

Silicon-enhanced resistance to rice blast is attributed to silicon-mediated defence resistance and its role as physical barrier

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Abstract A series of experiments were performed to study the effects of silicon (Si) on rice blast development, H_2O_2 accumulation and lipid peroxidation in a controlled rice—*Magnaporthe grisea* pathosystem. Rice plants supplied with Si as a single dose immediately after pathogen inoculation (–/+Si) exhibited the same high protection against disease as plants treated continuously with Si for the whole growth period (+/+Si), with disease severity indices of 20.8% and 19.6%, respectively, which were significantly lower than that for the control treatment with no Si supplied (63.7%). A single application of Si to rice plants before inoculation (+/–Si) conferred partial protection (disease severity index of 33.3%) compared with the control treatment. Silicon induced a rapid but transient burst of H_2O_2 at 24 h after inoculation. The addition of Si to rice plants significantly altered the activities of catalase and lipoxygenase and the concentration of malondialdehyde (indicative of lipid peroxidation) in rice plants. We propose that rice

plants may respond to Si by increased H_2O_2 accumulation and lipid peroxidation. In turn, these responses are linked to host defence mechanisms such as lignin production, oxidative cross-linking in the cell wall, phytoalexin production, and the hypersensitive reaction. Thus, the mechanisms of Si-stimulated plant disease protection may extend beyond its established role in physically strengthening cell walls.

Keywords Active oxygen species · Blast · Lipid peroxidation · Rice · Silicon

Abbreviations

| | |
|-----|---------------------------|
| AOS | active oxygen species |
| CAT | catalase |
| EC | electrolytic conductivity |
| HR | hypersensitive reaction |
| LOX | lipoxygenase |
| MDA | malondialdehyde |
| Si | silicon |

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Introduction

Rice blast, caused by *Magnaporthe grisea*, is one of the most widespread and destructive diseases of rice (*Oryza sativa* L.). Screening and breeding of blast-resistant rice cultivars is a key approach to protect rice from blast and also reduce the use of fungicides in this food crop. However, genetic resistance to blast

is often short-lived as the pathogen rapidly evolves to overcome resistance, particularly when conferred by single major genes. Therefore, it is necessary to develop alternative and novel technologies for controlling rice blast to maintain high productivity.

Silicon (Si) is the second most abundant element in soil. Although Si has not been considered an essential element for higher plants, it has been shown to be beneficial for healthy growth and development of many plant species exposed to abiotic and biotic stresses, especially grasses such as barley and maize (Liang 1999; Seebold et al. 2004). Furthermore, it has been established that Si has a role in protecting rice from diseases such as blast (Seebold et al. 2004; Rodrigues et al. 2004). The method of using Si for disease control on rice is both economically viable and environmentally friendly. However, the underlying mechanisms of disease control are poorly understood. In rice, a Si-accumulating plant species, Si is mainly located in epidermal cell walls, mid lamellae and the intercellular spaces of sub-epidermal tissues (Kim et al. 2002), and has furthermore been reported as an important component of papillae (Fawe et al. 2001). In the presence of Si, plant tissues can undergo silicification which, together with lignin, contributes to the strengthening of cell walls in leaves and xylem vessels (Ma et al. 2001). Therefore, it has been proposed that Si plays an important role in the formation of mechanical or physical barriers restricting the penetration of pathogens (Datnoff et al. 2001). However this is yet to be supported by direct evidence.

The hypothesis that Si provides a passive mechanical barrier to pathogen invasion of tissues has long been contradicted by other reports (Samuels et al. 1991; Rodrigues et al. 2004; Liang et al. 2005). Furthermore, there is increasing evidence showing that Si is associated with host defence responses (Rodrigues et al. 2004, 2005; Liang et al. 2005). Rodrigues et al. (2004) reported that application of Si increased resistance of rice to blast through accumulation of phenolic-like compounds and phytoalexins. It was subsequently shown that rice plants treated with Si had a more rapid hypersensitive response (HR) and earlier epidermal cell death in response to *M. grisea* infection than plants not receiving Si. These results provide evidence that Si has a wider role in affecting resistance of rice to blast rather than those associated with physical protection. However, these responses are still poorly understood.

