COMPARISON OF NON-DIRECT METHODS FOR ESTIMATION OF NITROGEN STATUS AND CANOPY STRUCTURE IN SPRING BARLEY

SROVNÁNÍ NEPŘÍMÝCH METOD PRO VYHODNOCENÍ VÝŽIVNÉHO STAVU A STRUKTURY POROSTU JEČEMENE JARNÍHO

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ABSTRACT

The main objective of this study was to compare numerous vegetation indices derived from reflectance measurements in range 350 – 2500 nm (using the instrument FieldSpec 3 equipped with fiber optics) with *in-vivo* measured flavonols and chlorophyll content and derived Nitrogen Balance Index (NBI) (using the instrument Dualex). The sensitivity of individual parameters to genotypic variability and canopy structure was tested in two spring barley varieties ('Barke' and 'Bonus') and three sowing densities (1, 3 and 5 million germinable seeds). Furthermore, the effect of UV and PAR radiation on flavonols content was studied within independent experiment to ensure robustness of the selected parameters for the diagnosis of nutritional status and structure of vegetation. The results show that content of flavonols provides more uniform outcome with less influence of sowing density, but with a certain effect of leaf age. In addition, within the variety 'Bonus' significant effect of lowest sowing density was found. It is also evident that at higher doses of nitrogen the effect on flavonol content is saturating.

Key words: spectral reflectance, vegetation indices, nitrogen, flavonols, UV radiation, PAR radiation

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INTRODUCTION

Spatial variation in soil conditions and temporal changes in weather, results in high spatio-temporal variability in nutrient availability and canopy structure with significant impact on crop productivity. In order to be able timely estimate this heterogeneity and prevent negative impact on crop productivity and canopy structure there is increasing demand for reliable method, which will enable to detect already small changes in crop physiology on relatively large areas. One of the most promising approaches in remote sensing is based mainly on spectral reflectance measurements. Vegetation indices that combine reflectance from few spectral bands have been developed for pigment retrieval and subsequently for nitrogen status estimation (Gitelson et al., 1996). Specific absorption coefficients of leaf pigments are high for blue and red wavelengths and the depth of light penetration into the leaf is very low (Merzlyak and Gitelson, 1995). As a result, even low amounts of foliar pigments are sufficient to saturate absorption. The widely applied Normalized Difference Vegetation Index (NDVI), due to its early saturation (Buschmann and Nagel, 1993), was found to be insufficiently sensitive to changes of medium and high chlorophyll/nitrogen content. For this reason, increased attention was later paid to the development of new vegetation and chlorophyll indices and new non-direct diagnostic methods for estimation of nitrogen nutrition status that would provide higher accuracy and reliable detection also at higher levels of nitrogen nutrition. One of the most promising approaches seems to be the detection of leaf polyphenolic compounds (flavonols) based on measurements of UV-screening of chlorophyll fluorescence (Cerovic et al. 2005). The main objective of this study was to compare numerous vegetation indices derived from reflectance measurements in range 350 – 2500 nm with *in-vivo* measured flavonols content and derived Nitrogen Balance Index (NBI). The sensitivity of individual parameters to genotypic variability and canopy structure was tested in two barley varieties and three sowing densities. Furthermore, the effect of UV and PAR radiation on flavonols content was studied within independent experiment to ensure robustness of the selected parameters for the diagnosis of nutritional status and structure of vegetation.

MATERIALS AND METHODS

Within the first experiment the pre-germinated seeds of two barley varieties ('Barke' and 'Bonus') were sown in pots filled with a mixture of siliceous sand (0 – 2 mm fraction) and a light peat substrate that contains no fertilizers and pH is adjusted to 5.5 – 6.5 (Base substrate, Klasmann-Deilmann, D). The sowing density was adjusted to 1, 3 and 5 million of germinable seeds (MGS). After 9 days since seeding (11 – 12 BBCH) were the pots fertilized with nitrogen ($CaNO_3$ dissolved in water) to achieve final nitrogen doses of 0, 5 and 100 kg $N.ha^{-1}$ respectively.

Within the second experiment two barley varieties ('Barke' and 'Bonus') were sown in one density 3 MGS and the nitrogen fertilization was carried out in two doses 0 and 200 kg N.ha^{-1}. At the growth stage BBCH 12 were the pots placed under two contrast radiation treatments. UV0 PAR50 represents the treatment with excluded UV radiation using UV filter (Lee Filters) and reduced PAR using shading net with approximately 50% transmittance of PAR. UV100 PAR100 treatment represents ambient UV and PAR conditions. Barley plants were cultivated under these treatments for 8 days.

Measurement of spectral reflectance in the range 350 – 2500 nm was done at BBCH 13 on the canopy level using the instrument FieldSpec 3 (ASD inc.) equipped with fiber optics. Spectral on plate was used as a reference before each measurement. Measurements of chlorophyll content and flavonols were carried out with the instrument Dualex (Force A) on the first and second leaf around 13 BBCH (within second experiment BBCH 12-13). Vegetation and chlorophyll indices were calculated as simple ratios or normalized indices based on commonly used formulas. Nitrogen Balance Index (NBI) was calculated as a ratio between chlorophyll and flavonols content. Data were analysed using statistical software Statistica 7.

