EGU23-1769, updated on 21 Feb 2024
https://doi.org/10.5194/egusphere-egu23-1769
EGU General Assembly 2023

Foliar water uptake as a source of hydrogen and oxygen in cellulose of vascular plants

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Where do oxygen and hydrogen atoms of organic matter from terrestrial plant, such as O and H in cellulose, come from? We already know that O in cellulose originates from water within plants, instead of CO_2 taken in via leaf stomata, and stable isotope tracers gave an answer to this question (Deniro & Epstein 1979, *Science*).

We know that all vascular plants acquire water from the soil via their roots. However, in recent years, we have learned that plants can also acquire significant amount of water directly through their leaves (Burgess and Dawson 2004, *Plant, Cell & Environment*; and others) and foliar-absorbed water can be a source of oxygen atoms of sugars in leaves (Lehmann et al. 2018, *New Phytologist*). Then, an interesting question comes up to our mind: where does the water that eventually become O and H in cellulose come from? Until recently, these water sources were thought to only be taken up by roots, as we have learned from our introductory biology textbook (Dawson 2022, *Tree Physiology*). However, validity of this common perception has recently been questioned, when, using hydrogen and oxygen isotope tracer in vapour or mist form, two researchers have demonstrated that foliar-absorbed water can be assimilated into organic matter (Studer et al. 2015, *Biogeosciences*; Lehmann et al. 2018). However, relative contributions of foliar-absorbed water and root-absorbed water assimilation into O and H in cellulose have remained an open question.

I therefore devised a labelling method that utilizes two different water sources, one enriched in deuterium and one enriched in oxygen-18, to simultaneously label both foliar-absorbed and root-absorbed water and quantify their relative contributions to plant organic matter (Kagawa 2022, *Tree Physiology*, https://doi.org/10.1093/treephys/tpac055). Using this new method, I will present evidence that, in the case of well-watered *Cryptomeria japonica*, hydrogen and oxygen incorporated into new leaf cellulose in the rainy season derives mostly from foliar-absorbed water (69% from foliar-absorbed water and 31% from root-absorbed water), while that of new root cellulose derives mostly from root-absorbed water (20% from foliar-absorbed water and 80% from root-absorbed water), and new branch xylem is somewhere in between (55% from foliar-absorbed water and 45% from root-absorbed water, see figure below). The novel dual labelling method first implemented in this study enables separate and simultaneous labelling of foliar-absorbed and root-absorbed water, and offers a new tool to study the uptake, transport, and assimilation processes of these waters in terrestrial plants. Thanks to our recent methodological

breakthroughs, this new tool will soon be publicly available, as it has become easy to enclose wet tissue samples from labelled trees into smooth wall tin capsules without significant leaks, and our laboratory can now routinely analyze H and O isotope ratios of 200 samples that are labelled with heavy water per week.