Study of Nitrate Uptake in Roots with Raman Microscopy

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Many different techniques exist to optically study physiologically relevant parameters in biological samples. Fluorescence microscopy coupled with fluorophores that change their behavior depending on certain environmental (physiological) parameters has allowed to make many important discoveries. Such fluorescent biosensors can be introduced in cells in different ways, for example by genetic encoding or allowing exgenous fluorophores to diffuse int the tissue. However, such biosensors may not be available for every physiologically relevant molecule. Another means to study such molecules is Raman micro-spectroscopy. As Raman spectra reveal molecular vibrational signatures of the samples under study, it is an attractive tool to detect and quantify the distribution of relevant molecules. As it is especially challenging to introduce biosensors in plant tissues (plant cell walls are much less permeable to such molecules than animal cell membranes), Raman microscopy is especially relevant to plant studies. In this work, we use a portable Raman spectrometer coupled to a home-built microscope to study the uptake and distribution of nitrate molecules in plant roots. Roots grow new cells at the root tip, and with increasing distance from the end of the root, the function and maturity of cells change [1]. While growing and dividing cells require (consume) nitrate as a nitrogen source to make proteins and other cellular components, maturated cells, that have their cell walls completed require much less nitrogen. The first few mm of a root consists of dividing and growing cells, which differentiate into cells with different functions when the root has grown a few mm. As cells in different phases of their life cycle have different nitrogen demands, it can be hypothesized that nitrate concentrations in the cells differ as a function of position. However, with conventional methods it is impractical to measure nitrate concentrations with a high spatial resolution as the amount of sample is very low and destructive analysis is required. Raman microscopy can overcome that limitation [2]. Our experimental results show that the nitrate concentration in root tissue is lowest at the root tip and increases to a maximum value at about 4-6 mm from the tip, and then levels off or decreases. This trend is consistent under different growth media conditions. We also observe that under nitrogen-starved conditions, the roots accumulate nitrate to several times the media concentration. Under more nitrogen-rich conditions, the accumulation of nitrate levels off, and turns into inhibition of nitrate to the tissue. These observations will allow us to perform more detailed studies of gene-regulation and understanding of plant responses to variations in nitrogen availability.

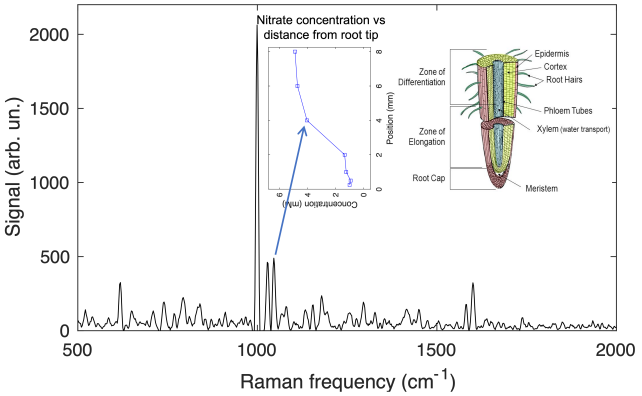

Figure 1: Raman spectrum of Arabidopsis root grown on agar plate medium. The peak at 1046 cm^{-1} scales linearly with the nitrate concentration. Inset: Measured concentration vs distance from the root tip. For details see text

References

[1] T Kagenishi, K Yokawa and F Baluška, Front Plant Sci. **7**, 79 (2016)

[2] A Fernández, D Sen, M C Lee, N Havrilchak, M Aleman, Z Han, B Strycker, Z Yi, J B West, A Sokolov, M O Scully and A J Verhoef, in: Proc. Conference on Lasers and Electro-Optics (CLEO) (May 15-20, 2022, San Jose, US), paper JW3A.21 (2022)