RESULTS AND DISCUSSION

The results of transmittance based measurement of chlorophyll content (Dualex) show relatively small changes with nitrogen dose with high variation of measured data. This method of measurement of chlorophyll content using transmittance is now considered as a standard among non-direct methods for detection of nitrogen nutrition status (e.g. SPAD or N-tester). Although this type of measurement is carried out at the leaf level, it is interesting that the results show a relatively conclusive effect of sowing density on these values. This is probably due to the competition of plants for nitrogen and light. Therefore with increasing density the chlorophyll content decreases. It is therefore evident that the canopy structure must be also taken into account during detection of nitrogen status. Similarly, there is also apparent effect of variety on the chlorophyll content. This shows the necessity of varietal calibration of this method.

Response of flavonols content on nitrogen dose shows generally opposite trend compare to chlorophyll content. At the higher dose of nitrogen the flavonols content decreases. Likewise, the sowing density affects the content of flavonols in the opposite direction than chlorophyll content. Generally, the content of flavonols provides more uniform results with less influence of sowing density, but with a certain effect of leaf age. In addition, within the variety 'Bonus' significant effect of lowest sowing density was found. It is also evident that at higher doses of nitrogen the effect on flavonol content is saturating.

Using the flavonol content for more accurate diagnosis of nitrogen nutrition in the form of NBI brings reduced variability and thus more accurate detection. Differences between sowing densities, however, remain almost unchanged and therefore the calculated nitrogen dose should be adjusted according to canopy structure.

If we compare NBI with the vegetation indices that provide the highest response, such as NDVI or NRERI, it is apparent that these indices are characterized by relatively high variability of the measured data, early saturation already at middle nitrogen dose, but surprisingly also by little response to sowing density. We can therefore assume that the combination of spectral reflectance measurement with UV-screening of chlorophyll fluorescence (flavonols content) may contribute to the more accurate results of diagnostic methods for nitrogen nutrition.

Based on the results of the second experiment, however, it is necessary to state that the amount of accumulated flavonols is also dependent on the radiation conditions and in particular on the amount of UV radiation. This can result in temporal variability of the content of flavonols, due to the change of the incident radiation. These measurements therefore require the development of appropriate methods of calibration or relative comparison that would limit the effect of different radiation conditions.

Fig. 1 Effect of nitrogen dose, sowing density and leaf age on chlorophyll content in two barley varieties. The chlorophyll content was determined non-destructively on the basis of transmittance measurements (Dualex, Force A, F). Means (points) and 95% confidence intervals (error bars) are presented (n≥4).

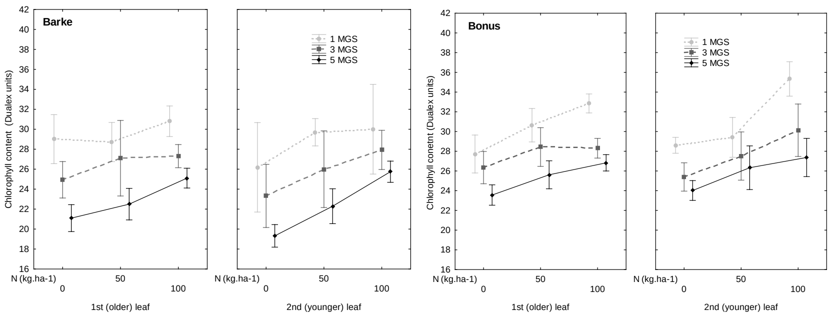


Fig. 2 Effect of nitrogen dose, sowing density and leaf age on flavonols content in two barley varieties. The flavonols content was determined non-destructively on the basis of measurement of UV-screening of chlorophyll fluorescence (Dualex, Force A, F). Means (points) and 95% confidence intervals (error bars) are presented (n≥4).

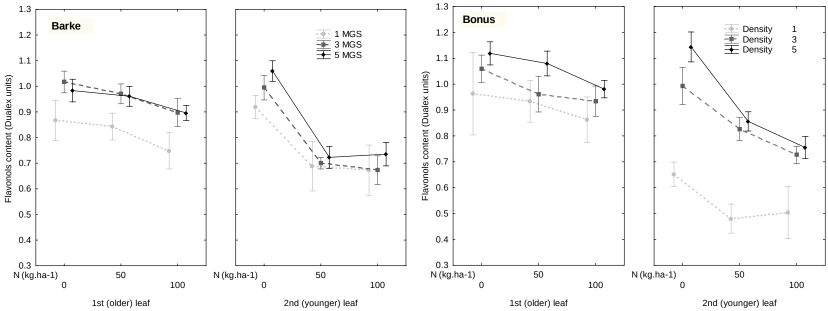


Fig. 3 Effect of nitrogen dose, sowing density and leaf age on Nitrogen Balance Index (NBI) in two barley varieties. The NBI was calculated as a ratio of chlorophyll and flavonols content both determined non-destructively with instrument Dualex. Means (points) and 95% confidence intervals (error bars) are presented (n≥4).

