



RESEARCH PAPER

# Uptake system of silicon in different plant species

Namiki Mitani and Jian Feng Ma\*

Faculty of Agriculture, Kagawa University, Ikenobe 2393, Miki-cho, Kita-gun, Kagawa 761-0795, Japan

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## Abstract

The accumulation of silicon (Si) in the shoots varies considerably among plant species, but the mechanism responsible for this variation is poorly understood. The uptake system of Si was investigated in terms of the radial transport from the external solution to the root cortical cells and the release of Si from the cortical cells to the xylem in rice, cucumber, and tomato, which differ greatly in shoot Si concentration. Symplasmic solutions of the root tips were extracted by centrifugation. The concentrations of Si in the root-cell symplast in all species were higher than that in the external solution, although the concentration in rice was 3- and 5-fold higher than that in cucumber and tomato, respectively. A kinetic study showed that the radial transport of Si was mediated by a transporter with a  $K_m$  value of 0.15 mM in all species, but with different  $V_{max}$  values in the order of rice > cucumber > tomato. In the presence of the metabolic inhibitor 2,4-dinitrophenol, and at low temperature, the Si concentration in the root-cell symplast decreased to a level similar to that of the apoplasmic solution. These results suggest that both transporter-mediated transport and passive diffusion of Si are involved in the radial transport of Si and that the transporter-mediated transport is an energy-dependent process. The Si concentration of xylem sap in rice was 20- and 100-fold higher than that in cucumber and tomato, respectively. In contrast to rice, the Si concentration in the xylem sap was lower than that in the external solution in cucumber and tomato. A kinetic study showed that xylem loading of Si was also mediated by a kind of transporter in rice, but by passive diffusion in cucumber and tomato. These results indicate that a higher density of transporter for radial transport and the presence of a transporter for xylem loading are responsible for the high Si accumulation in rice.

Key words: Passive diffusion, radial transport, silicon, transporter, uptake, xylem loading.

## Introduction

Silicon (Si) is a beneficial element for plant growth. Silicon helps plants to overcome multiple stresses including biotic and abiotic stresses (for a recent review, see Ma, 2004). For example, Si plays an important role in increasing the resistance of plants to pathogens such as blast on rice (Datnoff *et al.*, 1997) and powdery mildew on cucumber (Miyake and Takahashi, 1982*a, b*). Silicon is effective in preventing lodging in rice by increasing the thickness of the culm wall and the size of the vascular bundles (Shimoyama, 1958), thereby enhancing the strength of the stems. Silicon also alleviates the effects of other abiotic stresses including salt stress, metal toxicity, drought stress, radiation damage, nutrient imbalance, high temperature, and freezing (Epstein, 1999; Ma and Takahashi, 2002; Ma, 2004). These beneficial effects are mostly expressed through Si deposition in the leaves, stems, and hulls, although other mechanisms have also been proposed (Ma, 2004). Therefore, the Si effect is characterized by a larger effect associated with a greater Si accumulation in the shoots.

However, Si accumulation in the shoots varies considerably among plant species, ranging from 0.1% to 10% Si in the dry weight (Ma and Takahashi, 2002). Takahashi and coworkers made an extensive survey on the Si concentrations of nearly 500 plant species from Bryophyta to Angiospermae, grown under similar soil conditions (for the summary, see Ma and Takahashi, 2002). The results showed that there is a characteristic distribution of Si accumulation in the plant kingdom. In higher plants, only plants in Gramineae and Cyperaceae show high Si accumulation. Plants in Cucurbitales, Urticales, and Commelinaceae show intermediate Si accumulation, whereas most other plants species show low Si accumulation. The difference in Si accumulation has been attributed to the ability of the roots to take up Si (Takahashi *et al.*, 1990). Silicon is taken up in the form of an uncharged molecule, silicic acid (Takahashi and Hino, 1978). Three different modes of Si uptake have been proposed for plants having different degrees of Si accumulation, that is, active,

\* To whom correspondence should be addressed. Fax: +81 87 891 3137. E-mail: maj@ag.kagawa-u.ac.jp

passive, and rejective uptake (Takahashi *et al.*, 1990). Plants with an active mode of uptake take up Si faster than water, resulting in a depletion of Si in the uptake solution. Plants with a passive mode of uptake take up Si at a rate that is similar to the uptake rate of water; thus, no significant changes in the concentration of Si in the uptake solution are observed. By contrast, plants with a rejective mode of uptake tend to exclude Si, which is demonstrated by the increasing concentration of Si in the uptake solution. However, the mechanisms involved in the different uptake modes are not understood. The objective of this study was to examine the uptake systems of Si in rice, cucumber, and tomato, which represent high, intermediate, and low Si accumulation, respectively.

