Application Note 30270

TIMS and Ion Chromatography: $^{44}Ca/^{40}Ca$ Analysis in Plants

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Introduction

Characterization of the Co-occurring Geochemical and Biological Processes Affecting Ca Isotopes within Plants

Calcium is an essential mineral nutrient in plants where it is mainly involved in the stabilization of cell walls and membranes.[1,2] Calcium acts as a counter-ion for inorganic and organic anions in cell vacuoles (intracellular specialised sub-units) and as an intracellular messenger in the cytosol (intracellular liquid phase).[1] Calcium is taken up by the plant lateral roots by diffusion and/or by the water mass flux induced by foliar transpiration. Ca is subsequently transported to the leaves by the xylem sap. No translocation (transportation) from foliage to reproductive organs occurs through the phloem and, therefore, Ca supply is mainly supported by the xylem.[1–5] Along with nutrient elements, Ca is returned to litter and soil with the falling leaves or needles and subsequently mineralized. Ca is thus available again for vegetal uptake from soil solutions of organic-rich forest floor and underlying mineral soils of forested watersheds.[6] Mineral reserves prove often insufficient to sustain the nutrient requirements necessary to the development of the forest annual biomass, so that the vegetal Ca cycle constitutes an essential additional nutrient supply to forests.

Over the past decade, Ca isotopes have shown their potential in identifying and characterizing fractionation processes occurring within plants[7,8] or at the water-soil-plant interface of small forested watersheds (see reference 9 and references mentioned therein). The present study was initially intended at preparing germinated *Phaseolus vulgaris* L. bean organs to be used in hydroponic experiments designed to characterize the co-occurring geochemical and biological processes affecting the Ca isotopic compositions within plants.[7,8] Ca isotopes have been measured in *Phaseolus vulgaris* L. bean organs prior to and after germination. Due to small natural Ca isotopic fractionation within watersheds (~1.25‰/amu), it was mandatory to achieve an external reproducibility in the permil to sub-permil range in order to detect any Ca isotopic variability within the bean organs of *Phaseolus vulgaris* L. $^{44}Ca/^{40}Ca$ ratios cannot be measured to high-precision by multiple collector inductively coupled plasma source mass spectrometry (MC-ICP-MS) because of the large ^{40}Ar interference. Instead, Ca isotope analysis was achieved through high-precision mass spectrometry by TIMS and complete chromatographic separation of Ca from elements susceptible to induce isobaric interferences during mass spectrometry analysis (Mg, K, Sr). In particular, special attention has been paid to complete removal of K from the samples of *Phaseolus vulgaris* L bean organs, where $K/Ca \leq 40$.

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Analytical Protocol

Automated High Selectivity ICS and TIMS of Ca in Plants

Calcium isotopic analysis in plants remains an analytical challenge and cannot be operated routinely. The occurrence of isobaric interferences at masses 40 ($^{40}K^+$), 42 ($^{84}Sr^{2+}$), 43 ($^{24}Mg^{19}F^+$, $^{86}Sr^{2+}$) and 44 ($^{25}Mg^{19}F^+$, $^{88}Sr^{2+}$) requires a step of chromatographic clean-up before sample isotopic analysis by mass spectrometry. *Phaseolus vulgaris* L. bean organs (Figure 1) were crushed in a clean agate mortar and digested in Savillex™ vials in a hot oxidative acid medium (double distilled HNO_3, double distilled HCl, suprapure H_2O_2). Then, following the double spike method introduced for Ca isotopes[10] and modified for Ca multicollection TIMS analysis[11], 1 µg of ^{42}Ca-^{43}Ca double spike was added to 5 µg of sample prior to Ca purification. This ensured correction for any procedural or instrumental mass discrimination that would occur after addition of the double spike.

Classical Ca-exchange chromatography is not optimized for full recovery and complete separation of Ca from K and Sr (Figure 2A). Instead, the development of an automated high selectivity ionic chromatography separation protocol (Thermo Scientific Dionex™ ICS-3000 device with a high-capacity carboxylate-functionalized column Dionex CS16 and a CSRS-300 cation self-regenerating suppressor) ensures Ca full recovery and eliminates K and Sr tailing (Figure 2B,[12]).

NOTE: The Dionex ICS-3000 ion chromatography system was replaced by the Dionex ICS-5000 system in 2010.

