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ORIGINAL PAPER

Silicon isotopic fractionation by banana (*Musa* spp.) grown in a continuous nutrient flow device

S. Opfergelt · D. Cardinal · C. Henriet · X. Draye · L. André · B. Delvaux

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Abstract The determination of the plant-induced Si-isotopic fractionation is a promising tool to better quantify their role in the continental Si cycle. Si-isotopic signatures of the different banana plant parts and Si source were measured, providing the isotopic fractionation factor between plant and source. Banana plantlets (*Musa acuminata* Colla, cv Grande Naine) were grown in hydroponics at variable Si supplies (0.08, 0.42, 0.83 and 1.66 mM Si). Si-isotopic compositions were determined on a multicollector plasma source mass spectrometer (MC-ICP-MS) operating in dry plasma mode. Results are expressed as δ^{29}Si relative to the NBS28 standard, with an average precision of ± 0.08‰ (±2σ_D). The fractionation factor $^{29}\varepsilon$ between bulk banana plantlets and source solution is –0.40 ± 0.11‰. This confirms that plants fractionate Si isotopes by depleting the source solution in ^{28}Si. The intra-plant fractionation Δ^{29}Si between roots and shoots amounts to –0.21 ± 0.08‰. Si-isotopic compositions of the various plant parts indicate that heavy isotopes discrimination occurs at three levels in the plant (at the root epidermis, for xylem loading and for xylem unloading). At each step, preferential crossing of light isotopes leaves a heavier solution, and produces a lighter solution. Si-isotopic fractionation processes are further discussed in relation with Si uptake and transport in plants. These findings have important implications on the study of continental Si cycle.

Keywords *Musa* · Phytolith · Silicon · Si cycle · Si-isotopic fractionation · Si transport in plant

S. Opfergelt (✉) · C. Henriet · B. Delvaux
Soil Science Unit, Université catholique de Louvain, Place Croix du Sud 2/10, B-1348 Louvain-la-Neuve, Belgium
e-mail: opfergelt@sols.ucl.ac.be

D. Cardinal · L. André · S. Opfergelt
Department of Geology and Mineralogy, Musée Royal de l'Afrique Centrale, Leuvensesteenweg 13, B-3080 Tervuren, Belgium

X. Draye
Crop Physiology and Plant Breeding, Université catholique de Louvain, Place Croix du Sud 2/11, B-1348 Louvain-la-Neuve, Belgium

Introduction

The external silicon (Si) cycle is closely linked to the C cycle through CO_2 consumption by diatoms growth (Smetacek 1999), and silicate weathering (Raven and Edwards 2001). Plants readily contribute to weathering (Hinsinger et al. 2001), and induce a strong biological imprint on the terrestrial Si cycle (Lucas 2001). Only recent studies however concern the contribution of plants to the Si continental reservoir (Alexandre et al. 1997; Conley 2002; Derry et al. 2005). In this respect, the study of silicon stable isotopes is highly promising

(Conley 2002; Meunier 2003; Ding et al. 2005). Biogenic production of silica fractionates the three stable Si isotopes with a preferential uptake of light isotopes (Douthitt 1982; De La Rocha et al. 1997; De La Rocha 2003). Consequently, sea, lake and river waters display heavier Si-isotopic signatures (De La Rocha et al. 2000; Ding et al. 2004; Alleman et al. 2005) compared to igneous rocks (Douthitt 1982). Balancing the ^{28}Si-depletion in fresh and marine waters, opaline phytoliths may thus build up a ^{28}Si-enriched pool on continents, to which neoformed clays (Ziegler et al. 2005a) and silicified materials (Basile-Doelsch et al. 2005) also contribute. None of the existing isotopic studies on plant Si pool use the Si-isotopic composition of the source to estimate a fractionation factor between plant and source (Douthitt 1982; Ding et al. 2003, 2005; Ziegler et al. 2005b).

Plant Si content usually ranges between 0.1% and 10% dry weight (Ma and Takahashi 2002). Plants exhibit three distinct Si-uptake modes (Sangster and Hodson 1986; Takahashi et al. 1990): (1) rejection inducing Si-accumulation in soil solution (non Si-accumulating plants), (2) passive diffusion following mass flow (intermediate plants), (3) active uptake (Si-accumulating plants) involving Si-transporters (Tamai and Ma 2003; Ma et al. 2004; Mitani and Ma 2005). Though Si bio-accumulation is largely beneficial to plants (Ma et al. 2001), it is sill controversial whether silicon is essential for plant growth (Takahashi et al. 1990; Epstein 1999). Plants take up Si as silicic acid H_4SiO_4 (Raven 1983). Si precipitates in plant aerial parts through polymerization near transpiration termini as inter and/or intracellular particles of hydrated amorphous silica $SiO_2{\cdot}nH_2O$, i.e. opaline phytoliths (Jones and Handreck 1965, 1967; Sangster and Parry 1981; Raven 1983), which return to soil by decomposition of organic debris from plant material.

Banana (*Musa* spp.), a monocotyledon, is a fast-growing giant herb (2–9 m tall) with a pseudostem composed of leaf sheaths, and exhibiting high transpiration rate (3–6.3 mm/day depending on physiological stage and climatic conditions) caused by large broad leaves, high leaf area index (LAI) and a low water use efficiency (Robinson 1995). Banana is a very high mineral and water demanding crop (Lahav 1995), and banana roots readily mobilize nutrients from silicates through root-induced mineral dissolution (Hinsinger et al. 2001; Rufyikiri et al. 2004). Banana is Si-accumulating (Henriet et al. submitted), and contains opaline phytoliths mainly in the sheath cells of vascular bundles in leaves and pseudostem (Tomlinson 1969; Prychid et al. 2004). With such characteristics, banana is an ideal case study to quantify plant-induced Si-isotopic fractionation. Moreover, as Si nutrition is positive for crop yields (Ma et al. 2001), it is very relevant to study Si in banana, an important economical resource for developing countries.

