Bull. Fac. Life Env. Sci. Shimane Univ., 12：7－9, September 30, 2007

Susceptibility of a silicon uptake-deficient mutant *lsi1* of rice to *Magnaporthe grisea* infection

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Abstract Disease susceptibility of a silicon (Si) uptake-deficient mutant *lsi1* originating from cv. Oochikara of rice (wild-type) was investigated. When the mutant and wild type rice plants at three-leaf stages were inoculated with *Magnaporthe grisea*, blast lesion formation was rapidly enhanced in the leaves of the mutant *lsi1*, as compared to that in wild type rice cv. Oochikara. Si concentration in leaves of *lsi1* was lower than that of cv. Oochikara and its percentages per dry weight in *lsi1* and Oochikara were 1.2±0.6 % and 4.6±1.2 %, respectively. This study suggests that Si uptake deficiency is involved in enhanced susceptibility of *lsi1* mutant to seedling blast.

Key words: *Magnaporthe grisea*, Rice, Silicon uptake, Resistance

Introduction

Rice is one of the world most important food crops and rice blast disease, caused by *Magnaporthe grisea*, is the most destructive fungal disease to its production in the world. It is well known that rice (*Oryza sativa* L.) is the most effective silicon-accumulating plant. Si is the second most abundant element on the surface of the earth. Furthermore, Si 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. Si deposits in the leaves, stems, and hulls in the form of amorphous silica gel ($SiO_2 \cdot nH_2O$) and soluble silicic acid ($Si(OH)_4$). It is well known that there are many reports on the biological roles of Si (i.e., reproduction, alleviation of metal toxicity and nutrient imbalance, provision of structural rigidity, and increased resistance to fungal disease). However, the exact means by which Si influences these different physiological processes is unknown (Epstein 1999, Savant et al. 1997).

Recently, the rice mutant *lsi* (low Si rice, formerly GR1) was isolated from sodium azide-treated M_2 seeds of rice (cv. Oochikara) as a Si uptake-deficient mutant (Ma at al. 2002). This mutant accumulates less Si in the shoot throughout its growth period compared with the wild type. Ma et al. (2006) cloned the Low Si rice 1 (*Lsi1*) gene, which controls Si accumulation in rice. Physiological and molecular studies of the *lsi1* mutant of rice have contributed to elucidating the Si uptake system in plants and to creating new plants with high resistance to multiple stresses by genetic modification of the root's Si uptake capacity.

Epstein (1994) reported that Si 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. Although Si is an essential factor for high and sustainable production of rice, the molecular mechanism responsible for the uptake of Si has not yet been elucidated. In the present study, we investigated the responses of the mutant *lsi1* to seedling blast.

Materials and Methods

Plant and fungus

A wild type (cv. Oochikara) with blast resistance genes *Pik* and *Pita* and mutant (*lsi1*) (previous name GR1) of rice plant were used in this study. Mutant type rice *lsi1* was originated from cv. Oochikara by treatment with sodium azide (Ma et al. 2002). Seeds of these cultivars were germinated in water at 26-28℃ for 2-3 days and then sowed in seedling cases (5×10×15 cm, Fujimoto Kagaku Co.) containing 300 g (1g of $(NH_4)_2SO_4$, 0.2g of KCl and 1.5g of $CaH_4(PO_4)_2 \cdot H_2O$) of commercial soil. Rice plants were grown in a glasshouse

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and plants at three-leaf stages were used in this study. All experiments were conducted with three replicates in a glasshouse under natural daylight.

Fungal inoculation

A virulent strain 0528-2 (race 333.1) of *M. grisea* was cultured on rice bran agar medium at 26℃ for 14 days. The growth plates were kept at 26℃ for about 2 days with near-UV illumination after aerial hyphae on the medium were washed away by distilled water. Thus, synchronously formed spores were used as inocula. A spore suspension (3×10^5spores/ml) of *M. grisea* was sprayed on leaves of the rice plants at the three-leaf stages. Inoculated plant were incubated in a moist chamber for 24 h at about 26-28℃, and then kept in the glasshouse.

Determination of Si

The Si 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℃ for 3 days prior to analysis. The sample (0.1g) was digested in a mixture of 1.8ml of HNO_3 (69%), 1.8ml of H_2O_2 (30%), and 1.2ml of HF (46%) for 24 h and the digested sample (0.05ml) was diluted to 6.3ml with 4% (w/v) boric acid. The diluted of digested sample (0.1ml) was added to 2.3ml of H_2O_2, and then to 1.2ml of 0.25 N HCl, 0.16ml of 10% (w/v) $(NH_4)_6Mo_7O_{24}$, 0.16ml of 20% (w/v) tartaric acid and 0.16ml of reducing agent. The reducing agent was prepared by dissolving 1g of Na_2SO_3, 0.5g of $H_2NC_{10}H_5$ (OH) SO_3H and 30g of Na_2SO_3 in 200ml distilled water. After 1h, the absorbance was measured at 600 nm with a spectrophotometer (UV-1200; Shimadzu, Kyoto, Japan). A standard curve was prepared from Si standard solution (Wako Pure Chemical, Inc., Osaka, Japan).

