



Silicon reduces brown spot severity and grain discoloration on several rice genotypes

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ABSTRACT

This study aimed to investigate the effect of silicon (Si) on the reduction of brown spot severity (BSS), caused by *Bipolaris oryzae*, and grain discoloration in several rice genotypes. An experiment was conducted in a greenhouse where eight genotypes were evaluated for their response to Si rates and decrease on BSS. The relationship between Si rates and BSS was linear negative. Additionally, a field experiment was conducted including forty-eight genotypes which were grown in upland conditions and evaluated for their response to Si rates and reduction on grain discoloration. The relationship between Si rates and grain discoloration was quadratic negative. Weight of filled grains per panicle increased as the Si rates in the soil increased. Genotypic differences for Si concentration in husk were evident for both non-amended and Si-amended plots. While the Si concentration in husk increased to all genotypes, there was no relationship between grain discoloration and Si concentration in husk for plants from non-amended and Si-amended plots. The genotype CAN-7024 with the highest resistance to leaf brown spot also showed the lowest grain discoloration in comparison to the genotypes Casado and Caqui.

Key words: *Bipolaris oryzae*, *Oryza sativa* L., fungi associated to seeds, plant nutrition.

Grain discoloration caused by various fungal and bacterial pathogens is considered one of the major rice disease worldwide (Ou, 1985). In order of importance, the major pathogenic fungi causing grain discoloration in rice are *Bipolaris oryzae* (Breda de Haan) Shoemaker, *Phoma sorghina* (Sacc) Boerema, Dorenbosch & Van Kesteren and *Gerlachia oryzae* (Hashioka & Yokogi) Samuels and Hallett (Ou, 1985). Other fungi of sporadic occurrence include *Alternaria padwickii* (Ganguly) Ellis, *Pyricularia grisea* (Herbert) Barr, *Curvularia* spp., *Nigrospora* sp., and *Fusarium* spp., especially in upland rice ecosystems (Soave et al., 1997). Even though most of the bacterial diseases of rice cause grain discoloration, *Pseudomonas glumae* and *Pantoea ananatis* are the pathogens that attack primarily the glumes (Goto et al., 1988; Yan et al., 2010).

Grain discoloration starts at heading and continues until maturity, and is generally most severe under humid conditions (Ou, 1985). Glume blight, caused by *P. sorghina*, and brown spot, caused by *B. oryzae*, are the major fungal pathogens that reduce grain quality and weight in Brazil (Prabhu & Lopes, 1980). Under epidemic years of glume blight, the reduction in grain weight can range from 29 to 45% and head milling yield from 0 to 14% (Prabhu

& Bedendo, 1988). *Bipolaris oryzae* also causes grain discoloration in the United States (Marchetti & Peterson, 1984). In Brazil, *B. oryzae* has been shown to cause from 12 to 30% reduction in grain weight and from 18 to 22% in filled grains per panicle, depending upon on the level of resistance of rice cultivars (Prabhu & Lopes, 1980). Brown spot occurs on grains when plants are grown in nutrient-deficient soils, particularly those having low levels of calcium, iron, magnesium, manganese, potassium, and silicon (Webster & Gunnell, 1992). Soils fertilized with both high and low nitrogen levels can also increase rice susceptibility to brown spot under upland conditions (Faria & Prabhu, 1983). A number of commercial rice cultivars are known to exhibit different levels of resistance to grain discoloration (Malavolta et al., 2007).

Even though breeding for disease resistance is the most economical means to control grain discoloration, little success has been obtained because there are a number of different pathogens that may cause it. Grain discoloration is, in general, more severe when heading coincides with continuous rains and, as a consequence, fungicide efficiency is not very efficacious. Therefore, other cultural practices such as plant nutrition

management is sought to reduce the disease impact on yield to acceptable levels.

Silicon (Si) fertilization has been reported to increase plant growth and development with a corresponding increase in grain yield (Savant et al., 1997). In upland rice, yields increased linearly with the application of increasing Si rates, and were positively correlated with Si and Ca levels in the soil (Barbosa Filho et al., 2001). In addition, Si fertilization has increased rice resistance to several important diseases, including brown spot (Datnoff et al., 2007). Datnoff et al. (1997) reported reductions in brown spot severity by 14 and 18%, respectively, in treatments with residual Si in the soil and those that received new Si application. This result demonstrated that one year Si residual could significantly suppress brown spot development. In West Africa, the application of 18.7 g of Si/m², as sodium metasilicate, doubled Si uptake by plants and significantly reduced grain husk discoloration in upland rice grown on highly weathered Ultisols (Winslow, 1992). In studies conducted in the greenhouse with four different Si deficient savanna soils, Si increased total grain weight while decreasing grain discoloration regardless of the soil class (Korndörfer et al., 1999).

