SOYBEAN DI-NITROGEN FIXATION AFFECTING PHOTOSYNTHESIS AND SEED QUALITY CHARACTERS

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ABSTRACT

Di-nitrogen fixation by legume crops has recently gained in importance, as it provides nitrogen nutrition in a highly sustainable and economically competitive way thus contributing to environmentally sound agricultural production as well as to high quality crop products. Soybean is the major legume crop world-wide, and there is a growing interest in soybean production in Central Europe due to its high seed protein content of about 40%. However, as soybean nitrogen fixation may be limited by various environmental conditions, there is a need to optimize symbiotic fixation through agronomic and plant breeding research. As direct measurement of biological nitrogen fixation is difficult, we utilized the close physiological relationship between photosynthesis and nitrogen fixation in order to monitor genetic differences in nitrogen fixation and its effects on soybean seed quality. SPAD-meter readings of leaf chlorophyll content or leaf digital image analysis parameters determined at flowering time were significantly affected by the nitrogen fixation status of soybean, as established by comparing nodulating and non-nodulating genotypes. Subsequently, these relationships were confirmed in two field experiments with nodulating soybeans. In all experiments, the G (green) value of RGB image analysis (RGB colour space) and the SPAD-value describing chlorophyll content at flowering were correlated to soybean seed protein content as measured after harvesting. Therefore, these photosynthesis parameters could be utilized for determining the nitrogen fixation status of soybean crops and for predicting seed quality parameters, particularly for food-grade soybean production, which could contribute to optimizing biological nitrogen fixation for high quality crop production.

Key words: Soybean, biological nitrogen fixation, chlorophyll content, digital image analysis, seed protein content.

INTRODUCTION

Soybean (*Glycine max* [L.] Merr.) is the flagship legume crop on a world-wide scale grown on almost 100 mio ha recently (FAOSTAT, 2011). The interest in soybean is due to its high seed protein content of about 400 g/kg (dry matter basis) and an oil content of 200 g/kg, which make it a desirable raw material in a huge number of food, feed and non-food applications. While major soybean production takes place in the Americas and several Asian countries, a renewed interest in soybean growing is presently found in Central Europe. In contrast to world soybean production which concentrates on soybean as an oilseed crop, the European focus on soybean is in its high protein content. As soybean yield is closely related to nitrogen availability with a linear soybean grain yield increase of 13 kg per kg above ground nitrogen accumulation (Salvagiotti et al., 2008), N supply through biological di-nitrogen fixation is of high relevance for an economic and environmentally safe crop production. Moreover, N fixation has a significant impact on seed protein content (Vollmann, 2007) thus determining the value of the soybean crop harvested.

Photosynthesis and biological di-nitrogen fixation are the two key metabolic processes for legume growth, development and yield formation. In soybean, there is a close relationship between nitrogen supply and net leaf photosynthetic rate. In photosynthetically active leaves, half of the nitrogen content belongs to Rubisco which is the most relevant enzyme involved in carbon accumulation. Thus, the supply of N through nitrogen fixation can be a rate limiting process for photosynthesis as well as yield formation (Sinclair, 2004).

Determination of leaf chlorophyll concentration with hand-held chlorophyll meters such as the Minolta SPAD-502 has been widely used in recent years (Uddling et al., 2007), as it represents a rapid and non-destructive technique easily applicable to field experiments. Due to the close relationship between chlorophyll content and nitrogen concentration, SPAD-measurements can be interpreted as reflecting the plant's nutritional status. In addition, digital image analysis is another technique increasingly utilized in agricultural research. For instance, plant cover measurement is an easily applicable task in image analysis through segmentation of green colour on an image for canopy quantification (Richardson et al., 2001; Vollmann et al., 2010).

Recently, image analysis has been combined with SPAD metering: The G (green) value derived from RGB image analysis (RGB colour space) of soybean leaf images and SPAD values are highly correlated and can both be used for measuring chlorophyll content (Vollmann et al., 2011). Moreover, in a soybean population segregating for nodulation, biological N fixation as determined by comparison of nodulating and non-nodulating soybean lines affected leaf chlorophyll content and consequently a number of other plant characters such as leaf size, time to maturity, plant height, number of pods, 1000-seed weight and seed protein content. Thus, the measurement of chlorophyll content is of interest both for predicting seed protein content of a soybean crop and for estimating the status of N fixation. As the relationship between nitrogen fixation and other plant characteristics mentioned above have been determined in a qualitatively segregating population (nodulating vs. non-nodulating) only, there is an interest to confirm the findings in normal nodulation strains of soybean with a presumed quantitative variation in nitrogen fixation. Therefore, the goal of this study was to demonstrate the effect of varying leaf chlorophyll concentrations on soybean seed quality characters in early maturity breeding lines, which might represent quantitative differences in N fixation.

MATERIALS AND METHODS

The experiment on nodulating and non-nodulating soybean lines (*Rj1* vs. *rj1* alleles) was carried out at Vienna during the 2008 season and is described in full detail in Vollmann et al. (2011). Further experiments were carried out during the 2009 and 2010 seasons at the experimental station Gross Enzersdorf located east of Vienna. Experimental genotypes from four different crosses between early maturity soybeans were grown in single-row plots. Before sowing, soybean seeds were inoculated with a commercial preparation (Nodular G, Serbios, Badia Polesine, Italy) of soybean specific rhizobia (*Bradyrhizobium japonicum* [Kirchner] Jordan) for promoting nodule formation.

