



Rice grain resistance to brown spot and yield are increased by silicon

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ABSTRACT

Brown spot, caused by *Bipolaris oryzae*, is one of the most important diseases of rice and can cause a reduction in yield and grain quality. The effect of silicon (Si) on the resistance of rice grains to brown spot was investigated. Plants from cv. Oochikara and its mutant, defective in the *Lsi1* transporter (*lsi1* mutant), were grown in hydroponic culture either with Si (+Si; 2 mM) or without Si (-Si). Panicle inoculation with *B. oryzae* was carried out at the beginning of the milk-grain stage. Panicles were harvested at physiological grain maturity. The supply of Si significantly increased Si concentration in husks compared to -Si plants. Si concentration in husks from cv. Oochikara was up to three times greater than the *lsi1* mutant. In the presence of Si, brown spot severity was reduced by 88% in grains from cv. Oochikara and by 53% in grains from *lsi1* mutant. Brown spot severity was 77% lower for grains of cv. Oochikara than for the *lsi1* mutant, both plant types were grown in the presence of Si. Panicle inoculation reduced significantly the following yield components: number of grains per panicle, the weight of 1000 grains and the percentage of filled grains. Si significantly increased these yield components, especially for inoculated panicles. Considering kernel quality, the panicle inoculation with *B. oryzae* significantly reduced the yield of husked kernel, yield of whole kernel and kernel diameter, especially for grains from -Si plants. For panicles from +Si plants, the kernel quality was improved under inoculation, compared to -Si plants. Results from this study show that Si improved rice yield and kernel quality in panicles inoculated with *B. oryzae*. Furthermore the functional *Lsi1* gene contributed significantly for increasing the yield of whole kernel and kernel diameter, possibly due to the increasing Si concentration in husks.

Key words: *Bipolaris oryzae*, *Oryza sativa*, kernel quality, *Lsi1* gene, rice yield.

INTRODUCTION

Rice (*Oryza sativa* L.) is the main source of food for approximately half of the world's population (Maclean et al., 2002). For rice consumers, whole grains free from defects are preferred and this factor determines the price that growers will receive (Soave et al., 1984). Grain quality is affected by several abiotic and biotic factors (Ou, 1985). The biotic damage is related to fungal and bacterial incidence and injuries caused by insects (Marchetti et al., 1984). The fungus *Bipolaris oryzae* (Breda de Haan) Shoemaker, the causal agent of brown spot of rice, reduces yield and negatively impacts grain quality (Ou, 1985).

Leaf infection by *B. oryzae* did not significantly affect panicle number per plant or the number of spikelets per panicle, but caused a decline in yield by increasing the number of empty grains, reducing the number of grains per panicle and grain weight (Aluko, 1975; Prabhu et al., 1980). While the relation between *B. oryzae* infection on leaf and grain is controversial (Marchetti et al., 1984; Ba & Sangchote, 2006), the negative impact of grain infection causing discoloration and reduction in milling

quality is well documented (Marchetti et al., 1984; Soave et al., 1984). In Brazil, *B. oryzae* is one of the most common rice grain pathogens (Soave et al., 1984), which can infect the husks and even the inside area of the kernel (Ou, 1985). Brown spot symptoms are more commonly observed on leaves and glumes (grain husks), but sometimes may also appear on the coleoptiles, sheaths and panicle branches (Ou, 1985). The characteristic symptoms of brown spot on leaves and glumes include light reddish-brown lesions or lesions with a gray center surrounded by a dark to reddish-brown margin and then by a bright yellow halo (Dallagnol et al., 2009).

Currently, the major management strategies available for brown spot include the use of rice cultivars partially resistant, appropriate plant nutrition and fungicide application (Reunião Técnica da Cultura do Arroz Irrigado, 2012). Previously, the addition of silicon (Si) to the soil has been reported to provide a viable alternative to reduce brown spot severity (Nanda & Gangopadhyay, 1984; Datnoff et al., 1991; 1992; 1997; 2007; Dallagnol et al., 2009). The addition of Si to rice plants has also reduced other important diseases, such as blast, grain discoloration, leaf blight, leaf

scald, sheath blight and stem rot (Korndörfer et al., 1999; Datnoff et al., 2007). Seebold et al. (2000) reported that applying 1000 kg ha⁻¹ of Si increased rice yield up to 42% and reduced grain discoloration up to 70%.