Since a paucity of information exists about the response of Brazilian rice genotypes in accumulating Si and suppressing brown spot severity and grain discoloration, the purpose of the present study was to evaluate the effect of Si on brown spot development and grain discoloration in several rice genotypes under both greenhouse and field conditions.

For the greenhouse experiment, seeds of the rice genotypes BG 367-4, Caiapo, Caqui, Casado, Can-7024, Guarani, Metica-1 and Rio Paranaíba were sown in plastic trays (30 × 10 × 15 cm in size) containing 3 kg of perferic dark red latosol that was fertilized with 5 g of NPK (4-30-16), 1 g of zinc sulfate and 2 g of ammonium sulfate two days before sowing. An additional 2 g of ammonium sulfate was applied 20 days after seedlings emergence. The experiment was arranged in a split-plot design with four replications. The main plots corresponded to the Si rates of 0, 3, 6, 12 and 24 g per tray, which corresponded to 0, 486, 972, 1944 and 3888 kg/ha of Si, respectively. The eight genotypes were the sub-plots. Ten seeds of each genotype were sown in eight 10 cm long rows per tray. Fifty-five day old plants were inoculated with a conidial suspension of *B. oryzae* (6 × 10⁵ conidia/mL). This suspension was prepared by using a monospore isolate (CNPAF HO 82-1) of *B. oryzae*. The conidial suspension was sprayed onto rice leaves as a fine mist using a DeVilbiss N^o. 15 sprayer. The inoculated plants were kept in mist chamber for 48 hours (25°C and relative humidity of 90 ± 5%). After this period, plants were transferred to a greenhouse (temperature ranging from 25 to 29°C and relative humidity of 70 ± 5%). Brown spot severity (BSS) was evaluated on four leaves per plant at 10 days after inoculation by using a scale 1-9 (1 = 0%, 2 = less than 1%, 3 = 1-3%, 4 = 4-10%, 5 = 11-15%,

6 = 15-25%, 7 = 26-50%, 8 = 51-75% and 9 = 76-100%) based on the percentage of diseased leaf area (IRRI, 1996). Data analysis was performed by using ANOVA and GLM procedures of SAS (SAS Institute, Cray, NC). Means were compared by Tukey's test and regression and correlation analysis were done to determine the relationship between BSS and Si rates.

The field experiment was carried out in an experimental area with history of brown spot epidemics at EMBRAPA-Rice and Beans Research Center, Santo Antônio de Goiás city, Brazil, under upland conditions. The soil class in the experimental area was Si-deficient typic perferic dark-red latosol with plant-available Si of 10.2 mg dm⁻³. The chemical characteristics of the soil were: pH in H₂O (1:2.5) = 5.4; Ca²⁺ = 22 mmol_c dm⁻³; Mg²⁺ = 13 mmol_c dm⁻³; P = 1.3 mg dm⁻³ and K = 62.0 mg dm⁻³. The experimental design was a split-plot with three replications. Five Si rates (0, 188, 376, 564 and 752 kg/ha), using wollastonite (Wansil-10, CaSiO₃) as its source, were applied to the main plots. The wollastonite composition was: SiO₂ = 50% (Si = 24.2%); CaO = 42.1%, Al₂O₃ = 1.82%, MgO = 1.49%, Fe₂O₃ = 0.26%, Na₂O = 0.22% and MnO = 0.02%. Dolomitic lime (38.9% CaO and 12.7% MgO) was added to plots amended with 0, 188, 376, and 564 kg Si/ha to equilibrate the amount of Ca present in these treatments with the treatment containing 752 kg Si/ha before sowing. Forty-eight rice genotypes were sowed to the sub-plots. Each sub-plot consisted of 2.0 m long single row. Seeds were drill sowed with 0.40 m row spacing at the rate of 40 kg/ha. NPK fertilizer (4-30-10) was applied at sowing at the rate of 400 kg/ha in addition to 20 kg/ha of zinc sulfate. An additional 40 kg/ha of N, in the form of ammonium sulfate, was applied as top dressing 63 days after sowing.