## Materials and methods

### Plant materials and growth condition

Seeds of rice (*Oryza sativa* L. cv. Oochikara) were soaked in water overnight at 25 °C in the dark. The seeds were then transferred to a net floated on 0.5 mM CaCl<sub>2</sub> solution in a plastic container. On day 7, the seedlings were transferred to a 3.5 l plastic pot containing half-strength Kimura B solution. The composition of this nutrient solution is reported in the previous paper (Ma *et al.*, 2001). The pH of this solution was 5.6 and the nutrient solution was renewed every 2 d.

Seeds of cucumber (*Cucumis sativus* L. cv. Suyo) and tomato (*Lycopersicon esculentum* Mill. cv. Oogatahukujyu) were soaked in water for about 1 h and then placed in a refrigerator at 4 °C overnight. The seeds were transferred to a net floated on 0.5 mM CaCl<sub>2</sub> solution in a plastic container. On day 5, the seedlings were transferred to a 3.5 l plastic pot containing one-fifth Hoagland's solution. The nutrient solution contained the macronutrients 1.0 mM KNO<sub>3</sub>, 1 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 1.4 mM MgSO<sub>4</sub>, and 0.2 mM KH<sub>2</sub>PO<sub>4</sub>, and the micro-nutrients 20 μM Fe-EDTA, 3 μM H<sub>3</sub>BO<sub>3</sub>, 1.0 μM (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>, 0.5 μM MnCl<sub>2</sub>, 0.4 μM ZnSO<sub>4</sub>, and 0.2 μM CuSO<sub>4</sub>. The pH of this solution was adjusted to 6.0 and the nutrient solution was renewed every 3 d. All experiments were conducted at a temperature of 25 °C in a controlled growth chamber with natural lights.

### Si uptake experiment

Short-term Si uptake by rice, cucumber, and tomato was examined with three replicates. Two seedlings (30-d-old) were placed in a 180 ml black bottle containing half-strength Kimura B solution (for rice) or one-fifth Hoagland's solution (for cucumber and tomato) with 0.5 mM Si as silicic acid. Silicic acid was prepared by passing potassium silicate through cation-exchange resin (Amberlite IR-120B, H<sup>+</sup> form, Organo, Tokyo) (Ma *et al.*, 2001).

At different time points indicated in Fig. 1, 0.5 ml aliquots of uptake solution were taken for the determination of Si concentration. Transpiration (water loss) was also recorded at each sampling time. At the end of the experiment, roots and shoots were harvested separately and their fresh and dry weights were recorded.

### Collection of apoplasmic and symplasmic solutions

Apoplasmic and symplasmic solutions were extracted by centrifugation with slight modifications of the methods of Yu *et al.* (1999). For a time-course experiment, the root tips (0–1.5 cm) were excised from seedlings of rice (5-d-old) or cucumber and tomato (6-d-old) at time points indicated in Fig. 2 after exposure to 0.5 mM Si solution at 25 °C. For each sample, 40 roots for rice and 80 roots each for cucumber and tomato were used. The cut ends were washed in distilled water quickly and blotted dry. The tips were placed in

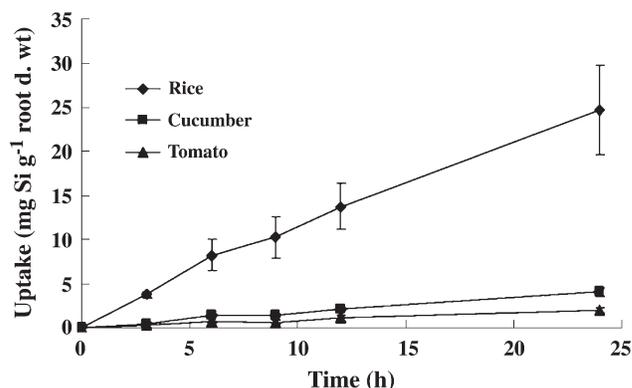


Fig. 1. Uptake of Si by rice, cucumber, and tomato. The uptake experiment was conducted in a nutrient solution containing 0.5 mM Si as silicic acid. Values are means  $\pm$ SD of three replicates.

