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Identification of the Silicon Form in Xylem Sap of Rice (*Oryza sativa* L.)

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Rice (*Oryza sativa* L.) is a typical silicon (Si)-accumulating plant, but the mechanism responsible for the translocation from the root to the shoot is poorly understood. In this study, the form of Si in xylem sap was identified by ^{29}Si-nuclear magnetic resonance (NMR) spectroscopy. In rice (cv. Oochikara) cultured in a monosilicic acid solution containing 0.5 mM Si, the Si concentration in the xylem reached 6 mM within 30 min. In the ^{29}Si-NMR spectra of the xylem sap, only one signal was observed at a chemical shift of –72.6 ppm, which is consistent with that of monosilicic acid. A ^{1}H-NMR study of xylem sap did not show any significant difference between the wild-type rice and mutant rice defective in Si uptake, and the components of the xylem sap were not affected by the Si supply. The Si concentration in the xylem sap in vitro decreased from an initial 18 mM to 2.6 mM with time. Addition of xylem sap to a solution containing 8 mM Si did not prevent the polymerization of silicic acid. All these results indicate that Si is translocated in the form of monosilicic acid through the xylem and that the concentration of monosilicic acid is high in the xylem only transiently.

Keywords: Nuclear magnetic resonance — Rice — Silicon — Xylem.

Abbreviation: NMR, nuclear magnetic resonance.

Introduction

Silicon (Si) is a beneficial element for plant growth, and important especially for rice (Savant et al. 1997, Ma 2003). The rice shoot contains Si at a several-fold higher concentration than essential macronutrients such as N, P and K (Epstein 1994, Epstein 1999, Ma and Takahashi 2002). Without Si, the growth of rice is significantly decreased and the productivity is markedly reduced mainly due to reduced fertility (Ma et al. 1989, Ma 2003). These beneficial effects of Si on the growth and productivity result from Si-enhanced resistance of rice to various stresses (Epstein 1994, Ma 2004). High accumulation of Si in the tissues helps alleviate the water stress of plants by decreasing transpiration (Matoh et al. 1991), and increases the photosynthetic activity by keeping the leaf blade erect and thereby improving light interception characteristics. Si accumulation also increases the resistance to diseases, pests and lodging, and restores nutrient imbalances (Epstein 1994, Savant et al. 1997, Epstein 1999, Ma et al. 2001, Ma 2004).

Si is taken up by the roots in the form of silicic acid as an undissociated molecule (Takahashi and Hino 1978). After uptake, Si is immediately translocated to the shoot together with the transpiration stream and then polymerized and accumulated on the cell surface of the rice leaf to form the silica–cuticle double layer and silica–cellulose double layer (Yoshida 1965). More than 90% of Si in the rice shoot is present in the form of silica gel (Ma and Takahashi 2002). The formation of these layers prevents excessive transpiration and increases the resistance to diseases and pests, which are important for the healthy growth of rice. Si is also accumulated in the bulliform cells, dumbbell cells, and long and short cells of the leaf epidermis and hulls, where the transpiration stream ends. In rice roots, the Si concentration is much lower than that in the shoot. These results indicate that the distribution of Si in rice is closely related to transpiration. Therefore, the high Si concentration in rice shoot is attributed to the high ability of the roots to take up Si (Takahashi et al. 1990).

The uptake of Si by rice roots consists of at least two processes; radial transport of Si from the external solution into the cortical cells and subsequent release of Si into the xylem (xylem loading). Both processes are important for high accumulation of Si in the rice shoot. A previous study showed that the Si uptake by rice roots is not affected by transpiration (Okuda and Takahashi 1962a), but is inhibited by metabolic inhibitors such as NaCN, 2,4-D (dichlorophenoxy acetate) and 2,4-dinitrophenol (Okuda and Takahashi 1962b), suggesting that Si uptake is an energy-dependent, active process. A recent study showed that the uptake of silicic acid by rice roots is mediated by a type of proteinaceous transporter (Tamai and Ma 2003). This transporter has a low affinity for silicic acid and contains cysteine residues but not lysine residues.