After Ca purification, Ca samples were dissolved in double-distilled HNO_3 and H_2O_2 and dried down to eliminate organic impurities originating from the sample and/or cation exchange resin breakdown. The Ca residues were subsequently re-dissolved in 1 µL 0.25 N HNO_3 and 6 µg Ca were subsequently loaded onto a single outgassed and partial-vacuum-oxidized Ta filament (99.995% purity). The Ca isotopic composition was measured by Thermal Ionisation Mass Spectrometry (TIMS) using a Thermo Scientific™ TRITON™ instrument operating in dynamic multi-collection mode.[11–12] The Ta filaments were typically heated until a $^{40}Ca^+$ ion beam intensity of 5.0×10^{-11} A, corresponding to a filament temperature ranging from 1380 to 1450 °C. 130 to 200 cycles of data were collected. Possible isobaric interferences from $^{40}K^+$ and $^{88}Sr^{2+}$ at masses 40 and 44 were systematically monitored for at masses $^{41}K^+$ and $^{88}Sr^+$. No interference was observed and, therefore, no interference correction was applied. The total procedural Ca blank varied between 50 ng and 150 ng (< 3% of the Ca sample amount) and had an isotopic composition of 0.5‰. Therefore the Ca blank contribution (< 0.02‰) was not accounted for.[8] One single measurement lasted 4–5 hours. One session of analysis typically consisted of 15 measurements over five days, including the analysis of one standard at the beginning and one standard at the end of a session. The analysis of most samples was replicated, including digestion, Ca purification by ion chromatography and TIMS analysis.

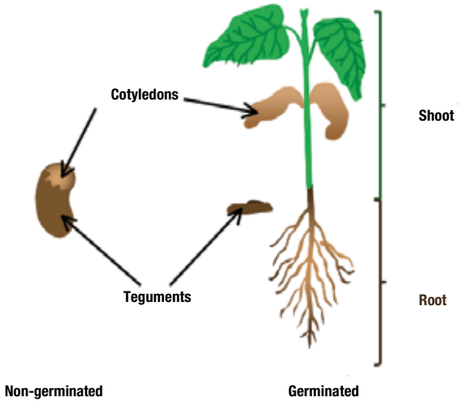

Figure 1. Bean organs of *Phaseolus vulgaris* L. prior to and after germination.

Bean seeds were placed in 100 g pre-cleaned vermiculite (5 days in ultrapure Millipore™ water), and germinated at 25 °C with no light exposure, for 7 days. Then, the plantlets were rinsed with ultrapure Millipore water[7] and aerial parts were separated from underground parts for analysis.

Non-germinated beans mainly consist of cotyledons, which constitute nutritional reserves for germination. Germinated beans consist of roots (underground part) and shoots (above ground part, other than the cotyledons). Teguments are present in both non-germinated and germinated beans and solely correspond to an envelope around the cotyledons, with no biological influence on germination.

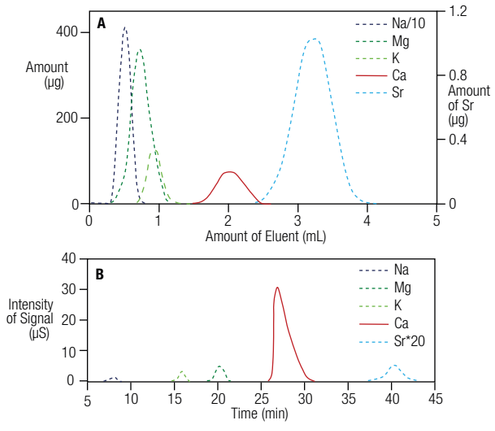

Figure 2. Elution curves for (A) classical Ca-exchange chromatography of a seawater sample (AG50W-X8, 200–400 mesh Biorad™ resin, 1.5 N HCl as eluent, recovery yield ~70%), and (B) automated high pressure ionic chromatography of a soil sample (Dionex CS16 cation-exchange column, methanesulfonic acid as eluent, recovery yield ~100%). The overall Ca blank contribution from the HPIC system was ~30 ng, i.e. ~0.5‰ of the sample Ca amount.[12] (Figure 2B modified after ref. 12).

Results

$^{44}Ca/^{40}Ca$ Isotopic Composition Long-term Reproducibility and Accuracy

$^{44}Ca/^{40}Ca$ isotope values are expressed as per-mil deviations relative to the NIST SRM™ 915a standard solution: $\delta^{44/40}Ca = \{[(^{44}Ca/^{40}Ca)_{sample}/(^{44}Ca/^{40}Ca)_{SRM915a}] -1\} \times 1000$ (ref. 13). The $\delta^{44/40}Ca$ values were corrected for instrumental mass bias using the exponential fractionation law and the Newton-Raphson iteration technique.[14] Data reduction was performed off-line using the optimization toolbox provided in the commercial Matlab™ software.

Long-term external reproducibility of $\delta^{44/40}Ca$ based on repeated measurements, over one year, of NIST SRM 915a was 0.09‰ (2 RSD, n = 24). The accuracy of the measurements was also tested by measuring two reference solutions used in other laboratories. $\delta^{44/40}Ca$ of seawater = 1.91 ± 0.10‰ (2 RSD, n = 8), $\delta^{44}Ca/^{40}Ca$ of CaF_2 = 1.45 ± 0.12‰ (2 RSD, n = 19) are indistinguishable from previously published data within uncertainties[15–16] and allow us to estimate an accuracy of 0.12‰.

