Effect of Rice Growth Stages and Silicon on Sheath Blight Development

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ABSTRACT

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The objective of this study was to determine the effect of silicon (Si) and rice growth stages on tissue susceptibility to sheath blight (*Rhizoctonia solani* Kühn) under controlled conditions. Rice plants (cv. Rio Formoso) were grown in pots containing low-Si soil amended with Si at 0, 0.48, 0.96, 1.44, and 1.92 g pot⁻¹ and inoculated with *R. solani* at the following days after emergence: 45 (four-leaf stage), 65 (eight-leaf stage), 85 (tillering), 117 (booting), and 130 (panicle exertion). For plants inoculated with *R. solani* at all growth stages, Si concentration in straw increased as rate of Si increased from 0 to 1.92 g pot⁻¹. Concentration of calcium in the straw did not differ among plant growth stages. Although incubation period was not affected by the amount of Si added to the soil, this variable was shorter at booting and panicle exertion stages. As the

rates of Si increased in the soil, the total number of sheath blight lesions on sheaths and total area under the relative lesion extension curve decreased at all plant growth stages. The severity of sheath blight was lower at booting and panicle exertion stages as the rates of Si increased in the soil. In general, plants grown in Si-nonamended pots and inoculated with *R. solani* were more vulnerable to infection at all growth stages, but especially at 45 days after emergence. Plant dry weights for inoculated plants increased as the Si rates increased from 0 to 1.92 g pot⁻¹. The greatest dry weight increases occurred for plants inoculated at booting and panicle exertion stages. Si fertilization is a promising method for controlling sheath blight in areas where soil is Si deficient and when cultivars that exhibit an acceptable level of resistance to sheath blight are not available for commercial use.

Additional keywords: calcium silicate slag, host resistance, *Oryza sativa*, *Rhizoctonia solani*.

Brazil is a major rice-producing country, with 5 million ha planted annually under upland or irrigated conditions. Sheath blight, caused by the fungus *Rhizoctonia solani* Kühn (*Thanatephorus cucumeris* (A. B. Frank) Donk), anastomosis group 1 IA (AG-1 IA) (33), is assuming economic importance among diseases of rice (*Oryza sativa* L.) in Brazil. Rice cultivation recently has been extended to the states of Tocantins and Mato Grosso do Sul in Brazil, where diseases have caused major yield losses. The rotation of rice with soybean in these regions has been associated with an increased incidence of sheath blight. High rates of nitrogen fertilizer, early-season planting, double cropping, high plant density per unit area, and planting of early maturing, short-stature, high-tillering, compact, susceptible cultivars are factors responsible for the increase in sheath blight in most rice-growing regions worldwide (5,25,34,39).

Strategies to control sheath blight are limited because cultivars with complete resistance are not currently available (26). Development of cultivars with a high level of partial resistance to sheath blight has been slow because of the wide host range of the pathogen and the lack of an adequate knowledge of the disease-yield loss relationship (13,34). Additionally, there is considerable variability in disease development from the influence of many environmental effects and host characteristics (25,39). In Brazil, rice cultivars that exhibit an acceptable level of resistance to sheath blight are not commercially available. Even though control

strategies for sheath blight have centered around the use of foliar fungicides (10), their use is limited due to perceived environmental problems, potential risk of emergence of pathogen population that become resistant, and expense. Many cultural and biological control methods have been investigated to minimize the intensity of sheath blight (1,4,30,38,47).

Silicon (Si) fertilization may offer a viable method to control sheath blight, especially where soils are low or limiting in plant-available Si. Some economically important diseases in rice, such as blast (*Magnaporthe grisea* (Hebert) Barr), brown spot (*Cochliobolus miyabeanus* (Ito & Kuribayashi) Ito) Drechs. ex Dastur), stem rot (*M. salvinii* Cattaneo), leaf scald (*Monographella albescens* Theum), and grain discoloration have been reduced by Si application (6-9,20,40,48). According to Mathai et al. (28), application of Si resulted in reduced severity of sheath blight even though the difference in disease severity between the high and low rates of Si was not statistically significant. The effect of Si on reducing sheath blight severity was shown to be greater in irrigated *indica* rice genotypes compared with *japonica* upland rice genotypes and intermediate groups (48). Si reduced the intensity of sheath blight on susceptible and moderately susceptible U.S. rice cultivars to levels comparable to those of cultivars highly or partially resistant to sheath blight and not amended with Si (35). For more effective management of sheath blight, it is necessary to determine plant growth stages that are most vulnerable to infection. Symptoms of sheath blight generally do not appear under field conditions until plants have reached the maximum tillering stage. At this growth stage, high humidity is maintained within the crop canopy and, consequently, contributes to favorable disease development (34,39). In contrast, Valdez (45)

