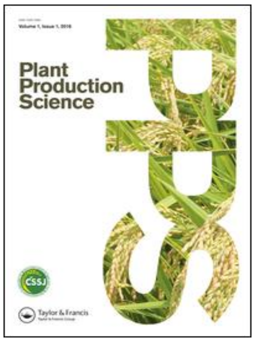

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Effects of Silicon on Transpiration and Leaf Conductance in Rice Plants (*Oryza sativa* L.)

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Abstract: To characterize the protection effect of silicon against excessive transpiration in rice plants (*Oryza sativa* L.), we investigated the factors responsible for transpiration, such as cuticular conductance (gc), leaf surface wax content (WC), and stomatal movement in response to environmental stimuli, using leaves of plants grown in nutrient solution with silicon (+Si) and without silicon (−Si). The rate of transpiration of whole plants (E) under field conditions was clearly reduced by application of silica. The gc of −Si leaves under precisely regulated conditions was 33% higher than that of +Si leaves, reflecting higher adaxial gc. Silicon has little effect on WC, a factor responsible for regulation of cuticular resistance to water flux in rice leaves. These results suggest that silicon is one of the main factors responsible for regulation of gc, but that its effect was not due to an increase in the WC. Leaf conductance (gl) of −Si leaves tended to decrease more slowly than that of +Si leaves over time after changes from high to low light intensity. Moreover, it was always higher over the entire duration of the experiment, irrespective of irradiation intensity. The gl of −Si leaves was higher at lower relative humidity (RH) and the initial response to a subsequent increase in RH was slower than that of +Si leaves. A comparison of gl and gc between +Si and −Si leaves showed that the higher gl of −Si leaves was due to higher stomatal conductance. These results indicated that the reduction in E by application of silica was mainly attributable to reduction in the rate of transpiration through stomatal pores.

Key words: Cuticular conductance, Leaf conductance, Rice, Silicon, Stomata, Transpiration.

Silicon plays an essential role in the normal growth and development of higher plants which accumulate considerable amounts of silicon. Silica deposits in rice (*Oryza sativa* L.) account for up to 20% of dry weight (Lewin and Reimann, 1969). In the absence of silica, the dry weight of shoots and the grain yield of rice plants are significantly reduced (Ma et al., 1989). Although many studies have clearly shown that silicon has positive effects on the growth of rice plants (Agarie et al., 1992 ; Elawad and Green jr, 1979 ; Lewin and Reimann, 1969 ; Ma et al., 1989), its physiological function is unclear.

Transpiration of rice plants is considerably reduced with applied silicon. Several investigators have postulated that this might be due to a reduction in the rate of transpiration through cuticular layers thickened by deposits of silica (Yoshida and Kitagishi, 1962 ; Yoshida et al., 1959). However, transpiration occurs mainly through stomatal pores, and transpiration through the cuticular layer represents a very small part of transpiration as a whole (Maruyama and Tajima, 1990 ; Yoshida and de los Reyes, 1976). Thus, previously reported differences in rates of transpiration between Si-treated and non-treated plants might have been due to differences in transpiration through the stomatal pores. However, there have been few studies on the effects of silicon on factors responsible for transpiration, including stomatal functions (Kawamitsu et al., 1989).

The stomatal behavior of plants has been extensively studied, and stomatal conductance can be readily predicted under a variety of steady-state conditions (Farquhar and Wong, 1984). However, stomata in a natural environment continually integrate various signals, which induce their opening and closing. Therefore, the analysis of transition of stomatal movement may provide useful information in elucidation of stomatal reactions in response to environmental stimuli.

In this study we investigated the factors responsible for transpiration, such as cuticular conductance, leaf surface wax content, and stomatal reactions in the leaves with different Si levels. In the study of stomatal reactions, we monitored the development of changes in leaf conductance over time after changes in light and humidity, in addition to the measurement of steady state response of leaf conductance to light.

