Reexamination of silicon effects on rice growth and production under field conditions using a low silicon mutant

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Abstract

Silicon (Si) is a beneficial element for healthy growth and high and sustainable production of rice, but the mode of action of the beneficial effects has not been well understood. We carried out field trials for four years at two different locations to re-examine the effects of Si on the growth and production of rice using a low silicon rice (*lsi1*) mutant. The mutant accumulated much lower Si at each growth stage compared with the wild-type rice (*Oryza sativa* L. cv Oochikara), but there was no difference in the accumulation of other nutrients including N, P, and K. Measurements at different growth stages showed that low Si in the mutant hardly affected the tiller

number, chlorophyll content (SPAD value), and root growth. The plant height and shoot dry weight of the wild-type rice were slightly higher than those of the mutant at a later growth stage, but the difference was not significant between the two lines. However, grain yield was reduced by 79-98%, depending on year, due to a low Si accumulation in the mutant, which showed the largest effect of Si on rice production among all studies reported so far. Among the yield components, the percentage of filled spikelets was mostly affected, being only 13.9 % of the wild-type rice in the mutant. The grain color of the mutant became brown because of excessive transpiration and infection of pathogens. These results indicate that Si increases rice yield mainly by enhancing the fertility of spikelets.

Introduction

Silicon (Si) is the most abundant mineral in soil and therefore all plants contain Si in their tissues. Plant roots take up Si in the form of silicic acid, a non-charged molecule under pH 9, from soil solution (Takahashi and Hino 1978). After uptake, Si is immediately translocated to the shoot via the transpiration stream and then polymerized and accumulated on the shoot tissues as silica (Ma and Takahashi 2002).

   A number of studies have shown that Si accumulated in plants exerts various

beneficial effects on plant growth. The most noticeable beneficial effect of Si is an enhanced resistance to both biotic and abiotic stresses (Epstein 1999; Ma 2004; Ma and Yamaji 2006). Silicon is especially important for healthy growth and high and sustainable production of rice, which is a typical Si-accumulating plant (Savant et al. 1997). Rice accumulates Si in the shoots several-times higher than macronutrients such as N, P, and K. High Si accumulation in rice enhances resistance of plants to diseases caused by both fungi and bacteria such as rice blast, sheath blight, and gray leaf spot (Datnoff and Rodrigues 2005; Fauteux et al. 2005) and to insect pests such as stem borers, brown planthoppers, and rice green leafhoppers (Sakamoto 1958; Savant et al. 1997). Silicon also increases the resistance of rice to abiotic stresses including chemical stress (salt, metal toxicity, nutrient imbalance) and physical stress (lodging, drought, radiation, high temperature, freezing, UV) (Epstein 1994, 1999, Savant et al. 1997; Ma and Takahashi 2002; Ma 2004; Ma and Yamaji 2006). In addition, high Si increases the canopy photosynthesis by keeping the leaf blades erect and thereby improving light interception characteristics. Therefore, Si in the form of silicate fertilizers has been applied to the paddy fields in many countries to gain a better rice yield. However, the process leading to these beneficial effects has not been well understood.

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Recently, we have isolated a rice mutant with low Si (*lsi1*) using Ge, a toxic analogue of Si (Ma et al. 2002).   Physiological studies have demonstrated that this mutant is defective in Si uptake, which is mediated by at least two transporters, Lsi1 and Lsi2. Lsi1 is an influx transporter, while Lsi2 is an efflux transporter of Si (Ma et al. 2006; 2007).   Both transporters are localized on the exodermis and endodermis of rice roots, where Casparian bands are located.   A single nucleotide substitution in the influx transporter gene Lsi1 was found in the mutant (Ma et al. 2006).   When this mutant was grown in the field, the yield was significantly reduced (Ma et al. 2006).   Therefore, this mutant provides a good material to examine the process leading to the beneficial effects of Si on rice growth and production.   In the present study, a number of growth parameters and yield components were compared between the mutant and the wild-type rice at different growth stages under field conditions.

Material and Methods

Plant materials and growth

Field trials were carried out for four years at two locations (2003 and 2004 at the Experimental farm of Kagawa University, 2005 and 2006 at the Experimental farm of Okayama University).   Seedlings were prepared by germinating seeds of wild-type rice

(*Oryza sativa* L. cv Oochikara) and a low Si mutant (*lsi1*) in the dark for two days and then pre-culturing in 1/2 strength Kimura B nutrient solution in a greenhouse for 20 days. The composition of the nutrient solution was reported previously (Ma et al. 2001) and the solution was renewed every 5 days.