After Si is taken up into the cortical cells, it must be released to the xylem for high accumulation in the shoot. The Si concentration in the xylem sap has been reported to be much higher than that in the external solution. For example, Okuda and Takahashi (1962a) reported that the Si concentration in rice xylem was about 10 mM in rice cultivated in 1.67 mM Si solution for 37 h. In another study, the Si concentration in the xylem sap was >5 mM in rice grown in a solution containing 0.15 mM Si (Ma et al. 2002). Silicic acid usually has a solubility of 2.0–2.3 mM at 25°C and polymerizes at higher concen-

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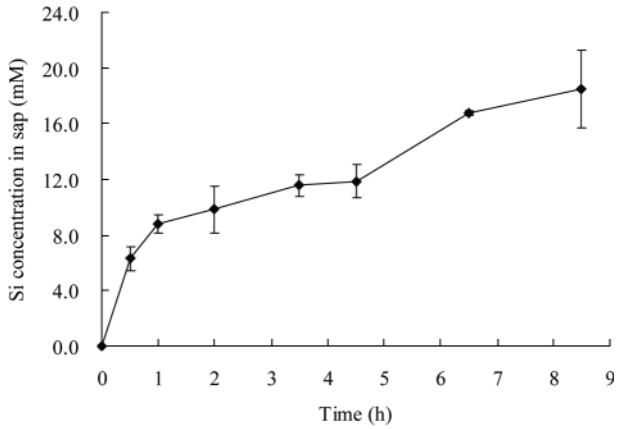

Fig. 1 Concentration of monosilicic acid in the xylem sap of rice cultured in Si solution for various durations. Seedlings (24 d old) were cultured in half-strength Kimura nutrient solution containing 0.5 mM Si in the form of silicic acid. The stem was severed at intervals and the xylem sap was collected for 30 min. Values are means ± SD of three replicates.

trations (Iler 1979). However, the concentration of silicic acid reported in the xylem sap is much higher than 2.0 mM, suggesting that Si in the xylem sap may exist in a chemical form other than silicic acid. In the present study, the form of Si in the xylem sap of rice was identified by ^{29}Si-nuclear magnetic resonance (NMR) spectroscopy.

Results

Xylem sap was collected within 30 min after decapitating the plant 1 cm above the roots. The average exudate rate was 1.6–2.8 nl plant^{-1} s^{-1} in all experiments. The pH of xylem sap ranged from 5.6 to 6.0. The concentration of Si as monosilicic acid was determined by reaction with molybdic acid immediately after the collection of xylem sap in all experiments. When the roots were exposed to a solution containing 0.5 mM silicic acid, the concentration of Si in the xylem sap reached 6.0 mM Si within 30 min, and 18 mM Si at 8.5 h (Fig. 1).

The form of Si in the xylem was identified by ^{29}Si-NMR spectroscopy. The xylem sap was collected from rice fed 1.0 mM of ^{29}Si-enriched silicic acid. A signal at a chemical shift of –72.6 ppm was observed (Fig. 2A), and the chemical shift was consistent with that of monosilicic acid (Fig. 2B). No other peaks were detected in the entire spectrum.

To search for compounds which may be coordinated with Si in the xylem sap, we subjected the fresh xylem sap to ^{1}H-NMR measurement. Signals ranging from chemical shifts of –1 to 11 ppm were observed, but there were no significant differences between xylem sap samples collected from the plants supplied with Si and those without Si (Fig. 3A, B). Although the peak intensity was altered slightly at some chemical shifts, the peak position was not shifted. A mutant rice (*lsi1*, formerly called GR1), which is defective in active Si uptake (Ma et al. 2002), was also examined for comparison. Again, the difference in the ^{1}H-NMR spectra between the wild-type rice and the mutant was small (Fig. 3C).