Materials and Methods

1. Plant material

Seeds of rice (*Oryza sativa* L., cv. 'Koshihikari') were sown in plastic petri dishes containing distilled water. The husks were removed from the seeds to prevent contamination by SiO_2, which is deposited in the husks. After two weeks, seedlings were transplanted to mats

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Abbreviations: A, CO_2 assimilation rate ; E, transpiration rate of whole plant ; gc, cuticular conductance ; gl, leaf conductance ; RH, relative humidity.

floating on the surface of water in culture tanks (480 L) set up in a greenhouse on the campus of Kyushu University, Fukuoka, Japan. The plants were cultured hydroponically from June to October in Yoshida's standard nutrient solution (Yoshida et al., 1976) containing 100 ppm SiO_2 or in the nutrient solution without SiO_2. The nutrient solution was renewed once a week, and the solution pH was adjusted to between 5.0 and 5.5 with 1 M NaOH and 1 M HCl. The uppermost, fully expanded leaves of the main culms of plants grown in the nutrient solution for 30 to 40 days were used for all experiments, with the exception that, in the measurement of steady-state gas exchange rate in leaves, the fourth leaves from the uppermost, fully expanded leaves were also used.

2. Determination of Si levels

Levels of Si in leaves and in culture solutions were determined by gravimetric analysis and by the colorimetric method with molybdenum blue (Yoshida et al., 1976), respectively. Four replicate plants were prepared for each measurement of SiO_2 level.

3. Measurements of diurnal course of changes in transpiration rate of whole plants (*E*)

The diurnal course of changes in *E* was measured by a gravimetric method. The plants grown in the 480-L culture tanks were transferred to a plastic pot, 18 cm in diameter and 20 cm in height, which contained the nutrient solutions, and the weight of whole plants plus the pots was monitored at 60-min intervals from 09 : 00 to 19 : 00 h. At the end of the measurements, the total leaf area of plants was measured. *E* was expressed in terms of the leaf area. Two replicate plants were prepared for the measurement. Temperature and photon flux density were measured using a meteorological survey system (GOLCON ; Shinnihon-sozai, Tokyo, Japan).

4. Measurement of cuticular conductance (gc)

The gc was measured according to the method described by Yoshida and de los Reyes (1976). To close the stomata, plants were placed in darkness for 2 h at 25°C. The gc of the adaxial and abaxial surfaces at the middle portions of leaves was measured at a leaf temperature of 25°C in darkness using a steady-state autoporometer. Four replicate plants were prepared for each measurement of gc.

5. Determination of leaf surface wax content (WC)

Wax content was determined by the colorimetric method described by Ebercon et al. (1977). The leaves used for the gc measurements were cut into squares of 1 cm^2. Twenty or thirty pieces were submerged in 10 mL of chloroform for 30 s. The extract was then evaporated in a hot-water bath, and 5 mL of $K_2Cr_2O_7$ were added to the pellet. The solution was heated again in the water bath for 30 min. After cooling, 5 mL of deionized water were added to the solution, and the optical density of the solution was measured at 590 nm. Concentrations of wax were calculated from a standard curve prepared with solutions of polyethylene glycol.

6. Measurements of gas exchange rates

Responses to illumination

Gas exchange rate was determined by two different procedures, one measuring changes over time of gas exchange rate after changes of illumination and the other determining the steady-state gas exchange rate under various intensities of illumination.

The steady-state gas exchange rate was determined using an open gas-exchange system according to the method described by Agata et al (1986) with the exception that vapor pressure difference was regulated manually, not automatically. Measurements were made at a leaf temperature of 30.0±0.1°C and a relative humidity of 52.4±6.6%. The intensity of light was varied from 1800 μmol m^{-2} s^{-1} to darkness in eight to nine steps by covering the chamber with cheesecloth and cloths, and by turning off the light. Measurements were made at least 10 min after the values had leveled off, requiring about 20 min for each measurement. The gas exchange rate was calculated as described by von Caemmerer and Farquhar (1981).