In the presence of pathogen infection, plant cells can produce active oxygen species (AOS). These compounds are considered to have direct antimicrobial effects and also to stimulate other defence mechanisms such as lignin production, oxidative cross-linking in the cell wall, lipid peroxidation, phytoalexin production, and the HR (Baker and Orlandi 1995; Lamb and Dixon 1997). Furthermore, the presence of these is likely to be correlated (Rustérucci et al. 1996). Lipid peroxidation, associated with increasing membrane damage during the development of the HR (Croft et al. 1990), can be due to lipoxygenase (LOX) activity leading to production of AOS. In turn, AOS can initiate enzyme-independent lipid peroxidation. However, induction of LOX activity and concomitant *in vivo* lipid peroxidation are associated with increasing membrane damage during the development of the HR (Croft et al. 1990). Malondialdehyde (MDA), a decomposition end product of polyunsaturated fatty acids, and relative electrolytic conductivity, have been frequently utilized as suitable biomarkers for lipid peroxidation and membrane permeability in plants exposed to stressful environments (Liang 1999; Mittler 2002). Therefore, as part of elucidating how Si elicits host defence responses, it is necessary to determine the effect of Si on AOS and lipid peroxidation in the rice-*M. grisea* pathosystem.

To our knowledge, there is currently no information available to link silicon with AOS metabolism and lipid peroxidation, and further with rice resistance to infection by *M. grisea*. The objectives of the present study were: 1) to show whether and how silicon enhances rice blast resistance by influencing AOS accumulation and lipid peroxidation, and 2) to give better insight into the roles of silicon in rice in response to *M. grisea*.

Materials and methods

Rice cultivation and silicon amendment

Rice (*Oryza sativa* L. cv. Lijiangxintuanheigu) was chosen for this study as it lacks any significant genetic resistance to any races of *M. grisea* in China (Wang et al. 2002). Plants were grown under controlled conditions with a photoperiod of 13 h, a light intensity of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and temperatures

of 25°C/20°C (day/night). Seeds were surface sterilized with 1% (v/v) NaOCl for 15 min, rinsed, and soaked in distilled water for 24 h at 33°C in dark, and then sown in plastic trays containing acid-washed and sterilized quartz sand. After 2 weeks, uniform seedlings were transplanted into 8.0 l plastic containers with full-strength Kimura B nutrient solution (Liang et al. 2006). The nutrient solution was prepared using purified (Milli-Q) water with final pH adjusted to 5.6. The solution was renewed every 4 d. Soluble Si was delivered to the plants by amending the nutrient solution with 1.7 mM $\text{NaSiO}_3 \cdot 9\text{H}_2\text{O}$, which was neutralized with HCl prior to addition (Liang et al. 2006). Control plants were continuously fed with full-strength Kimura B nutrient solution without silicon added.

Inoculation method

A virulent strain of *M. grisea* (“Sichuan-1”) was used for inoculation of rice seedlings. The pathogen was grown on oat tomato agar (30 g l⁻¹ oatmeal, 150 ml l⁻¹ tomato juice, 20 g l⁻¹ agar) in 15-cm petri dishes for 20 d. Suspensions of fungal conidia in water were made (5×10^5 conidia ml⁻¹), and Tween 20 (0.025%, v/v) added to aid adhesion to rice leaf blades. Inoculation took place at the time of emergence of the fifth leaf from the main tiller. Plants were inoculated with the conidial suspension until runoff. Non-inoculated plants were sprayed with a solution of Tween-sterile water (0.025%, v/v) until runoff. After inoculation, plants were transferred to a growth chamber and covered with a plastic bag. The relative humidity inside the growth chamber was maintained at >85% for the remainder of the experiment by using an atomizer (Defensor 3001, Axair Ltd., China). To promote pathogen infection, inoculated and non-inoculated plants were initially subjected to a 24 h dark period. After 48 h, plastic bags were removed from the plants.

Experimental design and sampling

Experiment A

This experiment was designed to study the effect of silicon application and inoculation with *M. grisea* on dry weight and silicon concentrations in rice plants. Plants were either amended or not amended with Si (+ and -Si) and, for both Si treatments, were inoculated or left uninoculated with *M. grisea* (+ and -*M. grisea*).

All treatments were arranged in a completely randomized design with three replications per treatment, and the entire experiment was repeated twice.

The shoots and roots of at least 60 rice seedlings per treatment were harvested separately 12 d after inoculation. After measuring fresh weight, shoots and roots were washed thoroughly with double-distilled H₂O and dried in an oven at 70°C to constant mass (about 3 d). After determining the dry mass, the tissue was ground (Ultra Centrifugal Mill; Retsch GmbH, Germany) to <0.5 mm and analysed colorimetrically for Si levels using the molybdenum blue method (Liang et al. 2006).

