Germanium-68 as an Adequate Tracer for Silicon Transport in Plants. Characterization of Silicon Uptake in Different Crop Species

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A basic problem in silicon (Si) uptake studies in biology is the lack of an appropriate radioactive isotope. Radioactive germanium-68 (68Ge) has been used previously as a Si tracer in biological materials, but its suitability for the study of Si transport in higher plants is still untested. In this study, we investigated 68Ge-traced Si uptake by four crop species differing widely in uptake capacity for Si, including rice (Oryza sativa), barley (Hordeum vulgare), cucumber (Cucumis sativus), and tomato (Lycopersicon esculentum). Maintenance of a 68Ge:Si molar ratio that was similar in the plant tissues of all four plant species to that supplied in the nutrient solution over a wide range of Si concentrations demonstrated the absence of discrimination between 68Ge and Si. Further, using the 68Ge tracer, a typical Michaelis-Menten uptake kinetics for Si was found in rice, barley, and cucumber. Compared to rice, the relative proportion of root-to-shoot translocated Si was lower in barley and cucumber and especially in tomato (only 30%). Uptake and translocation of Si in rice, barley, and cucumber (Si accumulators) were strongly inhibited by 2,4-dinitrophenol and HgCl2, but in tomato, as a Si-excluding species, both inhibitors produced the opposite effect. In conclusion, our results suggest the use of the 68Ge tracer method as an appropriate choice for future studies of Si transport in plants. Our findings also indicate that the restriction of Si from symplast to apoplast in the cortex of Si excluders is a metabolically active process.

Although silicon (Si) is the second most abundant mineral element in the Earth’s crust, it has not been proven to be essential for higher plants (Raven, 2003). However, beneficial effects of Si, mainly in alleviating various biotic (diseases and pests) and abiotic (nutrient deficiency, metal toxicity including aluminum, drought, and salt stress) stresses in many plant species, are well demonstrated in the literature (for review, see Epstein, 1999; Ma, 2004, 2005; Ma and Yamaji, 2006). These beneficial effects are mostly associated with the ability of plants to accumulate Si in specific tissues. The concentration of Si in shoots varies greatly between plant species (0.1%–10% of shoot on a dry weight basis), showing an extremely uneven distribution within the plant kingdom (Ma et al., 2001). Of the higher plants, typical Si accumulators include Cyperaceae and Poaceae. Some dicots (Cucurbitaceae and Utricaceae) and other monocots (Commelinaceae) accumulate moderate amounts of Si in their shoots, whereas in other families, Si accumulation is very low (Mitani and Ma, 2005, and refs. therein). It has been supposed that this variation is due mainly to different capacities for Si uptake by plant roots and/or xylem loading of Si in roots (Ma et al., 2001).

Plants take up Si in the form of the uncharged molecule of monosilicic acid, Si(OH)4 (Raven, 2001), which is present in soil solution in concentrations varying from 0.1 to 1.4 mM (Marschner, 1995). Takahashi et al. (1990) suggested three possible types of Si uptake for higher plants assessed in relation to water uptake: active (faster/higher Si uptake than water uptake), passive (similar to water uptake), and rejective (slower than water uptake). Plants with an active Si uptake mechanism cause a significant decrease of Si concentration in the uptake solution, whereas for plants taking up Si passively, it remains unchanged. Correspondingly, for plants with a rejective type of Si uptake with a tendency to exclude Si from their tissues, an increase in Si concentration in the uptake solution occurs over time. Uptake of Si by rice (Oryza sativa), a typical Si accumulator, is a metabolically active, transporter-mediated
process (Tamai and Ma, 2003; Mitani and Ma, 2005). Recently, Liang et al. (2005) suggested that Si uptake in cucumber (*Cucumis sativus*), which accumulates Si in the shoots to a much greater extent than other dicots, is also a metabolically active process, strongly inhibited by low temperature and the metabolic inhibitor, 2,4-dinitrophenol (2,4-DNP).

Based on kinetic studies, Mitani and Ma (2005) proposed two components involved in the radial transport of Si from the external medium to the cortex: (1) a transporter-mediated uptake (SIT1, a putative Si uptake transporter in root cortical cells); and (2) passive diffusion of Si(OH)₄ through the plasma membrane. An additional transporter, SIT2, is proposed to be involved in the metabolically active loading of Si from xylem parenchyma into the xylem vessels. In a study of three crop species, rice, cucumber, and tomato (*Lycopersicon esculentum*), Mitani and Ma (2005) concluded that the radial transport of Si was mediated by a transporter with the same *Kₘ* value in all species but with varying density of the transporter in the root cell membranes, hence resulting in different *Vₘₐₓ* values in the order of rice > cucumber > tomato. The lower Si accumulation in cucumber and tomato (prevailing passive Si transport) was thus accounted for by the lower density of the SIT1 transporter for radial transport, as well as by a possible defect in SIT2-transporter-mediated xylem loading of Si.