Changes in gas-exchange rates were measured at a leaf temperature of 28.6±0.02°C using a portable analytical system (LCA-2, PLC ; Analytical Development Co. Ltd., Hertford, England). Light intensity was varied from 540 μmol m^{-2} s^{-1} to 307μmol m^{-2} s^{-1} and from 158 μmol m^{-2} s^{-1} to darkness by covering the chamber with cheesecloth and cloth, and by turning off the light. The gas exchange rate was monitored at 10 to 15-s intervals after changes in illumination. There was little absorption or release of water vapor from the chamber.

Responses to humidity

Measurements were made at a leaf temperature of 28.3±1.3°C and a light intensity of 505±30.5μmol m^{-2} s^{-1} using the portable analytical system. For quick and precise regulation of the relative humidity (RH) in the chamber, the air supply was controlled through two different columns that contained wet cotton and silica gel to be humidified or dehumidified. The air with higher RH (75.7±1.0%) was supplied to the chamber until the value leveled off, and then the RH of the supplied air was changed quickly from that with 75.7±1.0% RH to 15.4±1.2% RH, and gas exchange rates were monitored at 10 to 15-s intervals for 10 min. After the values had leveled off, the air with 75.6±0.6% RH was quickly supplied to the chamber again. Gas exchange rates were monitored in the same manner. There was little absorption or release of water vapor from the chamber.

Results

1. Diurnal course of changes in *E*

In terms of dry weight, the +Si and −Si leaves used in the present study contained 12.6±0.38% and 0.29±0.06% SiO_2, respectively. Both +Si and −Si plants were placed under field conditions and the diurnal course of changes in *E* was monitored at 60-min intervals (Fig. 1). *E* changed in parallel with variations in temperature and photon flux density. Values were always higher in −Si plants than in +Si plants over the entire duration of the experiment.

2. gc and WC

Transpiration from leaves encompasses transpiration through the cuticular layer and through the stomatal pores. In order to characterize the mechanisms responsible for reduction in *E* in the presence of silica, we measured cuticular conductance (gc) and leaf conductance (gl) in +Si and −Si leaves, as an indicator of the transpiration through the cuticular layer and stomatal pores, respectively.

The gc was measured on both adaxial and abaxial surfaces in darkness, where stomata were presumably totally closed (Table 1). On the abaxial surface, gc was similar in +Si and −Si leaves, but on the adaxial surface gc was 33% higher in −Si leaves. Total gc of −Si leaves was higher than that of +Si leaves, reflecting higher adaxial gc.

Leaf surface wax content (WC), one of the factors responsible for the regulation of gc, was similar, in +Si and −Si leaves, indicating that silicon had little effect on WC (Fig. 2). There was a significant negative correlation ($P<0.05$; $r=-0.74$) between gc and WC in +Si leaves, but not in −Si leaves.

3. Reactions of stomata to environmental stimuli

Stomatal responses to light

Steady-state gl increased with the increase in the intensity of illumination in +Si and −Si leaves. The gl of −Si leaves was always higher than that of +Si leaves, irrespective of leaf position, over the entire range of intensities of illumination tested (Fig. 3B). The difference in gl between +Si and −Si leaves in darkness, when stomata were presumably closed, was lower than in the light. This suggests that differences in gl between +Si and −Si leaves in light reflect differences in the extent to which stomata opened in response to light. In upper leaves, the differences in gl between +Si and −Si leaves was most marked at a light intensity of around 500 μmol m^{-2} s^{-1} and the difference became small with increase of light intensity, but in lower leaves gl was always higher in −Si leaves above a light intensity of 500 μmol m^{-2} s^{-1}.

Although gl was apparently higher in −Si leaves, the CO_2 assimilation rate (*A*) was unchanged or reduced in the −Si leaves. In the uppermost, fully expanded leaves, *A* was similar, irrespective of exposure to Si, but in the fourth leaves of the main culms the maximum *A* of +Si leaves was about 80% higher than that of −Si leaves (Fig. 3A). The difference in *A* between +Si and −Si leaves disappeared when *A* was expressed in terms of the chlorophyll content of leaves (data not shown), indicating that the decline in *A* in −Si leaves was attributable to the destruction of chlorophyll.