Experiment B

The effects of timing the supply of Si to rice plants on disease were investigated. Treatments included a control (no Si supplied, -/-Si), continuous supply with Si before and after pathogen inoculation (+/+Si), supply with Si only before inoculation (+/-Si), and supply with Si only after inoculation (-/+Si). Pathogen inoculation and change in supply of Si to before/after or continuous (as appropriate for the treatment structure) occurred at emergence of the fifth leaf from the main tiller. Disease development was measured 10 days after inoculation. Each treatment had three replications and the entire experiment was repeated twice.

Experiment C

The effects of Si addition to rice on the activity of key enzymes, H₂O₂ and malondialdehyde contents, and membrane permeability were determined. As rice susceptibility to blast is affected by leaf age (Ou 1985), only the third and fourth leaves from the main tiller of each rice plant were collected to insure the use of leaves of the same age among all treatments. Leaves of rice plants amended with silicon (+Si) or not (-Si) were collected at seven different time points after inoculation with *M. grisea* and used for analysis. Treatments (rice plants amended with silicon or not) were arranged in a completely randomized design with three replications per treatment. As before, the experiment was repeated twice to ensure reproducibility of results.

Determination of silicon concentration

Samples (~ 0.3 g) were microwave-digested in a mixture of 3 ml of HNO₃ (62%), 3 ml of H₂O₂ (30%),

and 2 ml of HF (46%) for 2 h at 360°C. The digested samples were then diluted to 100 ml with 4% boric acid and Si in the digest solution determined by the colorimetric analysis (molybdenum blue method; Liang et al. 2006) against a calibration curve generated over a concentration range of silicon standard solutions (Merck, Germany).

Disease evaluation

Lesion types on rice leaves were scored from 1 (no symptoms) to 6 (typical susceptible lesions) according to a standard reference scale (Silue et al. 1992):

Grade 1 (HR): No visible symptoms; Grade 2 (R): small brown spots, lesion diameter (LD) <0.5 mm; Grade 3 (MR): small brown lesions, $0.5 \text{ mm} \leq \text{LD} < 1 \text{ mm}$; Grade 4 (MS): small whitish lesions encircled by a brown zone, $1 \text{ mm} \leq \text{LD} < 3 \text{ mm}$; Grade 5 (S): typical spindly lesions, $3 \text{ mm} \leq \text{LD}$; Grade 6 (HS): large lesions, $3 \text{ mm} \leq \text{LD}$, lesions even expanding and merging, making the leaves partially or completely die. Individuals with scores between 1 and 3 were considered to be resistant and individuals with scores from 4 to 6 were considered to be susceptible. Disease index (%) was calculated by the following formula:

$$\text{Disease index}(\%) = \{(1 \times N_{\text{HR}} + 2 \times N_{\text{R}} + 3 \times N_{\text{MR}} + 4 \times N_{\text{MS}} + 5 \times N_{\text{S}} + 6 \times N_{\text{HS}}) / 6N_{\text{t}}\} \times 100$$

Where: $N_{\text{HR} \sim \text{HS}}$ is the number of HR~HS index leaves and N_{t} is total number of leaves tested. Approximately 180 rice plants (three replications) were scored for each treatment.

Assays of catalase (CAT) and lipoxygenase (LOX) activities in rice leaves

CAT activity was assayed based on the decrease in the absorbance at 240 nm due to the degradation of H_2O_2 , as described by Cakmak and Marschner (1992). Catalase from bovine liver (EC 1.11.1.6, 1824 units/mg solid, (Sigma-Aldrich Company Ltd, USA) was used as the standard and CAT activity was expressed as units of CAT mg^{-1} protein in each sample. One unit is defined as the amount that will decompose 1.0 μmol of H_2O_2 per min at pH 7.0 at 25°C.

LOX enzyme extraction was carried out according to the method of Li and Wang (2005). Briefly, leaf samples (0.5 g) were homogenized in 4.0 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1% (w/v) PVP and centrifuged at 16,000 g for 20 min at 4°C. The supernatant was used for analysis of enzyme activity according to the method of Sekizawa et al. (1990). The absorbance of the reaction solution was recorded spectrophotometrically at 235 nm (U-2800 spectrophotometer; Hitachi Inc., Japan). Protein content in crude extracts was determined using the method of Bradford (1976) for all the enzymes, with bovine serum albumin (BSA) as standard.