Evaluation of grain discoloration was based on a sample of five panicles collected from each genotype 10 days before grain harvest. A modified visual rating diagrammatic scale (0, 25, 50, 75, and 100% of grain showing discoloration) developed by CIAT was used (CIAT, 1984) to assess grain discoloration (GD) on grains. Indeed, grain discoloration was evaluated by using a scale ranging from 0 to 4 where: 0 = no discoloration on grains, 1 = pin head spots on the grains, 2 = 25% of the area of grains with discoloration, and 4 = more than 50% of the area of grains with discoloration. Data was used to calculate disease severity index (DSI) from samples obtained from 200 grains by using the formula: $DSI = \frac{\sum (\text{class value} \times \text{frequency})}{\text{total number of grain} \times \text{the highest class value}}$.

The weight of filled grains (WFG) per panicle was based on a sample of 50 panicles per genotype. The grain weight was adjusted to 13% moisture. A bulked sample of 300 g of panicles collected from plants of non-amended and Si-amended (752 kg/ha) plots from the three replications was used for Si analysis on grain husk tissue. Concentration of Si in grain husk was determined according to the method described by Snyder et al. (1986).

Data analysis was performed by using ANOVA and GLM procedures of SAS (SAS Institute, Cary, NC). Data from DSI was transformed to $\arcsin\sqrt{x}$. Means were grouped according to Scott & Knott and regression and correlation analysis were performed with GD and WFG in function of the Si rates. Student's *t* test was applied at 5 or 1% level of probability to compare means of Si concentration in grain husk ($n = 48$) from panicles collected from plants of non-amended and Si-amended plots.

For the greenhouse experiment, only the factors Si rates rice and genotypes were significant. The relationship between BSS and Si rates was linear negative ($Y = 34.51 - 0.0021 x$, $R^2 = 0.91$). Casado and Caqui were the most susceptible genotypes to brown spot while the CAN-7024 was the least susceptible (Figure 1).

Soil analysis after rice harvest showed no significant increase in pH in H_2O ($0 = 5.5$, $188 \text{ kg/ha} = 5.5$, $376 \text{ kg/ha} = 5.6$, $564 \text{ kg/ha} = 5.5$ and $752 \text{ kg/ha} = 5.5$) and Ca ($0 = 22 \text{ mmol}_c \text{ dm}^{-3}$, $188 \text{ kg/ha} = 22 \text{ mmol}_c \text{ dm}^{-3}$, $376 \text{ kg/ha} = 24 \text{ mmol}_c \text{ dm}^{-3}$, $564 \text{ kg/ha} = 24 \text{ mmol}_c \text{ dm}^{-3}$ and $752 \text{ kg/ha} = 25 \text{ mmol}_c \text{ dm}^{-3}$), indicating no increase in Ca levels in the soil as the Si rates increased from 0 to 752 kg/ha.

Only the factors Si rates and rice genotypes were significant for GD and WFG and the factor rice genotypes for DSI (Table 1). Rice genotypes were significantly different for GD and DSI (Table 2). The DSI ranged from 0.81 for cultivar CNA 7420 to 2.64 for Pai-kan-tao. The upland rice genotypes, constituting one distinct group 'E', exhibited the lowest GD and DSI values and differed from the other four groups. The tall upland cultivars of medium duration, widely used as susceptible checks in routine disease resistance evaluations, such as Casado, Nenezão, Tongil, Mimoso and short duration ones such as Caqui, IAC 21, Cajueiro liso, T.S. Phoma pertaining to group 'B' showed the highest GD and DSI values. This result indicated that high and uniform disease pressure occurred in the field conditions. The semi-dwarf *indicas* IR 8, IR 36, Metica-1,

TKM 6, BG 90-2, BG 367-4 as well as the American long grain cultivars Lebonet and Dawn pertaining to the same group were also highly susceptible to grain discoloration under upland conditions. The rest of the *indica* and *japonica* rice genotypes pertaining to groups C and D showed moderate resistance to grain discoloration. Values for WFG varied from 0.10 to 2.22 g for the different genotypes (Table 2). The upland rice genotypes CNA 7024, Caiapo and Rio Paranaíba pertaining to group A had the greatest values for WFG. The lower values for WFG were observed for some irrigated genotypes such as IR-8, Lebonet, Chokoto, Kanto 51, IR-50 and Zenith pertaining to group I.