The gl of +Si and −Si leaves decreased gradually with time after changes in the intensity of illumination (Fig. 4) ; gl of −Si leaves was always higher than that

Table 1. Effects of silicon on cuticular conductance.

Si application	Cuticular conductance ($mmol\ m^{-2}\ S^{-1}$)		
	Abaxial	Adaxial	Total
+	23.6±2.7	23.3±4.2**	46.9±1.7*
−	29.1±4.2	41.2±1.4	70.4±5.7

Data represent means±SE of results from four replicates.
* and **, statistically significant at the 5% and 1% levels, respectively.

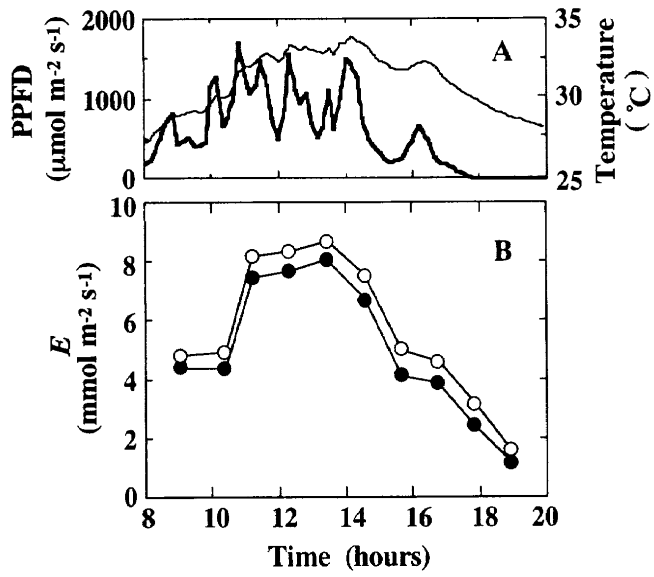

Fig. 1. Diurnal changes in air temperature (———, A), photosynthetic photon flux density (PPFD, ———, A), and the transpiration rate of whole plants (*E*, B) in +Si(●) and −Si(○) plants.

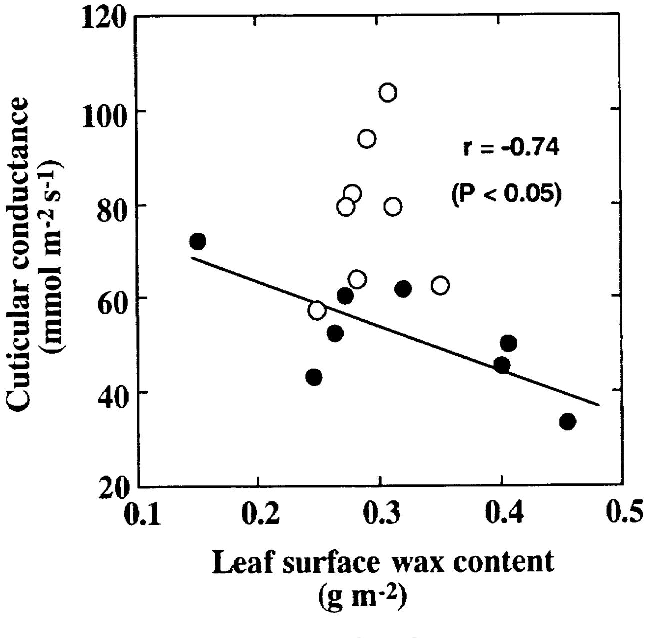

Fig. 2. Relationship between leaf surface wax content and cuticular conductance in +Si(●) and −Si(○) leaves. r, correlation coefficient. P, level of significance.

of +Si leaves over the entire duration of the experiment. The initial response of gl to changes in irradiation (for the first 120 s) was much more rapid in +Si leaves with an apparently biphasic decrease, whereas gl in −Si leaves decreased at a steady rate.