Rice accumulates Si in the shoots in concentrations several times greater than macronutrients such as nitrogen, phosphorus and potassium (Datnoff et al., 2007). Rice requires large concentrations of Si readily available in the root zone for vigorous growth and high yield (Ma et al., 2002). The high Si accumulation in rice shoots is caused by an active uptake system that utilizes the proteins involved in Si influx from the soil solution to the cytoplasm of the root cells (*Lsi1*), efflux from the cell cytoplasm to the xylem (*Lsi2*) and the subsequent distribution of Si in the rice shoots (*Lsi6*) (Ma et al., 2008; Yamaji et al., 2009). Mutation in the *Lsi1* gene of cv. Oochikara resulted in a mutant plant named low silicon rice 1 (*Lsi1*) that uptakes Si only through passive transport (Ma et al., 2002). Plant with the *Lsi1* mutation, in addition to expressing a significant reduction in yield under field conditions (Ma et al., 2002), also have the defense against brown spot development on the leaves compromised, suggesting that a minimum Si concentration is necessary to achieve an acceptable level of resistance against this disease (Dallagnol et al., 2009; 2011). Ma et al. (2006) reported that the Si uptake by the *Lsi1* mutant was significantly reduced compared to that of wild plants, resulting in grain production of one-tenth of that of wild-type rice and with a higher disease severity on the husks.

This study aimed to demonstrate the contribution of the *Lsi1* gene to increase rice grain resistance to brown spot and for improving yield and kernel quality under greenhouse conditions.

MATERIALS AND METHODS

Nutrient solution preparation

The nutrient solution used for the hydroponic growth of rice plants was prepared based on Hoagland & Arnold (1950) with some modifications, and included the following macronutrients: 1 mM KNO₃, 0.25 mM NH₄H₂PO₄, 0.1 mM NH₄Cl, 0.5 mM MgSO₄·7H₂O and 1 mM Ca(NO₃)₂·4H₂O; and the following micronutrients: 0.3 μM CuSO₄·5H₂O, 0.3 μM ZnSO₄·7H₂O, 11.5 μM H₃BO₃, 3.5 μM MnCl₂·4H₂O, 0.1 μM (NH₄)₆Mo₇O₂₄·4H₂O, 25 μM FeSO₄·7H₂O and 25 μM Bisodic EDTA. Si was supplied to the plants in the form of silicic acid, prepared by passing potassium silicate through a cation-exchange resin (Amberlite IR-120B, H⁺ form) (Sigma-Aldrich). The Si rates used were 0 or 2 mM. The pH of the nutrient solution was 5.5 and was not affected by the addition of the silicic acid.

Plant growth

Rice seeds from cv. Oochikara and the *Lsi1* mutant were surface sterilized in 10% (v/v) NaOCl for 1.5 min, rinsed in sterilized water for 3 min, and germinated on distilled-water-soaked germitest paper (Fisher Scientific)

in a germination chamber (MA-835/2106UR, Marconi) at 25°C and 12 h photoperiod (≈125 μmol photons m⁻²s⁻¹) for six days. The germinated seedlings were transferred to plastic pots (0.5 L) and were amended with a one-half strength nutrient solution without Si. After two days, the young seedlings were transferred to new plastic pots (30-cm diameter) containing 5 L of nutrient solution with or without Si. Six rice plants were grown in each pot. Every four days, the non-aerated nutrient solution was changed for new solution either with or without Si. The electrical conductivity and the pH of the nutrient solution were checked daily. The pH was maintained at approximately 5.5 using NaOH or HCl (1 M) as needed. The plants were grown in a greenhouse (relative humidity of 60±5%, temperature of 30±5°C) during the experiments. The relative humidity and temperature were measured using a thermo-hygrograph (TH-508, Impac). The photon flux density on sunny days inside the greenhouse (at midday), quantified with a Li 250A light meter (Li-Cor Environmental), was approximately 900 μmol photons m⁻²s⁻¹.