Determination of membrane permeability, H_2O_2 and malondialdehyde concentrations in rice leaves

Membrane permeability was determined by measuring relative electrolytic conductivity (EC%) as described by Yan et al. (1996). The H_2O_2 content was determined according to the method described by Jaleel et al. (2007). Malondialdehyde (MDA), an end product of lipid peroxidation, was estimated as thiobarbituric acid reactive substances (TBARS), as described by Zhao and Li (1999). The TBARS concentration was calculated according to its extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ and expressed as nmol mg^{-1} protein, according to the following formula: the concentration of MDA (μM) = $6.45 (A_{532} - A_{600}) - 0.56A_{450}$.

Statistical analysis

The reproducibility of data (disease incidence, disease severity, plant dry weight, Si, H_2O_2 and MDA concentrations) between the experiments was high and all replications of the experiments yielded similar results. Results are presented from individual experiments and all data shown are mean values of the three treatment replications in each experiment.

As the incidence of rice blast disease represents a discrete variable, the data was presented as percentage of plants infected and analysed by logistic regression assuming a binominal distribution. For comparison of discrete variables (percentages), odds ratios were

calculated, using the control plants ($-/-\text{Si}$ treated plants) as references (odds ratio=1.00). Odds is $P/(1-P)$, where P is the incidence of rice blast (percentage). Odds ratio is a relative measure of difference between the two probabilities compared: $[P2/(1-P2)]/[P1/(1-P1)]$ (Hiltunen et al. 2005). Significance of treatment effects were tested using the Wald statistic. Logistic analysis was performed using the SPSS 12.0 statistical software package.

Data obtained for frequency of grade 1–3 index leaves, disease severity, plant dry weight and Si concentration represents continuous variables and were analysed by one-way ANOVA, assuming a normal distribution. Means were separated by LSD-values at the 0.05 probability level. Means of H_2O_2 and MDA concentrations, CAT and LOX activities, and relative electrolytic conductivity were compared by Student t -test. All differences reported were considered significant if $P \leq 0.05$.

Results

Dry weight and silicon concentration

For both rice plants inoculated with *M. grisea* and non-inoculated controls, shoot dry weight of plants amended with 1.7 mM Si was significantly higher than for non-Si-amended plants (Table 1). The highest shoot dry weight was observed in Si-amended plants without inoculation ($+\text{Si}-M. grisea$). Inoculation with *M. grisea* significantly decreased shoot dry weight for both $-\text{Si}$ and $+\text{Si}$ plants. However, supplying the inoculated plants with silicon ($+\text{Si} +M. grisea$)

increased the shoot dry weight to similar levels as in the control plants. Inoculation with *M. grisea* significantly decreased root dry weight in both $-\text{Si}$ and $+\text{Si}$ plants compared with the non-inoculated controls and furthermore, supplying silicon to non-inoculated plants significantly increased root dry weight, but had no effect on inoculated plants (Table 1).

Silicon amendment significantly increased the Si concentration in shoots and roots of rice plants, with concentrations higher in shoots than in rice roots (Table 1). Inoculation with *M. grisea* also significantly increased the Si concentration in both shoots and roots of $-\text{Si}$ plants. However, there was no significant difference between inoculated and non-inoculated $+\text{Si}$ plants.

Disease symptoms and severity index

Four days after inoculation, many narrow to slightly elliptical lesions with necrotic centres and brown margins occurred on the leaves of rice seedlings. The lesions were larger and more frequent in leaves from plants in the $-\text{Si}$ (control treatment with no Si supplied) and $+\text{Si}$ (supply with Si only before inoculation) treatments. More and more symptoms in $-\text{Si}$ and $+\text{Si}$ treated plants appeared and, after 10 d, the lesions were large and expanding (Fig. 1). In severe cases, the lesions in $-\text{Si}$ and $+\text{Si}$ treated plants expanded and merged, resulting in partial leaf necrosis. For treatments receiving Si ($-/+ \text{Si}$ or $+/+ \text{Si}$), lesions were smaller, round to somewhat elongated, and had a restricted necrotic centre that was often surrounded with a very well-developed chlorotic halo. Frequencies of grades 1–3 index leaves in $-/+ \text{Si}$ and $+/+ \text{Si}$ treatments were 70.2% and 74.6% (Table 2),