Stomatal responses to humidity

The gl of both +Si and −Si leaves decreased with time after the decrease in RH, with an inflection point at around 80 seconds (Fig. 5A). The overall decrease was similar, in +Si and −Si leaves; however, the gl of −Si leaves was always higher than that of +Si leaves. The gl of both +Si and −Si leaves increased with time after the increase in RH, and leveled off at around 80 seconds (Fig. 5B). The initial response of gl of +Si leaves was more rapid than that of −Si leaves.

Discussion

The transpiration of whole plants (*E*) was higher in −Si plants than in +Si plants (Fig. 1). These results were consistent with those reported by other researchers (Yoshida et al., 1959; Yoshida and Kitagishi, 1962), who postulated that reduction in *E* in Si-treated plants might be due to a reduction in transpiration through cuticular layers thickened by silica deposits. Although this hypothesis has been widely accepted, it is not based on direct measurements of transpiration through the cuticular layer of single leaves, but on histochemical observations of deposited silica and measurements of transpiration from whole plants under field conditions. In the present study, we measured gc, as an indicator of the transpiration through the cuticular layer, for both adaxial and abaxial surfaces of leaves under precisely regulated conditions (Table 1). We found that the total gc was higher in −Si leaves than in +Si leaves, a reflection of the higher gc of the adaxial surface. To our knowledge, this is the first report on the effect of silicon in reducing cuticular transpiration in rice leaves by direct measurement of gas exchange rate. Ultrastructure obser-

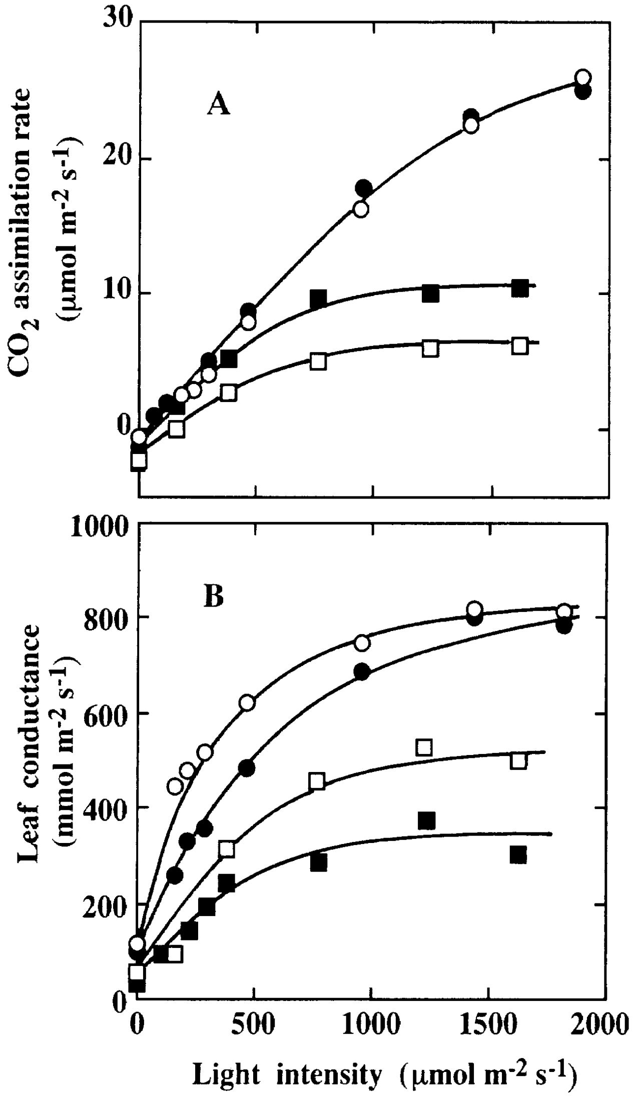

Fig. 3. Steady-state responses of photosynthetic rate (A) and leaf conductance (B) of upper(●, ○) and lower(■, □) leaves of +Si(●, ■) and −Si(○, □) plants. Upper and lower leaves were the uppermost, fully expanded leaves and the fourth leaves from the upper leaves, respectively.