Inoculation procedure

An isolate of *B. oryzae* (CNPAF-HO 82; Embrapa Arroz e Feijão, Santo Antônio de Goiás, GO, Brazil) obtained from symptomatic rice plants was preserved on pieces of filter paper in glass vials with silica gel at 4°C. Pieces of filter paper containing the fungus were transferred to Petri dishes containing potato-dextrose-agar (PDA). After three days, PDA plugs (0.5 cm²) containing fungal mycelia were transferred to new Petri dishes. Petri dishes were maintained in a growth chamber at 25°C with a 12 h photoperiod for 10 days. To obtain conidial suspension, 10 mL of sterilized water was added to Petri dishes containing fungal conidia, mixed using a Drigalski handle and then filtered through cotton gauze to remove mycelia. The suspension was adjusted to 5×10³ conidia mL⁻¹ using a hemocytometer and was applied as a fine mist to the panicles of each plant at the beginning of the milk-grain stage (IRRI, 1996) until runoff using a VL Airbrush atomizer (Paasche Airbrush Co.). Gelatin (1%, w/v) was added to the sterile water before inoculum preparation to aid conidial adhesion to the panicles. Only the panicles were inoculated with *B. oryzae*, while the leaves were protected with plastic during application of the conidial suspension. Immediately after inoculation, the plants were transferred to a mist chamber maintained at 25±2°C and 85±5% relative humidity, measured as described above. The plants with inoculated and non-inoculated panicles were exposed to an initial 24 h dark period, followed by 48 h of 12 h photoperiods (≈15 μmol photons m⁻²s⁻¹) provided by cool-white fluorescent lamps. The plants with non-inoculated and inoculated panicles were kept in separate mist chambers but were exposed to the same conditions. After 72 h, the plants were transferred to a greenhouse with the environmental conditions described above for the duration of the experiment.

Experimental design

Two 2×2×2 factorial experiments, consisting of the two Si rates (0 or 2 mM, referred to as -Si and +Si treatments, respectively), the plant types (cv. Oochikara and *lsi1* mutant), and the non-inoculated and inoculated panicles from both plant type, were arranged in a completely randomized design with three replications. Each experimental unit consisted of one plastic pot containing six rice plants. A total of 18 plants were used for each treatment and the number of panicles per plant ranged from four to six. The experiment was carried out twice.

Evaluations

At the stage of physiological maturity (IRRI, 1996), 40 days after inoculation, all inoculated and non-inoculated panicles were hand-harvested. At least 30 panicles for each replication of each treatment were used for the evaluations. Grains from the panicles of each plant were used to evaluate the severity of brown spot on grains, yield components, grain quality and the Si concentration in the husks.

Disease evaluation and calculation of brown spot index (BSI)

Brown spot severity on the grains of each panicle per plant was scored using a 0-7 scale, modified from IRRI (1996) as follows: 0 = no disease symptoms, 1 = less than 1%, 2 = from 1.1 to 5%, 3 = from 5.1 to 10%, 4 = from 10.1 to 25%, 5 = from 25.1 to 50%, 6 = 50.1 to 75%, and 7 = more than 75% of the grains' surface with disease symptoms. Data for brown spot severity were used to calculate the BSI based on the formula proposed by McKinney (1923) where: $BSI = [\sum (\text{rate of the disease scale} \times \text{number of grains receiving that rate}) / (\text{total number of grains} \times \text{the highest rate of the disease scale})] \times 100$.

Yield components

On each panicle, the total number of grains and the percentage of filled grains were determined. A grain was considered filled only if the endosperm was present. The filled and non-filled grains from all the plants in each replicate were used to determine the weight of 1000 grains of rice. To standardize nomenclature, the term grain was used for paddy rice and kernel for grains without husks.

Kernel quality measurements

Kernel quality was determined after stripping and burnishing the kernel (Houston, 1972). For the purpose of stripping, samples of 100 g of grain were processed in a mill for 1 min. Next, the kernel were polished and weighed. The weight values of husked kernel (broken and whole kernel) were considered to be the yield of husked kernel (YHK). Subsequently, the polished kernel were placed in a separator and processed for 30 sec in order to separate broken kernel from whole kernel. The remaining kernels in the sieve were weighed to obtain the weight of whole kernel, expressed in percentage as yield of whole kernel (YWK). Samples (≈ 100

kernels) of the whole kernel were used to obtain kernel diameter (in mm) using a digital caliper.