The general objective of this paper is to contribute to the Si isotopes dataset in terrestrial plants, as a mandatory step to further quantify the role of plants in the continental silicon cycle. More specifically, this paper aims to quantify the Si-isotopic fractionation from dissolved Si source to banana plant, and the Si-isotopic fractionation from roots to young leaves. The study is based on an experimental approach using banana plants grown in hydroponics in controlled conditions (Henriet et al. submitted).

Materials and methods

Hydroponics

Musa acuminata Colla, cv Grande Naine were grown in hydroponics during six weeks (Henriet et al. submitted). Banana plantlets issued from tissue culture were provided by Vitropic (Montpellier, France). Growth chambers conditions were fixed at 448 μE $m^{-2}s^{-1}$ photon density flux 12 h per day, 80% relative humidity, and 28/25°C day/night temperature. Dilute nutrient solutions were supplied continuously with peristaltic pumps at a rate of 104 ml $h^{-1}pot^{-1}$ (Rufyikiri et al. 2001). The composition of the nutrient solution was, in macroelements (mM): 0.9 $Ca(NO_3)_2$, 0.05 $CaSO_4$, 0.05 $CaCl_2$, 0.5 KCl, 0.25 K_2SO_4, 0.05 $MgCl_2$, 0.05 $MgSO_4$, 0.1 NH_4Cl, 0.05 $(NH_4)_2SO_4$, 0.05 NaH_2PO_4, and in microelements (μM): 80 H_3BO_3, 80 FeEDTA, 8 $MnCl_2$, 0.8 $ZnSO_4$, 0.8 $CuSO_4$, 5.6 $(NH_4)_6Mo_7O_{24}$, matching banana nutrient requirements and realistic ion concentrations in dilute solutions of tropical soils

(Rufyikiri et al. 2001). Four batches of five plants were treated with four different Si supplies (0.08, 0.42, 0.83 and 1.66 mM Si).

A blank experiment on five plantlets was conducted without Si supply (< 0.7 μM Si) and gave rise to very low Si concentrations in bulk plant (0.03 ± 0.01% SiO_2 dry wt) compared to final SiO_2 content in bulk plant at low Si supply (0.08 mM Si: 0.13 ± 0.01% SiO_2 dry wt). SiO_2 content and Si-isotopic compositions in plantlets before the hydroponic experiment are not available. However, it is very unlikely that such blank SiO_2 content would impact significantly the isotopic balance in the final plantlets, except maybe at low Si supply. This will be discussed along with the bulk fractionation factor.

Si was provided in the source solution as H_4SiO_4 obtained from sodium metasilicate after Na^+–H^+ exchange by using H^+-resin (Amberlite IR120). Si concentration was below solubility limit (≤1.66 mM Si) at given temperatures (≤28°C), and pH ranged between 5 and 6.5. Such conditions ensure that silicon was only as H_4SiO_4 (Stumm and Morgan 1996). Therefore, no initial Si-isotopic fractionation in the source solution between silicon forms has to be feared.

Sampling

After the six weeks growth period, each plant was split in five parts (Fig. 1): roots (R), pseudostem (PS), old leaves (OLv), young midribs and petioles (YMP), young lamina (YL). Old leaves were separated from young leaves in order to consider a Si-accumulation related to the age of leaf tissues (Motomura et al. 2004). The limit between old and young leaves was fixed after twelve growing days when a rapid increase in leaf area was observed. Plant materials were then dried at 60°C and grinded.

SiO_2 content analyses

The SiO_2 content in various plant parts was measured by inductively coupled plasma atomic emission spectrometry (ICP-AES), after borate fusion at 1000°C and dissolution of fusion beads in 10% HNO_3 (Chao and Sanzolone 1992). Detection limit in solution is 0.7 μM Si.

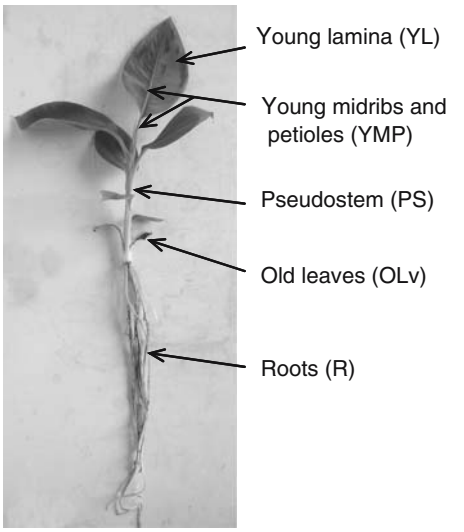

Fig. 1 Identification of banana plant parts separated for SiO_2 content and Si-isotopic analyses: roots, pseudostem, young midribs and petioles, old leaves, young lamina

SEM observations and EDX analyses of phytoliths

Dried and grinded samples of roots and shoots (aerial parts: pseudostem, midribs and petioles, lamina) were observed after organic matter digestion. About 0.1 g of dry matter was digested at 120°C in a concentrated suprapur HNO_3/H_2O_2 mixture in a 1:1 (v:v) ratio, then filtered on polycarbonate membranes (0.4 μm), and cleaned with demineralized water. Membranes were fixed on glass blades and coated with Au–Pd (1′30 at 0.05 mbar). Morphological studies of phytoliths by scanning electron microscopy (SEM) were achieved with a Leica Stereoscan 260, and their description follows the recent International Code for Phytolith Nomenclature (ICPN, Madella et al. 2005). Chemical analyses were realised by energy dispersive X-ray analysis (EDX) with an EDAX system. Working conditions were: accelerating voltage adjusted to 10 kV for SEM image, 25 kV for EDX analyses, working distance of 20 mm.

Silicon isotopes analyses

Recovery of Si from each plant aliquot consisted of: (1) organic matter digestion in hot concentrated suprapur HNO_3/H_2O_2 in a 2:1 (v:v) ratio, (2) opal dissolution by 0.2 M NaOH leaching at

100°C during 2 h (adapted from Ragueneau et al. 2005), (3) Si purification by triethylamine molybdate co-precipitation and combustion in covered Pt crucibles at 1000°C (De La Rocha et al. 1996), (4) Si dissolution in dilute suprapur HF-HCl mixture (Cardinal et al. 2003).

