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Photosynthesis and sugar concentration are impaired by the defective active silicon uptake in rice plants infected with *Bipolaris oryzae*

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The effect of soluble silicon (Si) on photosynthetic parameters and soluble sugar concentrations was determined in leaves of rice cv. Oochikara and mutant plants of Oochikara defective in active Si uptake [*low silicon 1* (*lsi1*)]. Plants were grown in hydroponic culture amended with 0 (−Si) or 2 mM Si (+Si), under either low or high photon flux density (PFD) and with or without inoculation with *Bipolaris oryzae*, the causal agent of brown spot of rice. Leaf Si concentration increased by 141 and 435% in +Si cv. Oochikara and by 119 and 251% in +Si *lsi1* mutant plants under high and low PFD, respectively, compared with −Si plants. Plant biomass accumulation was improved by Si regardless of PFD, especially plants for cv. Oochikara. Brown spot severity was highest in −Si plants for cv. Oochikara and *lsi1* mutant plants under low PFD. In the presence of Si, disease severity in plants grown under both low and high PFD was reduced, except for *lsi1* mutant plants under high PFD. Plant inoculation reduced the photosynthetic parameters measured regardless of plant material or Si supply. A decrease of net carbon assimilation rate (*A*) of inoculated plants under low PFD compared with non-inoculated plants was associated with damage in the photosynthetic apparatus, except for +Si cv. Oochikara in which stomatal restriction [low water vapour conductance (g_s)] contributed to *A* reduction. Under high PFD, damage to the photosynthetic apparatus of inoculated plants was the main reason for the reduction in *A* for +Si and −Si *lsi1* mutant plants. In addition, for −Si cv. Oochikara, a reduction in g_s contributed to reduced *A*. However, for +Si cv. Oochikara, g_s was the limiting factor for *A*. Inoculated plants of +Si cv. Oochikara had higher *A* values than +Si *lsi1* mutant plants, regardless of environmental conditions. Soluble sugars were not detected in leaf tissues of plants under low PFD. For high PFD, Si improved the hexose concentration in non-inoculated plants at 144 h after inoculation (hai) for *lsi1* mutant plants and from 96 hai onwards for cv. Oochikara compared with −Si plants. However, plant inoculation reduced hexose concentration compared with non-inoculated plants, mainly in +Si plants, regardless of plant material. Sucrose concentration increased in leaves of cv. Oochikara in the presence of Si whether inoculated or not. For +Si *lsi1* mutant plants, sucrose concentration increased only at 48 hai compared with −Si plants, whether inoculated or not. The results of this study show that a minimum Si concentration is needed in leaf tissues of rice plants to avoid the negative impact of *B. oryzae* infection on photosynthesis and sugar concentration. High leaf Si concentration resulted in an increased soluble sugar concentration and together, but in independent ways, soluble sugar and Si reduced brown spot severity of rice.

Keywords: brown spot, foliar disease, gas exchange, *Oryza sativa*, photon flux density, soluble sugars

Introduction

Brown spot, caused by *Bipolaris oryzae*, is an economically important disease in rice because it negatively impairs grain quality and reduces yield by up to 74% (Kohls *et al.*, 1987). Light reddish-brown lesions on leaf blades, or lesions with a grey centre surrounded by a dark to reddish-brown margin and a bright yellow halo, are the typical symptoms of this disease (Ou, 1985). These symptoms appear approximately 18 h after mycelia formed by the germinated conidia enter through stomata or penetrate directly into epidermal cells (Tullis, 1935). The non-selective toxins, known as ophiobolins A and B, produced by *B. oryzae* during conidial germination and within infected rice leaf cells are involved in symptom development (Xiao *et al.*, 1991). These toxins suppress the host defence response and cause browning of the infected cells and yellowing of neighbouring cells, resulting in large lesions that reduce leaf photosynthetic area (Tullis, 1935; Vidhyasekaran *et al.*, 1992). It was

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previously suggested that pathogen infection depresses the rate of photosynthesis in the infected leaf tissues by altering chloroplast structure and function, chlorophyll content, and enzymes directly involved in photosynthesis (Ou, 1985).

Brown spot severity is strongly influenced by environmental conditions. Dallagnol *et al.* (2011b) found that brown spot severity was highest in plants grown under low photon flux density (PFD) (15 μmol photons m^{-2} s^{-1}) and high temperature (32°C), whereas the lowest severity occurred on plants grown under high PFD (1000 μmol photons m^{-2} s^{-1}) and a wide temperature range (20–35°C). The soluble sugar concentration in rice plants grown under low PFD was lower in both inoculated and non-inoculated plants, suggesting that disease development was enhanced under low soluble sugar concentration conditions. High sugar concentration in rice leaves is unfavourable for brown spot development (Vidhyasekaran, 1974). Sugars are the direct products of photosynthesis, a primary form of energy storage, from which many other organic compounds found in plants are synthesized, such as those produced by secondary metabolic processes involved in host defence against pathogens (Scholes, 1992; Lawlor, 2001).

Strategies for brown spot management are limited. Commercial cultivars with high levels of resistance are not available to growers (Ou, 1985). Fungicide sprays and good plant nutrition have been the major management strategies for minimizing yield loss (Ou, 1985). It has long been known that rice plants grown in soil deficient in calcium, iron, magnesium, manganese, potassium and silicon are more susceptible to brown spot (Kaur & Padmanadhan, 1974). Silicon (Si) application reduced the intensity of important diseases in rice such as blast, sheath blight, stem rot, leaf scald, leaf blight, grain discoloration and brown spot (Datnoff *et al.*, 2007).