Table 1 Dry weight and silicon concentrations in shoots and roots of rice plants amended with silicon ($+\text{Si}$) or not ($-\text{Si}$) and inoculated with *M. grisea* ($+M. grisea$) or not ($-M. grisea$)

| Treatment | Dry weight (mg plant^{-1}) | | Si concentrations ($\text{mg g}^{-1} \text{DW}$) | |
|------------------------|---------------------------------------|--------|--|-------|
| | Shoots | Roots | Shoots | Roots |
| $-\text{Si}-M. grisea$ | 532.5b | 110.9b | 18.3c | 7.2c |
| $-\text{Si}+M. grisea$ | 302.6c | 82.0c | 38.9b | 7.6b |
| $+\text{Si}-M. grisea$ | 685.6a | 142.4a | 63.5a | 8.9a |
| $+\text{Si}+M. grisea$ | 488.4b | 85.8c | 64.7a | 8.6a |
| $\text{LSD}_{0.05}$ | 79.5 | 16.1 | 12.7 | 0.3 |

DW, dry weight. $-\text{Si}-M. grisea$: non-inoculated plants not amended with silicon; $+\text{Si}-M. grisea$: non-inoculated plants amended with silicon; $-\text{Si}+M. grisea$: inoculated plants not amended with silicon; $+\text{Si}+M. grisea$: inoculated plants amended with silicon. Different letters in each column represent significant differences at $P=0.05$



Fig. 1 Development of leaf blast symptoms at 10 d after inoculation with *Magnaporthe grisea* in rice plants continuously treated with (+/+Si) or without silicon (-/-Si)

respectively, and significantly higher than in the -/-Si and +/-Si treatments (30.9% and 51.3%, respectively). Moreover, the incidence of rice blast lesions in -/+Si and +/+Si treatments also significantly decreased.

The disease development in plants continuously grown in the nutrient solution amended with Si (disease severity index of 19.6%) was significantly lower than that in plants continuously grown in the -Si nutrient solution (63.7%) (Table 2). Rice plants only supplied with Si prior to pathogen inoculation (+/-Si) also exhibited severe disease symptoms (severity index 33.3%), which was significantly lower than that of the control treatment. Conversely, plants supplied with Si only after inoculation (-/+Si) exhibited a reduced level of symptoms, with a disease

severity index of 20.8%, which was similar to the plants continuously supplied with silicon (+/+Si).

H₂O₂ accumulation in rice leaves

H₂O₂ concentrations in the -Si plants were significantly higher than in +Si plants (Fig. 2a) at all time points, except at 0 and 24 h after inoculation (maximum value for +Si plants at 24 h). H₂O₂ concentration in -Si plants reached two peaks: 2.6 and 3.1 $\mu\text{mol g}^{-1}$ FW at 12 h and 72 h after inoculation, respectively.

On the other hand, catalase activity in leaves of +Si plants was higher than in -Si plants at 48–72 h, whereas the reverse situation occurred at 24 and 120 h (Fig. 2b). Maximum levels of enzyme activity occurred around 72–96 h after supplying Si.

Membrane lipid peroxidation in rice leaves

Lipoxygenase activity in +Si plants increased in steps until 48 h after inoculation, with a minor peak at 12 h. In -Si plants, a peak was seen at 24 h followed by a decline at 48 h and an increase to a relatively stable level at 1.4 $\text{A}_{235} \text{ min}^{-1} \text{ mg}^{-1}$ protein from 72 h (Fig. 3a). Lipoxygenase activity was significantly higher in +Si plants than in -Si plants at 12 and 48 h whereas at 24 h, the reverse situation occurred. Maximum lipoxygenase levels were significantly higher in +Si than in -Si plants (2.0 and 1.5 $\text{A}_{235} \text{ min}^{-1} \text{ mg}^{-1}$ protein, respectively). No differences in lipoxygenase

Table 2 Effects of four different Si treatments on rice blast development 10 days after inoculation

| Si-treatment | Incidence | | Severity index (%) ^b | Frequency of resistant reactions (grade 1–3 index leaves) (%) ^b |
|---------------------|----------------|-------------------------|---------------------------------|--|
| | Percentage (%) | Odds ratio ^a | | |
| -/-Si | 100.0 | 1.00 | 63.7 a | 30.9 c |
| +/-Si | 100.0 | 1.00 | 33.3 b | 51.3 b |
| -/+Si | 38.9 | 0.00* | 20.8 c | 70.2 a |
| +/+Si | 36.6 | 0.00* | 19.6 c | 74.6 a |
| LSD _{0.05} | | | 5.8 | 6.1 |

The treatments were: -/-Si, Plants treated with no Si at all, regardless of inoculation; +/-Si, Plants treated with silicon only before inoculation; -/+Si, Plants treated with silicon only after inoculation; +/+Si, Plants treated continuously with silicon in the nutrient solution before and after the inoculation

^a Odds ratios for comparison of treatments to the -/-Si treated (control) plants (-/-Si treatment used as a reference, odds ratio=1.00).