Si-isotopic compositions were measured using a Nu Plasma multicollector plasma source mass spectrometer (MC-ICP-MS) operating in dry plasma mode, with an external Mg doping to correct mass bias (Cardinal et al. 2003). To avoid matrix effects, purity of the samples (absence of major elements) was controlled by ICP-AES before MC-ICP-MS analyses. The resolution of the Nu Plasma MC-ICP-MS does not resolve the isobaric interference of $^{14}N^{16}O$ on ^{30}Si, therefore only ^{29}Si:^{28}Si ratios can be measured accurately. The data are reported relative to the NBS28 silica sand standard (National Institute of Standard and Technology RM #8546) for silicon isotopes (Carignan et al. 2004). Our results are thus expressed as $\delta^{29}Si$ versus NBS28 (‰) following:

$$\delta^{29}\mathrm{Si} = \left[\frac{\left(\frac{^{29}\mathrm{Si}}{^{28}\mathrm{Si}}\right)_{\mathrm{sample}}}{\left(\frac{^{29}\mathrm{Si}}{^{28}\mathrm{Si}}\right)_{\mathrm{NBS28}}} - 1 \right] \times 1000.$$

Single $\delta^{29}Si$ measurements were obtained by sample-standard bracketing technique and expressed with their standard error ($2\sigma_M$), which is the cumulated analytical error on Mg and Si-isotopic ratios measured on both the standard and sample propagated to $\delta^{29}Si$. Analytical and chemical replicates were carried out separately. Analytical replicates involved two determinations of the Si-isotopic composition in the same digestion solution of the same organ with MC-ICP-MS. Chemical replicates involved two different solid aliquots of the same plant part sample, which have undergone two separate complete chemical processes as well as MC-ICP-MS analysis. Average $\delta^{29}Si$ were expressed with the standard deviation ($2\sigma_D$) of the replicates. Assuming mass-dependent fractionation process under thermodynamic equilibrium, $\delta^{30}Si$ could be calculated from $\delta^{29}Si$ by a multiplying factor of 1.93 (calculated with molar masses, Young et al. 2002). Average precision and accuracy on $\delta^{29}Si$ was ± 0.08‰ ($\pm 2\sigma_D$).

Fractionation factors

The intra-plant fractionation between two organs is estimated by the difference between the Si-isotopic composition $\delta^{29}Si$ of these two organs. This difference is usually expressed as $\Delta^{29}Si$ (‰). The Si-isotopic fractionation between shoots and roots is thus calculated as follows:

$$\Delta^{29}\mathrm{Si}_{\mathrm{root-shoot}} = \delta^{29}\mathrm{Si}_{\mathrm{shoot}} - \delta^{29}\mathrm{Si}_{\mathrm{root}}.$$

$\delta^{29}Si_{root}$ is the Si-isotopic composition of the roots. $\delta^{29}Si_{shoot}$ is the average Si-isotopic composition of the aerial parts of the plant (the sum of the $\delta^{29}Si$ in each different aerial part balanced by the SiO_2 distribution in the plant as in Ding et al. 2005). In a similar way, $\Delta SiO_{2\ root-shoot}$ (%) expresses the difference in SiO_2 content (% dry wt) between roots and shoots.

The bio-fractionation process is usually quantified by computing a fractionation factor ($^{29}\varepsilon$ in ‰) representing the extent of Si-isotopic fractionation between biogenic silica (BSi, phytoliths) and the dissolved silicic acid source (DSi, nutrient solution). For small Si-isotopic fractionations, $\Delta^{29}Si$ offers a good approximation for $^{29}\varepsilon$ if an isotopic equilibrium has been reached between the dissolved and particulate phases (De La Rocha et al. 1997; Varela et al. 2004; Alleman et al. 2005). The fractionation factor $^{29}\varepsilon$ can thus be estimated by a difference of $\delta^{29}Si$ values as follows:

$$^{29}\varepsilon_{\mathrm{plant-source}} \sim \Delta^{29}\mathrm{Si} = \delta^{29}\mathrm{Si}_{\mathrm{plant}} - \delta^{29}\mathrm{Si}_{\mathrm{source}}.$$

In this expression, $\delta^{29}Si_{source}$ is the Si-isotopic composition of the source, while $\delta^{29}Si_{plant}$ is the bulk Si-isotopic composition of the plant (the sum of the $\delta^{29}Si$ in each different plant part, including roots, balanced by the SiO_2 distribution in the plant as in Ding et al. 2005).

Results

SiO_2 content

SiO_2 content in banana plantlets varied from 0.1% to 4.5% dry weight (Table 1). It exhibited a

Table 1 SiO_2 repartition in banana plantlets for each Si supply: mass fraction of each organ (% total biomass), SiO_2 content (% dry weight ± $2\sigma_D$), and SiO_2 distribution in each organ (% total silica)

	Mass fraction % total mass	SiO_2 content % dw ± $2\sigma_D$	SiO_2 % total silica	Mass fraction % total mass	SiO_2 content % dw ± $2\sigma_D$	SiO_2 % total silica
	1.66 mM Si			**0.83 mM Si**		
R	24	0.57 ± 0.10	8.5	25	0.44 ± 0.07	10.1
PS	31	0.78 ± 0.18	14.7	29	0.62 ± 0.13	16.5
YMP	8	1.12 ± 0.19	5.8	9	0.85 ± 0.10	6.9
YL	34	3.02 ± 0.43	63.5	34	1.93 ± 0.13	60.1
OLv	3	4.55 ± 4.20	7.5	3	2.76 ± 0.66	6.5
	0.42 mM Si			**0.08 mM Si**		
R	24	0.31 ± 0.05	12.5	25	0.10 ± 0.03	19.5
PS	31	0.33 ± 0.07	17.3	31	0.08 ± 0.01	18.6
YMP	10	0.44 ± 0.12	7.2	8	0.09 ± 0.01	6.3
YL	33	1.02 ± 0.31	57.4	32	0.19 ± 0.06	48.4
OLv	2	1.36 ± 0.51	5.5	3	0.35 ± 0.07	7.2

R = roots, PS = pseudostem, YMP = young midribs and petioles, YL = young lamina, OLv = old leaves. Data from Henriet et al. (submitted).

gradient with a progressive increase from roots to pseudostem to young midribs and petioles to young leaves. Old leaves were the most SiO_2 enriched plant parts. SiO_2 content was higher in plants grown with a higher Si supply. However, the intra-plant SiO_2 gradient was present for all Si supplies (Table 1; Henriet et al. submitted). The difference in SiO_2 content (% dry wt) between roots and shoots ($\Delta SiO_{2\ root-shoot}$) increased with Si concentration in the source solution.