Rice requires large amounts of Si for vigorous growth and high yield (Ma *et al.*, 2002). Rice plants can accumulate this element in shoots in concentrations several times higher than those of other macronutrients such as nitrogen, phosphorus and potassium (Savant *et al.*, 1997). Silicon is taken up by roots from the soil solution in the form of monosilicic acid (H_4SiO_4) (Takahashi & Hino, 1978), then translocated to shoots via the transpiration stream and rapidly polymerized forming silica (Ma *et al.*, 2006). In rice, the Si transporter genes *Lsi1* and *Lsi2* are responsible for the high capacity of lateral roots to take up Si in solution from the soil. *Lsi1* encodes an influx transporter protein for monosilicic acid, while *Lsi2* encodes an active efflux transporter protein, and both are localized in the root exodermis and endodermis (Ma & Yamaji, 2008). The gene *Lsi6* is involved in Si distribution in rice shoots (Yamaji & Ma, 2009). Mutations in these genes have enabled advances in knowledge of the importance of Si in rice physiology. Yields of *lsi1* mutant plants can be significantly reduced under field conditions (Ma *et al.*, 2002). A reduced Si concentration in tissues of *lsi1* mutant rice plants strongly affected their post-infection defence against brown spot, suggesting that a minimum Si concentration is needed for effective resistance to the disease (Dallagnol *et al.*, 2009, 2011a).

Yoshida *et al.* (1962) reported that a thick layer of silica is formed beneath the cuticle of rice leaves and sheaths after polymerization of monosilicic acid. This Si layer beneath the cuticle may be partially responsible for impeding pathogen penetration, consequently decreasing the number of lesions on leaf blades, or increasing the incubation period, as reported for the *Pyricularia grisea*– and *Rhizoctonia solani*–rice pathosystems (Rodrigues *et al.*, 2001; Seebold *et al.*, 2001). However, the soluble Si in plant tissue may be associated with an increase in rice resistance to blast and brown spot through increased production of phenolics and phytoalexins, and activation of some plant defence-related genes (with production of PR-proteins) (Rodrigues *et al.*, 2005; Datnoff *et al.*, 2007; Brunings *et al.*, 2009; Dallagnol *et al.*, 2011a).

The aim of this study was to investigate the effect of Si uptake on photosynthetic parameters and leaf sugar concentration in rice cv. Oochikara and in its *lsi1* mutant defective in the Si uptake grown under high or low PFD with or without *Bipolaris oryzae* inoculation.

Materials and methods

Nutrient solution preparation

The nutrient solution used in this study was prepared according to Dallagnol *et al.* (2011a). Silicon (2 mM) was supplied to plants in the form of silicic acid, which was prepared by passing potassium silicate through cation-exchange resin (Amberlite IR-120B; H^+ form; Sigma-Aldrich). The pH of the nutrient solution was 5·6 and was not affected by the addition of silicic acid.

Plant growth

Rice seeds of cv. Oochikara and the low-silicon-1 (*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 Co.) in a germination chamber (MA-835/2106UR; Marconi) at 25°C for 6 days. Germinated seedlings were transferred to 0·5-L plastic pots containing half-strength nutrient solution (Dallagnol *et al.*, 2011a) without Si. After 2 days, young seedlings were transferred again to new plastic pots with 5 L nutrient solution with or without Si. The non-aerated nutrient solution was changed every 4 days. 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. Plants were grown in a greenhouse with relative humidity of approximately 60% and a temperature of 30 ± 5°C. The photon flux density on sunny days inside the greenhouse was measured at noon with a Li 250A light meter (LI-COR Environmental) and provided a reading of approximately 900 μmol photons m^{-2} s^{-1}.

Inoculation procedure

Plants with eight leaves on the main tiller (Matsuo & Hoshikama, 1993) were inoculated with *B. oryzae* (isolate CNPAF-HO 82; EMBRAPA-National Center for Research on Rice and Beans, Goiânia, Goiás, Brazil) after 35 days of growth in hydroponic culture with treatments. Pathogen preservation and inoculum preparation were performed according to Dallagnol *et al.* (2011a). A conidial suspension of *B. oryzae* (5×10^3 conidia mL^{-1}) was applied as a fine mist to the leaves of each plant until run-off using a VL Airbrush atomizer (Paasche Airbrush Co.). Gelatin (1%, w/v) was added to the sterile water to aid conidial adhesion to the leaves.

Plants were transferred to a growth chamber (GC) or a plastic mist growth chamber (MGC) inside a greenhouse with favourable conditions for brown spot development immediately after inoculation. The MGC was built of wood (1 m wide, 1·5 m high and 2 m long, covered with transparent plastic). Plants in GC were maintained at 25 ± 2°C with an initial 24-h dark period, after which they were exposed to a 12-h photoperiod with PFD values ranging from approximately 15 to 6·0 μmol m^{-2} s^{-1} from the top to the base of plant canopies, respectively, provided by cool-white fluorescent lamps. The relative humidity inside the growth chamber, measured using a thermo-hygrograph (TH-508; Impac), was 95 ± 2% throughout the experiment. For MGC, there was no initial dark period and plants were exposed to temperatures ranging from 20 ± 2°C (night) to 30 ± 3°C (day). Relative humidity was maintained at 92 ± 3% using a misting system in which nozzles (model NEB-100; KGF Company) sprayed mist every 30 min above the plant canopy. The maximum natural PFD at plant canopy height was about 900 μmol m^{-2} s^{-1}. Non-inoculated plants were kept in separate chambers, but exposed to the same conditions as the inoculated plants (GC and MGC) during the experiments.

Brown spot severity (BSS) assessment

The fifth and sixth leaves of the main tiller of two plants per each replication were marked and used to evaluate BSS at 144 h after inoculation (hai) using a scale based on the percentage of diseased leaf area (IRRI, 1996).