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observed that rice is susceptible to infection by *R. solani* at all growth stages, but especially in the vegetative stage. The literature is conflicting about which is the most susceptible rice growth stage for sheath blight infection and reliable information is not available on the effect of Si on sheath blight intensity at different host growth stages. Consequently, the current study was undertaken to investigate the effects of Si and rice growth stages on tissue susceptibility to sheath blight under controlled conditions. A portion of this study was previously reported (36).

MATERIALS AND METHODS

Two five-by-five factorial experiments, consisting of five rice growth stages and five Si rates, were arranged in a randomized design with three replications in each experiment. Experiments were conducted under controlled conditions from January to July 1998 at the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil. All experiments were set in an Si-deficient typic acrustox soil, with plant-available Si at 14.6 mg dm^{-3} collected at a depth of 0 to 20 cm in the 'Triângulo Mineiro' area. Plastic pots were each filled with 2 kg of air dried, sieved (5 mm) soil. Wollastonite, used as the source of Si, (CaSiO_3 ; Vansil, EW-20, Ipiranga Chemical Co., São Paulo, Brazil) is composed of 24.3% Si and 42.1% calcium (Ca). Soil was mixed with wollastonite to obtain equivalent rates of elemental Si at 0, 0.48, 0.96, 1.44, or 1.92 g pot^{-1} . Dolomitic lime was added to pots treated with 0, 0.48, 0.96, or 1.44 g of Si to equilibrate the amount of Ca present in these treatments with the treatment that contained 1.92 g of Si. Pots with 1.44 g of Si received 1.44 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ and that with 1.92 g of Si received 2.88 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, to equilibrate magnesium among treatments. Soil in each pot was incubated 60 days (moist) before sowing 8 rice seeds (cv. Rio Formoso) and fertilized with 100 ml of a nutrient solution containing, in milligrams per kilogram of soil, 100 N, 300 P, 150 K, 40 S, 0.81 B, 1.33 Cu, 1.55 Fe, 3.66 Mn, 0.15 Mo, and 4.00 Zn (32). At 10 days after emergence, each pot was thinned to two plants. No modifications were made to the pots to allow for drainage, and plants were kept under flooded conditions until the end of the experiments.

A virulent isolate of *R. solani* (AG-1 IA), obtained from symptomatic plants (cv. Metica-1) in rice fields in the state of Tocantins and provided by the National Center for Research on Rice and Beans in Brazil, was used to inoculate the plants. The isolate was maintained on acid potato dextrose agar medium (APDA). Inoculum was obtained as follows: the isolate of *R. solani* was grown on APDA and incubated for 10 days at room temperature to produce mycelia. Wooden toothpicks, 1 cm in length, were placed in Erlenmeyer flasks containing a shallow layer of potato broth and autoclaved. After autoclaving, 15 toothpicks and five agar plugs, 5 mm in diameter, obtained from the margin of an actively growing colony of *R. solani* were transferred to APDA plates. These plates were allowed to incubate for 8 days at room temperature so *R. solani* could colonize the toothpicks.

Plants were inoculated at days 45 (four-leaf stage), 65 (eight-leaf stage), 85 (tillering), 117 (booting), and 130 (panicle exertion) after seedling emergence by placing a 1-cm toothpick colonized by *R. solani* into the lowest inner sheath of the main tiller. Immediately after inoculation, all plants were transferred to a glass mist chamber. Plants were kept at 27°C and 90% relative humidity. The 12-h relative humidity period was maintained within this chamber with a humidifier (Herrmidifier 500 Co., Inc., Lancaster, PA). Plants received 16-h photoperiod of approximately $162 \mu\text{E m}^{-2} \text{ s}^{-1}$ provided by cool-white fluorescent lamps.