Observations of silica deposits (SEM)

In shoots, phytoliths were observed as silica cells forming long chains (Fig. 2a, b) as described by Tomlinson (1969). They were characterized by a cone-shaped part and a basal part. The basal part was rectangular to squarish or boat-shaped with protuberances on surface, and the cone-shaped part was smooth truncated saddle-like (Mbida et al. 2001). They were rectangular in surface view

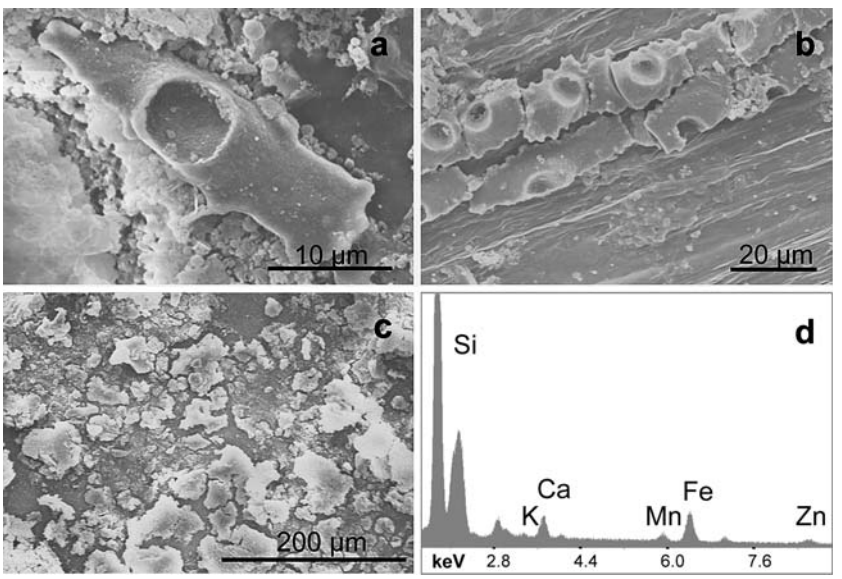

Fig. 2 Morphological studies in banana: (**a**) Shoots: SEM image of a typical cone shaped phytolith truncated saddle-like. (**b**) Shoots: SEM image of long phytoliths chain in pseudostem. (**c**) Roots: SEM image of the roots residual matter after organic digestion. (**d**) Roots: EDX analysis of the roots residue. Unlabelled peaks represent Au–Pd from the coating or secondary peaks

with central shallow depression (trough shaped) with spines projecting from base into pits in wall, present as one per cell over foliar vascular bundle sheath fibers (Prychid et al. 2004). Following ICPN (Madella et al. 2005), these phytoliths are ''rectangle cavate tuberculate sheath cell''. EDX chemical analysis confirmed SiO_2 as a major constituent of these phytoliths. On a dry weight basis, more phytoliths were observed in bulked shoots of banana plantlets supplied with 1.66 mM Si than with lower Si supplies.

As already reported (Tomlinson 1969; Prychid et al. 2004), no phytoliths were observed in banana roots. Root residual silica recovered after organic digestion (Fig. 2c) indicated the additional presence of Ca, K, Mn, Fe with Si (Fig. 2d).

Silicon isotopic compositions

Complete Si-isotopic compositions measured in banana plantlets are presented in Tables 2 and 3 and Fig. 3. Analytical and chemical replicates showed a very good reproducibility (smaller than 0.10‰, $2\sigma_D$, $n = 18$), close to 0.08‰ (Cardinal et al. 2005). Inter-plant variability (same organ of two plantlets at the same Si supply) was larger (0.16‰ $2\sigma_D$, $n = 51$), which might be explained by the larger representativeness (n number). Indeed, the two standard error on the mean are the same for analytical/chemical replicates and for inter-plant variability ($n = 18$, $2\sigma_M = 0.048$; $n = 51$, $2\sigma_M = 0.044$).

Si-isotopic composition of the source is + 0.06‰. Relative to this source, Si-isotopic signatures of banana plantlets were lighter (Fig. 3). The values of $\delta^{29}Si$ in banana plant parts varied mostly between –0.18 and –0.77‰ (Table 2). At 0.42, 0.83 and 1.66 mM Si supply, Si-isotopic fractionation relative to the aqueous silicon source was small in roots and larger in shoots. Within shoots, pseudostem were isotopically lighter than young leaves ($\delta^{29}Si_{PS} = -0.55‰$; $\delta^{29}Si_{YL} = -0.36‰$). Old leaves were among the lightest parts measured in shoots (–0.58‰; –0.70‰; Table 3). In banana plantlets, the average intra-plant fractionation $\Delta^{29}Si_{root-shoot}$ was –0.21 ± 0.08‰ (Table 4).