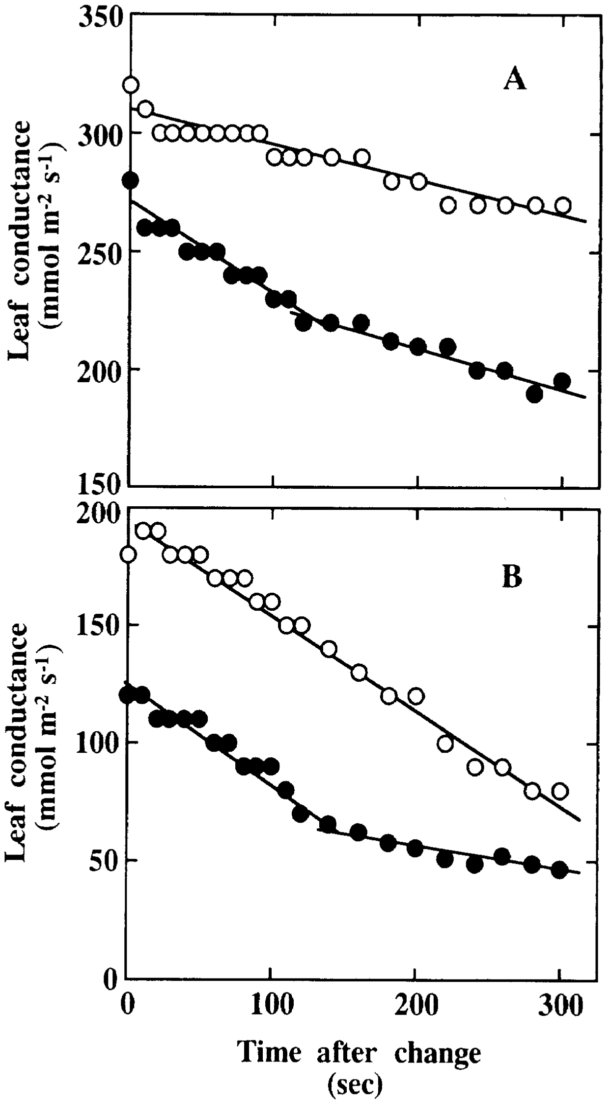

Fig. 4. Changes in leaf conductance in +Si(●) and −Si(○) leaves after changes in intensity of illumination from 540 μmol m^{-2} s^{-1} to 307 μmol m^{-2} s^{-1} (A) and from 158 μmol m^{-2} s^{-1} to darkness (B).

vations revealed that polymerized SiO_2 was predominant in the epidermal cells, in particular in the outer cell walls of these cells, but there appears to be little difference in the degree of deposition of polymerized SiO_2 between adaxial and abaxial surfaces (Agarie et al., 1998). An analysis of cuticular thickness in relation to sites of deposition and level of silica in leaves is required to elucidate the mechanisms responsible for increased gc in adaxial surfaces of −Si leaves.

To investigate the mechanism of regulation of gc by silicon, we compared WC, a factor responsible for the regulation of cuticular resistance to water flux in rice leaves (O'Toole et al., 1979), between +Si and −Si leaves (Fig. 2). Although there was little difference in WC between the +Si and −Si leaves, we found a negative correlation between gc and WC only in +Si leaves. These results suggest that silicon is one of the main factors responsible for regulation of gc, but its effect was not due to an increase in the WC of rice leaves. WC seems likely to play a role in the fine tuning of gc in leaves that have accumulated a certain amount of silicon.