Photosynthetic measurements and assessments

Gas exchange parameters were measured on the fourth leaf of two non-inoculated and two inoculated plants (144 hai) of each replication for each treatment. Inoculated and non-inoculated plants were kept in separate plots. The gas exchange measurements were performed on the same plants. Measurements were conducted under ambient CO_2 (370 ± 10 μmol mol^{-1}) and temperature conditions with artificial light (1200 μmol photons m^{-2} s^{-1} at the leaf level). The net carbon assimilation rate (A), stomatal conductance to water vapour (g_s), internal-to-ambient CO_2 concentration ratio (C_i) and transpiration rate (E) were measured throughout the morning (07·30–11·00 h) using a portable open-system infrared gas analyser (LI-6400; LI-COR Inc.). During photosynthetic measurements, mean values of air temperature and relative humidity were 33·1 ± 1·6°C and 77 ± 5%, respectively. Leaf temperature was similar to air temperature. In order to allow full activation of the photosynthetic apparatus, plants from GC (low PFD environment) were kept outside the mist chamber under full sunlight for approximately half an hour before measurements were recorded.

Carbohydrate assessments

Five leaf discs (~100 mg fresh weight) were collected from the fourth and fifth leaves of plants of each replication for each treatment at 48, 96 and 144 hai. For each time point, one plant per replication was sampled and was not used thereafter. The last sampling was done on the same plants used for gas exchange measurements after the measurements were performed. Leaf samples were also collected from non-inoculated plants at these same time points. Leaf discs were kept in liquid nitrogen during sampling and then stored at −80°C until analysis. Frozen leaf discs were homogenized with 800 μL 80% (v/v) aqueous ethanol, then incubated at 70°C for 90 min following centrifugation at 15 000 ***g*** for 10 min. Pellets were re-extracted, and the supernatants were combined and stored at −20°C for determination of soluble sugars. The concentrations of hexose (glucose + fructose) and sucrose were determined according to Praxedes *et al.* (2006).

Plant dry weight accumulation and Si concentration in leaf tissues

After samples for carbohydrate assessments were obtained, the remaining leaves and culms were collected at the end of each experiment and oven-dried for 72 h at 65°C to obtain dry weight. Leaves were ground with a Thomas-Wiley mill (Thomas Scientific) to pass through a 40-mesh screen and tissue Si concentration was determined by colorimetric analysis on 0·1 g dried and alkali digested tissue (Korndörfer *et al.*, 2004).

Experimental design and data analysis

A 2 × 2 × 2 × 2 factorial experiment, consisting of two Si concentrations (0 or 2 mM, referred to as −Si and +Si treatments, respectively), plant materials (cv. Oochikara and *lsi1* mutant), environmental conditions (GC and MGC), and inoculated and non-inoculated plants, was arranged in a completely randomized design with four replications. Each experimental unit consisted of one plastic pot containing six rice plants. The experiment was repeated once. Data were analysed by analysis of variance (ANOVA) followed by treatment mean comparisons using Tukey's test ($P \leq 0{\cdot}05$) or t-test by using SAS software (SAS Institute). For ANOVA,

it was considered to be a $2 \times 2 \times 2$ factorial experiment, consisting of two Si concentrations (Si), plant materials (PM) and environmental conditions (EC) for the variables foliar Si concentration, dry weight and BSS. For the hexose and sucrose variables, it was considered a $2 \times 2 \times 3 \times 2$ factorial experiment for ANOVA, consisting of two Si concentrations, plant materials, sampling times (ST) and non-inoculated or inoculated plants (I). For the variables A, g_s, C_i and E, a $2 \times 2 \times 2 \times 2$ factorial experiment was considered for ANOVA, which consisted of two Si concentrations, plant materials, environmental conditions, and non-inoculated or inoculated plants.

Results

Silicon concentration in leaf tissues

The factors EC, Si and PM, as well as some of their interactions, were significant (Table 1). Leaf Si concentration significantly increased in +Si plants compared with the −Si plants (Fig. 1). The Si concentration was significantly higher in plants under GC than in those under MGC conditions, regardless of Si treatment. Si addition increased leaf Si concentration in both cv. Oochikara (141%) and *lsi1* mutant plants (119%) for plants under GC. Greater increases in leaf Si concentration were found in cv. Oochikara (435%) and *lsi1* mutant plants (251%) under MGC than GC conditions. The Si concentration was always higher (97–200%) in cv. Oochikara than in *lsi1* mutant plants, regardless of Si treatment and plant growth conditions.

Plant dry weight accumulation

The factors EC, Si and PM, as well as some of their interactions, were significant (Table 1). Addition of Si improved plant biomass accumulation under both GC and MCG conditions for both cv. Oochikara and *lsi1* mutant plants (Fig. 2). Comparisons between +Si plant materials showed significantly higher plant biomass (28%) for cv. Oochikara than for *lsi1* mutant plants under MGC but not GC conditions. For the −Si treatment, plant biomass was significantly higher only for cv. Oochikara compared with *lsi1* mutant plants under MGC conditions. Plant biomass was significantly greater in plants grown under MGC than under GC conditions, regardless of treatments.

Table 1 Analysis of variance of the effects of environmental conditions (EC), silicon concentrations (Si) and plant materials (PM) on foliar Si concentration, dry matter and brown spot severity (BSS) in rice

		F values[a]		
Source of variation	df	Si (dag kg^{-1})	Dry matter (g plant^{-1})	BSS (%)
EC	1	685·36**	578·39**	56·37**
Si	1	1190·92**	258·80**	293·37**
PM	1	869·86**	138·17**	13·28**
EC × Si	1	23·24**	6·34*	41·61**
EC × PM	1	64·26**	40·55**	7·37**
Si × PM	1	272·60**	19·00**	27·12**
EC × Si × PM	1	0·06^{ns}	3·82^{ns}	0·04^{ns}
Replications	7	0·53^{ns}	1·53^{ns}	0·74^{ns}

[a]Levels of probability: ns, not significant; *, 5%;**, 1%.