In higher plants, unlike in the marine diatom *Cylindrotheca fusiformis* (Hildebrand et al., 1997, 1998), SIT gene family-encoding Si transporter(s) have not been characterized so far, although a gene controlling the xylem loading of Si has recently been mapped in rice, and work toward cloning SIT genes in rice is currently in progress (Ma et al., 2004). Very recently, Ma et al. made the discovery of *low-Si rice 1* (*Ls1*) gene in rice roots encoding Si accumulation; suppression of its expression resulted in reduced Si uptake (Ma et al., 2006; Ma and Yamaji, 2006). To our knowledge, the only current methods available for Si uptake studies are the depletion method of measuring Si concentration in aliquots of uptake solution over time (Tamai and Ma, 2003; Liang et al., 2005) and the measurement of Si concentration in the cell sap of roots (Ma et al., 2004; Mitani and Ma, 2005). Both these methods have limitations when used in efflux experiments in the study of radial transport and xylem loading of Si, particularly at low concentration of Si in the uptake medium. The absence of an adequate Si radioisotope is a major limitation in the methodology available for Si uptake/transport studies in plants; the half-life of $^{31}$Si is only 156 min and the price of $^{32}$Si is very high. However, germanium (Ge), a cognate element from the periodic table (group IV) with similar chemical properties to Si (e.g. atomic radius and outer shell electronic configuration), does have a commercially available radioisotope, germanium-68 ($^{68}$Ge), with a half-life of 288 d. From the literature, only a few studies using $^{68}$Ge as an analog tracer for Si transport in biological systems have successfully been carried out, i.e. in the diatoms *Navicula pelliculosa* and *Nitzschia alba* (Azam, 1974), *Xenopus laevis* oocytes injected with mRNA derived from the SIT1 clone of *C. fusiformis* (Hildebrand et al., 1997) and in rat tissues including brain (Mehard and Volcani, 1975; Taylor et al., 1992). Clear information as to the applicability of $^{68}$Ge as a tracer for Si uptake, and its possible extent of discrimination, is still lacking. Earlier studies by Takahashi et al. (1976a, 1976b) showed that Ge seems to be taken up by plant roots similarly to Si, and in a recent study using wheat (*Triticum aestivum*) plants, Rains et al. (2006) reported a direct competitive effect of Ge on Si uptake and vice versa. However, even at low concentrations (above 1 µg L⁻¹) in the nutrient solution, Ge induces toxic symptoms in Si accumulators such as rice and horsetail (Takahashi et al., 1976b), a feature that has recently been very effectively applied in the screening of rice mutants defective in Si uptake (Ma et al., 2002). From these findings, it would thus appear that to avoid any possible negative effects of Ge on plant growth, only trace amounts of $^{68}$Ge can be used, and even then only in the short-term experiments.

The main objective of the study reported here was to evaluate the use of $^{68}$Ge(OH)₄ as a tracer for Si uptake in rice, barley, cucumber, and tomato. In the course of a 6-h experiment, these species maintained the same $^{68}$Ge:Si ratio in tissues (both root and shoot) as that present in the nutrient solution, regardless of Si supply. This clearly shows the absence of discrimination between $^{68}$Ge and Si in the processes of uptake and root-to-shoot translocation of Si over the period of application. The Si uptake profiling for barley (Fig. 1) exemplarily illustrates the observed lack of discrimination between Si(OH)₄ and Ge(OH)₄. At the constant molar $^{68}$Ge:Si ratio (no increase in specific activity), the uptake of $^{68}$Ge(OH)₄ showed a typical saturation kinetics (Fig. 1A). When Si(OH)₄ in the nutrient solution was substituted by nonradioactive Ge(OH)₄ at the same concentrations (the same molar ratio of $^{68}$Ge:Si and thus specific activity; Fig. 1B), the uptake of $^{68}$Ge(OH)₄ showed the same kinetic pattern with a similar apparent *Kₘ* of about 0.45 µM. To minimize any toxic effect of Ge that may affect general metabolism and/or plasma membrane properties, and thus the uptake, all experiments shown in Figure 1 were shortened to 2 h. A