Results and Discussion

To compare the susceptibility between wild type rice (cv. Oochikara) and *lsi1* mutant originating from cv. Oochikara, each rice plant was grown in a nursery soil until three-leaf stages and then inoculated with *M. grisea* spores. After 7 days, typical blast lesions were formed on the leaves of the each rice. Especially, development of blast lesion by *M. grisea* was more promoted in *lsi1* mutant than that in wild type and then blast lesions developed in entire leaves (Fig. 1A). The number of

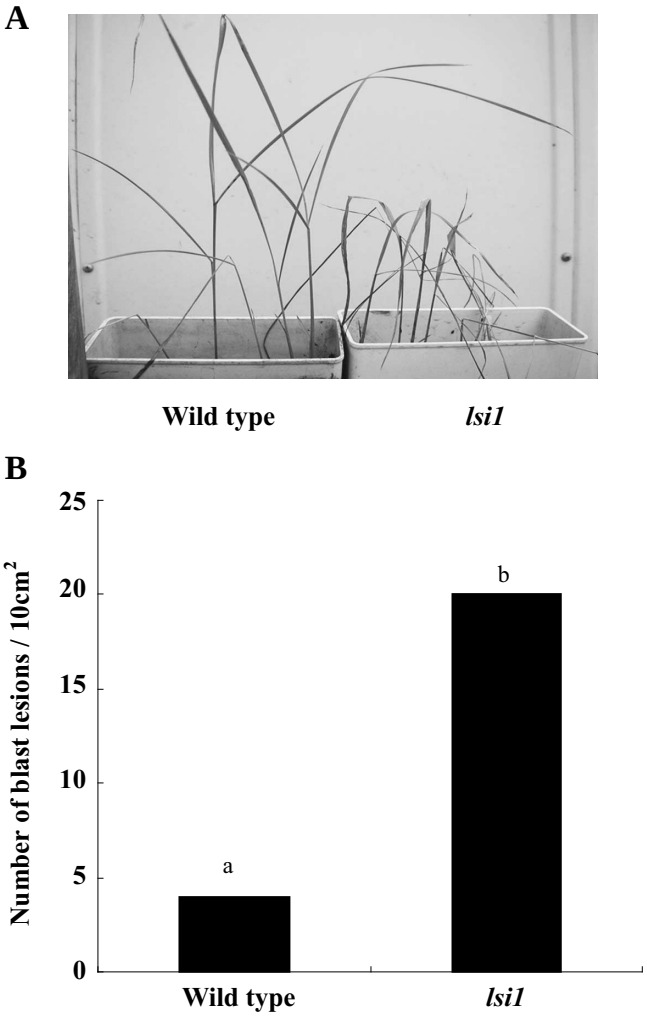

Fig. 1 Comparison of seedling blast susceptibility between wild type rice (cv. Oochikara) and *lsi1* mutant. Wild type rice and *lsi1* mutant were inoculated with a spore suspension of *M. grisea* and kept in a moist chamber for 24h at 26-28℃. After 7 days of inoculation, development of blast lesions (A) and number of blast lesions (B) were investigated.

blast lesions formed on the leaves of wild type rice and *lsi1* mutant was 4.0 and 20.0 per 10 cm^2, respectively (Fig. 1B).

Although Si is not universally recognized as an essential plant nutrient, many plants accumulate Si from 0.1 to 10% of dry weight (Epstein et al. 1994). It is well known that Si is accumulated at high levels in rice plant. In this study, percentages of Si concentration in the leaves of wild type and *lsi1* mutant at three-leaf stages were 4.6 ± 1.2 and 1.2 ± 0.6 % per dry weight, respectively (Fig. 2).

This study shows that seedlings of a Si uptake-deficient mutant *lsi1* are more susceptible than wild type rice cv. Oochi-

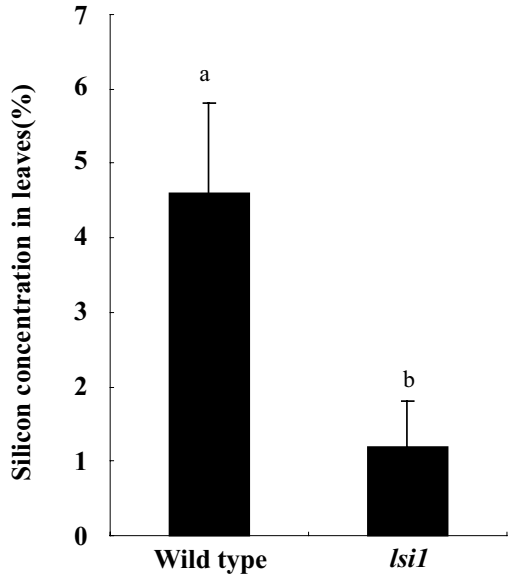

Fig. 2 Si concentrations in leaf blades of wild type rice (cv. Oochikara) and *lsi1* mutant at three-leaf-stages. Values are the means of three experiments. Values in each column followed by the same letter are not significantly different at the 5% level using Fisher's Protected LSD.

kara to seedling blast. There are many reports on the beneficial effects of Si for the cultivation of rice (Ando et al. 2002; Savant et al. 1997; Yoshida et al. 1962). Hayasaka et al. (2005) reported that the most effective level of the SiO_2 content of seedlings for control of seedling blast was estimated at about 5%. In this study, Si concentration was 4.6 % in wild type rice, but 1.2 % in *lsi1* mutant. This study suggests that Si-uptake deficiency is involved in enhanced susceptibility of *lsi1* mutant to seedling blast.

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