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Short communication

# Rice blast disease and susceptibility to pests in a silicon uptake-deficient mutant *lsi1* of rice

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**Abstract**

To elucidate the role of silicon more clearly in biotic stress such as pests and diseases, a silicon uptake-deficient mutant *lsi1* originating from wild-type rice (cv. Oochikara) was used. When the mutant was grown in a seedling case, silicon did not accumulate in leaves (about 50–80 mg $g^{-1}$ dry weight), regardless of the silicon amendment. In the paddy field, however, silicon increased three-fold (373 mg $g^{-1}$ dry weight) in leaves with silicon amendment, compared with those (117 mg $g^{-1}$ dry weight) with no silicon amendment. Lesion formation by *Magnaporthe grisea* was significantly suppressed in the leaves of the wild-type plant that had a high accumulation of silicon, but not in the leaves of the mutant that had a low silicon accumulation. Pest resistance was also observed in the leaves of the wild-type plant, but not in the mutant. These results demonstrated that silicon can protect rice plants from damage caused by biotic stresses.


*Keywords:* *Magnaporthe grisea*; Rice; Silicon uptake-deficient mutant; Resistance; Pest

Silicon (Si), the second most abundant element on the surface of the earth, is one of the most beneficial elements for several plants, although it is a common but generally minor element in the majority of living organisms. Silicon deposits in the leaves, stems, and hulls in the form of amorphous silica gel ($SiO_2 \cdot nH_2O$) and soluble silicic acid ($Si(OH)_4$). Although there are many reports on the biological roles of silicon (i.e., reproduction, alleviation of metal toxicity and nutrient imbalance, provision of structural rigidity, and increased resistance to fungal disease), the exact means by which silicon influences these different physiological processes is unknown (Epstein, 1999; Savant et al., 1997).

Rice (*Oryza sativa* L.), one of the most important food crops, is the most effective silicon-accumulating plant. It is well known that silicon absorbed in rice tissues contributes to enhance resistance to disease and insects, increases photosynthesis by keeping leaves erect, improves water use efficiency, and reduces the toxicity of heavy metals and cuticular transpiration (Epstein, 1994). Although silicon is an essential factor for high and sustainable production of rice, the molecular mechanism responsible for the uptake of silicon has not yet been elucidated. Recently, the rice mutant *lsi1* (low silicon rice, formerly GR1) was isolated from sodium azide-treated $M_2$ seeds of rice (cv. Oochikara) as a silicon uptake-deficient mutant (Ma et al., 2002). This mutant accumulates less silicon in the shoot throughout its growth period compared with the wild type (Ma et al., 2004). Ma et al. (2006) cloned the low silicon rice 1 (*Lsi1*) gene, which controls silicon accumulation in rice. Physiological and molecular studies of the *lsi1* mutant of rice have contributed to elucidating the silicon uptake system in plants and to creating new plants with high resistance to multiple stresses by genetic modification of the root's silicon uptake capacity. On the other hand, although rice is emphasized as a useful forage for domestic animals in Japan, rice accumulates silicon to the level of up to 10% of the shoot dry weight, which is often several times higher than that of essential macronutrients such as nitrogen,

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phosphate, and potassium (Ma et al., 2006). Because domestic animals cannot easily digest high silicon rice material, the silicon uptake-deficient mutant *lsi1* may become a useful plant for forage production.

It is well known that significant yield losses of rice products are mainly induced by disease and pest damage (Ou, 1980). However, neither disease resistance nor insect resistance has been investigated in this mutant. Therefore, in the present study, we investigated the responses of the *lsi1* mutant to *Magnaporthe grisea* infection and pest attack.