* $P < 0.05$

^b Different letters in each column represent significant differences at $P = 0.05$

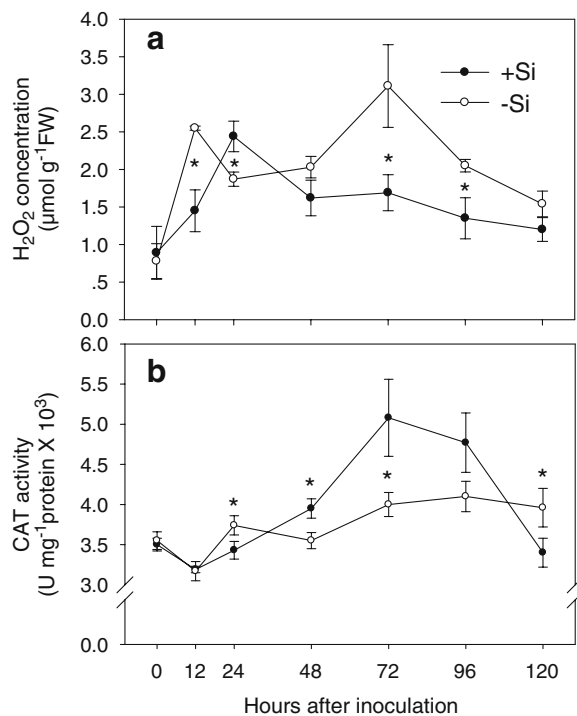


Fig. 2 Changes in H_2O_2 concentration and catalase (CAT) activity in leaves of rice seedlings amended with silicon (+Si) or not (-Si) after inoculation with *Magnaporthe grisea*. Vertical lines represent standard deviations. Asterisks denote significant differences between +Si and -Si treatments at a given time point

activities was observed between the +Si plants and the -Si plants from 72 h and onward.

Malondialdehyde concentration in rice leaves of the +Si plants rapidly increased after inoculation and reached a maximum ($124.4 \text{ nmol mg}^{-1} \text{ protein}$) at 48 h after inoculation (Fig. 3b), and this level was significantly higher than measured in -Si plants ($88.7 \text{ nmol mg}^{-1} \text{ protein}$). At 144 h after inoculation, the level was significantly higher in -Si than in +Si plants,

Membrane permeability in rice leaves

Relative electrolytic conductivity (EC%) in rice leaves of the +Si plants rapidly increased after inoculation and attained significantly higher levels than -Si plants at 24–48 h after inoculation, with a peak (20.7%) at 48 h (Fig. 4), being significantly higher than in the -Si plants (13.3%). Following this peak level, EC% in +Si plants declined to levels lower than in the -Si plants, even significantly lower at 96–120 h after inoculation (Fig. 4). EC% levels in -Si plants steadily increased to

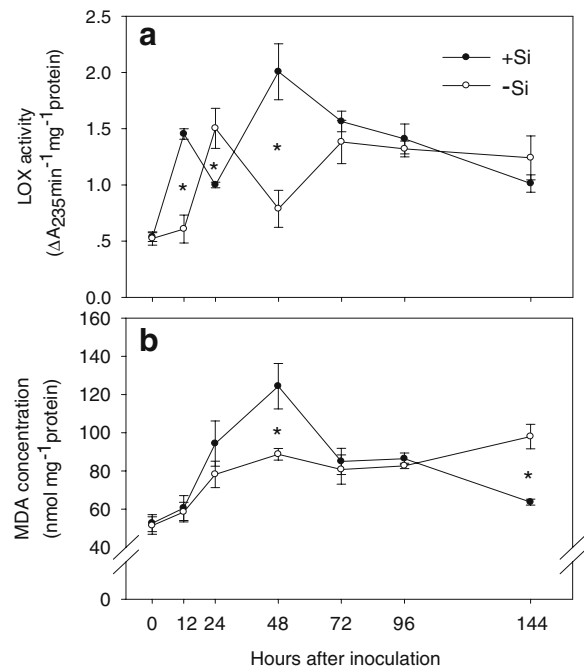


Fig. 3 Changes in lipoxygenase (LOX) activity and malondialdehyde (MDA) concentration in leaves of rice seedlings amended with silicon (+Si) or not (-Si) after inoculation with *Magnaporthe grisea*. Vertical lines represent standard deviations. Asterisks denote significant differences between +Si and -Si treatments at a given time point