In mid June, seedlings of the two lines were transplanted in the field. Each plot (0.7m x 0.7m) contained 36 seedlings and three replicates were made for each line. At the end of September, plants were harvested.

Measurement of growth parameters

After transplanting, the tiller number and plant height were recorded at different growth stages. Chlorophyll content was measured on the youngest fully expanded leaf with a SPAD meter (Minolta SPAD-502, Osaka, Japan). At the same time, the roots and shoots were harvested and dried at 70°C for at least 4 days before the measurement of the dry weights. The samples were ground to a powder for analysis of minerals as described below.

Determination of Si and other minerals

For Si determination, the ground plant samples were microwave-digested in a

mixture of 3 ml of HNO_3 (62%), 3 ml of H_2O_2 (30%), and 2 ml of HF (46 %) (Microwave Closed System 850, Hansen Co., LTD) and the digested solution was diluted to 100 ml with 4% boric acid. The Si concentration in the solution was determined by the colorimetric molybdenum blue method (Ma et al. 2001).

For determination of P and K, the samples were digested with nitric acid. The concentration of K in the solution was determined by flame atomic absorption spectrometry (Z-5000; Hitachi, Tokyo, Japan), after an appropriate dilution with 0.1 N HNO_3 and that of P determined by the colorimetric molybdenum blue method at a wavelength 882 nm. The concentration of N was determined by the CHN recorder (Yanaco MT-500, Kyoto, Japan).

Determination of yield components

At harvest, the panicle number was recorded. After air-drying, the spikelet number per panicle was counted. The percentage of filled spikelets was determined in a salt solution with a gravity of 1.06. After air-dried, the filled spikelets were weighed and the 1,000-grain weight was calculated. A portion of grains was de-husked with a small thresher (Kett TR-110) and the concentration of Si in the husk was determined as described above.

Results

The field trials were run for four consecutive years at two different locations, but since the trends were similar in all years, most of the results shown in this paper are those from the 2003 trial. The concentrations of Si and three essential macronutrients (N, P, and K) in the shoots were compared between the wild-type rice and the mutant at different growth stages. The concentrations of N, P, and K of the mutant were similar to those of the wild-type rice (Fig. 1A-C). The amounts of these minerals taken up by plants were also similar between the mutant and the wild-type rice at all growth stages (Fig. 2A-C). By contrast, both the concentration and uptake of Si were much higher in the wild-type rice than in the mutant (Fig. 1D, Fig. 2D). At harvest, the concentration of Si in the shoots of the wild type was 4.14 % of the dry weight, but that of the mutant was only 0.22 %. The Si concentration of the husk was also much higher in the wild type than in the mutant at either growth stage (Fig. 3).

Among the growth parameters measured, there was no significant difference in the tiller number or SPAD value between the mutant and the wild type at all growth stages (Fig. 4A, B). There was also no significant difference in the root dry weight between the two lines (Fig. 5A). The plant height and shoot dry weight were similar at the early growth stage between the two lines (Fig. 5B, C). At a later growth stage, the wild type had slightly higher plant height and shoot dry weight than the mutant (Fig. 6A), although these differences were no significant. Rice blast was observed in the mutant but not in the wild type (data not shown). Pest damage was also more serious in the mutant than in the wild type (Fig. 6B).

There was three-day difference in the heading time; the wild type headed on Aug. 24,

while the mutant on August 27. There were typhoons during the post-heading period. After typhoon, the panicle of the mutant became white head, while that of the wild type remained green (Fig. 6C). At harvest, the panicles of the mutant showed a dull brown color, whereas those of the wild type showed a golden-yellow color (Fig. 6D).

The grain yield of the mutant was significantly reduced by between 78.5 and 97.7%, depending on year, compared with the wild type (Fig. 7). Among the yield components measured in 2003, there was no difference in the panicle number and 1000 grain weight between the wild type and the mutant, but the spikelets number per panicle was about 20% lower in the mutant than in the wild type (Fig. 8). By contrast, the percentage of filled spikelets in the mutant was only 13.9 % of that in wild type (Fig. 8).