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**RESULTS**

Results presented in Table I clearly demonstrate the appropriateness of $^{68}$Ge as tracer for Si uptake in rice, barley, cucumber, and tomato. In the course of a 6-h experiment, these species maintained the same $^{68}$Ge:Si ratio in tissues (both root and shoot) as that present in the nutrient solution, regardless of Si supply. This clearly shows the absence of discrimination between $^{68}$Ge and Si in the processes of uptake and root-to-shoot translocation of Si over the period of application. The Si uptake profiling for barley (Fig. 1) exemplarily illustrates the observed lack of discrimination between Si(OH)₄ and Ge(OH)₄. At the constant molar $^{68}$Ge:Si ratio (no increase in specific activity), the uptake of $^{68}$Ge(OH)₄ showed a typical saturation kinetics (Fig. 1A). When Si(OH)₄ in the nutrient solution was substituted by nonradioactive Ge(OH)₄ at the same concentrations (the same molar ratio of $^{68}$Ge:Si and thus specific activity; Fig. 1B), the uptake of $^{68}$Ge(OH)₄ showed the same kinetic pattern with a similar apparent *Kₘ* of about 0.45 µM. To minimize any toxic effect of Ge that may affect general metabolism and/or plasma membrane properties, and thus the uptake, all experiments shown in Figure 1 were shortened to 2 h. A
control experiment (Fig. 1C) was performed to determine the tracer uptake kinetics when only radioactive 68Ge(OH)4 [without either Si(OH)4 or Ge(OH)4] was added at equal trace amount (radioactivity) as in the experiments A and B. At such low concentrations of 68Ge(OH)4, the tracer uptake increased linearly without any tendency toward saturation. When the nutrient solution contained solely trace amounts of radioactive 68Ge [without nonlabeled Ge(OH)4 or Si(OH)4], the uptake of tracer (i.e. the absolute radioactivity in the plant) was much higher than if the same 68Ge concentration in the nutrient solution was supplemented with either Si(OH)4 or Ge(OH)4. This shows that the 68Ge tracer uptake depends on the relative proportion to Si(OH)4 or Ge(OH)4 and that the roots are unable to distinguish between these two analogs.

While rice, barley, and cucumber showed typical Michaelis-Menten uptake kinetics, tomato plants showed nearly linear, nonsaturable kinetics, with an uptake rate nearly 20 times lower than in rice and 5 and 3 times lower than in barley and cucumber, respectively (Fig. 2). The $K_m$ value was similar for rice and barley (0.35 mM and 0.38 mM, respectively) and in cucumber was significantly higher (0.62 mM; Table II). However, the value of $V_{max}$ significantly differed among the three species (0.31, 0.12, and 0.05 mmol Si g$^{-1}$ root dry weight h$^{-1}$ for rice, barley, and cucumber, respectively). Consequently, all four plant species also differed in the relative root-to-shoot translocation of Si(OH)4 studied with radioactive tracer 68Ge(OH)4. Hence, during the 6-h uptake period, more than one-half of the Si taken up by roots was translocated to the shoots in rice, barley, and cucumber, whereas translocation was only 20-40% in tomato.

Table 1. Molar ratios between 68Ge and Si present in nutrient solution and in plant tissues after 6 h uptake of Si(OH)4 labeled with 68Ge(OH)4

<table>
<thead>
<tr>
<th>Species</th>
<th>Si Concentration in NS</th>
<th>68Ge:Si Molar Ratio</th>
<th>PT/NS Factor</th>
<th>68Ge:Si Molar Ratio</th>
<th>PT/NS Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>0.15 $10^{-5}$ M</td>
<td>5.97 $10^{-8}$</td>
<td>0.98</td>
<td>6.06 $10^{-8}$</td>
<td>1.00</td>
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<td></td>
<td>1.5 $10^{-5}$ M</td>
<td>5.79 $10^{-8}$</td>
<td>0.95</td>
<td>5.70 $10^{-8}$</td>
<td>0.94</td>
</tr>
<tr>
<td>Barley</td>
<td>0.15 $10^{-5}$ M</td>
<td>5.61 $10^{-8}$</td>
<td>0.91</td>
<td>5.38 $10^{-8}$</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>1.5 $10^{-5}$ M</td>
<td>6.02 $10^{-8}$</td>
<td>1.00</td>
<td>5.66 $10^{-8}$</td>
<td>0.94</td>
</tr>
<tr>
<td>Cucumber</td>
<td>0.15 $10^{-5}$ M</td>
<td>5.78 $10^{-8}$</td>
<td>0.96</td>
<td>5.87 $10^{-8}$</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>1.5 $10^{-5}$ M</td>
<td>6.01 $10^{-8}$</td>
<td>1.00</td>
<td>5.93 $10^{-8}$</td>
<td>0.99</td>
</tr>
<tr>
<td>Tomato</td>
<td>0.15 $10^{-5}$ M</td>
<td>5.59 $10^{-8}$</td>
<td>0.93</td>
<td>5.59 $10^{-8}$</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>1.5 $10^{-5}$ M</td>
<td>5.88 $10^{-8}$</td>
<td>0.98</td>
<td>5.67 $10^{-8}$</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Figure 1. 68Ge(OH)4 taken up by barley plants for 2 h in the presence of Si(OH)4 (A), in the presence of Ge(OH)4 instead of Si(OH)4 (B), and in the absence of either Si(OH)4 or Ge(OH)4 (C). The molar ratio of 68Ge:Si and 68Ge:Ge was kept constant at 3 $10^{-8}$ (specific radioactivity 5 $\mu$Ci mmol$^{-1}$ Si and Ge, respectively) in A and B. The 68Ge(OH)4 concentrations in C was calculated from radioactivity used in A and B. Data shown are means of two independent experiments with four replicates each.
barley, and cucumber (i.e. 97, 68, and 54%, respectively), whereas in tomato, only about 30% of Si was translocated to the upper parts (Fig. 3).

