**Spectral NPK stress discrimination in barley plants by use of three wavebands**

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**Abstract**

Discrimination of a nutrient stress condition is the essential step prior to estimating the actual nutrient status using remote sensing. This research introduces a new methodology able to discriminate amongst non-stressed barley plants and N, P, and K deficiency symptoms spectrally using just three narrow reflection bands utilising both the spectral and spatial dimension simultaneously. Nine spectral measurements were carried out on each plant using the directed sampling technique. The measuring regions were spatially located at the tip, middle and base of the three last fully developed leaves. This design generated a four-dimensional data set consisting of the specific plant, the spectral dimension, the plant leaf position, and the position on the leaf. The barley plants were grown under controlled conditions, certifying the establishment of the three target deficiency symptoms (N, P and K and the non-stressed control). Three measurement occasions were carried out at three early growth stages within a time window of two weeks. Based on the results from the four dimensional multiway partial least square regression models (N-PLS) using the full spectral range (450-1000 nm) for discrimination four central wavelengths were identified as essential in the spectral discrimination modelling. The 2 nm wide reflectance bands (R) identified and used in the discrimination model were R450, R700 and R810. These three wavebands correspond to earlier findings on leaf pigment and leaf structural quantification.

**Media summary**

Non-destructive nitrogen, phosphorus and potassium deficiency discrimination in barley plants is now possible by use of only three wavebands. This has a great potential for more accurate fertiliser application in the future.

**Key words**

NPK discrimination, nutrient stress, non-destructive, remote sensing, multiway partial least squares regression

**Introduction**

The application of nutrients to field crops is of critical importance to optimise crop yield and product quality. Farmers must balance the competing goals of supplying enough nutrients to their fields, and minimise input in order to avoid adverse environmental effects and economic penalties from reduced yield (Pedersen, 2003).

Spatial variability in nutrients and variations in soil features affect nutrient utilisation by the crop. In order to reduce nutrient losses at field level, information is needed about the variability of plant-available nutrients and hence plant’s nutrient status, to allow for variable rate fertilisation.

One of the real challenges in nutrient prediction through the canopy’s spectral reflectance is the ability to discriminate stress conditions. In order to predict a specific nutrient component’s content in a plant, one must be able to spectrally discriminate the target component from other influencing components in the obtained spectra. The main problem is that the majority of nutrient stress symptoms at canopy scale and even at leaf scale have very similar effect on the reflectance spectrum. This research presents a methodology able to non-destructively discriminate between three essential nutrient (N, P and K) deficiencies in spring barley by use of the reflectance signatures from just three wavebands.

## Materials and Methods

### *N, P and K stress symptoms establishment*

Unambiguous N, P and K deficiencies were established in spring barley (*Hordeum vulgare* L.), cultivar *Optic*, under controlled greenhouse conditions. Spring barley seeds were pre-germinated for ten days, in sphagnum containing all essential nutrients and water (Husted et al., 2002). The plants were then randomly transferred to 12 pots containing water-saturated, inorganic media (perlite). The plants were provided with sufficient amount of nutrients (Husted et al., 2002) with the exception of the target nutrient stress component (N, P or K). Control plants were further established according to the above procedure, but with all essential nutrients for optimal growth.

The first spectral reflectance measurements were carried out 20 days after the plants were transplanted into perlite. The second measurement occasion was 25 days after transplanting and the third measurement occasion 33 days after transplanting (Table 1). For each treatment and measurement occasion ten plants were randomly selected from three pots containing the specific treatment giving 120 plants in total.

**Table 1. Growth stage determination for nitrogen stressed, phosphorus stressed, potassium stressed and control plants. BBCH scale used (Lancashire et al., 1991).**

| | 1st measurement occasion | 2nd measurement occasion | 3rd measurement occasion |
|---|---|---|---|
| | | Days after transplanting | |
| Stress Symptom | 20 | 25 | 33 |
| Nitrogen | 12 | 13 | 13 |
| Phosphorous | 21 | 21 | 21 |
| Potassium | 21 | 23 | 26 |
| Control | 21 | 24 | 29 |

### *Equipment*

The equipment used for spectral reflectance measurements was a Zeiss monolithic miniature spectrometer (MMS 1) NIR enhanced, which is an OEM (Original Equipment Manufacture) multi operating spectrometer system by tec5 AG, Germany. The core sensor was a Zeiss MMS 1 NIR with a detection range of 306-1132 nm in 2 nm increments. Due to observed noise problems the range used in this research was reduced to 450-1000 nm. Dark frame subtraction was performed minimising the system's bias. A holder was designed to support the leaves and direct the light probe in a 45 degree angle to the leaves in order to avoid specular reflectance. A fixed distance of one cm between the probe and the leaves was used. A black block of aluminium was placed on top of each leaf when measured.

*Directed sampling measurements*

The reflectance measurements were carried out by use of *Directed Sampling Technique* (DST). The principle of DST is to obtain specific, spatial information and relate it to spectral information from the plant. This sampling technique was introduced by Christensen and J?rgensen (2003) in a nutrient discrimination context by remotely sensed data. The data acquisition was carried out using DST at three leaf positions (tip, middle and base) on each of the three latest fully developed leaves. The circular measuring area was 7 $mm^2$, with a diameter of 3 mm. The plants were randomly selected among the pots of same treatment at the three different measurement occasions. Reference spectra, from a barium sulphate plate were recorded at regular intervals throughout the measurements. The reference spectrum was used for adjusting spectral and temporal variations in the equipment's sensitivity.