Concentration of Si in husks

The grain husks were collected at the end of the stripping process, dried at 65°C for 72 h and ground with a Thomas-Wiley mill (Thomas Scientific) to pass through a 40-mesh screen. The Si concentration in the husks was determined by a colorimetric analysis on 0.1 g of dried and alkali-digested tissue (Korndörfer et al., 2004).

Data analysis

Cochran's test for homogeneity of variance indicated that the data from the two experiments could be pooled, so the data were pooled for analysis as a single six replications experiment. The experiment-treatment interactions were not significant ($P \geq 0.05$) when compared to the treatments main effects. The data were analyzed by an analysis of variance (ANOVA), significant interactions were sliced and the treatment mean comparisons were analyzed by F-test using SAS version 8.0 (SAS Institute).

RESULTS

Concentration of Si in husks

The factors Si rate and plant type as well as the interaction Si rate × plant type were significant (Table 1). Si concentration was significantly greater by 460 and 175%, respectively, for the husks of grains from cv. Oochikara and the *lsi1* mutant plants supplied with Si as compared to husks of grains from -Si plants (Table 2). Husks from grains of cv. Oochikara contained significantly greater (238% for +Si plants and 66% for -Si plants) Si concentration as compared to the *lsi1* mutant.

Brown spot index (BSI)

For the BSI index, the factor Si rate, plant type and the interaction Si rate × plant type were significant (data not showed). In the presence of Si, the BSI was significantly reduced by 88% and 53%, respectively, for the cv. Oochikara and *lsi1* mutant grain compared with the -Si treatment (Table 2). For the cv. Oochikara, the BSI was significantly reduced by 77% and 15% for +Si and -Si treatment, respectively, compared to the *lsi1* mutant.

Yield components

The factors Si rate and panicle inoculation were significant for the number of grains per panicle, weight of 1000 grains, and percentage of filled grain (Table 1). The interaction of Si rate and panicle inoculation was significant for the percentage of filled grain (Table 1). Si supply significantly increased by 22% and 12%, respectively, the number of grains per panicle and the weight of 1000 grains, compared to -Si plants (Table 3). On panicles inoculated with *B. oryzae*, the number of grains per panicle and the weight of 1000 grains were significantly reduced by 6.5% and 19%,

TABLE 1 - Analysis of variance for the effects of silicon rates (Sir), plant types (PT), and panicle inoculation (PI) on silicon (Si) concentration in the husks, number of grains per panicle (NGP), weight of 1000 grains (1000GW), filled grains (FG), yield of husked kernel (YHK), yield of whole kernel (YWK) and kernel diameter (KD).

Source of variation	d.f.	F values						
		Si	NGP	1000 GW	FG	YHK	YWK	KD
Sir	1	2,241.49*	180.13*	12.79*	51.59*	4.85*	47.28*	134.39*
PT	1	1,318.49*	2.38	1.01	0.82	1.07	11.48*	9.00*
PI	1	0.20	12.11*	43.90*	63.07*	14.00*	91.22*	50.78*
PT × PI	1	0.23	0.02	0.04	1.27	0.09	0.50	0.69
Sir × PI	1	3.20	0.06	0.40	16.96*	0.77	7.63*	46.04*
PT × Sir	1	884.45*	0.65	0.00	0.71	0.01	14.58*	17.56*
PT × Sir × PI	1	1.18	0.05	0.87	0.61	0.09	1.02	2.03
Error	40	-	-	-	-	-	-	-

* = significant at $p < 0.05$.**TABLE 2** - Silicon concentration (Si) and brown spot index (BSI) in the husk of rice grains from plants of cultivar Oochikara and *lsi1* mutant grown in hydroponic culture amended with 0 (-Si) or 2 (+Si) mM of silicon and subjected or not to panicle inoculation with *Bipolaris oryzae*.