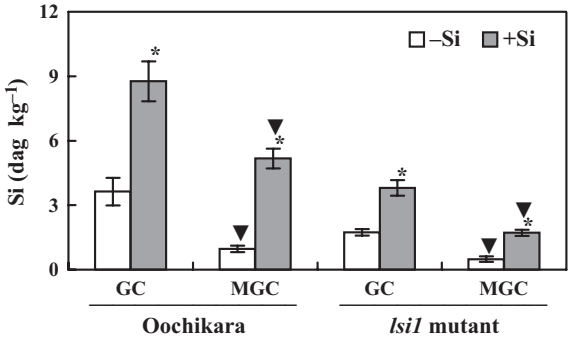

Figure 1 Silicon (Si) concentration in leaves of rice plants of cv. Oochikara and the *lsi1* mutant grown in hydroponic culture containing 0 (−Si) or 2 (+Si) mM silicon and placed in a growth chamber (GC) or mist growth chamber (MGC) inside a greenhouse. Error bars represent standard deviation of means. Means from −Si and +Si treatments, under each growing condition, followed by an asterisk (*) are significantly different ($P \leq 0{\cdot}05$) as determined using the *t*-test. Means from each treatment under MGC conditions followed by an inverted triangle (▼) are significantly different ($P \leq 0{\cdot}05$) from the respective treatment under GC conditions by *t*-test. Two experiments were conducted with consistent results and the data presented represent the second experiment. $n = 16$.

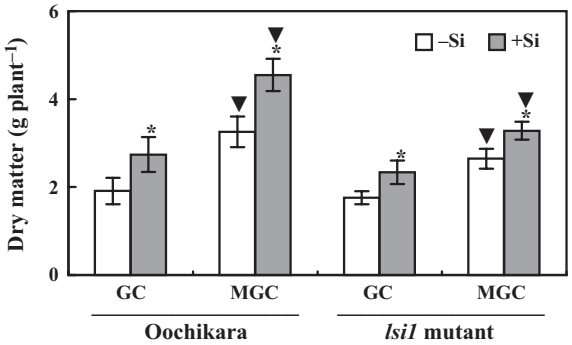

Figure 2 Plant dry matter accumulation of rice cv. Oochikara and *lsi1* mutant plants grown in hydroponic culture containing 0 (−Si) or 2 (+Si) mM silicon and placed in a growth chamber (GC) or mist growth chamber (MGC) inside a greenhouse. Error bars represent standard deviation of means. Means from −Si and +Si treatments, under each growing condition, followed by an asterisk (*) are significantly different ($P \leq 0{\cdot}05$) as determined using the *t*-test. Means from each treatment under MGC conditions followed by an inverted triangle (▼) are significantly different ($P \leq 0{\cdot}05$) from the respective treatment under GC conditions by *t*-test. Two experiments were conducted with consistent results and the data presented represent the second experiment. $n = 16$.

Brown spot severity (BSS)

The factors EC, Si and PM, as well as some of their interactions, were significant (Table 1). BSS was significantly higher in plants grown under GC conditions than MGC conditions for the −Si treatment (Fig. 3). The greatest BSS values were obtained in the −Si treatments for cv. Oochikara and *lsi1* mutant plants kept under GC conditions, with no significant difference between them (Fig. 3). In the −Si treatments, BSS values for both cv. Oochikara and *lsi1* mutant plants ranged from 14 to 15% for plants grown under MGC conditions and from 23 to 27% under GC conditions. Overall, BSS decreased significantly in +Si treated plants, particularly for cv. Oochikara (2·8% under MGC and 5·4% under GC conditions). For the *lsi1* mutant plants, Si treatment significantly reduced BSS under GC conditions compared with −Si plants, but did not significantly reduce BSS under MGC conditions (Fig. 3).

Photosynthetic measurements

The factors EC, Si, PM and I, as well as some of their interactions, were significant for *A* (Table 2). For g_s, only the main factors EC and I and some factor interactions were significant (Table 2). For C_i, only Si and I and some factor interactions were significant (Table 2). For *E*, the factors EC, Si and I, as well as some factor interactions, were significant (Table 2). Non-inoculated cv. Oochikara plants supplied with Si under GC conditions showed significantly enhanced *A* and *E* (Fig. 4a,g), but no apparent effect on g_s (Fig. 4c) and C_i (Fig. 4e), compared with −Si plants. Nevertheless, no significant differences between +Si and −Si plants among non-inoculated *lsi1* mutant plants were observed for gas exchange parameters (Fig. 4a,c,e,g) under GC conditions. Under GC conditions, inoculated plants of both cv. Oochikara and the *lsi1* mutant showed significantly decreased *A* (Fig. 4a,b), g_s (Fig. 4c,d) and *E* (Fig. 4g,h) compared with non-inoculated ones, regardless of Si supply. For C_i, no significant effect of inoculation was observed, except for +Si cv. Oochikara (Fig. 4e,f). However, inoculated plants supplied with Si showed significantly enhanced ($P \leq 0{\cdot}05$) *A* (Fig. 4b), g_s (Fig. 4d) and *E* (Fig. 4h) for cv. Oochikara and *A* (Fig. 4b) for the *lsi1* mutant compared with −Si plants under GC conditions.

The maximum *A* exceeded 40 μmol CO_2 m^{-2} s^{-1} for non-inoculated +Si cv. Oochikara plants under MGC conditions (Fig. 4a). However, non-inoculated cv. Oochikara and *lsi1* plants supplied with Si under MGC conditions did not show any significant increase in *A* (Fig. 4a), g_s (Fig. 4c), C_i (Fig. 4e) or *E* (Fig. 4f) compared with −Si plants. In inoculated plants of both genotypes, regardless of Si supply, *A* (Fig. 4b), g_s (Fig. 4d) and *E* (Fig. 4g) were reduced significantly compared with non-inoculated ones. However, *A* decreased significantly less in cv. Oochikara +Si (reduction of 42%) than in −Si plants (reduction of 56%) (Fig. 4a,b; Table 2). The decreases in g_s ranging from 67 to 84% for inoculated plants (Fig. 4c,d) compared with non-inoculated ones were accompanied by significant decreases in C_i (~17%), particularly in +Si cv. Oochikara (Fig. 4e,f), but not in the *lsi1* mutant plants (Fig. 4e,f). Inoculation also significantly reduced *E* compared with non-inoculated plants, regardless of Si supply or plant material (Fig. 4g, h; Table 2). Inoculated +Si cv. Oochikara plants displayed significantly higher *A* (22%) than +Si *lsi1* mutant plants (Fig. 4b; Table 2).