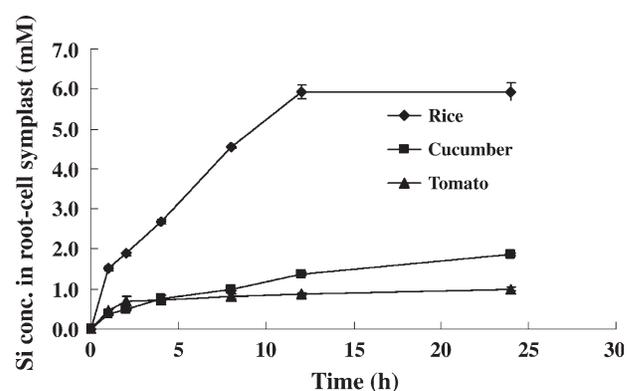


Fig. 2. Concentration of Si in the root-cell symplast of rice, cucumber, and tomato. Symplasmic solutions were extracted by centrifugation from the root tips exposed to 0.5 mM Si for various duration. Values are means  $\pm$ SD of three replicates.

a 0.22 μM filter unit (ULTRAFREER-MC, Millipore) with the cut ends facing down and centrifuged at 2000 g for 15 min at 4 °C to obtain the apoplasmic solution. After centrifugation, root segments were frozen at –80 °C for 2 h and then thawed at room temperature. The symplasmic solution was prepared from the frozen–thawed tissues by centrifugation at 2000 g for 15 min at 4 °C. The Si concentrations in the apoplasmic and symplasmic solutions were determined immediately as described below. In a preliminary experiment, it was confirmed that the freeze–thaw process did not affect the Si concentration.

To check the purity of the apoplasmic solution, the activity of malic dehydrogenase (MDH) in the apoplasmic and symplasmic solutions was determined according to Bergmeyer and Bernt (1974). The activity of MDH in the apoplasmic solution was below 1/20–1/40 that of the symplasmic solution.

In a kinetic study, seedlings of rice (5-d-old), cucumber and tomato (6-d-old) were cultured in nutrient solutions containing various Si concentrations. After an 8 h culture, the apoplasmic and symplasmic solutions were extracted as described above. All experiments were conducted with three replicates.

### Inhibitor and low-temperature experiments

To investigate the effect of metabolic inhibitors and low temperature on Si concentration in the apoplasmic and symplasmic solutions,

seedlings of rice (5-d-old), cucumber and tomato (6-d-old) were exposed to a nutrient solution containing 0.5 mM silicic acid in the presence or absence of 0.05 mM HgCl<sub>2</sub> or 0.5 mM 2, 4-dinitrophenol (DNP). 2, 4-Dinitrophenol was dissolved in ethanol before being added to the nutrient solution, with a final ethanol concentration of 0.3% (v/v). Preliminary experiments showed that this concentration of ethanol had no effect on Si uptake. The treatment period was 6 h.

For the low-temperature experiment, seedlings were exposed to a nutrient solution containing 0.5 mM silicic acid that had been precooled at 4 °C. After 6 h, Si concentration in the apoplasmic and symplasmic solutions was determined. All experiments were conducted with three replicates.

#### Xylem sap collection

Xylem sap was collected with a micropipette after decapitating at 1 cm above the roots. In a time-course experiment, seedlings (20-d-old) were exposed to the nutrient solution containing 0.5 mM silicic acid. At 0.5, 1, 2, 3, 4, 6, and 8 h after the decapitation, the xylem sap was collected for 20 min for tomato and cucumber and 30 min for rice and the Si concentration in the xylem sap was determined immediately.

A kinetic study was performed by culturing seedlings (20-d-old) in a 250 ml plastic bottle (4 or 2 seedlings per pot) containing various concentrations of Si. After uptake for 8 h, the stem was severed and the xylem sap was collected as described above.