Plant type	Si (g kg ⁻¹)			BSI		
	-Si	+Si	P	-Si	+Si	P
Oochikara	5.3	29.7	< 0.05	65.5	8.1	< 0.05
<i>lsi1</i> mutant	3.2	8.8	< 0.05	77.0	35.6	< 0.05
P	< 0.05	< 0.05		< 0.05	< 0.05	

The factor interaction effects were sliced and means compared by *F*-test. Data showed are from two pooled experiments.

respectively, compared to non-inoculated panicles (Table 3). The percentage of filled grains was significantly reduced by 28% and 8%, respectively, for inoculated panicles from -Si and +Si plants, compared to non-inoculated panicles (Table 4). The percentage of filled grains was significantly higher by 37% and 7%, respectively, for inoculated and non-inoculated panicles from +Si plants compared to panicles from -Si plants (Table 4).

Kernel quality measurements

The factors Si rate and panicles inoculation were significant for the yield of husked kernel (Table 1). For the yield of whole kernel and kernel diameter, the factors Si rate, plant type, and panicle inoculation and the interactions Si rate × plant type and Si rate × panicle inoculation were significant (Table 1). Yield of husked kernel increased significantly by 6% for +Si plants as compared to -Si plants (Table 5). Panicle inoculation reduced significantly by 7% the yield of husked kernel compared to non-inoculated panicles (Table 5). Yield of whole kernel significantly increased by 11% and 6% for cv. Oochikara and *lsi1* mutant plants supplied with Si as compared to -Si plants (Table 6). The yield of whole kernel for +Si cv. Oochikara was 8% higher than for +Si *lsi1* mutant, while there were not significant differences between plant types in the absence of Si. Panicle inoculation reduced by 10% and 9%, respectively, the yield of whole kernel for -Si and +Si plants compared to non-inoculated panicles (Table 6). Si supplied to rice plants increased the yield of whole kernel by 8 and 7%, respectively, for inoculated and

non-inoculated panicles compared to -Si plants (Table 6). Kernel diameter significantly increased by 7 and 11% for cv. Oochikara and *lsi1* mutant plants supplied with Si as compared to kernel from -Si plants (Table 7). Considering plant type, -Si cv. Oochikara produced kernel 6% larger in diameter compared to -Si *lsi1* mutant. On the other hand, there was no significant difference between plant types in the presence of Si (Table 7). Kernel diameter significantly increased by 15% and 4%, respectively, for +Si inoculated and +Si non-inoculated plants compared to kernel from -Si plants (Table 7). Panicle inoculation significantly reduced the kernel diameter by 10% for -Si plants compared to panicles from non-inoculated plants (Table 7). On the other hand, for the +Si plants there was no significant effect of panicle inoculation on the kernel diameter.

DISCUSSION

This study reports the influence of Si on the increase of rice grain resistance to brown spot and the beneficial effect of this element on rice yield and kernel quality. Additionally, this study reports that the functional *Lsi1* gene favored an increase in Si concentration in the husks. The impairment in the Si transporter for *lsi1* mutant reduced the Si concentration in the husks up to three times compared with the cv. Oochikara (Table 2), even when this element was available for plant uptake. According to Tamai & Ma (2008), the rice *lsi1* mutant was only affected in Si uptake capacity and the mutation on the *Lsi1* gene was related to

TABLE 3 - Number of grains per panicle (NGP) and 1000 grains weight (1000 GW), for rice grains from plants of cultivar Oochikara and *lsi1* mutant grown in hydroponic culture amended with 0 (-Si) or 2 (+Si) mM of silicon and subjected or not to panicle inoculation with *Bipolaris oryzae*.

Si rates	NGP	1000 GW (g)
-Si	76.5	32.7
+Si	93.7	36.5
<i>P</i>	< 0.05	< 0.05
Panicle inoculation		
Inoculated	80.1	31.1
Non-inoculated	85.7	38.1
<i>P</i>	< 0.05	< 0.05

The factor interaction effects were sliced and means compared by *F*-test. Data showed are from two pooled experiments.

TABLE 4 - Percentage of filled grains (FG) in rice panicles from plants of cultivar Oochikara and *lsi1* mutant grown in hydroponic culture amended with 0 (-Si) or 2 (+Si) mM of silicon and subjected or not to panicle inoculation with *Bipolaris oryzae*.

Si rates	FG (%)		<i>P</i>
	Inoculated	Non-inoculated	
-Si	53.4	74.5	< 0.05
+Si	73.1	79.8	< 0.05
<i>P</i>	< 0.05	< 0.05	

The factor interaction effects were sliced and means compared by *F*-test. Data showed are from two pooled experiments.