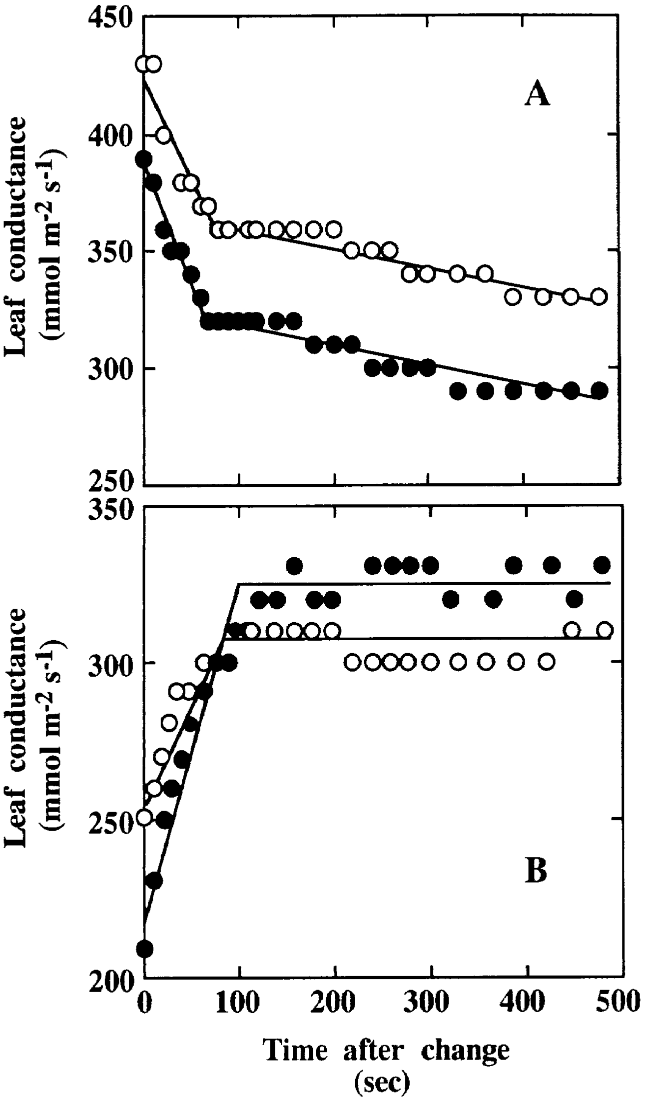

Fig. 5. Changes in leaf conductance in +Si(●) and −Si(○) leaves after changes in relative humidity from 75.7% to 15.4% (A) and from 15.4% to 75.4% (B).

The gc was 33% higher in −Si leaves than in +Si leaves ; however, the gc represented a small part of gl as a whole. For example, in the steady-state gas exchange measurements (Fig. 3), the gl of the upper leaves of +Si and −Si plants in darkness, were 96.6 and 117.2 mmol m^{-2} s^{-1}, respectively, being equivalent to 12.1%, and 14.3% of the highest gl at the saturating light, respectively. This indicated that about 90% of the total transpiration in leaves was attributable to the transpiration through stomatal pores. When the leaves were exposed to light, the difference in the gl between +Si and −Si leaves was between 140 and 190 mmol m^{-2} s^{-1}, whereas the value was 14 mmol m^{-2} s^{-1} in darkness (Fig. 3). These results suggest that differences in gl between +Si and −Si leaves in light reflect differences in the opening of the stomata in response to light. The stomata of −Si leaves appeared to open more sensitively in response to light and to close less sensitively with light reduction than those of +Si leaves. Considering the stomatal movement and opening in −Si leaves in response to light (Figs. 3, 4), the total amount of transpirative water loss from whole plants may be much higher in Si-deficient plants than in Si-treated plants under field conditions.

The differences between +Si and −Si leaves in the development of changes in gl over time after changes in light (Fig. 4) strongly indicate that silicon influences stomatal light reactions. Stomatal movement in response to light is considered to be the result of an integrated modulation of ion fluxes in guard cells (Macrobbie, 1987 ; Sharkey and Ogawa, 1987 ; Zeiger, 1983). This response is presumably mediated by four different phenomena : (1) signal perception ; (2) generation of an electrochemical gradient across guard cell membranes ; (3) transport of ions leading to changes in osmotic pressure ; and (4) hydraulic adjustments associated with stomatal movement. Our findings suggest that silicon influences these phenomena.