A wild-type (cv. Oochikara) and mutant (*lsi1*) rice plant were used in this study. Mutant-type rice *lsi1* originated from cv. Oochikara by treatment with sodium azide (Ma et al. 2002). These cultivars were grown in seedling cases for 30 d (four- to five-leaf stages) in a glasshouse. A virulent strain 92-06-2 (MAFF101530, race 337.1) of *M. grisea* was cultured on rice bran agar medium at 26 °C for 14 d (Kiyosawa, 1984; Yamada et al., 1976). The growth plates were kept at 26 °C for about 2 d with near-UV illumination after the aerial hyphae were washed away by distilled water. Thus, synchronously formed spores were used as inocula. A spore suspension ($3 \times 10^5$ spores $ml^{-1}$) of *M. grisea* was sprayed on the leaves of the rice plants at the four- to five-leaf stage. The inoculated plants were kept in a moist chamber for 24 h at about 26–28 °C, and then transferred to a glasshouse. Silica gel (Water silica: Fuji Silysia Chemical Ltd., Tokyo, Japan) was used as the silicon source. Seeds of wild-type or mutant rice were germinated in water at 26–28 °C for 2–3 d and the germinated seeds were then sown in a seedling case ($5 \times 10 \times 15$ cm, Fujimoto Kagaku Co.) with 300 g of nursery soil mixed with 0 ($Si^-$) or 20 g ($Si^+$) of silica gel. The rice plants were fertilized with 1 g of $(NH_4)_2SO_4$, 0.2 g of KCl, and 1.5 g of $CaH_4(PO_4)_2$–$H_2O$ per seedling case. All experiments were conducted with three replicates in a glasshouse under natural daylight. The silicon concentration was determined by the colorimetric molybdenum blue method as described by Okuda and Takahashi (1961). The rice leaves were dried in an oven at 70 °C for 3 d prior to analysis. The sample (0.1 g) was digested in a mixture of 1.8 ml of $HNO_3$ (69%), 1.8 ml of $H_2O_2$ (30%), and 1.2 ml of HF (46%) for 24 h and the digested sample (0.05 ml) was diluted to 6.3 ml with 4% (w/v) boric acid. The diluted digested sample (0.1 ml) was added to 2.3 ml of $H_2O_2$, and then to 1.2 ml of 0.25 N HCl, 0.16 ml of 10% (w/v) $(NH_4)_6Mo_7O_{24}$, 0.16 ml of 20%(w/v) tartaric acid, and 0.16 ml of reducing agent. The reducing agent was prepared by dissolving 1 g of $Na_2SO_3$, 0.5 g of 1-amino-2-naphthol-4-sulfonic acid, and 30 g of $Na_2SO_3$ in 200 ml distilled water. After 1 h, the absorbance was measured at 600 nm with a spectrophotometer (UV-1200; Shimadzu, Kyoto, Japan). A standard curve was prepared from a silicon standard solution (Wako Pure Chemical, Inc., Osaka, Japan). The field experiment was conducted in Kawamoto-cho, Shimane Prefecture, Japan, in 2005 and 2006. Seedlings of the wild-type and mutant rice plants were transplanted to a paddy field, and blast development and pest damages were investigated at designated times.

The amount of silicon in the leaf blades of the wild type and *lsi1* mutant grown in a soil amended with or without silicon was investigated. There was a large difference in silicon accumulation between the wild type and *lsi1* mutant. In the control soil ($Si^-$), silicon accumulation in the wild type was 117.9 mg $g^{-1}$ leaf dry weight, whereas that in *lsi1* was 58.1 mg $g^{-1}$ leaf dry weight (Fig. 1). The difference in silicon accumulation between the wild-type and mutant leaf blades became more significant with silicon amendment ($Si^+$). In the wild-type leaves, silicon accumulation was enhanced over three-fold (373.5 mg $g^{-1}$ leaf dry weight) compared with that of the control, but not in the *lsi1* mutant (84.2 mg $g^{-1}$ leaf dry weight). In the leaf-sheath, however, such a difference in silicon accumulation between the wild-type and mutant leaves or between $Si^-$ and $Si^+$ was not observed (data not shown). Ma et al. (2002) reported that the silicon content in the shoot of the *lsi1* mutant was much lower than that of the wild type in silicon-amended soil. The present study suggests that the *lsi1* mutant accumulates less silicon in leaf blades, though not in the leaf-sheath, even under field conditions with a high level of silicon. Therefore, the *lsi1* mutant could be a useful genetic resource for breeding low silicon forage for domestic animals.

To elucidate the role of silicon uptake on disease resistance in rice, blast lesion formation in *M. grisea*-infected leaves was investigated 7 d after inoculation. When wild-type and mutant rice grown in $Si^-$ soil were inoculated with a virulent strain of *M. grisea*, both plants were highly susceptible, with the formation of typical blast lesions on the leaves (Fig. 2A). The number of blast lesions on the leaves of the wild-type and mutant plants was 25.0 and 31.5 per plant, respectively (Fig. 2B). In the $Si^+$ soil, however, blast lesion formation was significantly inhibited in the wild-type (13.9 per plant) plants, but not in the mutant plants (34.0 per plant) (Fig. 2A). Hyphal growth of *M. grisea* was not inhibited in leaf-sheath

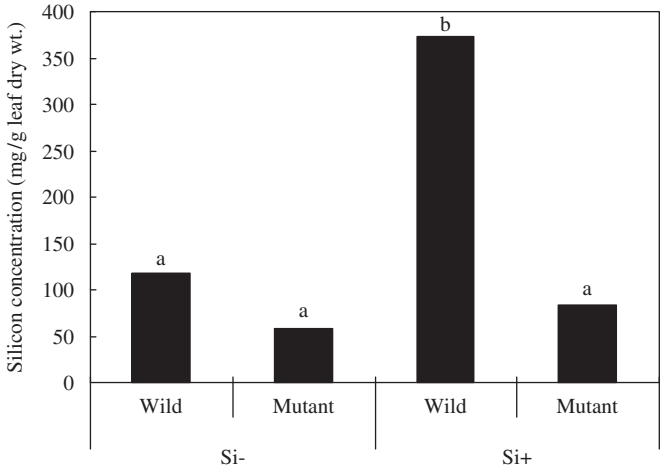


Fig. 1. Silicon concentration in leaf blades of wild-type (cv. Oochikara) and mutant (*lsi1*) rice grown in seedling cases in the presence ($Si^+$) or absence ($Si^-$) of silicon. Values are the means of three experiments. Values in each column followed by the same letter are not significantly different at the 5% level using Fisher's protected LSD.