Table 2 Analysis of variance of the effects of environmental conditions (EC), silicon concentrations (Si), plant materials (PM) and plant inoculated or non-inoculated with *Bipolaris oryzae* (I) for net carbon assimilation rate (*A*), internal CO_2 concentration ratio (C_i), stomatal conductance to water vapour (g_s) and transpiration rate (*E*)

		F values[a]			
Source of variation	df	*A*	g_s	C_i	*E*
EC	1	385·96**	154·69**	1·83^{ns}	710·12**
Si	1	83·32**	0·89^{ns}	10·51**	9·30**
PM	1	39·68**	3·95^{ns}	0·50^{ns}	2·28^{ns}
I	1	564·82**	230·31**	35·95**	209·90**
EC × Si	1	3·47^{ns}	7·0*	0·55^{ns}	0·30^{ns}
EC × PM	1	0·57^{ns}	0·01^{ns}	0·67^{ns}	0·26^{ns}
EC × I	1	20·96**	80·48**	8·28**	34·91**
Si × PM	1	7·21**	1·01^{ns}	2·80^{ns}	5·20*
Si × I	1	5·58*	6·79*	0·01^{ns}	0·02^{ns}
PM × I	1	1·10^{ns}	3·29^{ns}	13·77**	0·02^{ns}
EC × Si × PM	1	1·23^{ns}	0·77^{ns}	0·40^{ns}	0·03^{ns}
EC × Si × I	1	0·29^{ns}	6·92*	1·96^{ns}	0·04^{ns}
EC × PM × I	1	1·07^{ns}	0·83^{ns}	8·87**	0·15^{ns}
Si × PM × I	1	0·06^{ns}	0·01^{ns}	4·80*	0·51^{ns}
EC × Si × PM × I	1	0·25^{ns}	0·53^{ns}	1·96^{ns}	0·24^{ns}
Replications	7	0·09^{ns}	1·78^{ns}	2·28^{ns}	0·95^{ns}

[a]Levels of probability: ns, not significant; *, 5%; **, 1%.

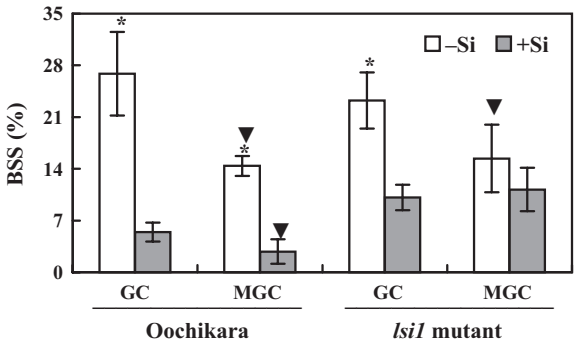

Figure 3 Brown spot severity (BSS) on leaves of rice plants of cv. Oochikara and the *lsi1* mutant grown in hydroponic culture containing 0 (−Si) or 2 (+Si) mM silicon and placed in a growth chamber (GC) or mist growth chamber (MGC) inside a greenhouse. Error bars represent standard deviation of means. Means from −Si and +Si treatments, under each growing condition, followed by an asterisk (*) are significantly different ($P \leq 0{\cdot}05$) as determined using the *t*-test. Means from each treatment under MGC conditions followed by an inverted triangle (▼) are significantly different ($P \leq 0{\cdot}05$) from the respective treatment under GC conditions by *t*-test. Two experiments were conducted with consistent results and the data presented represent the second experiment. $n = 8$.