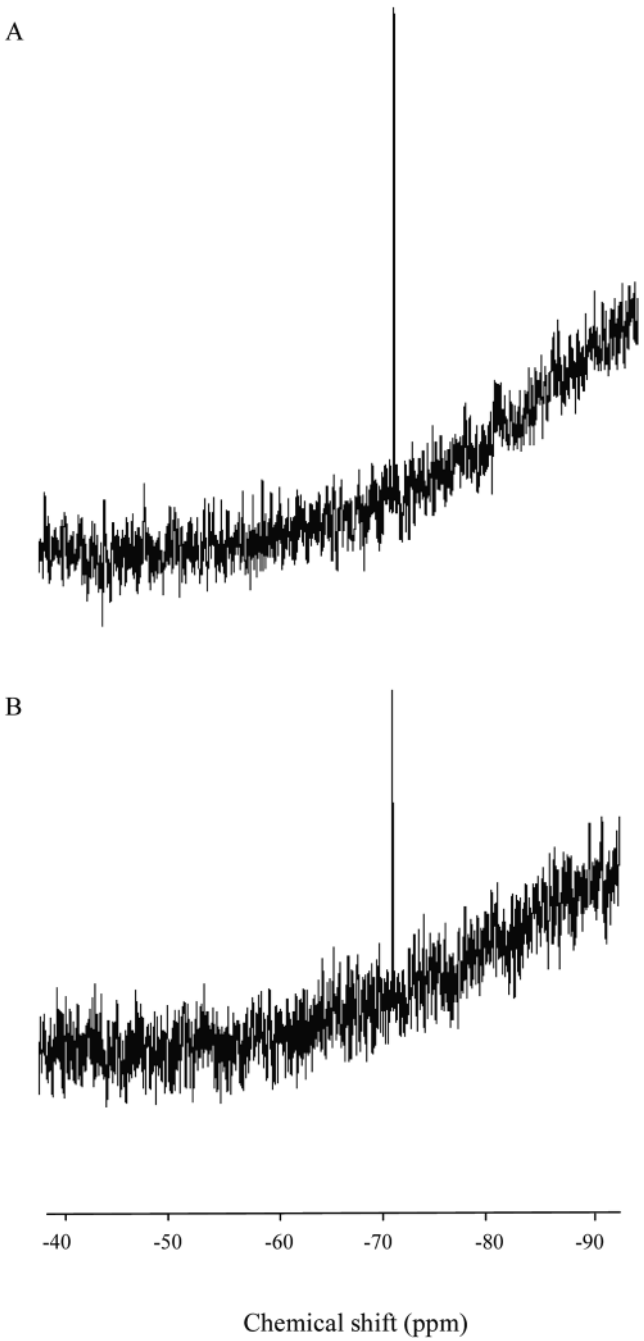

Fig. 2 The ^{29}Si-NMR spectra of xylem sap from rice. (A) Xylem sap collected from the seedlings cultured in 1.0 mM Si solution for 4 h. (B) 2 mM silicic acid. Spectra were measured at 99.36 MHz.

To examine whether the xylem sap contains any compounds that prevent the polymerization of Si, we prepared a solution with a high Si concentration and mixed it with water or xylem sap collected from the rice grown without Si at room

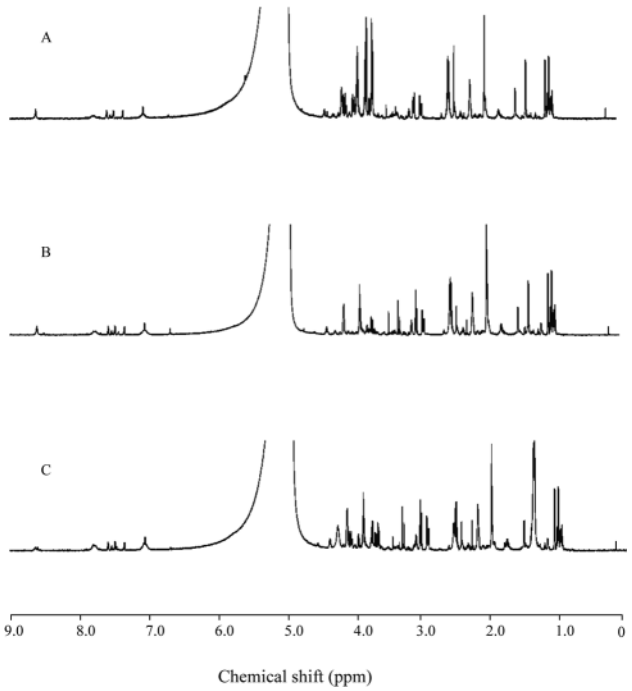

Fig. 3 The ^{1}H-NMR spectra of xylem sap from rice. Xylem sap was collected from seedlings of wild-type rice cultured in a solution containing (A) 1.0 or (B) 0 mM Si, and from the seedlings of a mutant rice cultured in a solution containing 1.0 mM Si (C). Spectra were measured at 750.13 MHz.