Table 2 $\delta^{29}Si$ versus NBS28 (‰) measured in different banana plantlets (1–5) grown in hydroponics

Sple ID	$\delta^{29}Si$ ‰ ± $2\sigma_M$	Sple ID	$\delta^{29}Si$ ‰ ± $2\sigma_M$
1.66 mM Si		**0.83 mM Si**	
R 3	–0.24 ± 0.07	R 3	–0.29 ± 0.07
R 4	–0.18 ± 0.06	R 4	0.00 ± 0.11
R 4**	–0.18 ± 0.09	R 5	–0.12 ± 0.11
PS 3	–0.64 ± 0.08	R 5**	–0.21 ± 0.07
PS 4	–0.76 ± 0.08	PS 3	–0.44 ± 0.06
PS 4*	–0.76 ± 0.12	PS 4	–0.27 ± 0.08
PS 4**	–0.62 ± 0.07	PS 5	–0.46 ± 0.06
YMP 3	–0.60 ± 0.08	YMP 3	–0.57 ± 0.07
YMP 4	–0.77 ± 0.07	YMP 4	–0.32 ± 0.10
YMP 4*	–0.63 ± 0.15	YMP 5	–0.52 ± 0.08
YL 1	–0.47 ± 0.08	YL 1	–0.23 ± 0.06
YL 2	–0.45 ± 0.07	YL 1*	–0.21 ± 0.11
YL 3	–0.35 ± 0.07	YL 2	–0.43 ± 0.07
YL 4	–0.55 ± 0.07	YL 3	–0.35 ± 0.07
YL 5	–0.37 ± 0.07	YL 4	–0.30 ± 0.08
OLv 4	–0.70 ± 0.06	YL 5	–0.46 ± 0.07
0.42 mM Si		**0.08 mM Si**	
R 1	–0.18 ± 0.08	R 2	0.12 ± 0.09
R 3	–0.20 ± 0.09	R 2*	0.16 ± 0.09
R 5	–0.18 ± 0.07	R 2*	–0.16 ± 0.08
PS 1	–0.55 ± 0.09	YMP 2	–0.43 ± 0.15
PS 3	–0.50 ± 0.11	YMP 3	–0.38 ± 0.09
PS 5	–0.61 ± 0.07	YMP 3*	–0.60 ± 0.07
YMP 1	–0.38 ± 0.08	YL 3	–0.45 ± 0.07
YMP 3	–0.47 ± 0.08	YL 3*	–0.23 ± 0.08
YMP 5	–0.59 ± 0.06	OLv 3	–0.58 ± 0.08
YL 1	–0.37 ± 0.08	Source solution	
YL 3	–0.36 ± 0.09	0.83 mM	0.08 ± 0.09
YL 5	–0.30 ± 0.08	1.66 mM	0.04 ± 0.09

The standard error ($2\sigma_M$) is the cumulated analytical error on Mg and Si-isotopic ratios measured both on the standard and sample propagated to $\delta^{29}Si$ (Cardinal et al. 2003). R = roots, PS = pseudostem, YMP = young midribs and petioles, YL = young lamina, OLv = old leaves; * = analytical replicates, ** = chemical replicates

Discussion

The bulk fractionation factor $^{29}\varepsilon$

In banana, the average fractionation factor $^{29}\varepsilon$ between the plant and the nutrient solution was –0.40 ± 0.11‰, phytoliths being isotopically lighter than nutrient solution (Table 4). Although the difference between $^{29}\varepsilon$ from two consecutive Si supplies is not significant, there is a general trend towards larger $^{29}\varepsilon$ with higher Si supplies (Table 4). Since the difference between the

Table 3 Average δ^{29}Si (‰ ± $2\sigma_D$) in each banana organ for each Si supply (mM)

	Average δ^{29}Si ‰ ± $2\sigma_D$	*n*	Average δ^{29}Si ‰ ± $2\sigma_D$	*n*
	1.66 mM Si		**0.83 mM Si**	
R	–0.20 ± 0.06	3	–0.16 ± 0.25	4
PS	–0.70 ± 0.15	4	–0.39 ± 0.21	3
YMP	–0.66 ± 0.18	3	–0.47 ± 0.26	3
YL	–0.44 ± 0.17	5	–0.33 ± 0.20	6
OLv	–0.70 ± n.d.	1	n.d.	0
	0.42 mM Si		**0.08 mM Si**	
R	–0.19 ± 0.03	3	0.04 ± 0.35	3
PS	–0.55 ± 0.12	3	n.d.	0
YMP	–0.48 ± 0.20	3	–0.47 ± 0.23	3
YL	–0.34 ± 0.07	3	–0.34 ± 0.31	2
OLv	n.d.	0	–0.58 ± n.d.	1

The standard deviations ($2\sigma_D$) for all replicates are given. R = roots, PS = pseudostem, YMP = young midribs and petioles, YL = young lamina, OLv = old leaves, n.d. = no data