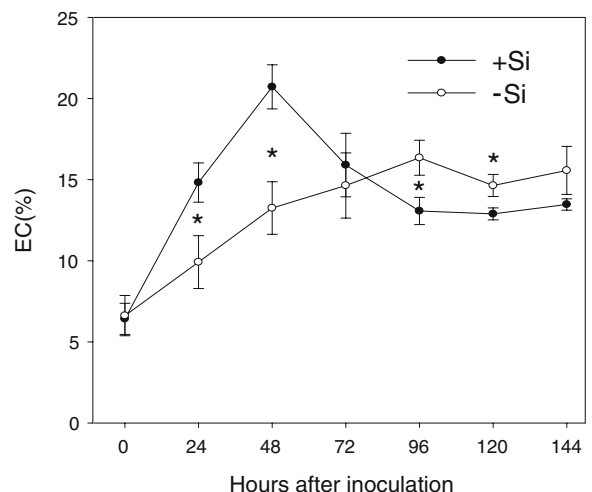


Fig. 4 Changes in relative electrolytic conductivity (EC%) in leaves of rice seedlings amended with silicon (+Si) or not (-Si), after inoculation with *Magnaporthe grisea*. Vertical lines represent standard deviations. Asterisks denote significant differences between +Si and -Si treatments at a given time point

a maximum level at 96 h (16.4%) and remained relatively constant thereafter (Fig. 4).

Discussion

Previously, it has been well documented that application of Si to rice plants increases resistance to blast disease (Seebold et al. 2004), however the mechanistic basis for this has been unclear. In the present study, rice plants initially grown in the presence of Si and then switched to a -Si nutrient solution prior to pathogen exposure still exhibited significant levels of resistance to blast disease development. However, this resistance was significantly lower than in the -/+Si and +/+Si treatments (Table 2). It is likely that Si deposited in the cell walls, which is immobilized and unavailable for redistribution, affects rice blast disease development by physically strengthening the cell wall. However, the deposited (immobile) Si may not be as important as the available (mobile) Si present in the cell at the time of infection (Samuels et al. 1991). Interestingly, in the present study the result of +/-Si treatment is contrary to previous findings from the cucumber–powdery mildew pathosystem (Samuels et al. 1991). Cucumber plants grown initially with Si and then switched to a -Si medium prior to inoculation entirely lost resistance to powdery mildew. Such discrepancies reported between investigators may be caused by the different plant species and pathogens tested. Rice used in the present study is a typical Si-accumulating grass species that can accumulate SiO_2 up to 10% in its shoots (Liang et al. 2006), whereas cucumber used in the study by Samuels et al. (1991) is a dicotyledonous plant species that accumulates far less Si (Liang et al. 2006).

As a physical barrier, increased silicon deposition occurring in the leaf cell walls of rice plants may play a role in preventing the subsequent penetration by the pathogen. However, in some non-accumulating plant species, such as tomato, the effect of Si on suppression of bacterial wilt was also significant (Dannon and Wydra 2004; Diogo and Wydra 2007). It was hypothesized that Si induced basal resistance and increased tolerance, interacting with resistance factors of the plants, and provided an indirect effect on bacterial growth and on the physiological status of the bacteria (Dannon and Wydra 2004; Diogo and Wydra 2007). On the other hand, rice plants that were

supplied with Si only after inoculation (-/+Si) exhibited the same high resistance to rice blast as plants treated continuously with Si (+/+Si) (Table 2). The results from the present study suggest an important physiological and biochemical role of Si in controlling rice blast.

Interestingly, inoculation with *M. grisea* also increased the Si concentration in shoots and roots of -Si plants. However, inoculation had no effect on Si concentration in +Si plants (Table 1). This is a novel and interesting finding and the possible mechanisms involved are not clear and worth exploring in further studies. An increased Si concentration in -Si plants caused by inoculation with *M. grisea* alone may be an indication of closer interrelationship between induced resistance and Si accumulation. However, the growth medium of the -Si treatment in the experiments still contained trace amounts of Si due to contamination from chemicals, purified water and even air dust etc., and was therefore not completely Si-free. In accordance with this, Epstein (1994) also believed that it was extremely difficult to create a Si-free environment in such experiments. Under such conditions, induced resistance resulting from inoculation with *M. grisea* will increase the requirements of plants for Si and potentially lead to increased uptake. By contrast, in tomato, a non-Si-accumulating plant species, inoculation of *Ralstonia solanacearum* did not influence Si uptake or distribution (Dannon and Wydra 2004). These findings highlight the important differences Si has between plant species and pathogens. For rice, Si is beneficial and important to both the growth of plants and the host resistance against infection by pathogens.