#### Determination of Si concentration

To avoid interference of Si measurement in the symplasmic solution, a small amount of cation resin (AG 50W-x8, 200~400 mesh, H<sup>+</sup> form, Biorad) was added to the symplasmic solution and mixed well. After 30 min, the solution was centrifuged for 3 min at 13 000 g. The Si concentration in the solution was determined by the colorimetric molybdenum blue method (Ma *et al.*, 2003). For rice, a 0.01 ml sample was diluted with 1.15 ml water, followed by the addition of 0.6 ml of 0.26 N HCl, 0.08 ml of 10% (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>, 0.08 ml of 20% tartaric acid, and 0.08 ml reducing agent. For cucumber and tomato, a 0.06 ml sample was diluted with 0.52 ml water, followed by the addition of 0.3 ml of 0.26 N HCl, 0.04 ml of 10% (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>, 0.04 ml of 20% tartaric acid, and 0.04 ml reducing agent. The reducing agent was prepared by dissolving 1 g Na<sub>2</sub>SO<sub>3</sub>, 0.5 g 1-amino-2-naphthol-4-sulphonic acid, and 30 g NaHSO<sub>3</sub> in 200 ml water. After 1 h, the absorbance was measured at 600 nm with a spectrophotometer (Jasco, Tokyo, Japan). A standard curve was prepared from Si standard solution (1000 mg l<sup>-1</sup>, Wako, Tokyo, Japan).

## Results and discussion

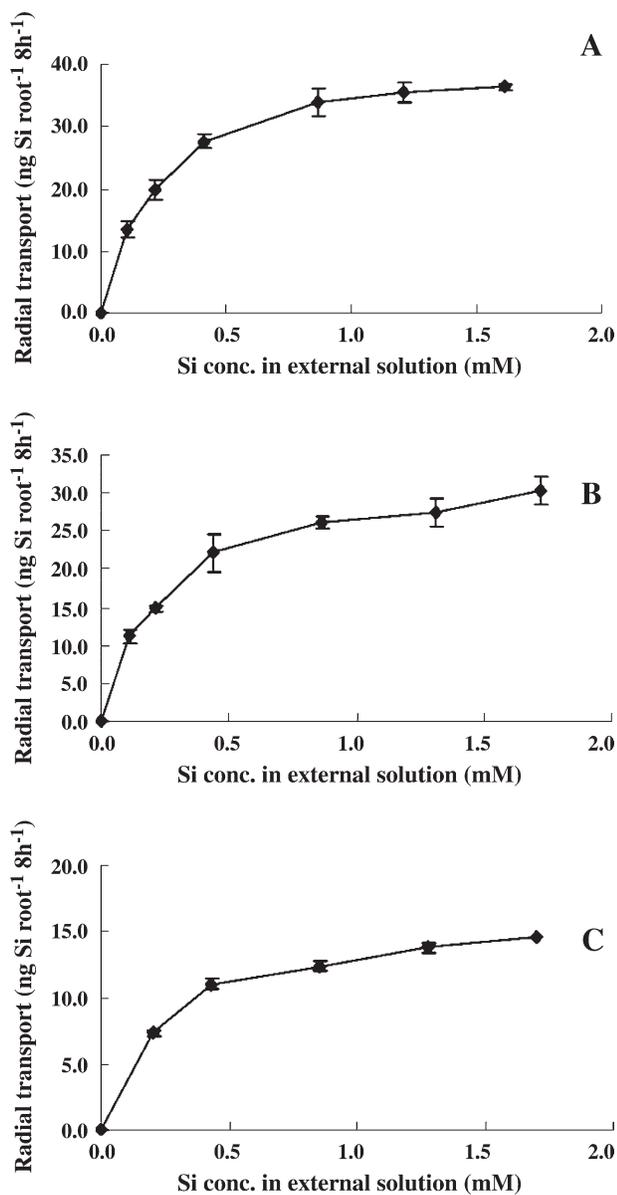
It was reported that rice, cucumber, and tomato contained 7.3, 2.3, and 0.2% Si in the shoot dry weight, respectively, when they were grown under similar conditions (Takahashi *et al.*, 1990). To investigate whether this difference is due to the difference in root uptake, a short-term (up to 24 h) uptake experiment was performed with the three species. When the plants were exposed to a solution containing the same Si concentration under the same conditions, the uptake of Si by rice was much higher than that by the other two species at each time point (Fig. 1). Si uptake by cucumber was also higher than that by tomato (Fig. 1). At 24 h, the Si uptake by rice was about 6-fold and 12-fold higher than cucumber and tomato, respectively. The Si concentration in the uptake solution for rice decreased

markedly with time (data not shown), but remained constant for cucumber and increased slightly for tomato. No significant difference was observed in the transpiration rate between the three species (data not shown). These results are in agreement with previous findings by Okuda and Takahashi (1962), who compared only rice and tomato in their study. The accumulation of Si in the shoots may be related to a number of factors such as transpiration, growth duration, growth rate, etc, but root uptake ability is the most important factor for determining Si accumulation in the shoots. These results confirmed a previous proposal that the difference in Si accumulation results from the ability of the roots to take up Si (Takahashi *et al.*, 1990).