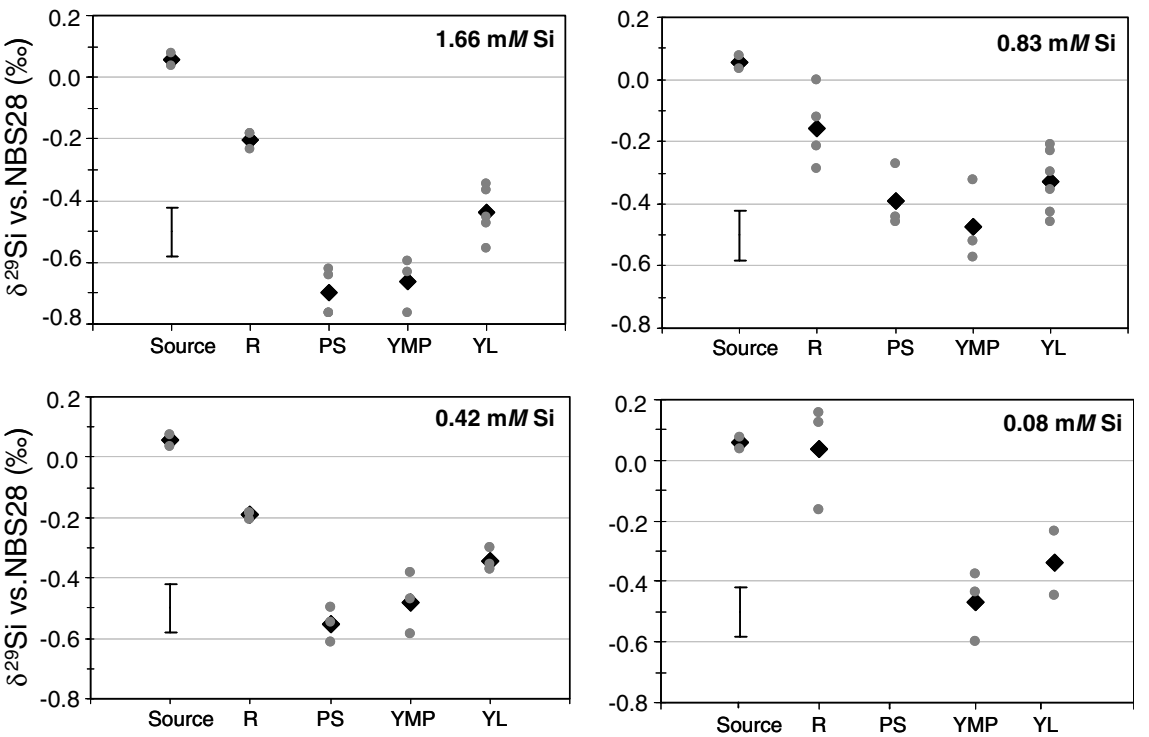

Fig. 3 Si-isotopic compositions in banana plantlets at four different Si supplies (0.08, 0.42, 0.83, 1.66 mM Si): single measurements (grey circles) and their average (black diamonds). Brackets show the average standard deviation of full replicates ($\pm 2\sigma_D$). Source = solution, R = roots, PS = pseudostem, YMP = young midribs and petioles, YL = young lamina

highest and the lowest supplies is relatively large (0.27‰), we cannot rule out a real variation of the fractionation factor as a function of Si supply. This plant–source fractionation may suggest that Si-isotopic fractionation could be established during plant Si-uptake, which seems to be, at least partly, an active process in banana (Henriet et al. submitted) as it is in rice (Mitani and Ma 2005). This is similar to the recent evidence in diatoms: Si-isotopic fractionation factor may be more variable (Varela et al. 2004) than previously thought (De La Rocha et al. 1997), and its extent could be

Table 4 Intra-plant fractionation $\Delta^{29}Si$ (‰ ± σ_D) in banana plantlets for each Si supply (mM) calculated by difference between $\delta^{29}Si_{shoot}$ (‰) and $\delta^{29}Si_{root}$ (‰); $^{29}\varepsilon$(‰ ± σ_D) approximated by a $\Delta^{29}Si$ between the bulked plant $\delta^{29}Si_{plant}$ (‰) and the source $\delta^{29}Si_{source}$ (‰)

	$\Delta^{29}Si$			$^{29}\varepsilon$		
	$\delta^{29}Si_{root}$ ‰	$\delta^{29}Si_{shoot}$ ‰	$\Delta^{29}Si_{root-shoot}$ ‰ ± σ_D	$\delta^{29}Si_{plant}$ ‰	$\delta^{29}Si_{source}$ ‰	$^{29}\varepsilon_{plant-source}$ ‰ ± σ_D
1.66 mM Si	–0.20	–0.47	–0.27 ± 0.10	–0.49	0.06	–0.55 ± 0.10
0.83 mM Si	–0.16	–0.29	–0.14 ± 0.21	–0.31	0.06	–0.37 ± 0.13
0.42 mM Si	–0.19	–0.33	–0.14 ± 0.05	–0.35	0.06	–0.41 ± 0.07
0.08 mM Si	0.04	–0.24	–0.28 ± 0.26	–0.23	0.06	–0.28 ± 0.15
Average	–0.13	–0.33	–0.21 ± 0.08	–0.34	0.06	–0.40 ± 0.11

affected by physiological and/or environmental conditions including Si availability (Cardinal et al. in press). As the present study is the first to highlight this effect in higher plants, further investigation is required to elucidate its causes. Alternatively the variation of $^{29}\varepsilon$ might also be partly explained by some initial isotopic difference in starting plantlets (no data).

Few Si-isotopic data are available in other plant species. Fractionation factor $^{29}\varepsilon_{plant-source}$ in different species was calculated for comparison with $^{29}\varepsilon_{plant-source}$ in banana (Table 5). The published $\delta^{30}Si$ data (Si-isotopic composition of the plant) were converted into $\delta^{29}Si$ values by applying a division factor of 1.93. The Si-isotopic composition of the source was measured for corn and wheat (Ziegler et al. 2005b) and estimated for rice (Ding et al. 2005). The fractionation factor in banana plantlets ($^{29}\varepsilon$ = –0.40 ± 0.11‰) is rather close to that calculated for wheat and corn grown in hydroponics ($^{29}\varepsilon$ = –0.52 ± 0.16‰; calculated from Ziegler et al. 2005b), and accords with that determined for rice cropped in field conditions ($^{29}\varepsilon$ = –0.53 ± 0.17‰; calculated from Ding et al. 2005). Moreover, it is worth noting that $^{29}\varepsilon$ in plants (banana, rice, corn and wheat) is of the same order as that in diatoms ($^{29}\varepsilon$ = –0.57 ± 0.21‰; De La Rocha et al. 1997).

Intra-plant fractionation

In bamboo, a high Si-accumulating plant, Si-isotopic composition becomes heavier from stem to leaf (Ding et al. 2003) as in banana. However, $\delta^{29}Si$ variations (standard deviation for the same organs of different bamboos) are large (0.96‰ on average) and vary with soil conditions. These variations are larger than those between banana organs (0.16‰) grown in controlled conditions in hydroponics (homogeneous supply of essential nutrients). Just as in banana, SiO_2 content in bamboo increases from stem to branch to leaf

Table 5 Si-isotopic compositions of different plants ($\delta^{30}Si$ data were converted to $\delta^{29}Si$ by a division factor of 1.93, see text)

	Plant	$\delta^{29}Si_{plant}$ ‰	$\delta^{29}Si_{source}$ ‰	$^{29}\varepsilon_{plant-source}$ ‰ ± σ_D
Douthitt (1982)	*Bambusa* sp.	–0.73	n.d.	
	Equisetum sp.	0.92	n.d.	
Ding et al. (2003)	Bamboo	–0.10	n.d.	
Ding et al. (2005)	Rice	–0.01	0.52	–0.53 ± 0.17
Ziegler et al. (2005b)	Corn, Wheat[a]	–0.62	–0.10	–0.52 ± 0.16
This study	Banana[a]	–0.34	+ 0.06	–0.40 ± 0.11

Comparison between $^{29}\varepsilon$ (‰) in different plants (calculated by difference between $\delta^{29}Si_{plant}$ and $\delta^{29}Si_{source}$). Si-sotopic composition of the source has been estimated for rice (Ding et al. 2005), and measured for corn and wheat (Ziegler et al. 2005b), and banana (this study). n.d. = no data; [a]Hydroponics

(Ding et al. 2003) but SiO_2 content is 20-fold higher in bamboo than in banana plantlets (bamboo = 38–72% dry wt; banana = 0.1–4.5% dry wt).