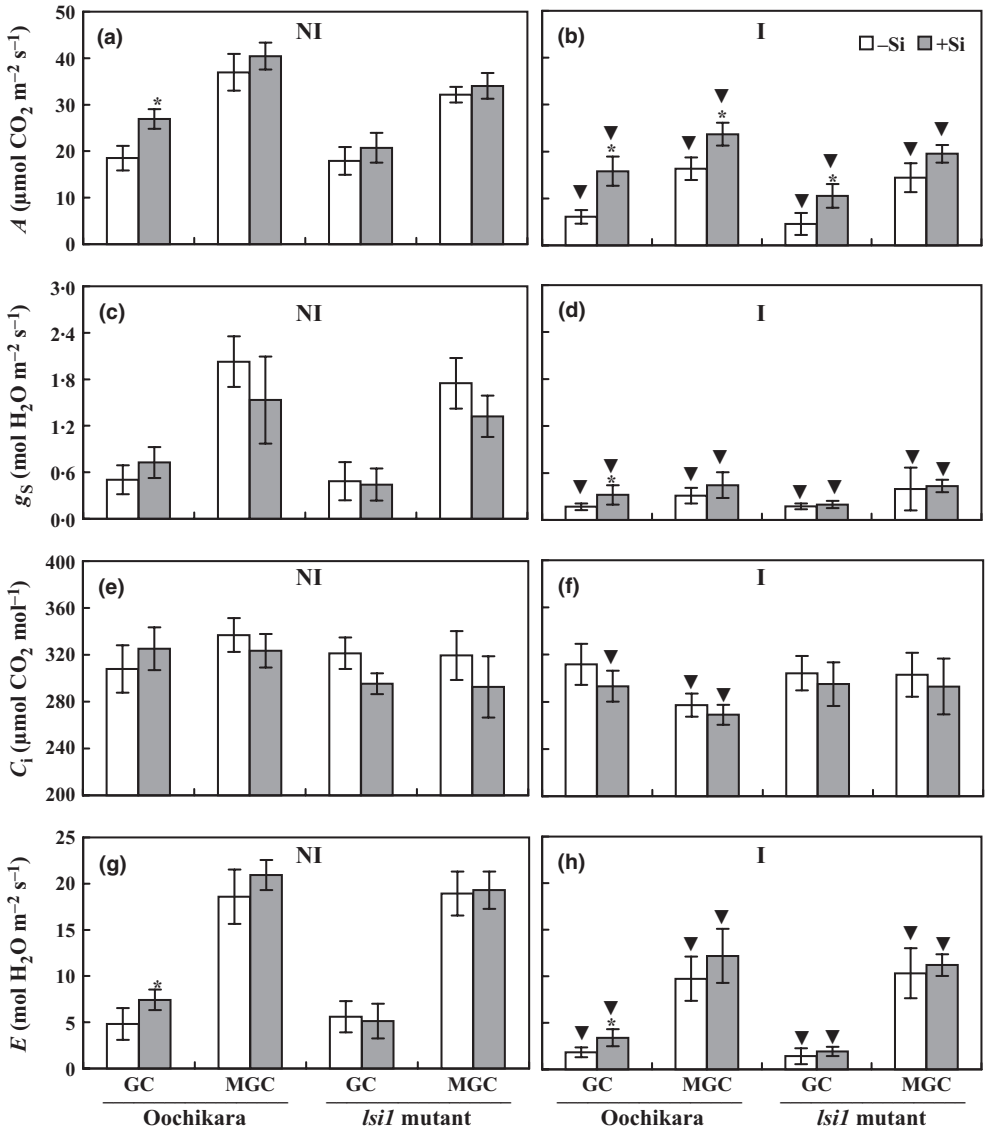

Figure 4 Net carbon assimilation rate (*A*) (a and b), stomatal conductance to water vapour (g_s) (c and d), internal CO_2 concentration (C_i) (e and f) and transpiration rate (*E*) (g and h) in leaves of rice cv. Oochikara and *lsi1* mutant plants grown under hydroponic culture containing 0 (−Si) or 2 (+Si) mM silicon, non-inoculated (NI) (a, c, e and g) or 144 h after inoculation (I) (b, d, f and h) with *Bipolaris oryzae*. Plants from all treatments were either placed in a growth chamber (GC) or mist growth chamber (MGC) inside a greenhouse. Error bars represent standard deviation of means. Means from −Si and +Si treatments, in each growing condition, followed by an asterisk (*) are significantly different ($P \leq 0{\cdot}05$) by *t*-test. Means from I treatments, under each growing condition, followed by an inverted triangle (▼) are significantly different ($P \leq 0{\cdot}05$) from the respective NI treatment as determined using the *t*-test. Two experiments were conducted with consistent results and the data presented represent the second experiment. $n = 8$.

Soluble sugars

Soluble sugars (hexoses and sucrose) were not detected in leaf tissues of plants grown under GC conditions.

For plants grown under MGC conditions, the factors Si, PM (except for hexoses), I and ST, as well as some of their interactions, were significant for hexoses and sucrose (Table 3). Hexose concentrations in +Si non-inoculated plants were significantly higher for *lsi1* mutant plants at 144 hai and from 96 hai onwards in cv. Oochikara than in −Si non-inoculated plants (Fig. 5). This effect was not seen in inoculated plants, in which no significant difference, regardless of plant material, was observed between −Si and +Si treatments, except for +Si *lsi1* mutant plants at 96 hai. Plants supplied with Si showed significantly lower hexose concentrations compared with non-inoculated plants for cv. Oochikara at 96 and 144 dai and for *lsi1* mutant plants at 144 hai (Fig. 5). In contrast to hexoses, sucrose concentrations were higher in +Si cv. Oochikara plants regardless of

Table 3 Analysis of variance of the effects of silicon concentrations (Si), plant materials (PM), plants inoculated or non-inoculated with *Bipolaris oryzae* (I), and sampling times (ST) for leaf concentrations of sucrose and hexoses in plants grown in a mist growth chamber

		F values[a]	
Source of variation	df	Hexoses	Sucrose
Si	1	75·06**	185·82**
PM	1	2·21^{ns}	57·80**
I	1	81·57**	39·50**
ST	2	178·37**	177·74**
Si × PM	1	18·98**	52·41**
Si × I	1	93·27**	0·08^{ns}
Si × ST	2	54·63**	34·43**
PM × I	1	38·03**	32·73**
PM × ST	2	66·45**	1·78^{ns}
I × ST	2	37·19**	80·76**
Si × PM × I	1	3·06^{ns}	0·88^{ns}
Si × PM × ST	2	51·09**	10·32**
Si × I × ST	2	30·22**	5·88**
PM × I × ST	2	14·75**	14·74**
Si × PM × I × ST	2	28·27**	11·31**
Replications	7	1·3^{ns}	1·11^{ns}

[a]Levels of probability: ns, not significant; *, 5%; **, 1%.

inoculation with *B. oryzae* (Fig. 6). However, for cv. Oochikara, plant inoculation significantly increased sucrose concentration at 48 hai and significantly reduced it from 96 hai onwards, regardless of Si supply, except for −Si at 144 hai. In *lsi1* mutant plants, sucrose concentrations were significantly higher in +Si plants than −Si plants, only at 48 hai, for both non-inoculated and inoculated plants (Fig. 6). Plant inoculation from *lsi1* mutant plants only significantly reduced sucrose concentration at 96 hai regardless of Si supply. Nevertheless, because sucrose concentrations were much higher than hexose concentrations, total soluble sugars (sucrose + hexoses) were significantly higher in +Si cv. Oochikara, but not in *lsi1* mutant plants, compared to –Si plants, irrespective of inoculation treatment (data not shown).