Active oxygen species (AOS), including superoxide, hydrogen peroxide, and the hydroxyl radical, could potentially affect many cellular processes involved in plant/pathogen interactions. Thus, H_2O_2 has been shown to be directly toxic to microorganisms, to drive oxidative cross-linking of cell wall (glyco) proteins (reducing their susceptibility to enzymatic degradation), to mediate phytoalexin and PR-1 accumulation, to induce systemic acquired resistance, to elicit gene activation as well as transcription-dependent defences and to orchestrate the hypersensitive cell-death response (Wojtaszek 1997). AOS that are maintained at a high level for a long time will cause oxidative damage in plants (Schützendübel and Polle 2002; Liang et al. 2003). In healthy plant cells, AOS is removed (and the

oxidative damage alleviated) by an AOS-scavenging system, including enzymatic antioxidants (e.g. superoxide dismutase, catalase and peroxidase) and non-enzymatic metabolites (e.g. glutathione and ascorbic acid) (Liang et al. 2003; Foyer and Noctor 2005). AOS can be difficult to monitor in plant cells because many species are short-lived and are exposed to cellular antioxidant. The AOS response during incompatible interactions has been reported to consist of two distinct phases (Baker and Orlandi 1995). In +Si plants, H_2O_2 could be anticipated to display two peaks and might have a peak from 0 to 12 h after infection by *M. grisea* (phase I), but this was not measured in the present study. However, a rapid and transient accumulation of H_2O_2 coupled with low catalase activity in leaves of the +Si rice plants was observed at 24 h after infection by *M. grisea* compared with the -Si plants (Fig. 2). In the advanced stages of infection by *M. grisea* (>48 h), silicon application significantly increased catalase activity and decreased accumulation of H_2O_2 , thereby resulting in decreased lipid peroxidation (lower MDA concentration) and membrane permeability (lower relative electrolytic conductivity).

In rice infected with *M. grisea*, Si has been linked with higher accumulation of antimicrobial compounds at infection sites, including diterpenoid phytoalexins (Rodrigues et al. 2004). In blast-susceptible rice lines, the lignin content of leaf tissue has been found to increase significantly with Si-treatment (Cai et al. 2008). Similarly, we also found that total soluble phenolics and lignin in rice leaves infected by *M. grisea* were significantly higher in Si-treated plants than in non-Si-treated plants (unpublished data). In addition, application of Si contributes to hypersensitive cell death (Rodrigues et al. 2005), as well as increasing epidermal cell wall thickness of rice leaves (Kim et al. 2002). These host defence mechanisms can also be induced and mediated by AOS in plant pathogenesis (Baker and Orlandi 1995; Lamb and Dixon 1997). Therefore, it is likely that Si may affect disease expression via mediating AOS accumulation and stimulation of host defence mechanisms.

Often, increases in LOX activity have been observed only in incompatible host-pathogen interactions, e.g., by *P. syringae* pv. *pisi* in cucumber (Keppler and Novacky 1987). In this interaction, increased LOX activity was hypothesized to occur in response to fatty acids released by AOS-induced lipid

peroxidation of membranes and therefore, it has been suggested that lipoxygenase might be involved in the development of the HR induced (Keppler and Novacky 1987). The increased membrane permeability observed during HR is due to peroxidation of membrane lipids. It was also suggested that AOS production, lipid peroxidation and hypersensitive necrosis were directly correlated (Rustérucci et al. 1996). Our results also showed that during the early stages of infection by *M. grisea*, elevation of lipid peroxidation (as indicated by malondialdehyde and lipoxygenase) and membrane permeability (relative electrolytic conductivity) in the +Si rice plants occurred earlier, faster, and became significantly higher than after -Si treatments (except for lipoxygenase activity at 24 h) (Figs. 3 and 4). It should be pointed out that LOX and CAT activities were all significantly higher in -Si plants than in +Si plants at 24 h (Figs. 2 and 3), which was in accordance with lower MDA concentrations (Fig. 3b), thus facilitating H_2O_2 accumulation in +Si plants. All this supports the model for Si-stimulated development of HR and Si-enhanced rice resistance to blast acting through lipoxygenase activity and lipid peroxidation processes.

In conclusion, the results of this study demonstrate the role of Si in enhancing rice resistance to blast. We propose that Si affects H_2O_2 accumulation and lipid peroxidation to induce host defence mechanisms, and plays a much larger role in disease resistance than its established role as a physical barrier.

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