Nitrogen Fixation

University of Saskatchewan

Mid infrared and soft X-ray spectroscopy techniques to understand the nitrogen fixation process in legumes

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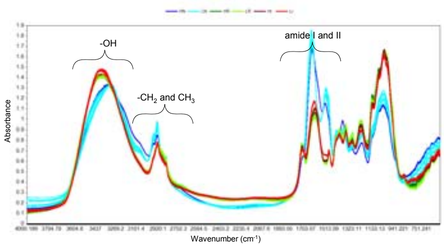
Picture source: http://www.wired.com/2012/08/nanoparticles-crop-pollution/

Objectives: Determine the advantages of mid-infrared and soft X-ray spectroscopy techniques as rapid methods to study nitrogen and other elemental (phosphorus, sulfur, boron) speciation in different parts of plant such as nodules, roots, and leaves.

Samples: Soybean plants, cultivar ‘Williams 82’ were grown. Inoculant is *Bradyrhizobium japonicum*. Plants supplied with 750 mL solution of nutrient solution every 5-6 days with variation in $MnSO_4$ (High: 22 mM $MnSO_4.H_2O$; Low: 2 mM $MnSO_4.H_2O$). The freeze-dried nodule, root, and leaf samples were ground to a fine powder and used for collecting mid-infrared and soft X-ray spectroscopy data.

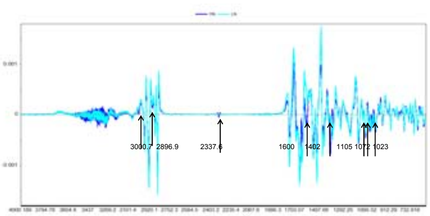
Mid Infrared Spectroscopy

The mid infrared Fourier Transform (FTIR) spectroscopy was performed at the Canadian Light Source Inc., using the glowbar source. Bruker - IFS 66V/S spectrophotometer (Bruker Optics, Ettlingen, Germany) with a liquid nitrogen cooled Deuterated triglycine sulphate (DTGS) detector was used for the transmission measurements of KBr pellet samples.

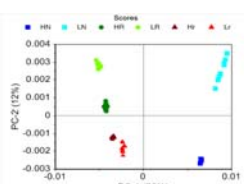


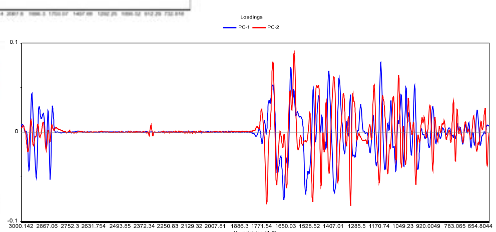
Mid infrared spectra of nodules, big roots, and small roots of high and low manganese treated soybean samples. Legend: HN – High manganese treatment and nodule samples; LN – Low manganese treatment and nodule samples; HR – High manganese treatment and big root samples; LR – Low manganese treatment and big root samples; Hr – High manganese treatment and small root samples; Lr – Low manganese treatment and small root samples.

There are clear differences in the absorption peaks between roots and nodules in the –OH, CH_2 and CH_3, and fingerprint (1800-800 cm^{-1}) regions.



Second derivatives of the mid infrared spectra of nodules of high and low manganese treated soybean nodule samples. Legend: HN – High manganese treatment and nodule samples; LN – Low manganese treatment and nodule samples.



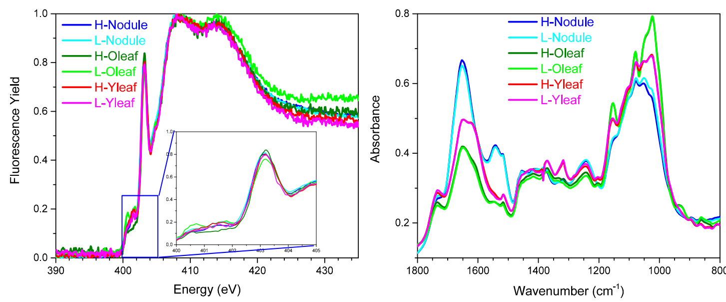


Loadings (left) and scores (right) plots of the principal component analysis of the mid infrared spectra of nodules of high and low manganese treated soybean nodule samples

The Principal Component Analysis of samples demonstrates that nodule and root samples could be distinguished from each other very distinctly. Further, the effect of high and low manganese treatments could also be very clearly distinguished.

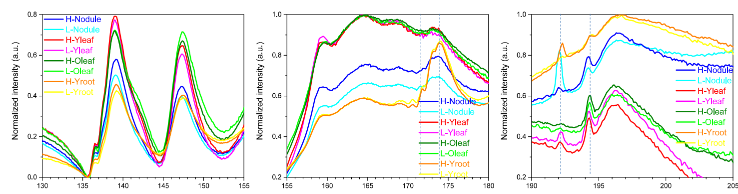
Soft X-ray Spectroscopy

The soft X-ray spectroscopy data were collected using the Spherical Grating Monochromator (SGM) and Variable Line Spacing-Plane Grating Monochromator (VLS-PGM) beamlines at the Canadian Light Source.



Nitrogen speciation in nodule and leaf samples collected using soft X-ray (left) and mid infrared (spectroscopy) techniques. Legend: H- high level of manganese; L – Low level of manganese; Yleaf – Young leaf; Oleaf – Old leaf; Yroot– Young root.

Differences in nitrogen speciation between nodules and leaves were clearly identified using mid infrared spectroscopy in the amide I (1650 cm^{-1}) and amide II (1550 cm^{-1}) regions. However, soft X-ray spectra were not able to reveal such differences.



Phosphorus (left), sulfur (middle), and boron (right) speciation measurements in soybean plant tissues (nodule, young leaves, old leaves, and young roots) treated with different levels of manganese.

The position and heights of peaks marked by vertical dotted lines reveal the different forms of sulfur and boron present in nodules, roots and leaves. No difference was observed in phosphorus speciation among different samples.

Acknowledgement

- NSERC
- Funding partners of the Canadian Light Source Inc.
- Saskatchewan Pulse Growers
- Agriculture Development Fund

Conclusions: The mid infrared spectroscopy data here highlights the extreme precision and wide scope of biochemical composition and changes identification in plant tissues. The bulk data collection of mid infrared spectra takes less than a minute with no sample alterations and minimal sample preparation. This makes it possible to characterize large sets of samples for fast screening and selection of germplasms, different treatment levels, and to track dynamic processes or response of plants. The soft X-ray spectroscopy data can be used to differentiate different chemical forms (speciation) of essential plant nutrients such as phosphorus, sulfur, and boron.

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