

Estimation of soybean seed protein accumulation by measuring canopy hyperspectral reflectance

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Soybean (*Glycine max* (L.) Merr.) is the major source of plant-based protein for both human nutrition and animal feeding. In Central European countries, interest in organic soybean production has recently grown due to increasing demands for high protein raw materials in food production. Consequently, dinitrogen fixation by symbiotic rhizobial bacteria is playing a crucial role for yield performance and harvest product quality. Additionally, high levels of symbiotic nitrogen fixation are particularly relevant for achieving a positive nitrogen balance as well as a beneficial crop rotation effect of soybean in organic production systems.

As direct methods of measuring nitrogen fixation under field conditions are tedious and not suitable for high-throughput screening of genotypes within plant breeding programs, the present research aimed at evaluating indirect phenotyping methods for field-based screening of soybean nitrogen uptake with seed protein content as a target trait. A set of soybean genotypes widely differ-

ing in seed protein content was tested in three environments (Tulln 2019, Tulln 2020, Raasdorf 2020) in the east of Austria in replicated single-row plots. Between the soybean developmental stages of full flowering (R2) and full seed (R6), *i.e.* the seed filling period, canopy hyperspectral reflectance data were collected at about weekly time intervals using a hand-held spectroradiometer (ASD HandHeld 2 Fieldspec, λ 325-1075 nm). Reflectance data at particular wavelength points were then utilized for calculating indices or regression models for predicting seed protein content.

In individual experiments, a level of seed protein content in the wide range of 285-489 g/kg was found. Reflectance data at particular wavelength regions were clearly correlated to seed protein content (Figure 1). From over 40 spectral reflectance indices calculated, nitrogen reflectance index (NRI), greenness index (GI) and different ratio vegetation indices (RVI) with optimized wavelengths for soybean canopies revealed highest correlations

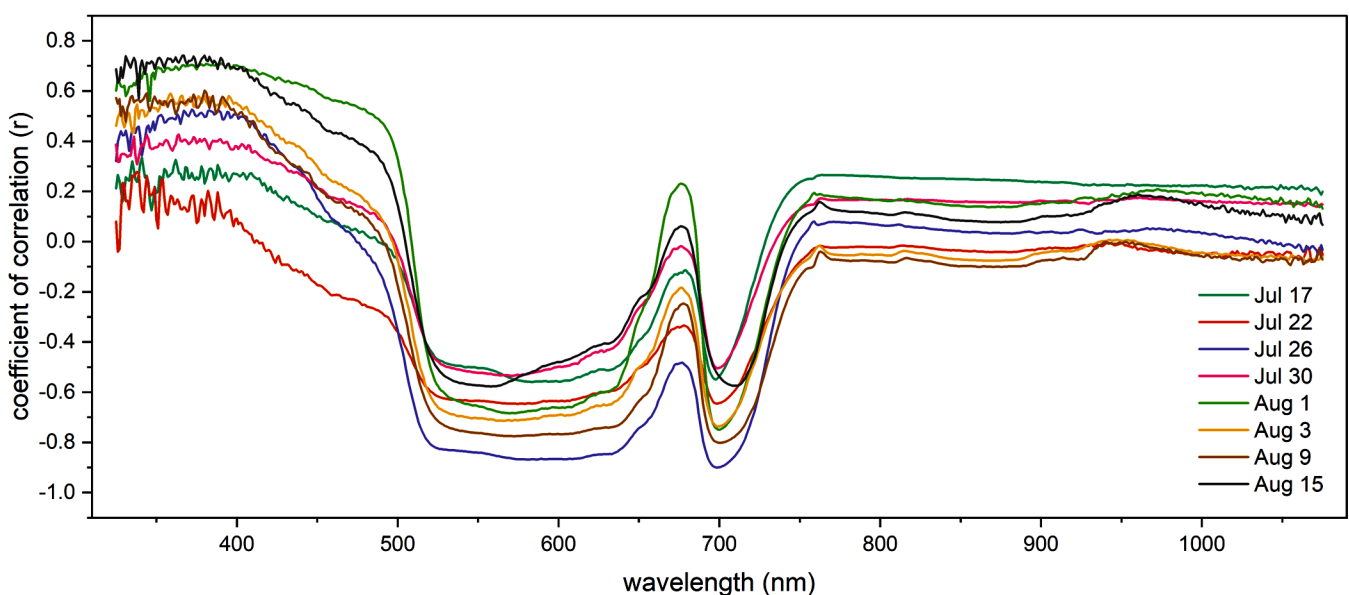


Figure 1 Relationship between hyperspectral reflection during the soybean seed filling period and seed protein content of the harvest product: Correlograms describing correlations between reflectance at given wavelengths (1 nm increment) and seed protein content for the Tulln 2019 subset A (nodulating vs. non-nodulating population) at 8 different spectral data collection dates.