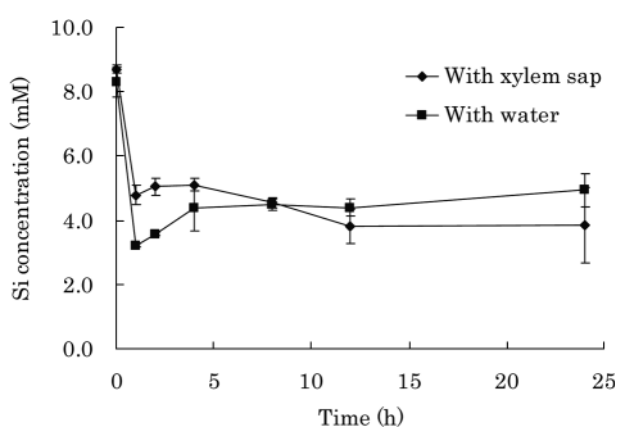

Fig. 4 Effect of xylem sap addition on the changes in the concentration of monosilicic acid. Solutions containing a high concentration of monosilicic acid were mixed with water or xylem sap collected from rice seedlings without Si supply and stored at room temperature for various periods. Values are means ± SD of three replicates.

temperature. The Si concentration decreased from the initial 8 mM Si to 3.2 mM Si within 1 h after mixing with water (Fig. 4). The results obtained with xylem sap were similar to those obtained with water.

The in vitro change in the concentration of monosilicic acid in the xylem sap was also investigated. The concentration

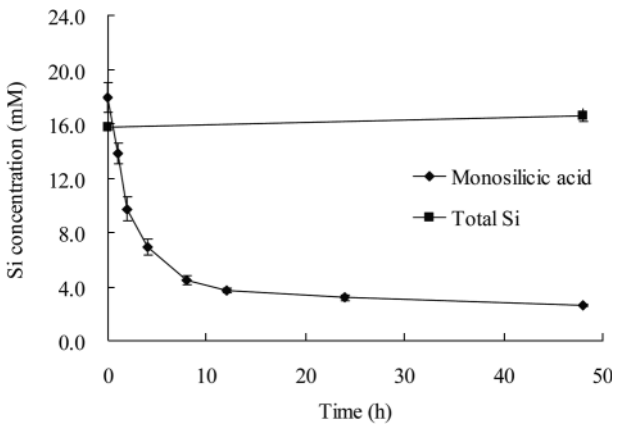

Fig. 5 In vitro change in the concentration of monosilicic acid in the xylem sap. Xylem sap collected from rice seedlings cultured in a solution containing 0.5 mM Si for 2 h was stored at room temperature for various periods. Values are means ± SD of three replicates.

of monosilicic acid in the xylem sap decreased from an initial 18 mM Si to 13.8 mM Si within 1 h after the collection of xylem sap (Fig. 5). The Si concentration decreased further to 4 mM Si at 12 h and gradually decreased thereafter to a stable level of 2.6 mM Si. However, the total Si concentration measured after digestion with hydrofluoric acid (HF) did not change with time (Fig. 5).

Discussion

Rice accumulates Si in the shoot up to 10% of dry weight (Ma and Takahashi 2002). A high ability to load Si into the xylem is supposed to be required to accumulate a large amount of Si in addition to a high ability to transport Si from the external solution to cortical cells. A time course experiment showed that the Si concentration in the xylem sap reached 12-fold that in the external solution within 30 min (Fig. 1), indicating that the xylem loading of Si is a very rapid process and that Si is loaded against a concentration gradient of Si. This result is in agreement with previous studies showing that the Si uptake by rice roots was significantly affected by metabolic inhibitors and low temperature treatments (Okuda and Takahashi 1962b, Ma et al. 2002).

The Si concentration in the xylem sap reached as high as 18 mM (Fig. 1), which is consistent with findings in previous studies (e.g. Okuda and Takahashi 1962a, Ma et al. 2002). As the Si concentration was determined by the reaction with molybdic acid, monosilicic acid rather than polymerized Si was quantified in the present study (Iler 1979). These findings led us to hypothesize that Si in the xylem sap is coordinated with some organic compounds (organosilicate complex) to prevent the polymerization. The form of Si in the xylem sap was then identified by using the ^{29}Si-NMR technique after the plants were enriched with ^{29}Si. If organosilicate complexes are present, a chemical shift would be expected between –98 and