*Data analysis*

Initially the mean spectra for each of the four nutrient conditions and the three temporal measurements were studied and analysed visually. The visual evaluation of the data included the spatial dimension also, consisting of reflectance measurements from the base, middle, and tip locations for the last three fully developed leaves. Visual inspection of the data revealing obvious grouping was identified, hence creating the basis for a sequential or stepwise discrimination of the four nutrient factors.

The model analysis was performed in a predefined sequence with increasing model complexity ending with a spectral reduction from hyperspectral to multispectral resolution.

Multidimensional partial least squares regression (N-PLS) was used to discriminate control and N, P, K deficiency across the three temporal measuring sessions. Multiway partial least square regression is a model, which uses latent structures for making predictions of dependant variables within empirical four-way data sets. The strength of N-PLS is that it summarises all latent information from a large N-way dataset of object variables (X) and relates it to a dependent variable (Y) using a relative low number of variables, which makes the prediction more robust compared to PLS-Unfold (Bro, 1996; Hansen, 2002).

In order to enable discriminating abilities to N-PLS a dummy response variable was introduced. The dummy variable can be either 1 or -1. Having only one response variable identifying control and K stress from N and P stress where training response variable was set to 1 for control and K and -1 for N and P then the predicted response values >0 were considered as TRUE for control and K and FALSE if the response <=0. The opposite was the case for the N and P group. Hence the group mentioned first was assigned the response values 1 and the second group mentioned was assigned the response values -1. For example separating N and P stressed plants, the N stressed plants were assigned with the response value 1 whereas the P stressed group was assigned response value -1. The procedure was similar to bidirectional PLS discrimination known as PLS-DISCRIM described by Esbensen (2001). Using only one dummy response variable, the separation of four treatments must be carried out in a sequence of two steps.

The four dimensions or modes (M1-M4) used in the N-PLS analysis are listed below and illustrated in Figure 1.

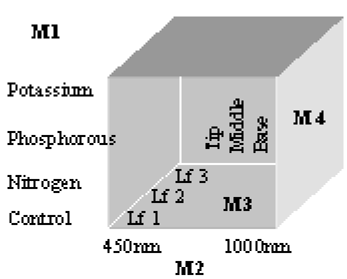

**Figure 1. Overview of the 4 dimensional N-PLS analysis. Dimension or mode 1 (M1): Treatment: control, N, P, K deficiency, M2: Spectral with the range 450-1000 nm in 2 nm bands. M3: Location on the plant; Leaf No. (Lf) 1, 2 and 3 where 1 one is the youngest leaf. M4: Location on the leaf; tip, middle and base.**

The four dimensional/mode analysis which orders the 3 *?* 3 spectra from a plant in a 3 dimension cube was termed N-PLS1-Fold. The plants with the different treatments as illustrated in Figure 1, added a fourth dimension to the mode (Figure 1, M1).

Three 2 nm bands important for the plant stress discrimination were selected based on a combination of loading weight interpretations (data not shown) and prior knowledge from literature on typical spectral areas related to changes in leaf pigments and leaf physiology. Hereafter N-PLS1-Fold was performed on the spectrally reduced multispectral dataset. The evaluation was based on full cross validation (leave-one-out).

## Results and Discussion

94% success rate was obtained using the two-step N-PLS1 discrimination procedure based on the plants' reflectance from the three wavelengths; 450 nm, 700 nm and 810 nm (Table 2, Step 1, 2a and 2b).

**Table 2. Two-step PLS1 discrimination model based on the mean spatial and spectral response from the wavelengths of 450 nm, 700 nm and 810 nm, measured through directed sampling technique.**

| | Wavelengths (nm) | Centred M1, Scaled M2 | PCs | RMSEP | Success rate (%) | Summary of Success rate (%) | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Step 1* Control, K vs. N, P | 450, 700, 810 | 1; 0 | 7 | 0.34 | 100 | | MO1 | MO2 | MO3 |
| *Step 2a* N vs. P | 450, 700, 810 | 1; 0 | 4 | 0.35 | 100 | C | 80 | 80 | 100 |
| | | | | | | N | 100 | 100 | 100 |
| *Step 2b* Control vs. K | 450, 700, 810 | 1; 0 | 6 | 0.75 | 88 | P | 100 | 100 | 100 |
| | | | | | | K | 100 | 80 | 90 |

Overall success rate: (112/119) ≈ 94 %

Step one in the two-step discrimination model (Table 2) separated the spectral responses from the control plants and K stressed plant from the spectral responses of the N and P stressed plants with a 100% success rate independently of the variable growth stages present in the three measurement occasions (Table 2, Step 1). The second step in the spectral discrimination model, distinguishing between the N and P stressed plants with also 100% success rate throughout the three measurement occasions (Table 2, Step 2a). However, a success rate of 88% was reached in the separation of the control plants and the K stressed plants (Table 2, Step 2b). The non-discriminative spectra were identified in measurement occasion 2 and 3 for the K stressed plants and measurement occasion 1 and 2 for the control plants (Table 2, ‘Summary of Success rate (%)’).

## Conclusions

Stepwise multiway partial least squares regression models (N-PLS) analysis with dummy response variables were able to correctly classify the four nutrient conditions with 94% success rate regardless of the respective growth stages within a time window of two weeks, using just three wavebands: R450 (predominant pigment absorb region), R700 (the maximum chlorophyll response) and R810 (plant cell structural response region).

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