In all three Si-accumulating plant species, rice, barley, and cucumber, the concentration of Si in the xylem exudate was 3- to 20-fold higher than in the nutrient solutions regardless of the Si supply to the nutrient solution (Table III). However, in tomato plants at both low (0.15 mM) and high (1.5 mM) Si supply, the Si concentrations in the xylem exudate were significantly lower than in the nutrient solution (ratio [Si]_{xylem}/[Si]_{NS} = 0.3–0.4).

HgCl₂ and 2,4-DNP significantly inhibited Si uptake by rice, barley, and cucumber plants (Fig. 4, A–C). However, Si uptake rate by tomato plants was not inhibited by either 2,4-DNP or HgCl₂, treated plants even taking up Si at a significantly higher rate as compared to the control untreated plants (Fig. 4D). Concomitantly, Figure 5 shows that both 2,4-DNP and HgCl₂ severely inhibited root-to-shoot translocation of ⁶⁸Ge-labeled Si(OH)₄ in rice, barley, and cucumber, whereas in tomato plants, the application of inhibitors led to an increased root-to-shoot translocation of Si (about 130% and 120% of the controls for 2,4-DNP and HgCl₂, respectively).

**DISCUSSION**

The use of radioactive isotopes provides a valuable tool in the study of the uptake and transport of mineral elements either as ions or molecules in plants; however, their reliability as tracers must sometimes be carefully verified. For instance, radioactive rubidium-86 with similar chemical properties to that of K⁺, which has often been used as a tracer for K⁺, can give misleading results under certain circumstances (Behl and Jeschke, 1982). In this study, both measurements of the radioactivity of ⁶⁸Ge and chemical analysis of Si in plant tissues were carried out to demonstrate that plant species with markedly different capacities for accumulating Si in their shoots are able to take up ⁶⁸Ge without discriminating between these two elements (Table I; Fig. 1). The clearly shown tendency of all the plant species (rice, barley, cucumber, and tomato) to maintain a ⁶⁸Ge:Si molar ratio in their tissues similar to that in the supplied nutrient solution (Table I) is a key condition for the applicability of the tracer (Maas and Leggett, 1968). It has been proposed that uncharged Si(OH)₄ is the only molecular species likely to cross the root plasma membrane at physiological pH (Raven, 2001). Therefore, taking into consideration the similar physicochemical properties of Si(OH)₄ and Ge(OH)₄ such as their pKₐ of about 9.3 to 9.5 (Pokrovski and Schott, 1998; Tossell and Sahai, 2000), it would be expected that the uncharged form of Ge(OH)₄ should also be able to cross the plasma membrane passively (by diffusion) and/or actively via Si transporter(s). The results shown in Figure 1, A and B unequivocally support this assumption. The uptake of both Si(OH)₄ and Ge(OH)₄ determined through the radioactivity of ⁶⁸Ge tracer showed saturable kinetics with similar apparent Kₘ values (Fig. 1, A and B). Furthermore, when ⁶⁸Ge(OH)₄ was applied solely as a trace amount in the range of 10⁻¹⁵ M (see Fig. 1C), the uptake pattern was completely different, showing no tendency toward
Table II. Kinetic parameters for Si(OH)$_4$ uptake by rice, barley, and cucumber

Values for $K_m$ and $V_{max}$ were obtained by Lineweaver-Burk’s transformation of data fitted to Michaelis-Menten equation. Data are means ($n = 4$) ± sd. Superscript letters denote significant differences between species at $P < 0.05$ according to Duncan's test.