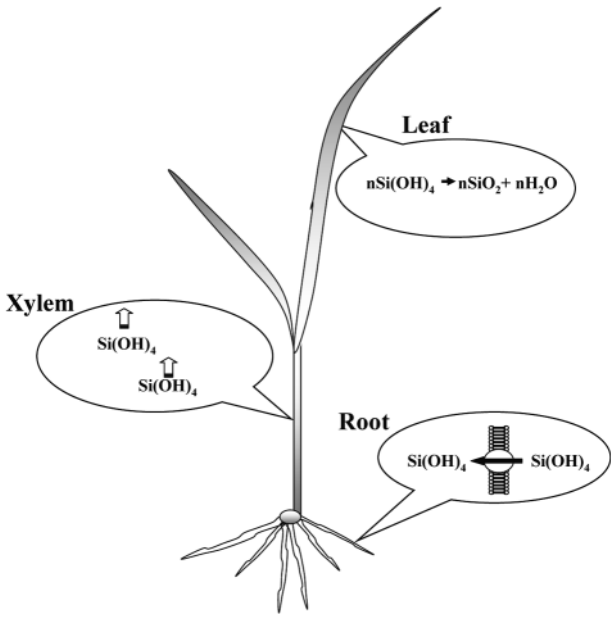

Fig. 6 Schematic presentation of the Si form from uptake to accumulation in rice. Si is taken up into the cortical cells in the form of silicic acid and then loaded into the xylem in the same form. After translocation into the shoot, Si in the form of silicic acid is concentrated with water loss (transpiration) and polymerized into silica.

–110 ppm for pentaoxosilicon species and from –135 to –143 ppm for hexaoxosilicon species (Kinrade et al. 1999, Kinrade et al. 2001). However, in the ^{29}Si-NMR spectra, only one signal which corresponds to monosilicic acid was observed at the chemical shift of –72.6 ppm (Fig. 2). Recently, Casey et al. (2003) also reported that the main form of Si in xylem sap of wheat was monosilicic acid. They also observed a minor signal at a chemical shift of –80 ppm, which corresponds to a dimer of silicic acid. However, in the present study, this signal was not observed although the Si concentration in xylem sap was much higher in rice than in wheat (Fig. 1, Casey et al. 2003). Since they measured the samples 10 h after the collection of xylem sap, part of silicic acid might have polymerized to the dimer during the transportation of the samples to the laboratory (Fig. 5). However, we measured the sample immediately after collection and completed the measurement within 30 min, which was before the polymerization of silicic acid proceeded (Fig. 5). Another possibility is that the chemical species of Si in the xylem sap differs slightly between rice and wheat.

To examine further the possibility of the existence of organosilicate complexes in the xylem, ^{1}H-NMR spectra were compared between rice plants supplied or not with Si and between a wild-type rice and a mutant rice (*lsi1*) defective in active Si uptake. The Si concentration in the xylem sap was much lower in the mutant than in the wild-type rice (Ma et al. 2002). However, in the ^{1}H-NMR spectra, no characteristic signals were observed in the xylem sap of wild-type rice supplied with Si (Fig. 3). An in vitro study also showed that the xylem sap did not contain the compounds that prevent the polymerization of silicic acid (Fig. 4). All these results consistently indicate that Si is present in the form of monosilicic acid in rice xylem.

Silicic acid from xylem sap showed properties of autopolymerization. After collection of xylem sap, the concentration of monosilicic acid decreased with time to a certain level although the concentration of total Si remained at the same level (Fig. 5). This result suggests that the existence of monosilicic acid at a high concentration in the xylem is transient. After monosilicic acid is loaded in the xylem, it may be rapidly translocated to the leaves with the transpiration stream without polymerization, and be polymerized in the leaves. This idea is supported by the fact that the concentration of monosilicic acid in the leaves is <3 mM (data not shown) and >90% of total Si is present in the form of silica (Ma and Takahashi 2002).

In conclusion, Si is translocated in the form of monosilicic acid which is transiently present at a high concentration in xylem. The change of Si form from uptake to accumulation in rice is summarized in Fig. 6. Si is taken up by the roots in the form of silicic acid and then loaded into the xylem in the same form. After Si is translocated into the shoot, Si as silicic acid is concentrated with water loss (transpiration) and polymerized into silica. Silica is deposited in the apoplast as described above and plays an important role in protecting the plants from various stresses.

Materials and Methods

Plant materials

Seeds of rice (*Oryza sativa* L. cv. Oochikara) were soaked in water overnight at 25°C in the dark. A rice mutant (low Si 1, *lsi1*, formerly called GR1) was also used (Ma et al. 2002). The seeds were then transferred to a net floated on a 0.5 mM $CaCl_2$ solution in a plastic container. On day 7, the seedlings were transferred to a 3 liters plastic pot containing one-half-strength Kimura B solution (pH 5.6). The composition of the nutrient solution was as reported previously (Ma et al. 2001). The solution was renewed every 2 days. All experiments were conducted with three replicates in a glasshouse at 25°C under natural daylight.