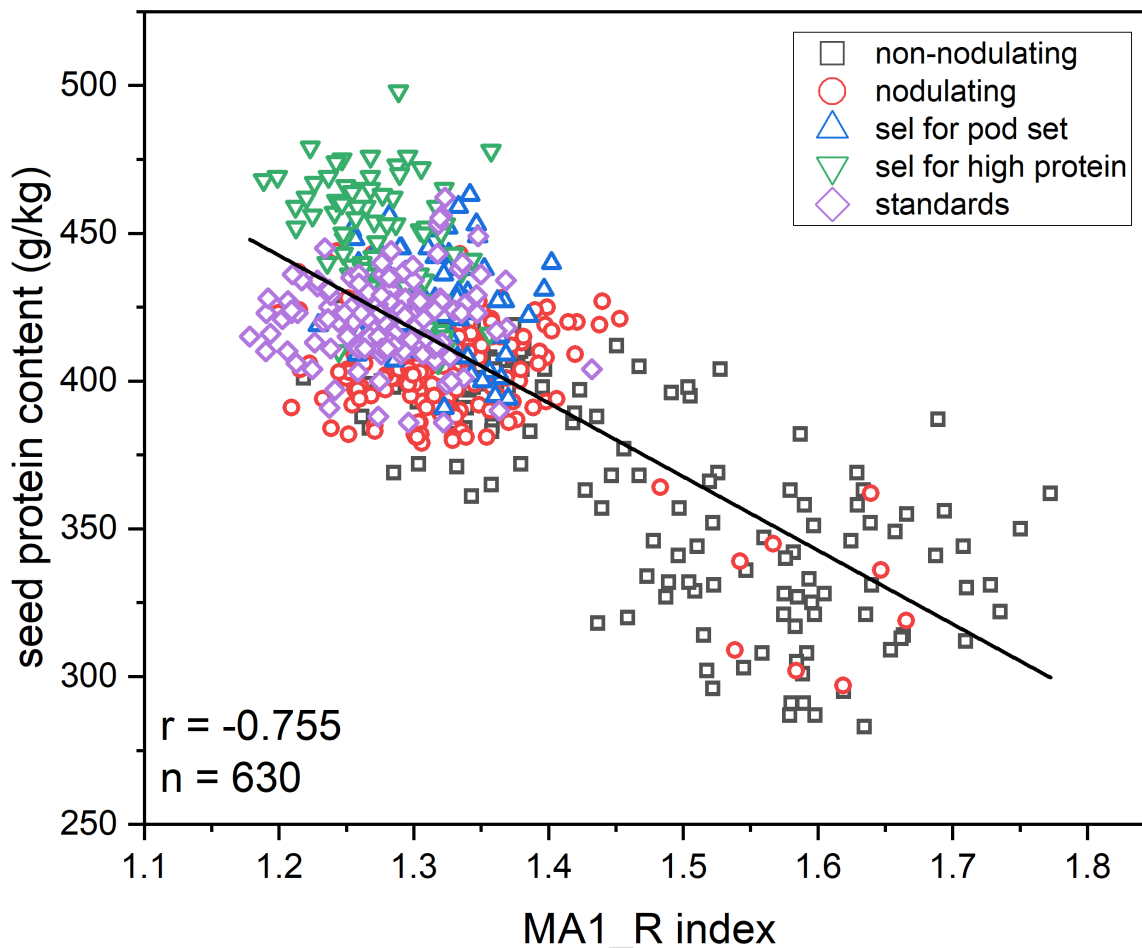


Figure 2 Relationship between hyperspectral reflection during the soybean seed filling period and seed protein content of the harvest product: Regression of the MA1_R index term on seed protein content for all genotypes and across all environments.

($r = 0.88$) to seed protein content within particular environments. These indices were also highly correlated with leaf chlorophyll content (SPAD-meter-values). The ratio vegetation index term MA1_R showed the highest correlation to seed protein content across all three environments and all genotypes (Figure 2). In a second approach utilizing the whole range of spectral data available and partial-least-square regression modelling (PLSR), correlations of up to $r = 0.91$ (model calibration; $r = 0.89$ for model validation) were achieved for seed protein content data across all three environments. In addition to seed protein content which is directly related to nitrogen fixation, hyperspectral reflectance data appeared to be useful for prediction of additional traits such as time to maturity, oil content or 1000-seed weight as well. Moreover, on the level of individual genotypes, particular indices could be utilized for better characterization of genotypes in terms of water use efficiency, biomass production, and nitrogen metabolism.

While further modelling and application research will be required for optimizing the screening procedures, the present results reveal a considerable potential of hyperspectral reflectance measurement for characterizing soybean traits related to nitrogen content. This could contribute to selection for improved nitrogen fixation capacity and better harvest product quality in organic soybean production.

Keywords

Field phenotyping · *Glycine max* · hyperspectral reflectance · nitrogen fixation · seed protein content · soybean breeding

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