Sulfur isotope mass balance reveals $^{32}S/^{34}S$ fractionation during sulfate uptake and translocation in rice

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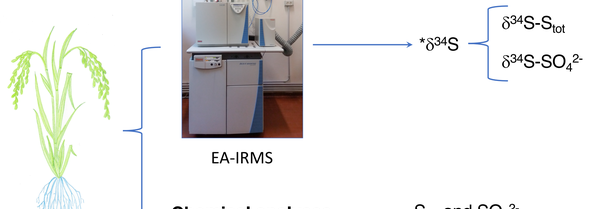
Background

Four stable isotopes of sulfur exist (^{32}S, ^{33}S, ^{34}S, ^{36}S) whose natural isotopic percentage abundances are 0.94499, 0.0075, 0.0425 and 0.0001 atom fraction, respectively. The most abundant isotopes – ^{32}S and ^{34}S – are now commonly measured using elemental analyzers coupled with isotope ratio mass spectrometers (EA-IRMS). Such an approach is based on the complete transformation of total S to SO_2, which is subsequently analyzed by the mass spectrometer with regards to masses 64 ($^{32}S^{16}O_2$) and 66 ($^{34}S^{16}O_2$ or $^{32}S^{16}O^{18}O$) atomic mass units. S stable isotopes have been used to trace the movements of the related compounds in plants, in testing S flux models, and in identifying and determining the impact of natural and anthropogenic S sources on the environment. However, the isotope technique applied for S metabolism investigations, as well as for sulfate transport and allocation within the plants, is limited by our current knowledge of the potential $^{32}S/^{34}S$ isotope discrimination that may occur during both S metabolism and sulfate transport.

Experimental design and methods

Isotope analyses



Rice (*Oryza sativa* L.; cv Vialone Nano) plants were grown in a complete hydroponic solutions containing 100 μM SO_4^{2-} as sole sulfur source. The $\delta^{34}S$ signature of the S source was 4.49 ± 0.04 ‰. At different times both plants and residual solutions were sampled and analyzed.

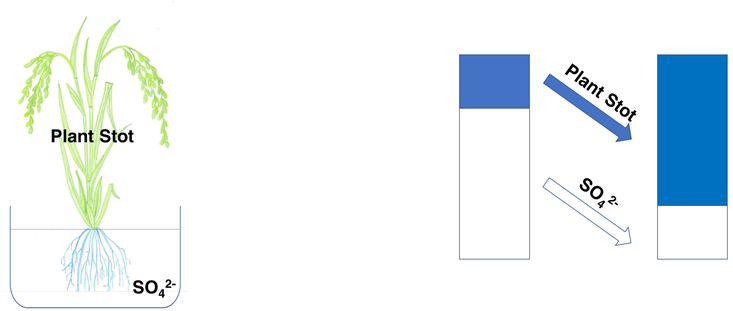
The relative ^{34}S abundance were quantified by EA-IRMS. Values were expressed using the δ notation versus the international standard VCDT (Vienna-Canyon Diablo Troilite):

$$* \delta^{34}S = (R_{sample} - R_{standard})/R_{standard}$$

were R is $^{34}S/^{32}S$ isotope ratio.

We approached the topic of $^{32}S/^{34}S$ fractionation in rice by investigating the hypothesis that the $\delta^{34}S$-tot of the whole plant could be determined by the activities of the sulfate transporters involved in sulfate uptake (**i.e. sulfate transporters discriminate between ^{32}S and ^{34}S**).
In particular, we considered a close system in which a limited amount of substrate (i.e., the sulfate ions in the nutrient solution) is continuously removed from the solution - by the activity of the high-affinity sulfate transporters of the roots - and converted in a final product (i.e., the Stot of the plant).