The uptake of Si involves at least two processes: radial transport of Si from the external solution to the cortical cells and the release of Si from the cortical cells into the xylem (xylem loading). In rice, it has been demonstrated that radial transport of Si is mediated by a type of transporter with a  $K_m$  value of 0.15 mM Si (Tamai and Ma, 2003; Ma *et al.*, 2004). In the present study, this process was also examined in cucumber and tomato. For comparison, rice was also investigated under the same experimental conditions. A time-course experiment showed that the Si concentration in the root-cell symplast increased with time in all species (Fig. 2). However, the Si concentration in the symplast was much higher in rice, followed by cucumber and tomato, although the Si concentration in the symplast in all species was higher than that in the external solution. After exposure to 0.5 mM Si for 12 h, the Si concentration in the root symplast was 6.0, 2.0, and 0.9 mM for rice, cucumber, and tomato, respectively (Fig. 2).

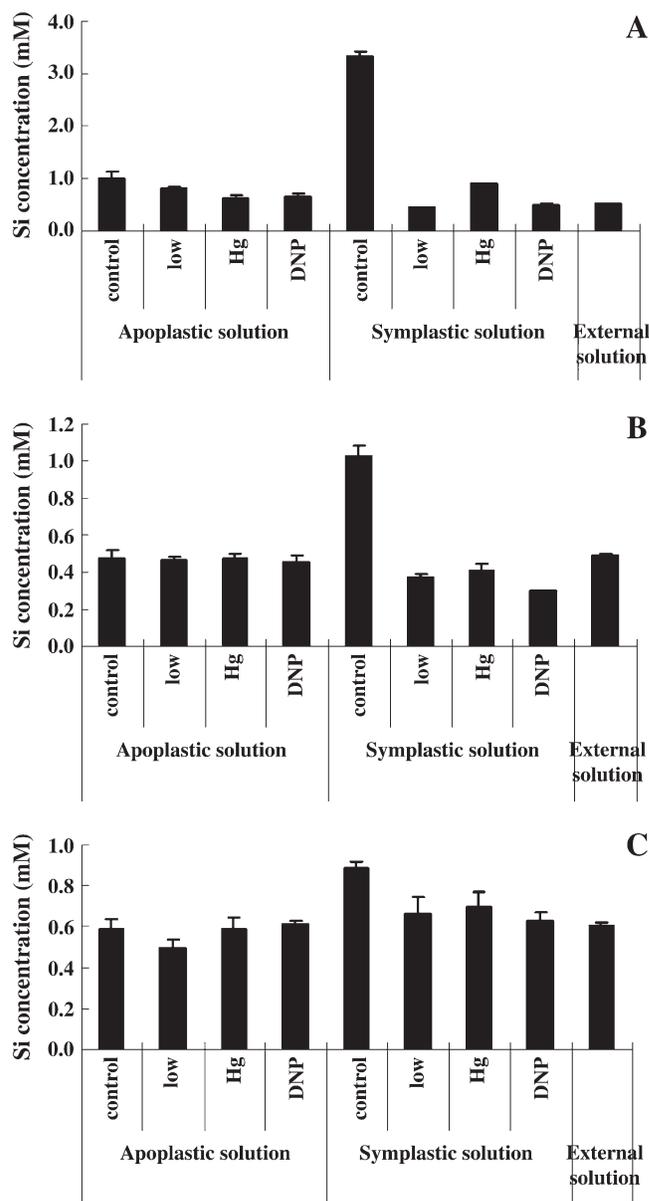
A kinetic study showed that the Si concentration in the root-cell symplast increased with increasing external Si concentrations, but saturated at a higher concentration, although the Si concentration in the root-cell symplast differed greatly between the three species (Fig. 3A, B, C). Based on these curves, the  $K_m$  value was estimated to be 0.16, 0.15, and 0.16 mM for rice, cucumber, and tomato, respectively. The value of  $V_{max}$  was 34.5, 26.9, and 13.3 ng root<sup>-1</sup> 8 h<sup>-1</sup>, respectively, for rice, cucumber, and tomato. These results suggest that the radial transport of Si from the external solution to the cortical cells in the three plant species is mediated by a transporter that shows a similar affinity to silicic acid. However, the difference in the  $V_{max}$  value suggests that the density of the Si transporter on the root cell membranes differs among the plant species, following the order of rice>cucumber>tomato.