$^{44}Ca/^{40}Ca$ Isotopic Composition of *Phaseolus vulgaris* L. Bean Organs

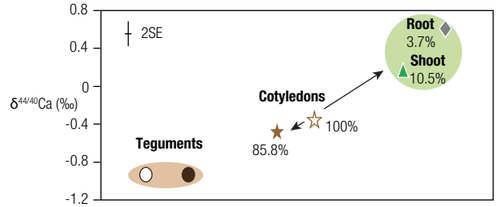

Figure 3. Calcium isotopic variations between germinated (full symbols) and non-germinated (empty symbols) bean organs. Germinated cotyledons present a $\delta^{44/40}Ca$ composition intermediate to those of non-germinated cotyledons and of roots and shoots.

External reproducibility of $\delta^{44/40}Ca$ based on replicate sample measurements was 0.12‰ (2 RSD, n = 80). The Ca isotopic analysis of *Phaseolus vulgaris* L. organs resolve natural $^{44}Ca/^{40}Ca$ isotopic variations in the sub-permil range between teguments and non-germinated cotyledons, and in the sub-permil to permil range between non-germinated cotyledons and germinated organs (Figure 3). Non-germinated and germinated teguments have an identical Ca isotopic composition. In contrast, germinated cotyledons ($\delta^{44/40}Ca$ = -0.49‰) are enriched in the light ^{40}Ca isotope whereas shoot ($\delta^{44/40}Ca$ = 0.20‰) and roots ($\delta^{44/40}Ca$ = 0.68‰) are depleted in the light ^{40}Ca isotope compared to the non-germinated cotyledons ($\delta^{44/40}Ca$ = -0.37‰).

$^{44}Ca/^{40}Ca$ Isotopic Fractionation During the Germination of *Phaseolus vulgaris* L.

Ca isotopic variability observed in *Phaseolus vulgaris* L. organs evidences fractionation mechanisms. Ca isotopic variability between teguments and non-germinated cotyledons could result from Ca isotopic fractionation during physiological processes leading to the formation of seeds. As expected, teguments, which do not participate in the germination, do not show Ca isotopic fractionation during this process. On the contrary, Ca isotopic variability between non-germinated cotyledons and germinated cotyledons, shoot and roots, shows that isotopic fractionation occurred during the germination process, i.e during the development of roots and shoots from the cotyledons. The Ca amount of rinsed vermiculite has been checked to be negligible compared to the Ca amount of the bean seeds and, as such, is not expected to contribute the Ca isotopic composition of the bean organs. The dry-mass-weighted calculated average $\delta^{44/40}Ca$ of the germinated organs (cotyledon, shoot and roots) equals that of the non-germinated cotyledons ($\delta^{44/40}Ca$ = -0.37‰), confirming that all the Ca mobilized during germination originated from the cotyledons and not from the germination medium (vermiculite) itself[7].

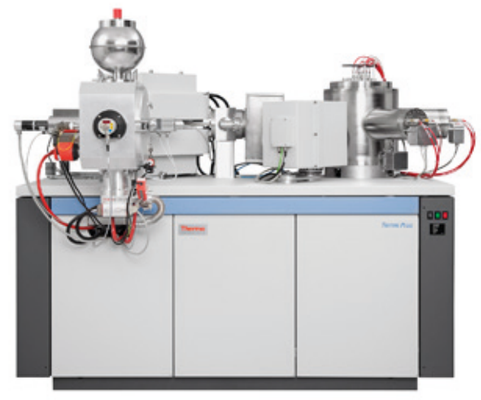

Figure 4. Thermo Scientific TRITON *Plus* Thermal Ionization Mass Spectrometer (TIMS).

Figure 5. Thermo Scientific Dionex ICS-5000 Reagent-Free™ HPIC™ system (replacing, since 2010, the Thermo Scientific Dionex ICS-3000 Reagent-Free™ HPIC™ system).

Conclusion

Flexible Analytical Protocols and Measurement Routines for High-precision Ca Isotopic Analysis

Coupling Dionex ICS-3000 and ICS-5000 ion chromatography and TRITON/TRITON *Plus* TIMS analysis improved the purity of Ca samples and ensured a long-term 2RSD $\delta^{44/40}$Ca external reproducibility of 0.12‰ on plant samples. This unraveled natural Ca isotopic variability down to the sub-permil range within plant organs and assessed the extent of Ca isotopic fractionation during vegetal germination. Note that the TRITON/TRITON *Plus* system offers diverse analytical protocols for Ca isotopic analysis, combining different loading techniques and measurement routines. With Ca sample amounts in the µg range, large ^{40}Ca ion beams (>40 V with ^{40}Ca measured on a 10^{11} Ω resistor amplifier and >400 V with ^{40}Ca measured on a 10^{10} Ω resistor amplifier) can provide 2RSD external reproducibility on ^{40}Ca/^{44}Ca ratios better than 50 ppm.[17–18]

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