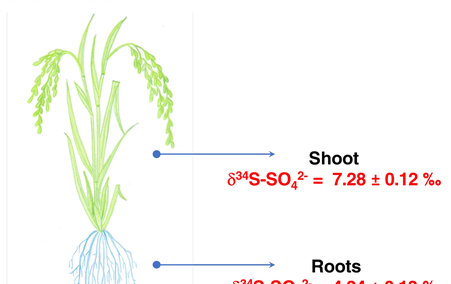
Substrate (external SO_4^{2-}) → Product (rice Stot)



In such a system, if fractionation against ^{34}S-SO_4^{2-} occurs, the $\delta^{34}S$-Stot of the plant (product) is expected to be less than the $\delta^{34}S$-SO_4^{2-} of the external solution (substrate). Moreover, fractionation will also cause the $\delta^{34}S$-SO_4^{2-} of the remaining substrate to increase over the time, and thus a corresponding increase in the $\delta^{34}S$-Stot of the product is expected.

Results and discussion

The $\delta^{34}S$ signature of the total biomass produced by a plant generally reflects that of the available sulfate in the soil solution, indicating the fractionation against ^{34}S during sulfate acquisition negligible. However, our preliminary results **(Fig. 1)** revealed some disparity amongst $\delta^{34}S$ value of the S source and the values of total S measured in rice total biomass, suggesting that substantial $^{32}S/^{34}S$ isotope effects occurred during sulfate uptake. Moreover, the $\delta^{34}S$-SO_4^{2-} values measured in root and shoot were significantly different, indicating that other fractionation events may occur during sulfate translocation and/or assimilation.



S source (SO_4^{2-} in the hydroponic solution) $\delta^{34}S$-SO_4^{2-} = 4.49 ± 0.04 ‰ ≠ **S tot in the whole plant** $\delta^{34}S$-Stot = 3.24 ± 0.04 ‰

Figure 1. Natural isotope composition ($\delta^{34}S$) of total sulfur (Stot), measured in the whole plants, and sulfate, measured in roots and shoot of rice plants grown in hydroponic solution.

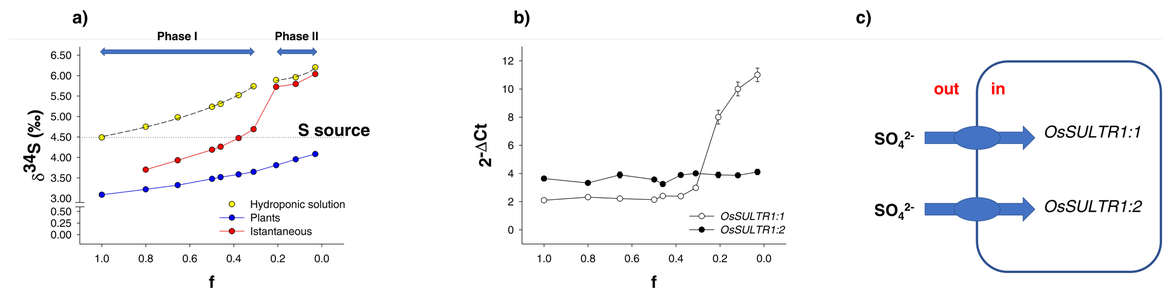


Figure 2. a) S isotope dynamics in the closed system (hydroponic solution + plants). **b**) Dynamics of *OsSULTR1:1* and *OsSULTR1:2* transcript in the roots. **c**) Main sulfate trasporter genes involved in root sulfate uptake.

The $\delta^{34}S$-SO_4^{2-} value measured for the sulfate ions in the external medium increased over the time with respect to initial $\delta^{34}S$-SO_4^{2-} of the source **(Fig. 2a)**. It is worth to note that – because of mass balance – the $\delta^{34}S$-Stot of the rice plants tends to $\delta^{34}S$-SO_4^{2-} of the original source as the sulfate concentration in the external medium approaches zero. Data analysis also reveals that a dual phase Reyleigh fractionation occurred during root sulfate absorption. In the first phase of the process ($f > 0.3$) a significant isotope fractionation against ^{34}S-SO_4^{2-} occurred ($\varepsilon = 1.02$ ‰), whilst in the final part of the process ($f < 0.3$) a less evident isotope effect ($\varepsilon = 0.16$ ‰) was associate to sulfate uptake (please, see the difference between $\delta^{34}S$-SO_4^{2-} in hydroponic solution and instantaneous products). Transcriptional analysis clearly shows that the transition from phase I to phase II was associated to changes in the ratio between the transcript levels of *OsSULTR1:1* and *OsSULTR1:2*, the two main genes involved in root sulfate uptake **(Fig. 2b,c)**.