Sheath blight progress on the inoculated and upper three sheaths of the main tiller from plants inoculated in all growth stages were evaluated as a ratio of lesion extension on sheaths, measured vertically (cm), to the total sheath lengths (cm). In experiment 1, vertical lesion extension on sheaths was evaluated daily from 4 to

21, 4 to 24, 4 to 26, 3 to 28, and 3 to 25 days postinoculation with *R. solani* at the following days after seedling emergence: 45 (four-leaf stage), 65 (eight-leaf stage), 85 (tillering), 117 (booting), and 130 (panicle exertion), respectively. In experiment 2, vertical lesion extension on sheaths was evaluated daily from 4 to 19, 4 to 22, 4 to 25, 3 to 27, and 3 to 26 days postinoculation with *R. solani* at the following days after seedling emergence: 45 (four-leaf stage), 65 (eight-leaf stage), 85 (tillering), 117 (booting), and 130 (panicle exertion), respectively. Data from each sheath in each plant, replication, treatment, and experiment were used to construct the relative lesion extension curve. The area under the relative lesion extension curve (AURLEC) for each sheath in each plant, replication, treatment, and experiment was computed using the trapezoidal integration of relative lesion extension curve according to the formula proposed by Shaner and Finney (41). This was conducted over a period of 20 to 28 days postinoculation with *R. solani*, depending on the plant growth stage inoculated with *R. solani* in each experiment. The total AURLEC represented the sum of the AURLEC from four sheaths.

The severity of sheath blight was scored using a scale that ranged from 0 to 9 based on relative lesion height on the whole plant (18): 0 = no infection, 1 = lesions limited to lower 20% of the plant height, 3 = lesions limited to lower 20 to 30% of the plant height, 5 = lesions limited to lower 31 to 45% of the plant height, 7 = lesions limited to lower 46 to 65% of the plant height, and 9 = lesions more than 65% of the plant height.

Incubation period was scored as the time, in hours, from inoculation until the appearance of the first lesion on the inoculated sheath for all growth stages. Sheath blight lesions that developed on the four sheaths of the main tiller were counted at the end of the experiments.

Plant dry weight was determined 20 to 28 days after inoculation with *R. solani*, depending on the rice growth stage in each experiment. Leaves and culms from each replication were dried and ground to pass through a 40-mesh screen with a Thomas-Wiley mill (Thomas Scientific, Swedesboro, NJ). Si in leaf and culm tissue was determined by automated colorimetric analysis on 0.1 g of dried and digested tissue (11). Results obtained were expressed in grams of Si per kilograms of plant tissue. Ca concentration (gram per kilogram) in tissue was determined after perchloric digestion by atomic absorption spectrophotometry (19).

Data for each variable from the two experiments were pooled for statistical analysis because homogeneity of variances was confirmed by Bartlett's test (14). Moreover, the experiment-treatment interactions were not significant ($P \geq 0.05$) when compared to the main effects of treatments. Data from each variable were analyzed by analysis of variance (ANOVA) using the GLM procedure of SAS (version 6.12). An equation and graphic that fit the data for each variable were generated using Table Curve 3D (SPSS, Inc., Chicago).

RESULTS AND DISCUSSION

Rice growth stages and rates of Si had significant effects ($P < 0.05$) on the severity of sheath blight, total area under the relative lesion extension curve, total number of sheath blight lesions, plant dry weight, and Si concentration in the straw (Table 1). The incubation period was affected ($P < 0.05$) only by rice growth stages. The interaction between rice growth stages and rates of Si was only significant ($P < 0.05$) for severity of sheath blight (Table 1).

The concentration of Si in the straw increased as rates of Si increased from 0 to 1.92 g pot^{-1} (Fig. 1). Concentration of Si was greatest in plants inoculated with *R. solani* at 45 days after emergence (four-leaf stage), and decreased sharply as plants grew older (booting and panicle exertion stages). The greater concentration of Si in tissue from plants inoculated with *R. solani* at 45 days after emergence (four-leaf stage) was from the reduced dry matter production as a result of the high intensity of sheath

blight. Silicon is classified as an immobile element and is not retranslocated upon deposition as opal, which represents more than 90% of Si present in the plant tissue (24). Ca concentration in rice straw ranged from 2.65 to 3.35 g kg⁻¹ among plant growth stages inoculated with *R. solani* with no significant difference ($P \geq 0.05$) among them. Ca protects the middle lamella of host cell walls against enzymes produced by some pathogens (16). However, in this study, the level of Ca was carefully equilibrated in all treatments for each experiment so that only variations in Si levels could account for the difference in disease intensity. Datnoff et al. (7) analyzed leaf samples from Si-treated plots and found Si, but not Ca, to increase as calcium silicate fertilization was increased. Silicon has been reported to be the only element that has increased significantly in plant tissue of rice grown over a 3-year period in organic soil amended with calcium silicate slag (43). Uptake of Si also may depress the absorption of Ca by the rice plant, and this results in a decrease of the amount of Ca accumulated in shoots (27).