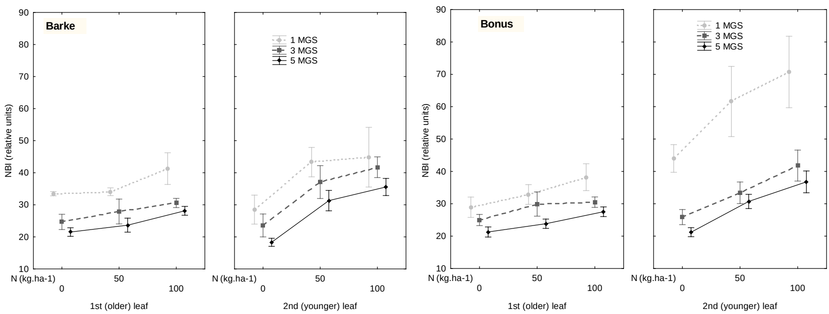


Fig. 4: Effect of nitrogen dose and sowing density on selected vegetation indices (NDVI and NRERI) in two barley varieties. The spectral reflectance measurements were made on the canopy level using spectroradiometer FieldSpec 3. Means (points) and 95% confidence intervals (error bars) are presented (n=4).

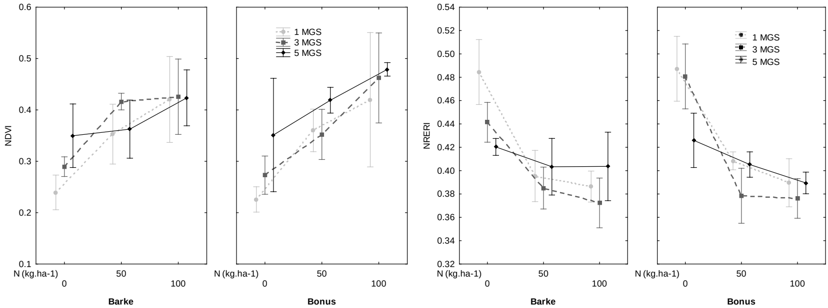
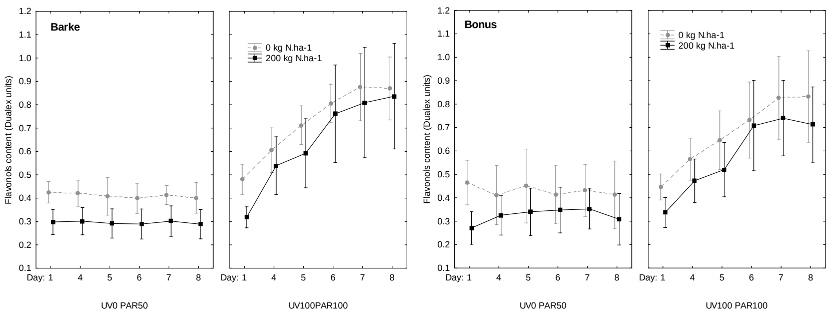


Fig. 5: Changes in flavonols content during short-term exposition to two levels of UV and PAR radiation treatments (UV0 PAR50 represents UV exclusion and reduction of PAR to 50%; UV100 PAR100 represents ambient UV and PAR conditions) in response to nitrogen dose. Means from two leaves (1st and 2nd leaf) are shown separately for individual barley varieties. The flavonols content was determined non-destructively on the basis of measurement of UV-screening of chlorophyll fluorescence (Dualex, Force A, F). Means (points) and 95% confidence intervals (error bars) are presented (n≥4)



CONCLUSIONS

The chlorophyll content shows relatively small changes with nitrogen dose and with high variation of measured data. Interestingly, the data show a relatively conclusive effect of sowing density on chlorophyll content. Response of flavonols content on nitrogen dose shows generally opposite trend compare to chlorophyll content. Generally, the content of flavonols provides more uniform results with less influence of sowing density, but with a certain effect of leaf age. Using the flavonol content for more accurate diagnosis of nitrogen nutrition in the form of Nitrogen Balance Index (NBI) brings reduced variability and thus more accurate detection. However, the amount of accumulated flavonols is also dependent on the radiation conditions and in particular on the amount of UV radiation. The vegetation indices that provide the highest response to nitrogen dose (e.g. NDVI or NRERI) are characterized by relatively high variability of the measured data, early saturation already at middle nitrogen dose, but surprisingly also by little response to sowing density. We can assume that the combination of spectral reflectance measurement with UV-screening of chlorophyll fluorescence (flavonols content) may contribute to the more accurate results of diagnostic methods for nitrogen nutrition.

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