A higher Si concentration in the root-cell symplast than in the external solution (Fig. 1) suggests that silicic acid is transported against a concentration gradient from the external solution to the cortical cells, which would be energy-dependent. To confirm this, the effect of a metabolic inhibitor (2,4-DNP) and low temperature on the radial transport of Si was investigated. In the absence of the inhibitor, the Si concentration in the apoplasmic solution in all species was



**Fig. 3.** Kinetics of radial transport of Si from external solution to the root cell. Symplasmic solution was extracted from root tips of rice (A), cucumber (B), and tomato (C) cultured in a nutrient solution containing various concentrations of Si for 8 h. Values are means  $\pm$ SD of three replicates.

almost the same as that in the external solution, whereas the Si concentration in the root-cell symplast differed among the three plant species, being 5-, 2-, and 1.5-fold higher than that in the external solution, respectively, for rice, cucumber, and tomato. In the presence of 2,4-DNP or under a low temperature (4 °C), the Si concentration in the root-cell symplast was decreased to a level similar to that in the apoplast and the external solution (Fig. 4). These results suggest that Si uptake involves two components: a transporter-mediated component as described above and a passive trans-



**Fig. 4.** Effect of 2,4-dinitrophenol, HgCl<sub>2</sub>, and low temperature on Si concentration in the root-cell symplast. Seedlings of rice (A), cucumber (B), or tomato (C) were exposed to a nutrient solution containing 0.5 mM Si as silicic acid for 6 h in the presence and absence of 0.05 mM HgCl<sub>2</sub> or 0.5 mM 2,4-dinitrophenol or under low temperature (4 °C). Values are means  $\pm$ SD of three replicates.

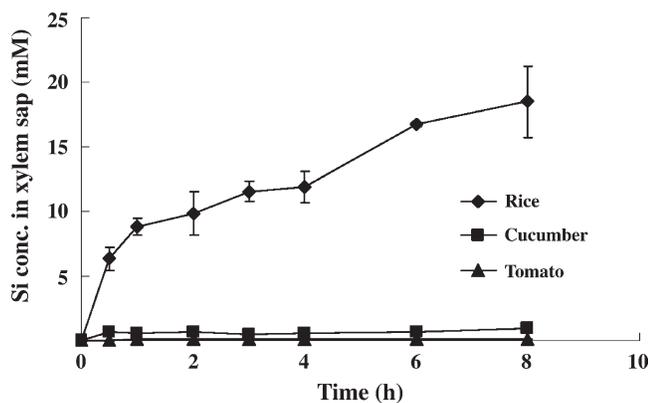
port by diffusion. Different from most other minerals, Si is present in the form of an uncharged molecule, silicic acid, at a pH below 9. This uncharged form is probably permeable across the plasma membranes in the roots (Raven, 2001).

Recently, it was reported that Si uptake by rice was significantly inhibited by HgCl<sub>2</sub>, (Tamai and Ma, 2003). Further, this inhibition is independent of water inhibition caused by HgCl<sub>2</sub> because the Hg-induced inhibition of Si uptake occurred earlier than that of water uptake and Hg-inhibited water uptake was completely recovered in

the presence of 10 mM 2-mercaptoethanol, while Hg-inhibited Si uptake was not. In the present study, the effect of HgCl<sub>2</sub> on the radial transport of Si was therefore compared among rice, cucumber, and tomato. In the presence of HgCl<sub>2</sub>, the Si concentration in the root-cell symplast decreased to a similar level as that in the apoplast in all three species (Fig. 4). It is suggested that HgCl<sub>2</sub> blocks water channels via the oxidation of cysteine residue(s) proximal to the aqueous pore and the subsequent occlusion of the aqueous pore by the large mercury ion (Maurel, 1997). Therefore, these results suggest that the transporter responsible for the radial transport of Si from the external solution to the cortical cells contains a Cys residue.