<table>
<thead>
<tr>
<th>Species</th>
<th>$K_m$ (mM)</th>
<th>$V_{max}$ (mmol Si g$^{-1}$ root dry weight h$^{-1}$)</th>
<th>Regression Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>0.35 ± 0.05$^a$</td>
<td>0.31 ± 0.03$^a$</td>
<td>0.975</td>
</tr>
<tr>
<td>Barley</td>
<td>0.38 ± 0.07$^a$</td>
<td>0.12 ± 0.04$^b$</td>
<td>0.996</td>
</tr>
<tr>
<td>Cucumber</td>
<td>0.62 ± 0.02$^b$</td>
<td>0.05 ± 0.01$^c$</td>
<td>0.988</td>
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</table>

saturation. These observations, together with the results of other authors (e.g. Azam, 1974; Taylor et al., 1992; Hildebrand et al., 1997), confirm that $^{68}$Ge can be considered as an adequate tracer for Si transport in different biological materials. Moreover, the straightforward study of Mehard and Volcani (1975), in which $^{68}$Ge and $^{31}$Si were directly compared, showed that both isotopes were equally good tracers for Si uptake in rat tissues. Because there is no discrimination between Ge and Si uptake by plant roots, any decrease in a $^{68}$Ge:Si ratio (measured as specific activity) of the uptake solution at constant tracer uptake rate (data not shown), which has also been demonstrated in a culture of marine diatoms (Azam, 1974). Hence, it is important to note that keeping a specific activity of the uptake solution at constant value is a prerequisite for the reliability of the concentration kinetic studies.

Even though they have similar chemical properties, Ge is more reactive than Si (Greenwood and Earnshaw, 1984), and, unlike Si, becomes toxic to plants after medium-term supply (characteristic symptoms of brown spots on the leaves; Ma et al., 2002). The range of the Ge:Si ratio used in this study ($3–6 \times 10^{-5}$) is indeed representative of real environmental conditions, as this value in most crustal rocks is about $10^{-5}$ (Mortlock and Froelich, 1987). Furthermore, in such trace amounts, no toxic effects of Ge are to be expected, even in Si-accumulating species such as rice, because the leaf $^{68}$Ge concentration, calculated through the radioactivity, was below 1 $\mu$g kg$^{-1}$ dry matter. The method might hence also be suitable for long-term studies of Si uptake and translocation for plants growing in soil.

In this study, Si uptake was characterized using $^{68}$Ge tracer in rice, barley, cucumber, and tomato. From recently reported studies, these crop species have different Si uptake properties (Tamai and Ma, 2003; Liang et al., 2005; Mitani and Ma, 2005). Of these species, however, only rice is generally accepted as a plant with a prevailing metabolically active Si uptake, which has received additional recent confirmation by the discovery of a mutant defective in Si uptake (Ma et al., 2004). Our kinetic study clearly demonstrated that barley also has a typical concentration-dependent Si uptake with a $K_m$ value of 0.38 mM, which was very similar to that found in rice (0.35 mM) but with a significantly lower $V_{max}$ as compared to rice (Table II). On the other hand, the $K_m$ value for cucumber was significantly higher than in rice and barley with a significantly lower $V_{max}$ value. Considering radial transport of Si from the external solution to the root cells by measurement of Si concentration in the cell sap from frozen-thawed roots, Mitani and Ma (2005) found similar $K_m$ values of about 0.16 mM not only for rice and cucumber but also for tomato plants. In another not directly comparable study, Tamai and Ma (2003) using a depletion technique, reported a higher $K_m$ value in rice of 0.32 mM, which was closer to our findings. It has been proposed that radial transport of Si from the external solution to cortical cells includes both transporter-mediated Si uptake and passive diffusion in rice, cucumber, and tomato, the variation in density of Si transporter of the root cell membranes (SIT1) between the crop species resulting in different $V_{max}$ values, decreasing in the order rice, cucumber, tomato (Mitani and Ma, 2005; also see introduction). Furthermore, a lower accumulation of Si in cucumber and tomato shoots was explained by passive diffusion of Si(OH)$_4$ due to a defect in the SIT2 transporter in xylem parenchyma of these two species during xylem loading (Mitani and Ma, 2005). However, considering the net uptake of $^{68}$Ge-labeled Si(OH)$_4$ by the whole plant in the study presented, it can be hypothesized that both mechanisms, active and passive, coexist not only in rice and barley but also in cucumber, their relative contribution being dependent