TABLE 5 - Yield of husked kernel (YHK) for rice grains from plants of cultivar Oochikara and *lsi1* mutant grown in hydroponic culture amended with 0 (-Si) or 2 (+Si) mM of silicon and subjected or not to panicle inoculation with *Bipolaris oryzae*.

Si rates	YHK (%)
-Si	73.6
+Si	77.8
<i>P</i>	< 0.05
Panicle inoculation	
Inoculated	73.5
Non-inoculated	79.0
<i>P</i>	< 0.05

The factor interaction effects were sliced and means compared by *F*-test. Data showed are from two pooled experiments.

reduction in the grain yield under field conditions. Results from the present study indicated that the reduction of Si concentration in the husks of grains from the *lsi1* mutant reduced grain's resistance to brown spot and also the yield of whole kernel from inoculated panicles (Tables 2 and 6).

The importance of the *Lsi1* gene in increasing brown spot resistance in the grains by Si uptake was indicated by the greater reduction in BSI for grains from cv. Oochikara with Si than that found for the *lsi1* mutant. Although not determinate in this study, the reduction on the BSI may be

associated to the state of priming (anticipation and increase in plant defense under pathogen attack) caused by the presence of soluble Si in the plant tissue as previously proposed (Gareeb et al., 2011; Dallagnol et al., 2012; Van Bockhaven et al., 2012). The earlier activation of plant defenses by Si against *B. oryzae* infection on rice leaves was previously reported by Dallagnol et al. (2011) and involved higher activity of chitinases and peroxidases, greater concentration of phenolic compounds and lignin, lower lipid peroxidation and less electrolyte leakage. Additionally to priming plant defenses, the higher concentration of Si beneath the cuticle may avoid or delay the delivery of pathogenicity and aggressiveness factors into the plant cell by the pathogens as pointed out by Bélanger et al. (2012), thus contributing to the reduction on host tissue colonization by the pathogen and could also explain the lower BSI on plants from cv. Oochikara supplied with Si.

The lower BSI in the glumes of the plants supplied with Si, mainly for the cv. Oochikara, compared with the plants without Si resulted in a higher yield and better kernel quality after milling (Tables 3, 6 and 7). Even though no significant differences were found between cv. Oochikara and *lsi1* mutant for the majority of the grain variables evaluated, regardless of Si supply, in a previous study Tamai & Ma (2008) reported that under field conditions where the plants were exposed to typhoons and the occurrence of disease on leaves, plants of *lsi1* mutant showed significant reduction in the number of grains and in the percentage of filled grains. The absence of differences between plant types for the grain yield components in the present study may be associated to the less stressful environment under greenhouse in which only pathogen inoculation on panicles was the stress that the plants were exposed. Furthermore, no disease symptoms were observed on leaves because they were protected during panicle inoculation. According to Tamai & Ma (2008), the beneficial effects of Si might have been underestimated under greenhouse conditions due to a less stressful environment as compared to experiments carried out under field conditions.

Although no significant differences were found between the *lsi1* mutant and cv. Oochikara for the number of grains per panicle, the weight of 1000 grains and the percentage of filled grains, both plant types supplied with Si showed an increase in these yield components (Tables 3, 4, 5, 6 and 7). This indicates that increasing Si concentration in the shoots and husks may improve rice yield when plants are grown in a stress-free environment. On the other hand, the number of grains per panicle was negatively affected on panicles inoculated with *B. oryzae* regardless of plant types (Table 3). This result indicates that infection by *B. oryzae* during milk-grain stage can reduce the number of grains per panicle, contradicting the previous studies that also reported reduction in the number of grains per panicle due to *B. oryzae* infection during panicle development, but only when infection occurred before the grain-filling stage (Aluko, 1975; Prabhu et al., 1980). However, Si supplied to

TABLE 6 - Yield of whole kernel (YWK) of rice grains from plants of cultivar Oochikara and *lsi1* mutant grown in hydroponic culture amended with 0 (-Si) or 2 (+Si) mM of silicon and subjected or not to panicle inoculation with *Bipolaris oryzae*.