There were significant differences among genotypes for Si concentration in grain husks for plants from non-amended and Si-amended plots (Table 2). Si concentration in grain husks significantly increased at the rate of 752 kg/ha and this averaged for all genotypes from 0.9 to 1.48 dag/kg. The increase in Si concentration ranged from 17.3 to 135% for Veneza Roxo and Lebonet, respectively, for the Si-amended plots compared to the non-amended plot. While the Si concentration varied for the different genotypes, there was no relationship between DSI and Si concentration in the husk for both Si-amended ($r = -0.15$, $P = 0.05$) and non-amended treatments ($r = 0.029$, $P = 0.05$) (data not shown).

The relationship between GD and Si rates was quadratic negative ($Y = 63.62 + 0.0612 x - 0.0005 x^2$, $R^2 = 0.95$). The correlation between GD and DSI was positive and significant ($r = 0.69$, $P = 0.01$, $n = 720$). GD significantly reduced WFG ($r = -0.39$, $P = 0.01$, $n = 720$). The relationship between WFG and Si rates was positive and linear ($Y = 0.82 + 0.0004 x$, $R^2 = 0.97$).

Silicon was effective in decreasing BSS and GD for a number of rice genotypes. The decrease in BSS by the Si rates indicates that higher leaf Si concentration increased genotype's resistance to brown spot as previously reported by Dallagnol et al. (2009). Similar results also were

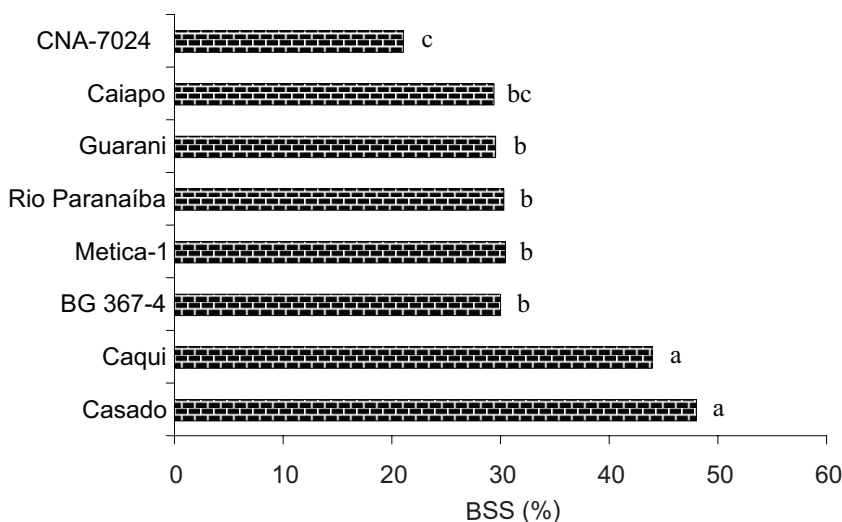


FIGURE 1 - Brown spot severity (BSS) on eight rice genotypes. Mean for each rice genotype was based on the average of five Si rates. Bars with the same letter do not differ significantly as determined by Tukey's test ($P = 0.05$).

TABLE 1 - Analysis of variance of the effect of silicon rates and rice genotypes on grain discoloration (GD), disease severity index (DSI) and weight of filled grains (WFG) per panicle

Sources of variation	df	Mean squares			F values ^a		
		GD	DSI	WFG	GD	DSI	WFG
Replications	2	4976.6	1534.8	31.1	9.0**	0.2 ^{ns}	3.0*
Silicon rates (Si)	4	7213.9	18543.7	202.7	12.9**	2.2 ^{ns}	19.7**
Error (a)	8	550.4	8364.8	10.3	-	-	-
Genotypes (G)	47	5481.6	21069.0	431.0	27.2**	17.4**	85.9**
Si x G	188	232.8	1358.4	4.5	1.2 ^{ns}	1.1 ^{ns}	0.9 ^{ns}
Error (b)	470	201.5	1222.0	5.0	-	-	-
Total	719						

^a * and ** = significant at 0.05 and 0.01 levels of probability, respectively. ^{ns} = non significant. - = not determined.

reported under field conditions in the US, where brown spot was significantly reduced by Si, especially in moderately susceptible and susceptible rice cultivars (Datnoff et al., 1991). The effect of genotype resistance to leaf brown spot and grain discoloration was found to be similar for some genotypes under both greenhouse and field conditions. Rice genotypes with the highest leaf brown spot resistance also had the lowest DSI. The genotype CAN-7024 had the lowest BSS and DSI in comparison to the genotypes Casado and Caqui.