The incubation period for sheath blight development ranged from 48 to 96 h regardless of the rice growth stage inoculated with *R. solani*. However, plants inoculated at 45 (four-leaf stage) and 65 (eight-leaf stage) days after emergence showed the longest incubation period, which decreased sharply for plants inoculated at day 117 (booting), and day 130 (panicle exertion) after emergence (Fig. 2). As reported by Kozaka (22), 5- to 6-week-old leaf sheaths were more susceptible than 2- to 3-week-old sheaths. For plants inoculated with *R. solani* at all growth stages, there was no significant effect of Si rates on incubation period. Considering that *R. solani* has the capacity to penetrate through stomatal openings (34), this may have favored the early appearance of the first lesion on inoculated sheaths because inoculum was placed within the inner sheath. Stomatal invasion seldom occurs on the outer surface of the sheath, but is quite common on the inner surface (13). Seebold et al. (40) did not find significant differences in the incubation period of *Magnaporthe grisea* among susceptible and partially resistant rice cultivars amended with Si; however, the incubation period of a resistant cultivar was significantly longer than for the other cultivars.

The total number of sheath blight lesions was higher on rice plants grown in pots without Si amendment and inoculated at 85 (tillering) and 117 (booting) days after emergence (Fig. 3). The number of sheath blight lesions was somewhat less on plants inoculated at 45 (four-leaf stage) and 65 (eight-leaf stage) days after emergence, and slightly less on plants inoculated at days 85 (tillering), 117 (booting), and 130 (panicle exertion) after emergence as the Si rates ranged from 0 to 1.92 g pot⁻¹ (Fig. 3).

Plants grown in soil not amended with Si, and inoculated at 85 (tillering) and 117 (booting) days after emergence, showed the greatest total area under the relative lesion extension curve (TAURLEC) (Fig. 4). Plants inoculated with *R. solani* at 45 (four-leaf stage) and 65 (eight-leaf stage) days after emergence and grown at the highest rate of Si exhibited the lowest TAURLEC

compared with plants inoculated at day 117 (booting) and day 130 (panicle exertion). Additionally, plants inoculated at 85 (tillering) and 117 (booting) days after emergence showed lower values for TAURLEC as the rates of Si ranged from 0 to 1.92 g pot⁻¹ (Fig. 4).

The exact mechanism or mechanisms by which Si reduces the intensity of rice sheath blight is still, to date, unknown. Silicon-mediated resistance to rice blast has been associated with an increased in the density of silicified cells in the epidermis or the presence of a cuticle-silica double layer formed upon deposition and polymerization of silicic acid beneath the cuticle (34,49). This physical barrier created after Si deposition and polymerization on leaf sheath might have affected the penetration (lower number of lesions) and colonization (decrease in vertical lesion expansion over time) by *R. solani*. Another possibility is that Si also may enhance rice resistance to sheath blight by stimulating plants to react more rapidly and efficiently to infection. In cucumber, there is clear evidence that Si mediates a number of cell defense reactions that confer protection against *Pythium ultimum* and *Sphaerotheca fuliginea*, such as the accumulation of phenolic compounds and a significant increase in chitinase, β -1,3-glucanase, peroxidase, phenylalanine ammonia-lyase, and polyphenol-oxidase activities (2,12,29). Zeyen et al. (51) demonstrated that soluble Si was present in considerable amounts during and immediately following papilla deposition in barley epidermal cells

Fig. 1. Effect of rice growth stages and rates of silicon (Si) on concentration of Si in the straw. The equation for the response surface is $z = 49.68 + 9.06x - 1.81x^2 - 0.768y + 0.00351447y^2$, $R^2 = 0.89$, where $z = \text{Si (g Kg}^{-1}\text{)}$, $x = \text{rate of Si (g pot}^{-1}\text{)}$, $y = \text{plant age (days)}$, and $R^2 = \text{coefficient of determination}$. Dots connected by a vertical line represent the six replications from the pooled experiments.