Discussion

Numerous studies have reported beneficial effects of Si on rice growth and production based on both pot (solution and soil culture) and field experiments (for a review, see Ma and Takahashi 2002). However, the beneficial effects of Si might have been underestimated in previous studies. Because of the role of Si in alleviating abiotic and biotic stresses, the effect of Si on plant growth becomes more pronounced under stress conditions, but may not be noticeable under non-stressed conditions. In solution culture experiments, although Si is easily removed from the nutrient solution in the control treatment, the experiments are usually performed inside greenhouses, where the environmental conditions are not as stressful as often encountered in the fields. On the other hand, in soil culture including pot and field experiments, although plants may be exposed to various stresses, the effect of Si can only be

evaluated by applications of silicate fertilizers at different rates. In the control treatment receiving no silicate fertilizers, plants can still acquire a substantial amount of Si from the soil because it has a large reservoir of Si. It is therefore impossible to evaluate the "real" effect of Si on rice growth and production in these experiments. In the present study, we took the advantage of using a low Si mutant (*lsi1*) to re-examine the effect of Si on rice growth and production in the field. The mutant did not differ in the uptake capacity of N, P and K from the wild-type rice (Figs. 1, 2), but had a much lower Si concentration in the shoot. Therefore, this mutant allows us to examine the effect of Si on rice growth and production under natural stress conditions in the field. The results of the field trials showed a dramatic reduction in grain yield, by 79-98%, due to low Si accumulation, irrespective of experimental years and locations (Fig. 7). This is the largest beneficial effect of Si on rice yield ever reported, and demonstrates conclusively that a high Si accumulation is required for high rice yield.

It is interesting to notice that Si hardly affected the tiller number, SPAD value and root dry weight (Figs. 4, 5). This result is in agreement with a previous study by Ma et al. (1989), who found that removal or addition of Si during the vegetative stage did not have an effect on rice growth and production. Different from essential nutrients, Si does not affect the photosynthetic rate *per se*, because it has not been demonstrated to be involved in the metabolism of plants (Takahashi et al. 1966; Ma and Takahashi 2002). A slight increase in the shoot dry weight resulted from Si-alleviated stresses such as pest damage, rice blast, water stress and others (Fig. 6). High Si accumulation in the shoots acts as a physical barrier, which can mechanically impede penetration by fungi and insects (Fauteux et al. 2005; Ma and Yamaji 2006).

In contrast, the rice grain yield of the mutant was remarkably reduced due to a low Si accumulation (Fig. 7). Analysis of yield components showed that the percentage of filled spikelets was much lower in the mutant than in the wild type (Fig. 8), whereas other components were less affected by low Si. The low Si-induced sterility may mainly result from two factors. Firstly, low Si caused an excessive transpiration of the spikelets. An important factor for normal development of spikelets is to keep a high moisture condition within the husk (Seo and Ohta 1982). Different from leaves, husk does not have stomata and the transpiration occurs only through the cuticle. Silicon is deposited beneath the cuticle, forming a cuticle-silica double layer (Yoshida 1965; Ma and Takahashi 2002), thereby preventing excessive transpiration. Previous study has shown that high Si in the husk decreased the transpiration of the spikelets by 20-30% at both milky and maturity stages (Ma and Takahashi 2002). This beneficial effect of Si is especially important if rice crop encounters typhoon during the spikelet-filling period (Fig. 5). The wild type rice accumulated 21 times more Si in the husk than the mutant (Fig. 3), resulting in a high percentage of filled spikelets (Fig. 8). The second factor may result from pathogen infection. The dull brown color of the mutant panicles might be caused by infection of various pathogens such as panicle blast and Curvularia (Kiyoshi Ishiguro, personal communication). A high deposition of Si on the husk can prevent the infection of pathogens, thereby protecting the spikelets for normal development.

The effect of low Si on grain yield varied between years (Fig. 7). This differential effect of Si may be attributed to the growth conditions (climate, incidence of diseases and pests, etc.) in different years. The effect of Si was larger in the years with more unusual climate (such as typhoon, hot and low temperatures in summer, etc.) and with

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more other stressful conditions.

In conclusion, our study using the low Si mutant convincingly demonstrated that a high Si accumulation is required for high grain yield of rice. Silicon mainly functions to increase the percentage of filled spikelets by decreasing excessive transpiration and preventing pathogen infection in the panicles.

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Figure legends

Fig. 1 Concentrations of minerals in the shoots of the wild-type rice (WT) and the mutant (*lsi1*). A, N; B, P; C, K and D, Si. Samples were taken at different growth stages after transplanting. Data are means±SD (n=3).

Fig. 2 Accumulation of minerals in the shoots of the wild-type rice (WT) and the mutant (*lsi1*). A, N; B, P; C, K and D, Si. Samples were taken at different growth stages after transplanting. Data are means±SD (n=3).