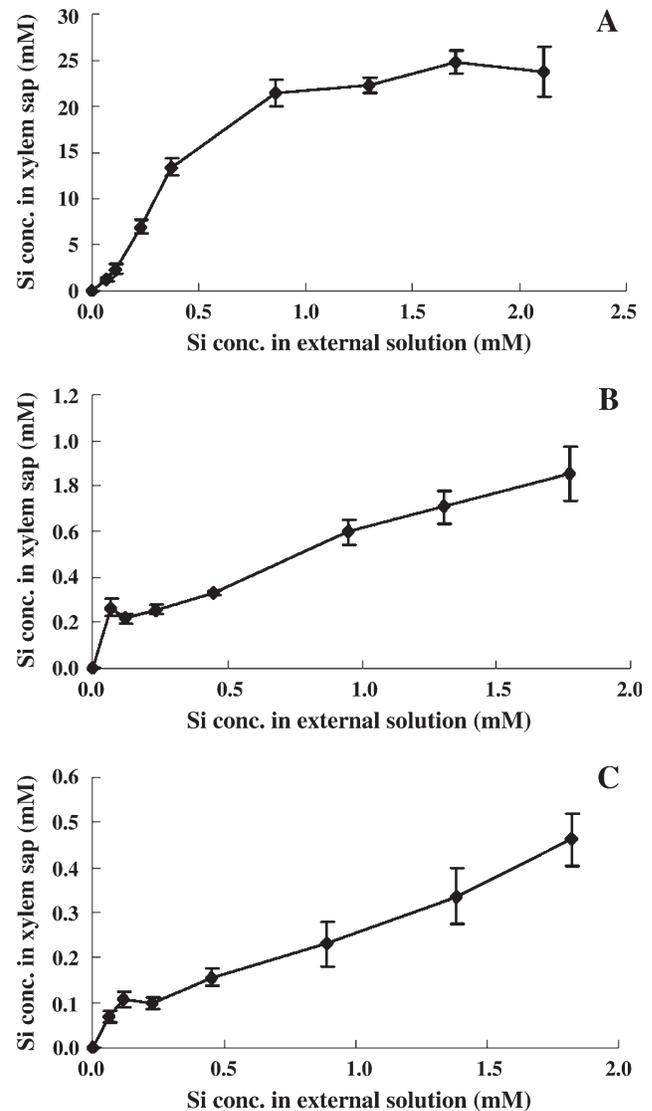
The subsequent process, i.e. the release of Si from the cortical cells to the xylem (xylem loading) was then investigated in cucumber and tomato. For comparison, rice was also investigated under the same experimental conditions, although the xylem loading process has been characterized in rice (Ma *et al.*, 2004; Mitani *et al.*, 2005). The average exudation rate of xylem sap was 3–5  $\mu\text{l plant}^{-1} 30 \text{ min}^{-1}$  for rice, and 40–50  $\mu\text{l plant}^{-1} 30 \text{ min}^{-1}$  for cucumber and tomato. When the roots were exposed to a nutrient solution containing 0.5 mM Si, the concentration of Si in the xylem sap of rice reached 6.0 mM Si in 30 min and 18 mM in 8.5 h (Fig. 5), suggesting that the release of Si to the xylem is a very rapid process and that Si is loaded against a concentration gradient in rice as reported previously (Ma *et al.*, 2004; Mitani *et al.*, 2005). By contrast, in cucumber and tomato, the Si concentration in the xylem sap was much lower than that in rice (Fig. 5). The Si concentration in the xylem sap of cucumber was 0.6 mM after 30 min, and remained stable throughout the experiment period. In tomato, the Si concentration in the xylem sap was lower than that of the external solution throughout the experiment.

