.93b	0.53a	2.57b	0.52a	0.52a	11.00b	12.80a	1.80a
CV (%)	8.73	3.94	8.37	10.28	9.26	15.28	16.63	11.77

Means between -Si and +Si treatments and between noninoculated and inoculated plants treatments, within each column, followed by the same letter are not significantly different according to *F* test ($p \leq 0.05$). CV = coefficient of variation.

Table 2. Foliar concentrations of zinc (Zn), iron (Fe), manganese (Mn), and sulfur (S) in rice plants grown in nutrient solution containing 0 (-Si) or 2 mmol.L⁻¹ (+Si) silicon (Si) and noninoculated or inoculated with *Pyricularia oryzae*. $n = 12$.

Treatments	Foliar concentrations							
	Zn (mg·kg ⁻¹)		Fe (mg·kg ⁻¹)		Mn (mg·kg ⁻¹)		S (mg·kg ⁻¹)	
	-Si	+Si	-Si	+Si	-Si	+Si	-Si	+Si
Noninoculated plants	56.43 aA	31.98 aB	110.10 aA	69.68 bB	303.53 aA	166.65 aB	0.24 aA	0.23 aA
Inoculated plants	46.43 bA	30.01 aB	84.81 bA	72.16 aA	185.25 bA	109.51 bB	0.25 aA	0.20 bB
CV (%)	9.22		10.93		12.76		10.32	

For each nutrient, means within each column followed by the same lowercase letter or means within each row followed by the same uppercase letter are not significantly different ($p \leq 0.05$) as determined by Tukey's test. CV = coefficient of variation.

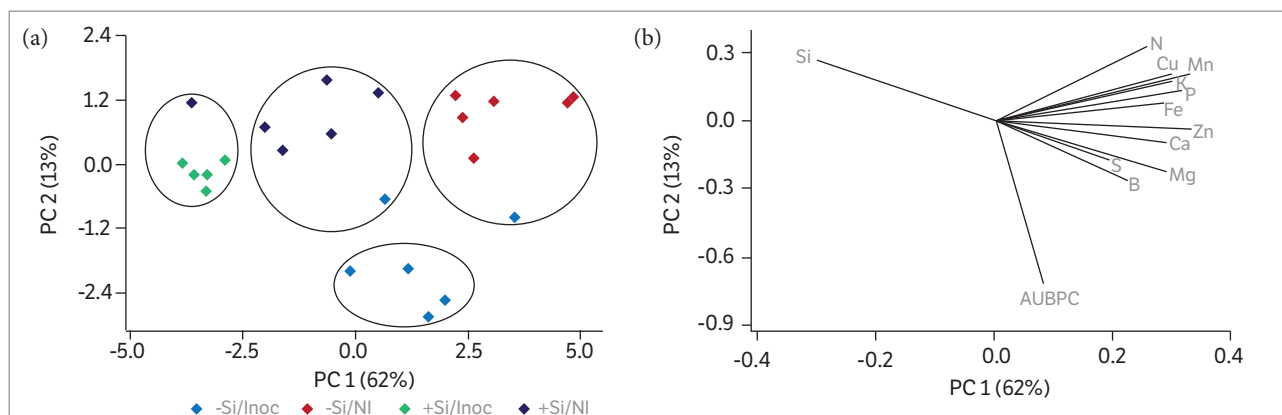


Figure 1. Score (a) and loading (b) plots of principal component analysis comparing the area under blast progress curve (AUBPC) and the foliar concentrations of silicon (Si), nitrogen (N), copper (Cu), manganese (Mn), potassium (K), phosphorus (P), iron (Fe), zinc (Zn), calcium (Ca), magnesium (Mg), sulfur (S), and boron (B) for rice plants nonsupplied (-Si) or supplied (+Si) with Si and noninoculated (NI) or inoculated (Inoc) with *Pyricularia oryzae*. Groups were generated from cluster analysis with complete linkage and Pearson distance.

B and negative scores for foliar Si concentration (Fig. 1b). The foliar Si concentration was inversely proportional to the concentrations of other nutrients regardless of inoculation with *P. oryzae*.