Xylem sap collection

Xylem sap was collected from the cut end for 30 min with a micropipet after decapitating the plant 1 cm above the roots. For time course experiments, seedlings (24 d old) were cultured in nutrient solution containing 0.5 mM Si in the form of silicic acid for various periods before collecting the xylem sap. Silicic acid was prepared by passing potassium silicate through a cation-exchange resin (Amberlite IR-120B, H^+ form).

Measurement of ^{29}Si- and ^{1}H-NMR

$^{29}SiO_2$ (98.7% enrichment) was purchased from Shokotsuso (Tokyo, Japan). A portion (0.1 g) of $^{29}SiO_2$ was dissolved in 2 M NaOH with a microwave. The digested solution was diluted and passed through cation-exchange resin as described above before use. The seedlings (40 d old) were allowed to take up ^{29}Si-enriched silicic

acid (1.0 mM) for 4 h and then decapitated. The xylem sap collected was subjected immediately to ^{29}Si-NMR measurement in 5 mm NMR tubes.

The ^{29}Si-NMR spectra were obtained at 99.36 MHz (DMX-500 spectrometer; Bruker BioSpin GMBH, Germany). The observation parameters for ^{29}Si-NMR were as follows: frequency range, 27.8 kHz; data point, 64 k; acquisition time, 1.18 s; relaxation delay, 3.2 s; number of scans, 512. tetramethylsilane (TMS) at 1% was used as an external reference for calibration of the chemical shift (0 ppm). The ^{29}Si-NMR spectrum of a 2 mM ^{29}Si-enriched silicic acid was also recorded.

For measurement of the ^{1}H-NMR spectrum, xylem sap was collected from rice supplied or not with 1.0 mM Si as silicic acid and from a rice mutant (*lsi1*) supplied with 1.0 mM Si. After adding D_2O, the ^{1}H-NMR spectra of the sap were recorded on a DMX-750 spectrometer operated at 750.13 MHz (Bruker BioSpin GMBH, Germany). The observation parameters were as follows: frequency range, 8.3 kHz; data point, 32 k; acquisition time, 1.97 s; relaxation delay, 2.0 s; number of scans, 128. 3-(trimethylsilyl)propionic acid-d_4 sodium salt (TSP) was used as an external reference for calibration of the chemical shift (0 ppm).

In vitro change of the concentration of monosilicic acid in xylem sap

In the experiment shown in Fig. 4, the high Si solution was prepared by passing potassium silicate through cation-exchange resin within seconds. It was then immediately mixed with xylem sap or water at room temperature and the concentration of monosilicic acid was determined at intervals as described below. Xylem sap was collected from seedlings (34 d old) without Si supply.

In the experiment shown in Fig. 5, the xylem sap was collected from the seedlings (34 d old) cultured in the nutrient solution containing 0.5 mM Si for 2 h. The concentration of monosilicic acid in the collected xylem sap was determined at intervals as described below. The concentration of total Si after digestion with HF was also determined at the start and the end point of the experiment.

Determination of monosilicic acid and total Si concentration

The concentration of monosilicic acid in the xylem sap was determined by the colorimetric molybdenum blue method immediately after collection. Briefly, a 0.01 ml sample was added to 1.15 ml of H_2O, then 0.6 ml of 0.26 M HCl, 0.08 ml of 10% $(NH_4)_6Mo_7O_{24}$, 0.08 ml of 20% tartaric acid and 0.08 ml of reducing agent were added. The reducing agent was prepared by dissolving 1 g of Na_2SO_3, 0.5 g of 1-amino-2-naphthol-4-sulfonic acid and 30 g of $NaHSO_3$ in 200 ml of water. After 1 h, the absorbance was measured at 600 nm with a spectrophotometer (Jasco, Japan). The concentration of total Si was determined after digesting with HF. Xylem sap (up to 20 µl) was mixed with 20 µl of HF–HCl (5 M HCl : 11.5 M HF at a ratio of 1 : 2) solution and left to stand overnight at room temperature. To the mixture, 0.4 ml of 3.2% boric acid was added and left to stand overnight. Then 0.5 ml of a reagent containing 0.08 M H_2SO_4 and 2% $(NH_4)_6Mo_7O_{24}$ at a 1 : 1 ratio was added. Thirty minutes later, 0.5 ml of 3.3% tartaric acid and subsequently 0.5 ml of 0.4% of ascorbic acid was added. Ater 1 h later, the absorbance was measured at 600 nm.

Acknowledgments

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