TABLE 1. Analysis of variance of the effect of rice growth stages (GS) and rates of silicon (Si) on six disease and plant components

Source of variation	df	F values ^a					
		Incubation period	Severity of sheath blight ^b	Total area under the relative lesion extension curve ^c	Total number of sheath blight lesions	Plant dry weight	Silicon concentration in straw
GS	4	24.55**	25.78**	4.06**	18.81**	16.23**	73.39**
Si	4	0.30 ^{ns}	40.44**	2.98*	12.89**	70.38**	44.29**
GS × Si	16	0.75 ^{ns}	3.34**	1.00 ^{ns}	1.67 ^{ns}	1.81 ^{ns}	1.56 ^{ns}

^a Levels of probability: ns = nonsignificant, * = 0.05, and ** = 0.01.

^b The severity of sheath blight was scored using a scale that ranged from 0 to 9 based on relative lesion height on the whole plant.

^c Sheath blight progress on the inoculated and upper three sheaths of the main tiller from plants inoculated in all growth stages were evaluated as a ratio of vertical lesion extension (cm) to sheath length (cm). The relative lesion extension curve was constructed from plant, sheath, replication, treatment, and experiment data. The area under the relative lesion extension curve (AURLEC) for each sheath in each plant, replication, treatment, and experiment was computed using the trapezoidal integration of relative lesion extension curve in a period of time ranging from 20 to 28 days postinoculation with *Rhizoctonia solani*, depending on the plant growth stage in each experiment. The total AURLEC represented the sum of the AURLEC from four sheaths.

infected by *Erysiphe graminis*. This finding suggests that Si plays more than a passive role during attempted fungal penetration.

The shorter length of sheaths on plants inoculated with *R. solani* at 45 (four-leaf stage) and 65 (eight-leaf stage) days after emergence might have affected the number of lesions formed on those sheaths. Increased thickness of epicuticular wax appears to be correlated with resistance to sheath blight and with decreased production of lobate appressoria and infection cushions by *R. solani* (15). Rodrigues et al. (35) considered reduction in lesion extension to be the most important component of resistance to sheath blight, especially in moderately susceptible and susceptible U.S. rice cultivars. Volk et al. (46) hypothesized that lesion size of rice blast could be reduced by organosilicon compounds in the walls of epidermal cells. Inanaga et al. (17) later showed that

silicic acid may compete with Ca for binding sites on the cell wall after some complexes are formed with polyhydric alcohols, organic acids, lignin, and phenol-carbohydrate.

Plants grown in pots without Si amendments and inoculated with *R. solani* at any growth stage exhibited the highest values for severity of sheath blight (Fig. 5). Severity of sheath blight reached lower values on plants inoculated with *R. solani* at 117 (booting) and 130 (panicle exertion) days after emergence as the rates of Si increased from 0 to 1.92 g pot^{-1} .

The information available in the literature on which rice growth stage is the most susceptible to infection by *R. solani* is somewhat contradictory. A sheath blight epidemic occurs rapidly in the early heading and grain filling growth stages (39). Young plants seem to be more resistant than older plants and the greatest susceptibility

Fig. 2. Effect of rice growth stages and rates of silicon (Si) on incubation period (IP). The equation for the response surface is $z = 863.05 - 141.81\text{Ln}(y) - 2818.30\text{Ln}(y/y)$, $R^2 = 0.65$, where $z = \text{IP (h)}$, $y = \text{plant age (days)}$, and $R^2 = \text{coefficient of determination}$. Dots connected by a vertical line represent the six replications from the pooled experiments.

Fig. 3. Effect of rice growth stages and rates of Si on total number of sheath blight lesions (TNSBL) on sheaths. The equation for the response surface is $z = -3.53 - 5.14x + 1.64x^2 + 0.3877y - 0.00199y^2$, $R^2 = 0.69$, where $z = \text{TNSBL}$, $x = \text{rate of Si (g pot}^{-1})$, $y = \text{plant age (days)}$, and $R^2 = \text{coefficient of determination}$. Dots connected by a vertical line represent the six replications from the pooled experiments.