In the current study, the mean Si concentration in the husk of the rice genotypes increased for the Si treatment with significant differences among the genotypes. This result suggests that genotypes probably differ in their ability to accumulate Si especially in the husk. While Si concentration for the different genotypes varied, there was no detectable correlation between Si concentration on husk and DSI among them. However, the relationship between Si rates and GD was quadratic with the lowest GD values obtained for the highest Si rate. In studies conducted in Africa, a significant negative and linear correlation was obtained between husk discoloration and Si concentration (Winslow, 1992; Winslow et al., 1997). The results in the present study suggest that while Si reduces grain discoloration, the differential response of the rice genotypes to GD was not evident because the highest Si rate utilized was limited to 752 kg/ha. It is possible that these genotypes might show a better response in suppressing GD at higher Si rates. According to Snyder et al. (1986), the rice tissue must contain at least 3% of Si for better growth and great yield. Others have reported a Si tissue concentration of 5% or greater for optimizing yield (Savant et al., 1997; Dallagnol et al., 2009). Si concentrations between 3 and 5% may be the minimum values needed for disease control in rice (Datnoff et al., 1997). The Si concentration in the rice husk of the genotypes appeared to be low and the differences in Si were specific to the type of plant tissue such as leaves or husks. In the present study, Si concentration in husk was much lower as compared to what has been reported for leaves, i. e. reaching up to 8 dag/kg (Dallagnol et al., 2009; Ma et al., 2002).

Besides Si concentration in husk, the genetically controlled genotypic resistance may also influence grain discoloration. This was found in the present study to which no detectable relationship was observed between Si concentration in husk and GD among the genotypes. Deren et al. (1994) demonstrated a significant and negative correlation between plant Si concentration and brown spot development among rice genotypes. Although there was a general trend across genotypes for decreasing brown spot development with increasing tissue Si concentration, there was one exception for the rice genotype Rico 1. Therefore, a genotype with a greater Si concentration may not necessarily be more disease resistant than a genotype that has a lower Si concentration when grown under the same Si fertility level (Kozaka, 1965). This result suggests that other genetic resistance factors are probably involved and similar findings were observed in the present study. Nevertheless, great genetic resistance and the ability to accumulate Si in the husk for the same genotype will be a viable measure to reduce GD. Reducing GD will also be reflected in a lower DSI as indicated by the positive correlation between these two variables. This resulted in yield increase as indicated by the negative correlation between GD and WFG. This finding is in agreement with studies conducted with upland rice genotypes in Colombia in relation to decrease grain discoloration caused mainly by *B. oryzae* (Winslow et al., 1997).

WFG varied significantly among the different genotypes. The upland rice genotypes showed the greatest values for WFG while the lowest values were observed for some irrigated genotypes which could be attributed to high grain sterility under upland conditions.

The mechanism(s) by which Si reduces grain discoloration has not been previously studied as with *M. grisea* or *B. oryzae* on leaves (Rodrigues et al., 2005; Brunings et al., 2009; Dallagnol et al., 2011). However, the overall decrease in grain discoloration of the rice genotypes with increasing Si rates possibly could be attributed to either the mechanical barrier formed after the polymerization of monosilicic acid below the cuticle or biochemical changes as previously reported for leaf brown spot (Savant et al.,

TABLE 2 - Grain discoloration (GD), disease severity index (DSI), weight of filled grains (WFG) per panicle and silicon (Si) concentration in husk for several rice genotypes grown in soil non-amended (0) or amended (752 kg/ha) with Si