The present study brings new insights regarding the nutritional status of rice plants supplied with Si and infected by *P. oryzae*. Blast severity was reduced on +Si plants and corroborate the findings of other researchers (Debona et al. 2017). The increase of rice resistance to blast by Si was associated with lower foliar concentrations of macro and micronutrients. Common bean plants supplied with Si and infected by *Colletotrichum lindemuthianum* showed high concentrations of K and S and low concentrations of Ca, Fe, Mg, and Mn in the infected tissues based on X-ray microanalysis (Cruz et al. 2014). The effect of the nutrients on diseases development is related to the amount used and their sources, plant species, cultivars, and pathogens' lifestyle (Wang et al. 2013). According to Cruz et al. (2014), Si can positively regulate macro and micronutrients uptake by common bean plants. A high foliar concentration of N can increase the susceptibility of some crops to diseases because of the physiological changes that favor pathogens' aggressiveness (Huber and Thompson 2007). The susceptibility of rice to leaf and neck blast increased due to high foliar N concentration (Bhat et al. 2013). In the present study, it was possible to postulate that a high foliar N concentration for plants nonsupplied with Si contributed to blast symptoms development. The high foliar P concentration for plants nonsupplied with Si contributed to their susceptibility to blast. High foliar P concentration can either increase or reduce diseases development (Prabhu et al. 2007 b). A high

foliar P concentration on rice plants was positively correlated with an increase in panicle blast severity (Filippi and Prabhu 1998). A shorter latent period of blast on rice was linked to an increase in foliar P concentration (Prabhu et al. 2007 b). The foliar concentrations of Ca, Mg, and S decreased for plants supplied with Si. The effect of Ca on host resistance to diseases is linked to its role in the strength of cell walls that affect pathogens colonization (Rahman and Punja 2007). Mg is a component of structural tissues and participates in many physiological processes of the primary and secondary metabolisms, while S is found associated with some defense compounds (Huber and Jones 2012). Changes in the balance among the foliar concentrations of Mg, Ca, and S can increase the susceptibility of some crops to pathogens (Prabhu et al. 2007 a). Araujo et al. (2016) showed reduced symptoms of leaf scald in rice plants supplied with Si without any apparent alteration on the concentrations of Ca, Mg, and S at the fungal infection sites. According to Gao et al. (2011), rice resistance to blast potentiated by Si was associated with an adjustment on nutrients status on the infected leaf tissues. The concentrations of K, Ca, Mg, and Fe on rice leaves of plants supplied with Si and infected by *P. oryzae* was significantly reduced (Gao et al. 2011). In the present study, alterations on the foliar concentrations of Ca, Mg, and S for plants supplied with Si was not associated with their susceptibility to blast. For plants nonsupplied with Si, the foliar concentrations of micronutrients were greater in comparison to plants supplied with this element regardless of fungal infection. The lower foliar concentrations of micronutrients seemed not to affect rice resistance to blast. According to Cruz et al. (2014), the foliar concentrations of micronutrients were

→

lower for common bean plants supplied with Si even though anthracnose symptoms decreased. Reduction in diseases intensities by B was associated with high tissue lignification and an increase in phenolics production (Stangoulis and Graham 2007). The micronutrients Cu, Fe, Mn, and Zn are cofactor of some enzymes involved in host resistance against diseases (Thompson and Huber 2007).

It can be concluded that Si stood itself in reducing blast symptoms on rice plants without the need of high foliar concentrations of either macro or micronutrients.

AUTHOR'S CONTRIBUTION

Conceptualization, Domiciano G. P. and Rodrigues F. A.; Methodology, Domiciano G. P., Rodrigues F. A. and Einhardt A. M.; Investigation, Domiciano G. P., Araujo L., Duarte H. S. S., Freitas C. C. and Rodrigues F. A.; Writing – Original Draft, Domiciano G. P., Araujo, L., Duarte H. S. S. and Einhardt A.

M.; Writing – Review and Editing, Rodrigues F. A.; Funding Acquisition, Rodrigues F. A.; Resources, Rodrigues F. A.; Supervision, Rodrigues F. A.