Xylem loading of Si has been reported to be a transporter-mediated process in rice (Ma *et al.*, 2004). A kinetic study of xylem loading in the present study confirmed this



**Fig. 5.** Concentration of Si in the xylem sap of rice, cucumber, and tomato exposed to Si solution for different times. Seedlings were cultured in a nutrient solution containing 0.5 mM Si as silicic acid. The stem was severed at each sampling time and xylem sap was collected for 20–30 min. Values are means  $\pm$ SD of three replicates.

conclusion in rice (Fig. 6A). By contrast, in cucumber and tomato, the Si concentration in the xylem sap increased gradually with increasing Si concentration in the external solution (Fig. 6B, C), but the concentration was lower than that in the external solution. The Si concentration in the xylem sap of cucumber was 2–3-fold higher than that of tomato (Fig. 6B, C). These results suggest that different from rice, xylem loading of Si in cucumber and tomato is mediated by passive diffusion. In this experiment, xylem sap was collected 8 h after exposure to Si. At this stage of exposure, the Si concentration in xylem sap of cucumber and tomato by diffusion did not reach the level of the external solution (Fig. 6). However, with prolonged exposure, the Si concentration eventually approaches to that of the external solution. This has been demonstrated

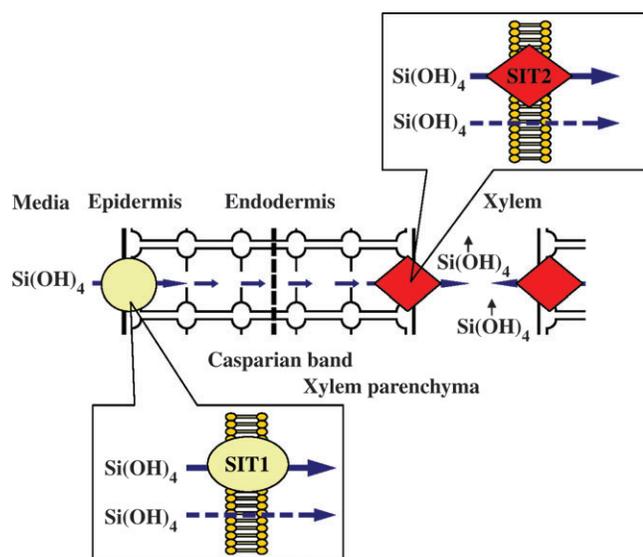


**Fig. 6.** Concentration of Si in the xylem sap of rice (A), cucumber (B), and tomato (C) cultured in Si solution at various concentrations. Seedlings were cultured in a nutrient solution containing various concentrations of Si. The stem was severed after 8 h and the xylem sap was collected for 30 min. Values are means  $\pm$ SD of three replicates.

previously in tomato (Okuda and Takahashi, 1962), in which the Si concentration in xylem sap became similar to that in the external solution by 37 h after the initiation of Si treatment. A higher Si concentration in the xylem sap of cucumber compared with that of tomato may be attributed to the higher density of transporter for radial transport of Si in cucumber (Fig. 3B, C), resulting in a higher concentration of Si in the root cells, and subsequently more Si in the xylem sap by diffusion.

Figure 7 presents a scheme for an Si uptake system, modified from that of Ma *et al.* (2004) based on results obtained in the present study. There are two components in the radial transport of Si from the external solution to the cortical cells: transporter-mediated transport (SIT1) and passive diffusion. Further, the density of this transporter differs among plant species, following the order of rice>cucumber>tomato, although the affinity of this transporter to silicic acid was similar in these three species. Xylem loading of Si was mediated by a putative transporter (SIT2) in rice, but by diffusion in cucumber and tomato. Recent studies (Ma *et al.*, 2002, 2004) comparing wild-type rice and a mutant defective in Si uptake showed that xylem loading is the most important step leading to a high accumulation of Si in the shoots. Therefore, the much lower accumulation of Si in cucumber and tomato could be explained by a lower density of SIT1 and a defect of SIT2.

Silicon is abundant in soil. However, most plants, especially dicots, are unable to accumulate a large amount of Si in the shoot from the soil. Therefore, they do not



**Fig. 7.** Schematic representation of Si uptake system in different plant species. Radial transport of Si includes transporter-mediated transport and passive diffusion in rice, cucumber and tomato. Xylem loading of Si is mediated by a kind of transporter in rice, but by diffusion in cucumber and tomato. SIT1, Si transporter from external solution to cortical cells; SIT2, Si transporter for xylem loading.

benefit from Si. Recently, a gene controlling the xylem loading of Si has been mapped to chromosome 2 of rice (Ma *et al.*, 2004). Cloning of this gene from rice may be useful in genetically modifying the Si uptake ability of other plant species, thereby enhancing the resistance of plants to multiple stresses.

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