In rice, SiO_2 content increases from roots to husks and decreases in grains (roots = 6.0% dry wt; husks = 10.6% dry wt; grain = 0.032% dry wt; Ding et al. 2005) rising to a variation between roots and leaves similar to banana ($\Delta SiO_{2\ root\text{-}leave}$: rice = 1.6%; banana = 1.2%). In parallel, Si-isotopic composition becomes regularly heavier from roots to grains ($\delta^{29}Si$ = –0.14 to + 1.39‰; Ding et al. 2005). Focusing on the shoots, rice and banana provide a similar increase in $\delta^{29}Si$ (Fig. 4), with isotopic compositions getting heavier from rice stem-leaf to husk, and from banana pseudostem to leaves. In roots, there is a significant difference between rice and banana (Fig. 4): rice roots are isotopically lighter than the rest of the plant ($\Delta^{29}Si_{leaves–roots}$ = +0.06‰) whereas banana roots are isotopically heavier than the rest of the plant ($\Delta^{29}Si_{leaves–roots}$ = –0.21‰). Intra-plant fractionation in rice has been interpreted as a Rayleigh fractionation process, considering the plant as a closed system, getting heavier from roots to shoots (Ding et al. 2005). As banana roots are isotopically heavier than the rest of the plant, intra-plant fractionation in banana cannot be interpreted as a Rayleigh fractionation process. Moreover, rice roots are much more SiO_2-enriched than banana roots (rice = 6.0% dry wt; banana = 0.1% dry wt). No phytoliths were observed in banana roots but some Gramineae (Poaceae) have phytoliths in their roots (Geis 1978). Hence, the possible presence of phytoliths and/or the large SiO_2 content in rice roots, in contrast to banana, might explain the Si-isotopic difference in roots. Despite this difference, we may reasonably assume global similar trend of intra-plant fractionation for these two monocotyledons (Fig. 4).

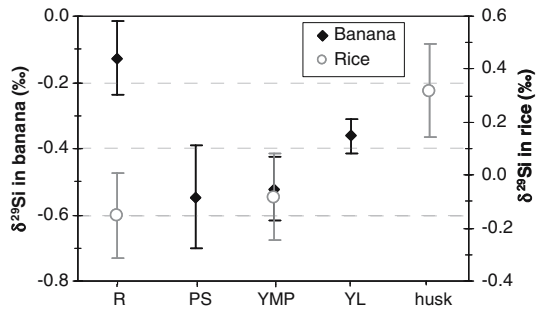

Fig. 4 Comparison between Si-isotopic compositions ($\delta^{29}Si$ (‰)) in banana (this study) and rice (Ding et al. 2005). Brackets show the standard deviation. R = roots, PS = pseudostem, YMP = young midribs and petioles, YL = young lamina, husk = in rice (PS-YMP-YL are represented by one point Stem-Leaf in rice)

Banana plant fractionation model

Our results clearly indicate that Si isotopes are fractionated by banana, both at the root level and within the shoots. This multi-site fractionation would indicate the presence of Si selection sites at different places in the plant. This could be transporters: e.g. during Zn transport in higher plants, light Zn isotopes were shown to be favoured by ions channels and/or electrogenic pumps (Weiss et al. 2005). Si transporters (SITs) were recently identified in rice (Ma et al. 2004; Mitani and Ma 2005). Since rice and banana are both Si-accumulating species where an active Si uptake takes place (Mitani and Ma 2005; Henriet et al. submitted), and since intra-plant fractionation trend is similar in banana and in rice (Fig. 4), we propose to interpret Si transport and induced Si-isotopic fractionation in banana taking into account these recent advances on silicon transport in the rice plant.

Root-step fractionation

In banana, there is a large Si-isotopic fractionation between the external solution and the plant. This must result from a discrimination mechanism, whereby light isotopes enter the root more readily than heavy isotopes. In rice roots, Si uptake is mediated by a silicon transporter SIT 1 located in the epidermis (Ma et al. 2004; Mitani and Ma 2005). As Si-uptake in banana involves an active process (Henriet et al. submitted), the Si-isotopic discrimination observed in banana may be reasonably ascribed to a similar process (Fig. 5).

The solution entering the root moves radially in the root tissues towards the central cylinder where it is loaded in xylem vessels and transported to the shoots. The Si-isotopic composition of the solution entering the roots should be

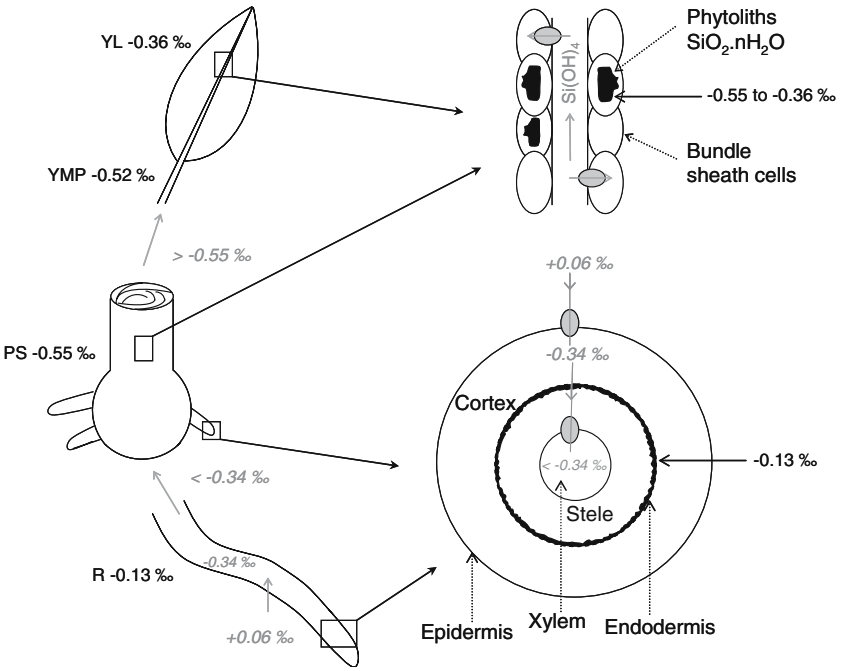

Fig. 5 Proposed schematic model of global Si-isotopic fractionation in banana. Black = Si-isotopic composition of biogenic silica; grey and italic = Si-isotopic composition of H_4SiO_4 ($\delta^{29}Si$). R = roots, PS = pseudostem, YMP = young midribs and petioles, YL = young lamina