Genotypes ^a	GD ^b	DSI ^b	WFG per panicle (g)	Si (dag/kg) ^c	
				0	752 kg/ha
Pai-kan-tao ^d	63.2 a	2.64 a	0.65 g	0.99 c	1.42 c
Caqui ^e	59.2 b	2.15 b	1.43 c	1.04 b	1.53 b
Casado ^e	58.2 b	2.04 b	1.29 d	0.96 c	1.48 c
IR 8 ^d	56.6 b	2.01 b	0.11 i	0.69 c	1.24 c
IR 36 ^d	55.2 b	1.98 b	0.56 g	0.65 c	1.06 c
Metica 1 ^d	55.6 b	1.98 b	0.93 f	0.85 c	1.06 c
Nenezão ^e	55.8 b	1.98 b	1.33 d	0.90 c	1.42 c
Tongil ^d	55.7 b	1.96 b	1.13 e	0.86 c	1.60 b
TKM 6 ^d	54.2 b	1.95 b	0.41 h	0.74 c	1.14 c
Mimoso ^e	51.2 b	1.88 b	1.13 e	1.03 b	1.25 c
Ram Tulasi ^d	51.2 b	1.88 b	1.01 e	1.14 b	1.69 b
BG 90-2 ^d	51.2 b	1.88 b	0.68 g	0.73 c	1.11 c
Lebonet ^d	51.4 b	1.87 b	0.17 i	0.77 c	1.81 b
Dawn ^d	51.7 b	1.86 b	0.40 h	0.62 c	1.24 c
BG 367-4 ^d	51.7 b	1.86 b	0.87 f	0.63 c	1.21 c
Chokoto ^d	51.8 b	1.84 b	0.23 i	0.94 c	1.22 c
Cajueiro Liso ^e	52.1 b	1.84 b	1.04 e	0.84 c	1.75 b
T. S. Phoma ^e	52.8 b	1.84 b	0.99 e	1.60 a	2.88 a
Kanto 51 ^d	49.7 c	1.79 c	0.19 i	0.73 c	1.18 c
Veneza Roxo ^e	49.2 c	1.79 c	1.05 e	1.44 a	1.69 b
IR 50 ^d	48.9 c	1.77 c	0.10 i	0.77 c	1.34 c
Branco Três Meses ^e	47.2 c	1.76 c	1.21 d	0.88 c	1.73 b
Três Marias ^d	47.5 c	1.76 c	1.32 d	1.10 b	1.83 b
Colômbia 1 ^d	46.9 c	1.75 c	0.75 g	0.75 c	1.22 c
IAC 21 ^e	45.8 c	1.75 c	1.11 e	0.93 c	1.63 b
Labelle ^d	45.2 c	1.71 c	0.44 h	0.87 c	1.29 c
IRI 342 ^d	46.9 c	1.71 c	0.86 f	0.87 c	1.71 b
IAC 165 ^e	47.3 c	1.68 c	0.37 h	0.73 c	1.63 b
IRAT 127 ^e	47.9 c	1.67 c	0.68 g	0.88 c	1.38 c
Shin 2 ^d	46.8 c	1.59 c	1.01 e	0.76 c	1.23 c
Ceysovoni ^d	42.4 d	1.52 d	0.52 h	0.90 c	1.30 c
IR 24 ^d	41.6 d	1.50 d	0.72 g	0.94 c	1.35 c
CNA 7449 ^e	40.7 d	1.48 d	1.66 b	0.85 c	1.58 b
Zenith ^d	40.4 d	1.45 d	0.28 i	0.58 c	1.00 c
IRAT 140 ^e	39.9 d	1.41 d	0.95 f	0.74 c	1.37 c
IRI 344 ^d	39.2 d	1.41 d	1.03 e	0.88 c	1.63 b
Arroz de Guerra ^e	38.7 d	1.41 d	1.27 d	0.84 c	1.18 c
Myliang 30 ^d	38.4 d	1.40 d	0.44 h	0.77 c	1.47 c
IRAT 104 ^e	37.2 d	1.34 d	1.82 b	0.83 c	1.12 c
Guarani ^e	36.4 d	1.23 d	0.78 f	1.21 b	2.14 b
Pérola ^e	35.6 d	1.18 d	1.62 b	0.84 c	1.55 b
Carajás ^e	26.7 e	1.12 e	0.83 f	1.09 b	1.69 b
Rio Paranaíba ^e	27.8 e	1.05 e	2.02 a	1.18 b	1.81 b
IAC 47 ^e	27.9 e	1.01 e	1.48 c	1.17 b	1.42 c
Araguaia ^e	26.7 e	0.99 e	1.75 b	1.04 b	2.09 b
Iguape Redondo ^e	25.4 e	0.92 e	1.23 d	0.78 c	1.53 b
Caiapó ^e	24.6 e	0.91 e	2.11 a	0.97 c	1.55 b
CNA-7024 ^e	25.6 e	0.81 e	2.22 a	0.96 c	1.48 c

^a Means for rice genotypes followed by different letters within each column are significantly different based on the Scott & Knott test ($P = 0.05$).

^b GD and DSI= values represent mean of the five Si rates.

^c Mean Si concentration in husk between non-amended (0.90 ± 0.28) and Si-amended (752 kg/ha) (1.48 ± 0.41) treatments differ by Student's t test ($t = 20.44$, $P = 0.01$).

^{d, e} Irrigated and upland rice genotypes, respectively.

1997; Dallagnol et al., 2011). The impact of Si to reduce grain discoloration may be of utmost importance to rice growers in Brazil since current commercial rice cultivars exhibiting satisfactory level of resistance to this disease are not available and fungicide management may be erratic at best.

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