Si rates	YWK (%)			YWK (%)		
	Oochikara	<i>lsi1</i> mutant	<i>P</i>	Inoculated	Non-inoculated	<i>P</i>
-Si	55.1	53.9	0.623	52.0	58.0	< 0.05
+Si	61.4	57.0	< 0.05	56.3	62.1	< 0.05
<i>P</i>	< 0.05	< 0.05		< 0.05	< 0.05	

The factor interaction effects were sliced and means compared by *F*-test. Data showed are from two pooled experiments.

TABLE 7 - Kernel diameter (KD) of rice grains from plants of cultivar Oochikara and *lsi1* mutant grown in hydroponic culture amended with 0 (-Si) or 2 (+Si) mM of silicon and subjected or not to panicle inoculation with *Bipolaris oryzae*.

Si rates	KD (mm)			KD (mm)		
	Oochikara	<i>lsi1</i> mutant	<i>P</i>	Inoculated	Non-inoculated	<i>P</i>
-Si	2.61	2.47	0.001	2.40	2.67	< 0.05
+Si	2.78	2.75	0.401	2.76	2.77	0.809
<i>P</i>	< 0.05	< 0.05		< 0.05	< 0.05	

The factor interaction effects were sliced and means compared by *F*-test. Data showed are from two pooled experiments.

the rice plants may provide a viable alternative to reduce the damage caused by *B. oryzae* on panicles and, consequently, on the number of grains produced per panicle.

Although there was a significant increase on the number of grains per panicle on the Si+ plants, the weight of 1000 grains and the percentage of filled grains did not improve as much when compared to the Si- plants (Tables 3 and 4). That may be due to the absence of leaf symptoms or to the lower number of grains per panicle in the -Si plants, which resulted in a reduced number of photoassimilate sinks, thus increasing grain weight for the -Si plants. However, in the inoculated panicles of the cv. Oochikara and *lsi1* mutant, especially on plants without Si which showed the highest BSI values, a reduction of grain weight and of the percentage of filled grains occurred, probably because of a disruption in the translocation of photoassimilates to the developing grain. Husk infection affected grains weight and the percentage of filled grains due to loss of sink function. Prabhu et al. (1980) reported that the variation in grain weight was mainly explained by grain husk infection by *B. oryzae*, with a negative linear relationship between brown spot severity in the grains and the percentage of filled grains. Thus, the lower values for BSI in the grains from panicles from plants supplied with Si, especially of the cv. Oochikara, had less of an impact on grain weight and the percentage of filled grains as compared with the grain obtained from the panicles of the plants without Si.

The increase in Si concentration was also important for the production of grains with better quality, characterized by yield of both husked and whole kernel, after the milling process (Tables 5 and 6). The yield of whole kernel obtained after grinding and sieving is one of the most important variables related to value in rice marketing (Oliveira et al., 1998). Well-filled rice grains are more resistant to shock and vibration occurring during the

harvest and the milling process, resulting in commercial grain of the highest quality (Castro et al., 1999). Mechanical damage to the grains, affecting the yield of intact kernel, occurs from the indirect action of several biotic and abiotic stresses, such as the lack or the excess of rain, insects and diseases (Castro et al., 1999). Diseases, such as brown spot, affect rice quality by causing grain discoloration and affecting grain filling and ripening, therefore accelerating the drying process and resulting in a greater incidence of cracked grains. The functional *Lsi1* gene for cv. Oochikara increased the concentration of Si in the husks, thus favoring an increase in whole kernel yield and grain diameter by reducing disease severity and transpiration, while keeping high moisture content within the husk that is important for normal grain development (Seo & Ota, 1982). As husks do not have stomata, the transpiration occurs through the cuticle and the double layer of cuticle-silica beneath the cuticle prevents excessive transpiration reducing it by 30 to 40% (Yoshida, 1965; Ma & Takahashi, 2002), allowing a normal and continuous process for grain filling.

In conclusion, the importance of Si for increasing the production of high quality grains under stressful biotic conditions was demonstrated by growing plants either without Si or using the *lsi1* mutant. Clearly increasing the concentration of Si in the rice husks may be a strategy to reduce brown spot while achieving better yield and grain quality. The *Lsi1* gene played a pivotal role to increase the whole kernel yield under *B. oryzae* infection.

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