In contrast to stomatal responses to light, the response of stomata to humidity seems generally to be independent of metabolic reactions since responses generated a water gradient simultaneously with application of the stimulus (Lösch, 1977 ; Maier-Maercker, 1983 ; Schulze et al., 1987 ; Sheriff, 1979). Thus, it is possible that silicon influenced the response of gl to humidity via some involvement in the physical and mechanical properties of epidermal tissues. In some species, even when bulk leaf water potential is unchanged, stoma respond directly to humidity of the ambient air (Maier-Maercker, 1983 ; Schulze et al., 1987). In this case the cuticular layer plays an important role in stomatal regulation by limiting the transport of water from xylem vessels to transpiration site and the rate of water loss from guard cell complexes to ambient air (Nonami et al.,1991) ; when water status in epidermal cells surrounding stomata is maintained appropriately under dried conditions, water flux into guard cells is facilitated, with more rapid resultant swelling of guard cells after the increase in RH. This response is expected to be detected by analysis of development of changes in gl over time after changes in humidity.

In the present study gc was always higher in −Si leaves than in +Si leaves. In addition, the gl of +Si leaves was lower under less humid conditions (Fig. 5A) and its initial response to an increase in RH was more rapid than that of −Si leaves (Fig. 5 B). In rice, silica is mainly deposited in the leaf epidermal system (Elawad and Green jr., 1979), forming double cuticular-silica layers at the surface of epidermal cells beneath the cuticle (Clarkson and Hanson, 1980; Yoshida and Kitagishi, 1962), which regulate the transpiration through the cuticular layers (Yoshida and Kitagishi, 1962; Yoshida et al., 1959). Our findings suggested that silica, in combination with the cuticle, influences stomatal regulation by limiting water conditions in the epidermal cell, such as regulation of water transport in the epidermal system and water loss from the guard cell complexes.

The present findings suggest that, the stomata of −Si leaves open to light and RH in response more sensitively, and close in response to light reduction and increase of RH less sensitively than those of +Si leaves. Cuticular transpiration accounts for only a small part of the whole transpiration (Figs. 3, 4), and its changes in response to environmental stimuli are generally much smaller than that through stomatal pores. We concluded therefore that the higher E of −Si leaves was mainly due to higher transpiration through the stomatal pores resulting from the inappropriate opening of stomata in response to environmental stimuli.

Increased stomatal opening was generally in parallel with the increase of photosynthetic CO_2 uptake. In the present study, however, the increase of gl of −Si leaves was not accompanied by the increased A (data not shown). In the −Si leaves the higher gl resulted in an increase of transpiration rate, but CO_2 uptake was similar to that of +Si leaves. Consequently, water use efficiency, ratio of A and transpiration, was always higher in +Si leaves than in −Si leaves, irrespective of measurements. These results indicate that excessive transpiration occurs in −Si leaves resulting from inappropriate gl in terms of productive balance between water loss and CO_2 uptake.

High rates of transpiration can induce local water stress in leaves and reduced mesophyll capacity for the CO_2 assimilation rate even when water is adequately supplied to the root system (Sharkey, 1984). If the leaves are injured severely by stress, and cannot be repaired, the aging process is accelerated. In Si-deficient plants, wilting and necrosis of leaves are often observed as typical symptoms, and are conspicuous under conditions of high temperature with low humidity (Lewin and Reimann, 1969). These observations support the hypothesis that transpiration-induced water stress might also occur in rice leaves with Si-deficiency.

Our previous study revealed an increase in growth rate and in dry-matter production on application of silicon, showing that these changes were attributable to maintenance of a high rate of photosynthesis and to protection of chlorophyll from destruction in aged leaves (Agarie et al., 1992, 1993). Therefore, reduced E, resulting from increased stomatal sensitivity and cuticular resistance, appears to be the main reason for maintenance of photosynthetic activity, and, thus, increased dry matter production in Si-treated plants.

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