![Figure 3. Portion of root-to-shoot translocated Si of rice, barley, cucumber, and tomato exposed to 1 mM Si(OH)$_4$ and 5 $\mu$Ci $^{68}$Ge ($^{68}$Ge:Si molar ratio of 3 $\times$ 10$^{-5}$) for 6 h.](image-url)
of external Si supply (Fig. 4D; see also Liang et al., 2005, 2006). Contrary to the findings of Mitani and Ma (2005), we observed a Si concentration in the xylem sap of cucumber 3- to 4-fold higher than the Si concentration in the external solutions (after 6 h; Table III; see also Liang et al., 2005). This observation, considered together with the results of the relative proportion of root-to-shoot translocated Si (Fig. 3), indicates that a metabolically active mechanism might also be involved in xylem loading of Si in cucumber, however, to a lesser extent than in barley, and especially than in rice. Thus, our conclusions do not differ greatly from those of Mitani and Ma (2005) that transporter density could be lower in the cucumber cortex compared to that in rice. However, in considering xylem loading of Si, our results do not exclude the existence of a metabolically active component.

Rice is recognized to be a high Si-accumulating species able to translocate more than 90% of Si taken up by the roots, thereby continuously maintaining roots at a relatively low Si status for the efficient operating of a metabolically active transporter-mediated uptake (Fig. 3; van der Vorm, 1980). The relative proportion of root-to-shoot translocated Si was lower in barley and cucumber (68 and 54%, respectively) and especially in tomato (about 30%), which, together with the results from Figure 2D, clearly indicate that the uptake system in tomato differs greatly from that in the other plant species examined. In contrast to the kinetic study of Mitani and Ma (2005) for radial Si uptake in the cortex, tomato exhibited no tendency toward saturation in total Si uptake. As, at the same time, the Si concentration in the xylem sap was lower than in the nutrient solution regardless of the external Si concentration (Table III; see also Mitani and Ma, 2005), the proposal of these workers of an active component of Si uptake in the cortex of tomato thus seems to be unlikely.

Passive transport of uncharged Si(OH)₄ by diffusion across the lipid component of the plasma membrane and/or by facilitated diffusion via proteinaceous channels (i.e. aquaporin-like membrane proteins as reported by Ma et al., 2006) is a concentration-dependent component of Si uptake, which is present in all plant species regardless of their ability to accumulate Si (Raven, 2001). However, in Si-accumulating plants, a metabolically active, concentration-independent component of Si uptake prevails and is responsible for rapid transport of Si to the shoots, as for instance in rice. Yet, the nature of metabolically active transport of Si(OH)₄ in higher plants is not sufficiently clear, although a H⁺/Si(OH)₄ cotransport and involvement of H⁺-ATPase in energizing this secondary active transport seems to be most likely (Liang, 1999; Raven, 2001). The application of 2,4-DNP, a common metabolic inhibitor that is believed to inhibit the formation of ATP by uncoupling oxidative phosphorylation, caused a severe decrease of Si uptake in accumulating- and intermediate-type species in the order rice > barley > cucumber (Fig. 4, A–C), confirming the prevalence of an energy dependent, active component of Si uptake in all three species albeit more prevalent in rice. The application of an aquaporin-like channel inhibitor, HgCl₂, in rice, barley, and cucumber also caused the same decrease of Si uptake as the application of 2,4-DNP (in agreement with Mitani and Ma, 2005). This inhibitory effect of HgCl₂ on Si uptake is probably due to the direct target of Cjys residues in Si transporter(s) and/or poison-induced change in the general metabolic status of plants (Maurel and Chrispeels, 2001; Tamai and Ma, 2003). It is of interest that this mercury-induced inhibition of Si uptake was not caused by the inhibition of water uptake (Tamai and Ma, 2003; Mitani and Ma, 2005), suggesting a difference between aquaporin-like Si transporter(s) and water channels. Furthermore, the Lsi1 gene, which is constitutively expressed at the plasma membranes of both exodermal and endodermal root cells and hence controls xylem loading of Si in rice (expression of Lsi1 in Xenopus oocytes results in Si but not in water transport activity), is found to belong to the aquaporin family (Ma et al., 2006). Surprisingly, in tomato, a Si-excluding species (see Table III; Helne et al., 2005), the application of both 2,4-DNP and HgCl₂ even caused an increase of uptake and root-to-shoot translocation of Si (Figs. 4D and 5, A and B). The application of ⁶⁸Ge tracer for Si demonstrates the existence of a system of metabolically active Si exclusion in tomato, not possible to be observed by the methods of determination of Si in the root cell sap used in the previous studies of Mitani and Ma (2005). The nonaccumulators actually exclude Si(OH)₄ from their roots, because they contain

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**Table III. Concentration of Si in the xylem sap of rice, barley, cucumber, and tomato grown in the nutrient solution (NS) supplied with two levels of Si(OH)₄**

The plants were decapitated after 6 h, and xylem sap was collected during 1 h. Data are means (n = 4) ± sd.

