



Hyperspectral root imaging: Methods and added-value of spectral phenotyping soil-grown root systems.

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Spectral imaging makes use of different wavelength to infer on plant properties and processes. In the context of plant phenotyping, spectral imaging mostly uses multispectral sensors with defined broad-band wavelength in then VIS (400-700 nm), NIR (700-1100 nm) and SWIR (1100-2500 nm) regions. Hyperspectral imaging on the contrary captures the entire spectrum with up to several hundred narrow-band channels.

It is expected that the comprehensive spectral signature obtained from hyperspectral imaging can provide deeper insights into plant properties of potential use for structural-functional phenotyping. On the other hand the resulting spatial-spectral datasets are substantially larger compared to multi-spectral images, targeting defined wavelength to obtain spectral indices (e.g. NDVI), and require adequate methods to extract information from the data cloud.

Here we present the application of hyperspectral imaging to the root zone of plants grown in soil filled rhizoboxes. Essential steps in processing the hyperspectral datasets to obtain structural and functional information on the root system are demonstrated and implications for phenotyping application are discussed.

Plants of *Triticum durum* are grown in soil (silty loam topsoil, 2 mm sieve-size) filled rhizoboxes (30 x 1000 x 1 cm) at optimum moisture (field capacity) for imaging via a transparent mineral glass side. Spectral images are taken via a spectral scanner (1000-1700 nm, 222 bands, spatial resolution 0.1 mm).

Different pre-processing, dimensionality reduction and segmentation algorithms for separating root foreground and soil background pixels are discussed. Chemometric analysis of the segmented root images is exemplified for spectral distinction of root regions.

Results demonstrate that pre-processing of spectral images is the most important step for classification of root vs. soil pixels. Thereby the heterogeneity of the root axes as well as the soil background (water content, surface morphology) can be significantly reduced. As an example, polynomial de-trending with subsequent scatter correction via standard normal variate increases the Bhattacharyya distance between pixel histograms of root vs. soil from 0.47 for raw data to 2.76 for pre-processed data, with maximum distinction at a wavelength of 1462 nm. Based on pre-processed images and identification of most distinctive wavelength, segmentation (e.g. via fuzzy clustering) provides accurate binary images of the root system that can be further analysed with different chemometric approaches. This is exemplified by identifying central vs. boarder regions on the root axes showing different spectral signature. It is hypothesized that these spectral feature represents the distinction between parts belonging to the central cylinder with water conducting xylem (lower reflectance at water sensitive bands) vs. the cortex region.

First results demonstrate that hyperspectral imaging can provide novel insights into the root zone via distinctive spectral characteristics of different domains. Due to the heterogeneous biophysical and biochemical nature of the root zone, a key requirement for successful application of hyperspectral imaging to plant phenotyping is the use of efficient image processing tools in order to extract features of interest capturing root structure and functionality.