Discussion

The results of this study show that *lsi1* mutant rice plants, in addition to having greater susceptibility to brown spot, probably as a result of reduced Si uptake (Dallagnol *et al.*, 2009, 2011a), had lower photosynthetic rates and soluble sugar concentrations under stress conditions, such as low PFD and upon infection by *B. oryzae*, than did plants of cv. Oochikara. These results are supported by the data that was obtained in a series of preliminary experiments that showed similar trends (data not shown). Similar to results reported by Ma *et al.* (2006) and Dallagnol *et al.* (2009, 2011a), leaf Si concentration was lower in *lsi1* mutants than in cv. Oochikara plants regardless of Si treatment. The higher Si concentrations for both cv. Oochikara and *lsi1* mutant plants grown under GC conditions as compared with MGC conditions was probably caused by dilution of the element as a result of the greater plant biomass accumulation under MGC conditions. The differential stimulation under MGC compared with GC conditions was the result of a higher PFD intensity in

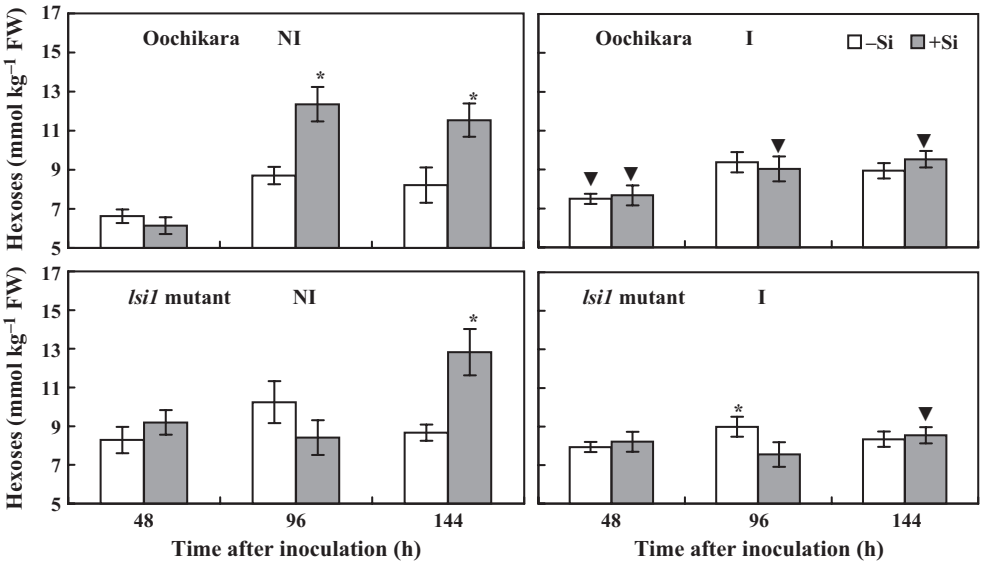

Figure 5 Concentrations of hexoses (glucose + fructose) in rice leaves of cv. Oochikara and *lsi1* mutant plants grown under hydroponic culture containing 0 (–Si) or 2 (+Si) mM silicon. Plants non-inoculated (NI) or inoculated (I) with *Bipolaris oryzae* were placed in a mist growth chamber inside a greenhouse. Error bars represent standard deviation of means. Means from –Si and +Si treatments, at each time after inoculation, followed by an asterisk (*) are significantly different ($P \leq 0{\cdot}05$) by *t*-test. Means from I treatments, at each time after inoculation, followed by an inverted triangle (▼) are significantly different ($P \leq 0{\cdot}05$) from the respective NI treatment as determined using the *t*-test. Two experiments were conducted with consistent results and the data presented represent the second experiment. $n = 8$. FW = fresh weight.

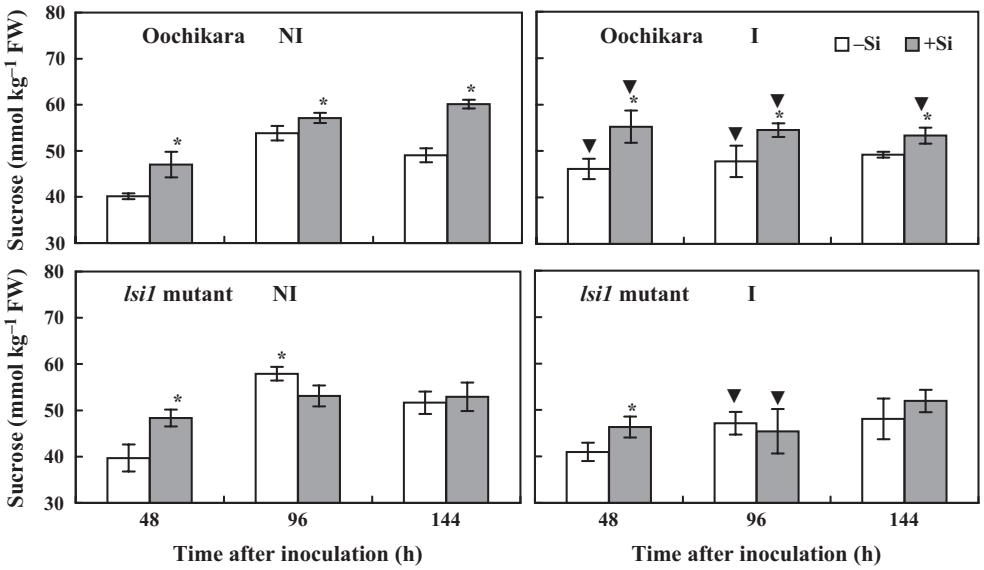

Figure 6 Concentration of sucrose in rice leaves from cv. Oochikara and *lsi1* mutant plants grown under hydroponic culture containing 0 (−Si) or 2 (+Si) mM silicon. Plants non-inoculated (NI) or inoculated (I) with *Bipolaris oryzae* were placed in a mist growth chamber inside a greenhouse. Error bars represent standard deviation of means. Means from −Si and +Si treatments, at each time after inoculation, followed by an asterisk (*) are significantly different ($P \leq 0{\cdot}05$) as determined using the *t*-test. Means from I treatments, at each time after inoculation, followed by an inverted triangle (▼) are significantly different ($P \leq 0{\cdot}05$) from the respective NI treatment by *t*-test. Two experiments were conducted with consistent results and the data presented represent the second experiment. $n = 8$. FW = fresh weight.