ORCID IDs

G.P. Domiciano

 <https://orcid.org/0000-0003-4389-3585>

L. Araujo

 <https://orcid.org/0000-0003-4793-4751>

H.S.S. Duarte

 <https://orcid.org/0000-0002-8128-5428>

C.C. Freitas

 <https://orcid.org/0000-0002-5807-7081>

A.M. Einhardt

 <https://orcid.org/0000-0001-7741-9094>

F.A. Rodrigues

 <https://orcid.org/0000-0002-3091-0000>

REFERENCES

- Araujo, L., Paschoalino, R. S. and Rodrigues, F. A. (2016). Microscopic aspects of silicon-mediated rice resistance to leaf scald. *Phytopathology*, 106, 2, 132-141. <https://doi.org/10.1094/PHYTO-04-15-0109-R>
- Bhat, Z. A., Ahangar, M. A., Sanghera, G. S. and Mubarak, T. (2013). Effect of cultivar, fungicide spray and nitrogen fertilization on management of rice blast under temperate ecosystem. *International Journal of Science, Environment and Technology*, 2, 3, 410-415.
- Battaglia, O. C. and Raji, B. V. (1990). Eficiência de extratores na determinação de boro em solos. *Revista Brasileira de Ciência do Solo*, 14, 25-31
- Cruz, M. F. A., Araujo, L., Polanco, L. R. and Rodrigues, F. A. (2014). Aspectos microscópicos da interação feijoeiro *Colletotrichum lindemuthianum* mediados pelo silício. *Bragantia*, 73, 3, 284-291. <https://doi.org/10.1590/1678-4499.0139>
- Debona, D., Rodrigues, F. A. and Datnoff, L. E. (2017). Silicon's role in abiotic and biotic plant stresses. *Annual Review of Phytopathology*, 55, 85-107. <https://doi.org/10.1146/annurev-phyto-080516-035312>
- Filippi, M. C. and Prabhu, A. S. (1998). Relationship between panicle blast severity and mineral nutrient content of plant tissue in upland rice. *Journal of Plant Nutrition*, 21, 8, 1577-1587. <https://doi.org/10.1080/01904169809365505>
- Gao, D., Cai, K., Chen, J., Luo, J., Zeng, R., Yang, R. and Zhu, X. (2011). Silicon enhances photochemical efficiency and adjusts mineral nutrient absorption in *Magnaporthe oryzae* infected rice plants. *Acta Physiologiae Plantarum*, 33, 3, 675-682. <https://doi.org/10.1007/s11738-010-0588-5>
- Huber, D. M. and Thompson, I. A. (2007). Nitrogen and Plant Disease. In Datnoff, L. E., Elmer, W. H. and Huber, D. M. (Eds), *Mineral Nutrition and Plant Disease*. (p. 31-44), Saint Paul: APS.
- Huber, D. M. and Jones, J. B. (2012). The role of magnesium in plant disease. *Plant and Soil*, 368, 1-2, 73-85. <https://doi.org/10.1007/s11104-012-1476-0>
- [IRRI] International Rice Research Institute (1996). *Standard Evaluation System for Rice*. Manila: International Rice Research Institute.
- Kim, S. G., Kim, K. W., Park, E. W. and Choi, D. (2002). Silicon-induced cell wall fortification of rice leaves: A possible cellular mechanism of enhanced host resistance to blast. *Phytopathology*, 92, 10, 1095-1103. <https://doi.org/10.1094/PHYTO.2002.92.10.1095>

- Ma, J. F. and Yamaji, N. (2006). Silicon uptake and accumulation in higher plants. *Trends in Plant Science*, 11, 8, 392-397. <https://doi.org/10.1016/j.tplants.2006.06.007>
- Malavolta, E., Vitti, G. S. and Oliveira, S. A. (1997). Avaliação do estado nutricional das plantas: princípios e aplicações. Piracicaba: POTAFOS.
- Moore, K. J. and Dixon, P. M. (2015). Analysis of combined experiments revisited. *Agronomy Journal*, 107, 2, 763-771. <https://doi.org/10.2134/agronj13.0485>
- Ou, S. H. (1985). *Rice Diseases*, 2nd. Kew: Commonwealth Mycological Institute.
- Prabhu, A. S., Fageria, N. K., Huber, D. M. and Rodrigues, F. A. (2007 a). Potassium and Plant Disease. In L. E. Datnoff, W. H. Elmer and D. M. Huber (Eds), *Mineral Nutrition and Plant Disease*. (p. 57-78). Saint Paul: APS.
- Prabhu, A. S., Fageria, N. K., Berni, R. F. and Rodrigues, F. A. (2007 b). Phosphorus and Plant Disease. In L. E. Datnoff, W. H. Elmer and D. M. Huber (Eds). *Mineral Nutrition and Plant Disease*. (p. 45-55). Saint Paul: APS.
- Rahman, M. and Punja, Z. K. (2007). Calcium and Plant Disease. In L. E. Datnoff, W. H. Elmer and D. M. Huber (Eds). *Mineral Nutrition and Plant Disease*. (p. 79-93). Saint Paul: APS.
- Stangoulis, J. C. R. and Graham, R. D. (2007). Boron and Plant Disease. In L. E. Datnoff, W. H. Elmer and D. M. Huber (Eds), *Mineral Nutrition and Plant Disease*. (p. 207-214). Saint Paul: APS.
- Tamai, K. and Ma, J. F. (2008). Reexamination of silicon effects on rice growth and production under field conditions using a low silicon mutant. *Plant and Soil*, 307, 1, 21-27. <https://doi.org/10.1007/s11104-008-9571-y>
- Thompson, I. A. and Huber, D. M. (2007). Manganese and Plant Disease. In L. E. Datnoff, W. H. Elmer and D. M. Huber (Eds). *Mineral Nutrition and Plant Disease*. (p. 139-153). Saint Paul: APS.
- Wang, M., Zheng, Q., Shen, Q. and Guo, S. (2013). The critical role of potassium in plant stress response. *International Journal of Molecular Science*, 14, 4, 7370-7390. <https://doi.org/10.3390/ijms14047370>