Fig. 3 Concentration of Si in the husk of the wild-type rice (WT) and the mutant (*lsi1*). Samples were taken at different growth stages after heading. Data are means±SD (n=3).

Fig. 4 Growth parameters in the wild-type rice (WT) and the mutant (*lsi1*). A, tiller number; B, SPAD value. Data were collected at different growth stages after transplanting. Data are means±SD (n=6).

Fig. 5 Effect of low Si on root dry weight (A), plant height (B) and shoot dry weight (C) at different growth stages. WT, wild type rice; *lsi1*, low Si mutant. Data are means±SD (n=3).

Fig. 6 Phenotype of the wild-type rice (WT) and the mutant (*lsi1*) grown in the field. A, shoot at harvest; B, pest damage in the mutant; C, white head in the mutant after

typhoon; and D, panicles at harvest.

Fig. 7 Grain yield of the wild-type rice (WT) and the mutant (*lsi1*) grown in the field cultivated from 2003 to 2006 at two different locations.   Data are means±SD (n=3).

Fig. 8 Yield components of the wild-type rice (WT) and the mutant (*lsi1*) grown in the field in 2003.   Relative value of the wild-type rice (as 100) was presented.   Data are means±SD (n=3).
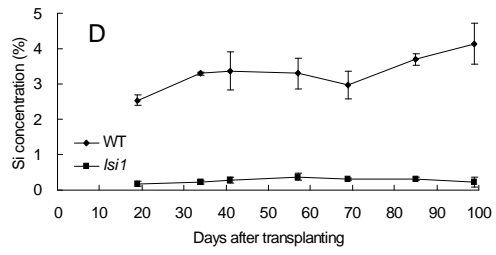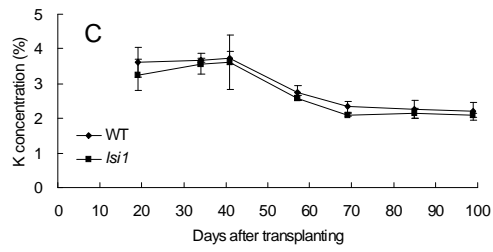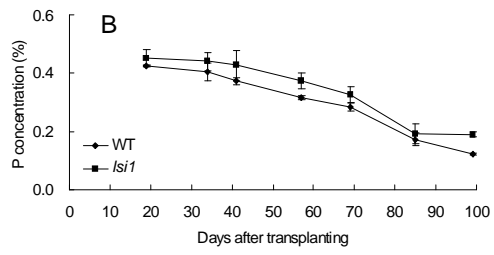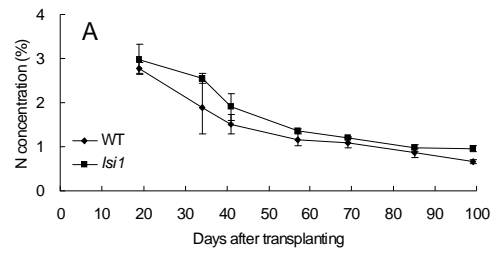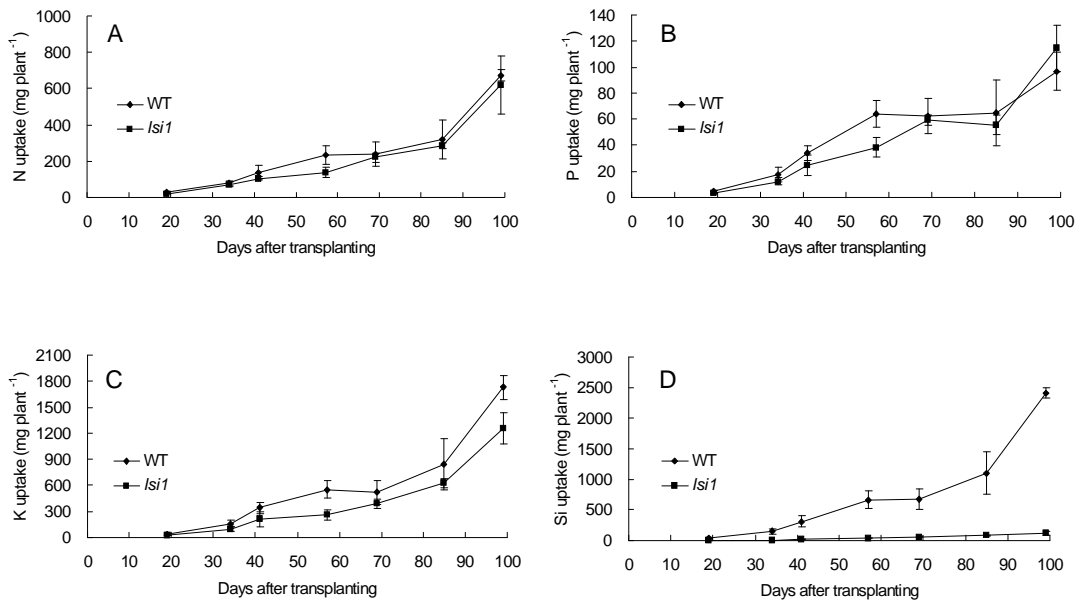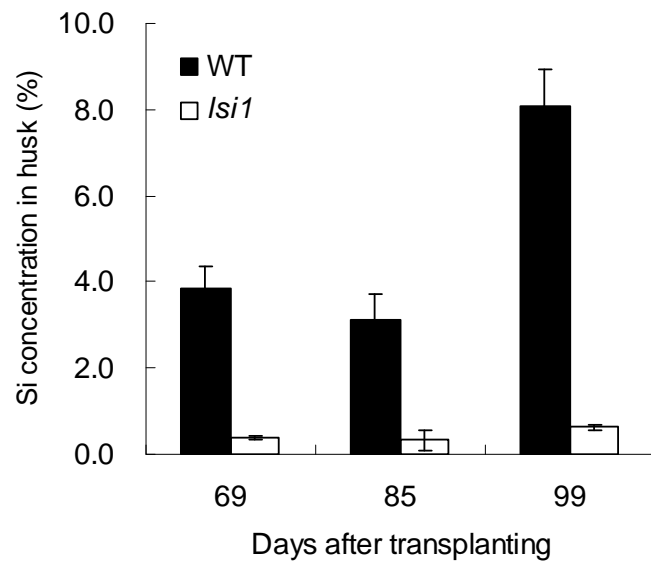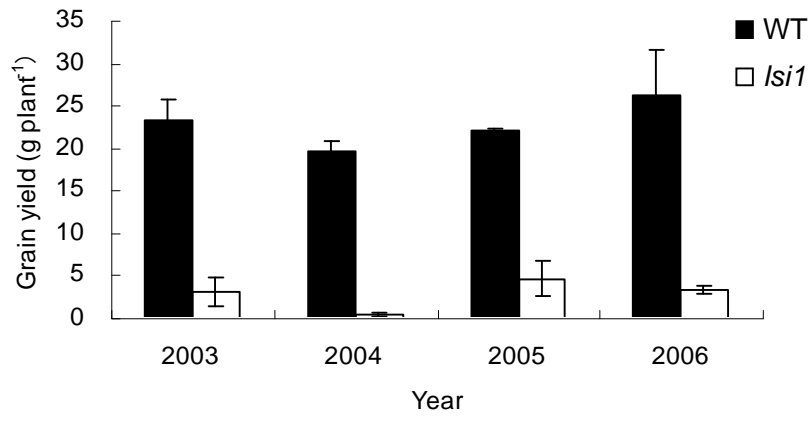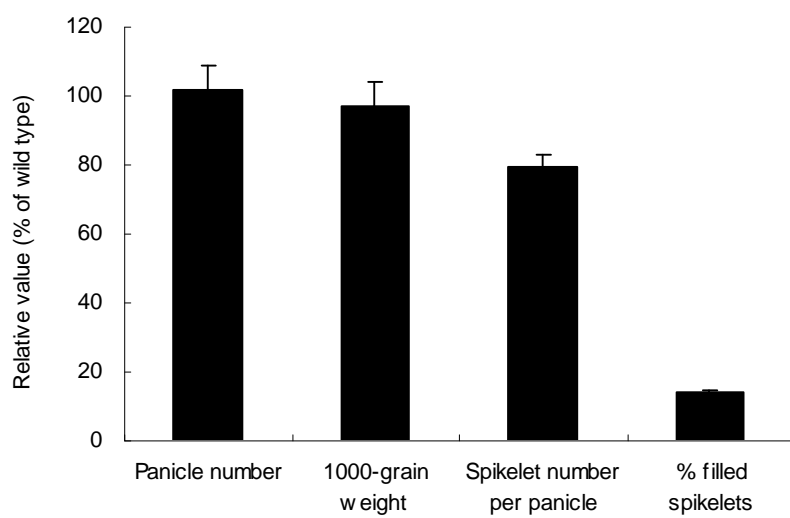
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