MGC, which was more favourable for plant growth. Moreover, low PFD under GC conditions negatively impacted plant biomass accumulation, even for +Si plants, for both cv. Oochikara and the *lsi1* mutant. However, regardless of MGC or GC conditions, +Si treatment improved plant biomass accumulation compared with the −Si treatment.

The addition of Si reduced BSS to a greater extent in cv. Oochikara than in *lsi1* mutant plants. Regardless of Si treatment, BSS for cv. Oochikara increased by up to 93% in plants kept in GC (from 2·8 to 5·4%) and MGC conditions (from 14·4 to 26·95%), even when leaf Si concentrations were higher under GC conditions. This result indicates the interdependence of host and environmental factors on rice defence responses against infection by *B. oryzae*.

High BSS values, as seen in −Si cv. Oochikara plants and *lsi1* mutant plants with or without Si, may also negatively affect biochemical processes involved in CO_2 fixation in addition to decreasing their capacity to use solar energy for photosynthesis. Evidence for this effect is shown by non-stomatal limitations, as indicated by declines in A but not C_i, despite significant decreases in g_s.

A pronounced effect of Si on photosynthetic parameters was observed in plants kept in GC conditions; in non-inoculated +Si cv. Oochikara plants compared with −Si non-inoculated ones, A reached higher values without significant changes in g_s or C_i, indicating an improvement in the photosynthetic apparatus when these plants were exposed to high PFD. This effect was not observed in non-inoculated +Si *lsi1* mutant plants, suggesting that a decrease in Si uptake may limit increases in A. On the other hand, A rates were less impaired by fungal infection in +Si *lsi1* mutant plants inoculated with *B. oryzae*, although to a lesser extent than in cv. Oochikara plants, compared to −Si *lsi1* mutant plants. This result suggests that a small increase in Si concentration in leaf tissues of *lsi1* mutant plants could improve CO_2 fixation rates when they are exposed to associated biotic and abiotic stresses.

The effect of Si on photosynthetic parameters under MGC conditions was only significant in inoculated plants. However, despite the lower BSS values in MGC than GC conditions, a sharp reduction in A was observed for both cv. Oochikara and *lsi1* mutant plants, regardless of Si supply. For inoculated −Si cv. Oochikara and both +Si and −Si *lsi1* mutant plants, the reduction in A was probably the result of photosynthetic apparatus impairment, as observed for plants grown under GC conditions. However, in inoculated +Si cv. Oochikara plants, the fact that g_s decreased to a greater extent than A, with a concomitant decline in C_i, compared with non-inoculated plants, suggests that stomatal limitations may play a key role in constraining A in inoculated ones. Additionally, the reduction in A in these plants may have been associated with an increase in sugar pools in infected tissues that, in turn, might transiently suppress the expression of photosynthesis-related genes in leaf tissues with low BSS. According to Herbers *et al.* (1996), the same minimum leaf sugar concentration required for induction of plant

defence-related genes was also responsible for the repression of genes associated with photosynthesis.

The decrease in *A* associated with low PFD under GC conditions was accompanied by low soluble sugar concentrations. In contrast, the soluble sugars concentration was elevated in plants grown at MGC under high PFD and supplied with Si, especially for cv. Oochikara. Inoculation with *B. oryzae* induced a slight reduction in sucrose concentration and a large reduction in hexose pools in these plants. Therefore, it is proposed that changes in soluble sugars could affect the expression of host defence response genes that ultimately reduce BSS, as seen in +Si cv. Oochikara plants, but not in *lsi1* mutants, because no significant changes in soluble sugar pools were seen in the latter regardless of Si and *B. oryzae* inoculation.

The activation of host defence responses by soluble sugars, especially sucrose, has been reported in the literature. In transgenic rice plants expressing the PR*ms* gene from maize, high concentrations of sucrose act as an endogenous signal to activate host defence responses through priming against infection by *B. oryzae*, *Magnaporthe oryzae* and *Fusarium verticillioides* (Gomez-Ariza *et al.*, 2007). However, in the present study, the lower BSS values observed in +Si cv. Oochikara plants were caused not only by the induction of defence responses by soluble sugars, but also by the high Si concentrations in leaf tissues. This was clearly illustrated by the low BSS values in +Si cv. Oochikara plants under GC conditions where no sucrose was detected. These data indicate that Si *per se* may stimulate signalling pathways that lead to the activation of host defence responses through an unknown mechanism that is independent of sugar pools. In contrast, the activation of host defence responses in the *lsi1* mutants was impaired in two ways: by accumulation of soluble sugars and by the effect of high concentrations of soluble Si.

In conclusion, the inefficiency of *lsi1* mutant plants to actively take up Si negatively affected their resistance against *B. oryzae* because of the low concentrations of Si and soluble sugars. The results of this study raise the hypothesis that any increase in Si concentration in leaves under multiple stress conditions (e.g. low PFD and infection by *B. oryzae*) would improve growth and reduce disease severity. Under conditions of a single stress (e.g. infection by *B. oryzae* under adequate PFD), the beneficial effects of Si were increased with increasing Si concentrations in leaf tissues. The results of the present study also suggest that rice resistance to brown spot is independently and additively affected by Si and soluble sugar concentrations in leaf tissues.

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