Fig. 4. Effect of rice growth stages and rates of silicon (Si) on total area under the relative lesion extension curve (TAURLEC). The equation for the response surface is $z = -192.99 - 149.20x + 26.76y - 0.14322y^2$, $R^2 = 0.70$, where $z = \text{TAURLEC}$, $x = \text{rate of Si (g pot}^{-1})$, $y = \text{plant age (days)}$, and $R^2 = \text{coefficient of determination}$. Dots connected by a vertical line represent the six replications from the pooled experiments.

Fig. 5. Effect of rice growth stages and rates of silicon (Si) on severity of sheath blight (SSB). The equation for the response surface is $z = 14.79 - 1.11x + 1.13x^2 + 0.52x^3 - 0.139284y + 0.00069y^2 + 0.0000001325y^3$, $R^2 = 0.78$, where $z = \text{SSB}$, $x = \text{rate of Si (g pot}^{-1})$, $y = \text{plant age (days)}$, and $R^2 = \text{coefficient of determination}$. Dots connected by a vertical line represent the six replications from the pooled experiments.

occurs at panicle emergence (34). Seedlings may be infected easily when in contact with sclerotia produced by the fungus (13). Lakpale et al. (23) observed that transplanted seedlings 20 and 30 days old showed the highest severity of sheath blight compared with seedlings 40, 50, and 60 days old. The authors also reported that the maximum tillering stage was more suitable for disease development compared with booting and milk stages. Similarly, Roy (37) observed that *R. solani* caused maximum disease and, consequently, yield loss on rice plants inoculated at the maximum tillering stage, less at exertion of tillering, and the least at booting stage. Yoshimera and Nishizawa (50) also found that inoculation with *R. solani* at the maximum tillering stage was the most suitable for varietal testing. In contrast, Sharma et al. (42) observed that rice was more susceptible to sheath blight at booting and flowering stages than at tillering and panicle exertion stages. Cu et al. (3) demonstrated that the incidence of sheath blight was great when plants were inoculated at panicle exertion, booting, or flowering stages, and significantly lower at tillering and grain-filling stages. Tsai (44) inoculated, under controlled conditions, rice plants at different stages of growth to determine the relationship between intensity of sheath blight and yield losses. Plants inoculated at 15 days after sowing were quickly killed by *R. solani*. Sheath blight intensity also reached high values at stages of maximum tillering and booting. In this study, however, we observed that rice plants grown in pots without Si amendment and inoculated at any growth stage, but particularly at 45 (four-leaf stage) days after emergence, exhibited the highest values for disease intensity as a whole when compared with Si-treated plants. Unlike the controlled conditions in a greenhouse, in the field the rice growth stage most susceptible to sheath blight may vary markedly by climatic conditions. As reported by Kozoka (21), rice plants become more susceptible to sheath blight as they grow older due to an adequate microclimate within the crop canopy which greatly affects the incidence and the damage caused by this disease.

Plant dry weight increased as the rates of Si ranged from 0 to 1.92 g pot⁻¹, and reached the highest values at 117 (booting) and 130 (panicle exertion) days after emergence (Fig. 6). For plants grown in pots without Si, plant dry weight increased somewhat as plants grew older, although the values were lower when compared with those obtained from plants grown at the highest Si rate. Increases in dry matter accumulation could be attributed to a decrease in the intensity of sheath blight by Si application. Infection

by *R. solani* in rice changes dramatically the dynamic balance of synthesis and decomposition of chlorophyll pigments on leaves and also the rate of photosynthesis (31), due to the formation of many expanding lesions on leaf sheaths and blades.

The results presented in this study provide clear evidence that Si can be used successfully to enhance the level of rice resistance to sheath blight. Fertilization with Si could become important in areas where soil is Si deficient and when cultivars that exhibit an acceptable level of resistance to the pathogen are not available for commercial use.

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Fig. 6. Effect of rice growth stages and rates of silicon (Si) on dry weight (DW) of two inoculated plants. The equation for the response surface is $z = -1.16 + 14.64x - 4.56x^2 + 0.06604y$, $R^2 = 0.75$, where $z = DW$ (g), $x =$ rate of Si (g pot⁻¹), $y =$ plant age (days), and $R^2 =$ coefficient of determination. Dots connected by a vertical line represent the six replications from the pooled experiments.

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