–0.34‰ in accordance with the average Si-isotopic composition of the banana plant ($\delta^{29}Si_{plant}$ = –0.34‰; Table 4). However, the much heavier Si-isotopic composition recovered from banana root tissues (–0.13‰) indicates that a mechanism confines heavier isotopes in root tissues between the epidermis and the xylem. Recent studies in rice suggest the existence of a second silicon transporter SIT 2, responsible for xylem loading (Ma et al. 2004; Mitani and Ma 2005). Such a transporter might constitute the fractionation site that causes the ^{29}Si enrichment recognized in banana roots. This Si-isotopic fractionation model fits also the current knowledge of Si incorporation and fractionation in diatoms. Indeed, in diatoms a gene family encoding for silicon transporters (SITs) through the cell membrane has been identified (Hildebrand et al. 1997; Martin-Jézéquel et al. 2000), and Si isotopes are fractionated by this transport step, and not by Si efflux or by Si polymerisation (Milligan et al. 2004). However, similar genes (molecular probes of diatoms) were not found in rice (Ma et al. 2004).

In summary, this fractionation model invokes two fractionation levels in roots, each inducing preferential passage of light isotopes. As a result, the solution leaving the root tissues (xylem loading) is lighter than the solution entering the roots, and Si residing in the roots is isotopically heavier than in the shoots (Fig. 5). Si confined in root tissues (between the epidermis and the xylem), not under phytoliths chains (Fig. 2c), may accumulate in the stele, or as a parietal deposition in the endodermis (Fig. 5) as in bamboo and sorghum (Lux et al. 2003a, b). These roots depositions only result from entering solution, and not from Si return from shoots to roots, as H_4SiO_4 mobility in the phloem is very restricted (Raven 1983).

Shoot-step fractionation

As explained above, the xylem solution leaving the roots entering the base of the shoots is likely to be lighter than the solution entering the roots (< –0.34‰, in accordance with source = + 0.06‰ and $^{29}\varepsilon$ = –0.40‰; Table 4). This is consistent with the aerial parts globally isotopically lighter than the roots. However, a Si-isotopic fractionation occurs between banana shoot parts: as in rice (Ding et al. 2005), the Si-isotopic composition tends to be heavier from pseudostem to leaves (from Table 3, average $\delta^{29}Si$ (‰): PS = –0.55; YMP = –0.52; YL = –0.36).

Phytoliths were observed as intra-cellular morphotypes in vascular bundle-sheath cells (Prychid et al. 2004) of pseudostem (leaf sheaths) and leaves (Fig. 2a, b). Transported as H_4SiO_4 in the xylem (Mitani et al. 2005), Si leaving the xylem enters bundle sheath cells where it accumulates in the form of $SiO_2.nH_2O$ polymers (Fig. 5). We observed lighter Si-isotopic compositions in pseudostem than in leaves. This should result from a similar mechanism as previously discussed in root epidermis and for xylem loading (SITs): discrimination of heavy isotopes by preferential passage of light isotopes at the sites of xylem unloading. As a result in shoots, xylem sap will progressively exhibit heavier Si-isotopic composition in the youngest plant parts, and phytoliths formed in pseudostem will be lighter than the ones formed later on in lamina (Fig. 5). All of the Si reaching the lamina will remain there in the transpiration termini, since Si is essentially phloem-immobile (Raven 1983). Therefore, nothing can be inferred from Si-isotopic ratios in lamina about Si mass selectivity along transportation. The resulting Si-isotopic composition of the lamina will be heavier than the rest of the shoots.

Conclusions

This experimental study using banana plantlets in hydroponics confirms that plants fractionate Si isotopes by depleting its parent solution in ^{28}Si ($^{29}\varepsilon = -0.40 \pm 0.11‰$), which is an important contribution to understand the silicon continental cycle. A similar fractionation level in diatoms might indicate a general characteristic feature of photosynthetic organisms.

Besides, Si-isotopic compositions of the various plant parts indicate that a similar mechanism of heavy isotopes discrimination should occur at three levels in the plant: at the root epidermis, for xylem loading and for xylem unloading. At each step, preferential passage of light Si isotopes contribute to a progressive isotopic fractionation of the solution moving from the uptake sites in roots to the transpiration termini in lamina, and result in reproducible Si-isotopic compositions of the plant organs consistent with their position along the trajectory of the solution. This indicates that precise study of isotopic compositions within plants can contribute to the understanding of plant physiological mechanisms.

More precise localisation of silica bodies in banana, especially in roots, and studies of Si transporters (SITs) would be valuable to follow Si pathway in the plant. To understand the impact of plants on the continental Si cycle, further study should be carried out in situ on contrasting soils to compare the impact of soils and soil solutions as Si source on the Si-isotopic composition of the plant (Opfergelt et al. 2006). Besides, studying Si in the soil–plant system and induced Si-isotopic fractionation is required to develop an isotopic tracer of continental processes involved in the Si cycle. In particular, Si uptake by plants impacts the Si transfer into rivers (Conley 2002; Cary et al. 2005; Derry et al. 2005) and hence to the coastal ocean, inducing significant modifications for Si-requiring biota in those habitats. The consequences of this bio-continental Si cycle on the Si-isotopic budget are unknown and remain to be undertaken.

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