<table>
<thead>
<tr>
<th>Species</th>
<th>Si Concentration in Xylem Sap</th>
<th>Ratio [Si₅] xylem/[Si₅]NS</th>
<th>Si Concentration in Xylem Sap</th>
<th>Ratio [Si₅] xylem/[Si₅]NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>3.12 ± 0.81</td>
<td>20.8</td>
<td>21.39 ± 2.07</td>
<td>14.3</td>
</tr>
<tr>
<td>Barley</td>
<td>1.02 ± 0.32</td>
<td>6.8</td>
<td>6.13 ± 0.86</td>
<td>4.1</td>
</tr>
<tr>
<td>Cucumber</td>
<td>0.43 ± 0.05</td>
<td>2.9</td>
<td>4.98 ± 0.30</td>
<td>3.3</td>
</tr>
<tr>
<td>Tomato</td>
<td>0.06 ± 0.03</td>
<td>0.4</td>
<td>0.42 ± 0.12</td>
<td>0.3</td>
</tr>
</tbody>
</table>
less Si in the shoots than would be expected if there was nonselective passive influx with water (Liang et al., 2005, 2006). Metabolically active exclusion of Si against the concentration gradient in the root cortex might be responsible for lower uptake of Si by tomato at high concentration of Si in the external media (Figs. 2D and 3). Therefore, it can be hypothesized that in the Si-excluding plants such as tomato, passive diffusion of Si(OH)4 into the root cortex obviously coexists with a transporter-mediated Si exclusion from the root cortical cells into the apoplast, which might further explain the rejective type of Si uptake as postulated by Takahashi et al. (1990).

Although 32Si (very long half-life of 134 years) has been reported to be suitable for measuring silica production in the ocean (Brzezinski and Phillips, 1997), its practical application is constrained by the high price (approximately 2,000 U.S. dollars for 1 μCi; J. Brockmann, Vira-Pharma GmbH, personal communication). However, this study showed that 68Ge can be used as an adequate tracer for Si uptake experiments in higher plants in the short-term studies without any discrimination over a wide range of Si concentrations in the uptake solution and for plant species differing in Si uptake mechanisms. Also, there are no experimental difficulties and limitations in using this method, which

Figure 4. Effect of inhibitors on net uptake of Si(OH)4 determined using radioactive tracer 68Ge(OH)4 by rice (A), barley (B), cucumber (C), and tomato (D). The experiment was carried out in the nutrient solution containing 1 mM Si(OH)4 with constant 68Ge:Si molar ratio of 3 × 10^-8 (specific radioactivity 5 μCi mmol^-1 Si) for 4 h. 2,4-DNP and HgCl2 were added at final concentrations of 500 and 50 μM, respectively. No inhibitors were added in the control. Data are means (n = 4) ± sd.

Figure 5. Effect of 2,4-DNP (A) and HgCl2 (B) on relative root-to-shoot translocation of 68Ge-labeled Si in rice, barley, cucumber, and tomato plants. In the control treatment (none of either 2,4-DNP or HgCl2), absolute shoot amount of Si was denoted as 100%. Data are means (n = 4).
is highly sensitive and easy in terms of analysis of radioactivity.

In conclusion, our results compared to those obtained by other methods currently available, i.e. direct measurement of Si concentration in the cell sap and/or depletion technique (e.g. Liang et al., 2005; Mitani and Ma, 2005), suggest the $^{68}$Ge tracer method as an appropriate choice for future studies of Si transport in plants by efflux experiments, which are suitable for both radial transport and xylem loading of Si. These results also suggest for the first time that in Si excluders (e.g. tomato), the restriction of Si from symplast to apoplastic in the root cortex is coupled with metabolically derived energy, presumably mediated by an efflux type of Si transporter in root cortical cells.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

After soaking in 1 M CaSO$_4$ overnight, seeds of rice (Oryza sativa L.) cv Shanyou number 6, barley (Hordeum vulgare L.) cv Chinese long, and tomato (Lycopersicon esculentum Mill.) cv FER were germinated in quartz sand moistened with saturated CaSO$_4$. The 5-d old seedlings were then transferred to a full-strength nutrient solution (four plants/2.5-L plastic pot) containing (in mM): 0.7 K$_2$SO$_4$, 0.1 KCl, 2.0 Ca(NO$_3$)$_2$, 0.5 MgSO$_4$, 0.1 K$_2$HPO$_4$, and (in μM): 0.5 MnSO$_4$, 0.5 ZnSO$_4$, 0.2 CuSO$_4$, 0.01 (NH$_4$)$_6$Mo$_7$O$_2$$_4$, and 1 or 10 H$_2$BO$_3$ for monocots and dicots, respectively. Iron was supplied as Fe(III)EDTA at 80 μM. Plants were grown under controlled environmental conditions in a growth chamber with a light/dark regime of 16/8 h, temperature regime of 24°C/20°C, photon flux density of 450 μmol m$^{-2}$ s$^{-1}$ at plant height, and relative humidity of about 70%.

