

## Publication

## Improving the extraction and purification of leaf and phloem sugars for oxygen isotope analyses

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**RATIONALE:** The oxygen isotopic composition (here shown as the  $\delta^{18}\text{O}$  value) of soluble sugars in leaves and phloem tissue holds valuable information about plant functions in response to climatic changes. However,  $\delta^{18}\text{O}$  analysis of sugars is prone to error and thoroughly tested methods are lacking. **METHODS:** We performed three experiments to test if sample preparation modifies the  $\delta^{18}\text{O}$  values of sugars. In experiment 1, we tested effects of oven- vs freeze-drying, while in experiment 2 we focused on the extraction and purification of leaf sugars. In experiment 3, we investigated the exudation and purification of twig phloem sugars as a function of exudation time and different ethylenediaminetetraacetic acid (EDTA) exudation media. **RESULTS:** Freeze-drying produced more consistent  $\delta^{18}\text{O}$  values than oven-drying for sucrose, but not for phloem sugars. Extraction and purification of leaf sugars can be performed without a significant modification of its  $\delta^{18}\text{O}$  values. Yet, purified leaf and phloem sugars had higher  $\delta^{18}\text{O}$  values than the fraction of water-soluble compounds, highlighting the necessity for purification of extracted sugars. Moreover, the exudation time significantly modulated the  $\delta^{18}\text{O}$  values of phloem sugars, which is probably related to changes in sugar composition. EDTA addition did not improve the determination of phloem sugars'  $\delta^{18}\text{O}$  values. **CONCLUSIONS:** We show that sample preparation of plant sugars for reliable determination of  $\delta^{18}\text{O}$  values requires a strict protocol that we provide in this manuscript. We recommend for phloem sugar a maximum exudation time of one hour to reduce the degradation of sucrose and minimise oxygen isotope exchange reactions between the resulting hexoses and water. This article is protected by copyright. All rights reserved.

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