Si Uptake Experiments

All short-term (up to 6 h) Si uptake experiments were carried out in 150-mL plastic pots (one plant per pot), exposing 10-d-old plants to a standard nutrient solution with various concentrations of Si (ranging from 0.05-2.5 mM). Si was applied as Si(OH)$_4$, freshly prepared by passing Na$_2$SiO$_3$ through a column filled with cation-exchange resin (Amberlite IR-120, H$^+$ form, Fluka). In all uptake experiments, $^{46}$Ge(OH)$_4$ was used as a radioactive tracer. $^{46}$GeCl$_4$ (Perkin Elmer; specific activity 2,443.07 mCi mg$^{-1}$; 1 mCi = 37 MBq) was converted to $^{46}$Ge(OH)$_4$, with 0.001 mCi KOH, mixed with a solution containing Si(OH)$_4$, and left to equilibrate over 1 h with continuous stirring. The molar ratio of $^{46}$GeSi was kept constant at 3 $\times$ 10$^{-8}$ and 6 $\times$ 10$^{-8}$ (specific radioactivity of 5 and 12.5 μCi mmol$^{-1}$ Si, respectively).

Ge Uptake Experiment

A short-term (2 h) Ge uptake experiment was performed to compare concentration-dependent kinetic of Si(OH)$_4$ and Ge(OH)$_4$. GeCl$_4$ (Sigma) was carefully dissolved in 1 M HCl to give a final Ge concentration of 50 mM and subsequently converted to Ge(OH)$_4$ by neutralization with 1 M KOH. Fresh solution was labeled with $^{46}$Ge(OH)$_4$ (prepared as described above) to reach a specific activity of 5 μCi mmol$^{-1}$ Ge. Uptake experiments were carried out in 150-mL plastic pots (one plant per pot), exposing 10-d-old plants to a standard nutrient solution. The concentrations of Ge, which ranged from 0.05 to 2.5 mM, were equal to the concentrations of Si.

Determination of $^{68}$Ge Radioactivity

At the end of all uptake experiments, roots were washed for 20 min in the ice-cold desorption solution to remove free $^{68}$Ge from the root surface (apoplast). Plants were separated into roots and shoots (leaves + stems), oven dried at 70°C, ashed at 550°C, ash dissolved in 1% HCl, and the radioactivity determined by liquid scintillation counting (Wallac 1414 Win Spectral, Wallac Oy).

Application of Inhibitors

The effects of 2,4-DNP (500 μM) and HgCl$_2$ (50 μM) on Si uptake were also examined in the $^{46}$Ge uptake solution containing 1 mCi Si(OH)$_4$. The stock solutions of 2,4-DNP and HgCl$_2$ were dissolved in ethanol and methanol, respectively. The final concentration of both ethanol and methanol in the uptake solutions of 0.2% (v/v) did not affect Si uptake (Ma et al., 2002; Tamai and Ma, 2003).

Collection of Xylem Sap

Xylem sap was obtained by exudation after plants were decapitated at the stem about 2 cm above the root base. Soft rubber tubes were fixed over decapitated stem, and xylem sap was collected by micropipette for 1 h after discarding the exudates obtained during the first few minutes.

Determination of Si

In some experiments, to verify a $^{46}$Ge tracer method, the Si concentration in xylem sap, plant tissues, and nutrient solution was also determined. After oven drying, the plant material was microwave digested with 3 mL HNO$_3$ + 2 mL H$_2$O$_2$ for 2 h. Samples were diluted with about 15 mL water, transferred into 25-mL plastic flasks, 1 mL H$_2$O$_2$ was added, and left overnight. After addition of 2.5 mL 2% (w/v) H$_2$BO$_3$ flask volume was adjusted to 25 mL with water and Si was determined by atomic absorption spectroscopy using a nitrous oxide-acetylene flame. To avoid any Si polymerization in both xylem sap and nutrient solution, Si was determined immediately in the fresh samples by the colorimetric molybdenum blue method at 700 nm (Liang et al., 2005).

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Germanium-68 as Tracer for Silicon Transport in Plants

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