In each year, plants from 240 individual plots were analysed for leaf and seed quality characters. At the R3 stage of soybean development (full bloom, approx. mid of July), fully developed leaves from five plants per row were detached. The SPAD-502 chlorophyll meter (Konica Minolta Sensing, Osaka, Japan) was used to determine chlorophyll content of the leaves. For imaging, JPEG pictures of the plain leaves were taken using a Sony DSC F707 digital still camera (Sony Corp., Tokyo, Japan). For image analysis, Sigma Scan Pro Vers. 5.0 (SPSS Inc., Chicago, IL, USA) software and a Visual Basic macro (Karcher and Richardson, 2005) were utilized. For each leaf image, an average R (red), G (green) and B (blue) value (RGB color model) as well as H (hue), S (saturation) and B (brightness) values (HSB color model) were calculated from the total leaf area. After harvest, near-infrared reflectance spectroscopy (NIRS) using a Bruker Matrix-I Fourier-Transform NIRS instrument and OPUS spectroscopy software (Bruker, Ettlingen, Germany) was applied to determine seed quality parameters on finely ground samples. Soybean seed protein and oil content were then expressed in g per kg on a dry matter basis.

Quantitative experimental data were collected and analysed according to the respective experimental design, i.e. as randomized block design for the 2008 experiment, augmented design in 2009 and generalized lattice design in 2010, respectively. PLABSTAT software (Utz, 2005) and the SAS statistical software package and its procedures (SAS, 1988) were used at different stages of statistical data analysis.

RESULTS AND DISCUSSION

In the nodulating vs. non-nodulating experiment (2008), clear differences in leaf colour were visible from the early flowering stages onward, which was due to differences in the availability of nitrogen and subsequent chlorophyll synthesis (Fig. 1). In the 2009 and 2010 experiments dealing with nodulating genotypes only, colour differences were also visible but less pronounced than the ones illustrated in Fig. 1.

Fig. 1 Soybean leaflets from non-nodulating (left) and nodulating (right) plants differing in chlorophyll content

In image analysis of soybean leaf pictures, picture segmentation was easily achieved when using the HSB colour space on leaf images taken on a red background and hue values between 33 and 110, as shown in Fig. 2.

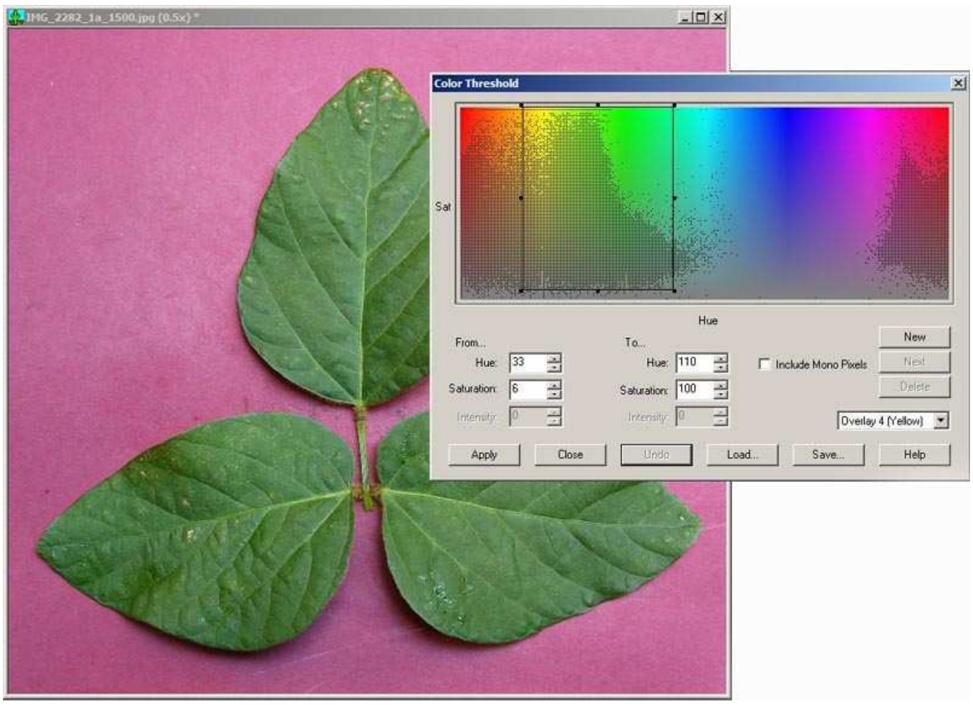

Fig. 2 Setting of colour thresholds (HSB colour model) for image segmentation in Sigma Scan Pro

The results of the the nodulating vs. non-nodulating experiment (2008) were clearly indicating the effects of biological nitrogen fixation on various characteristics of soybean crops. As summarized in Vollmann et al. (2011), nodulating soybean lines had a significantly larger leaf size and higher chlorophyll content at full flowering stage, later maturity, greater plant height, higher number of pods, increased 1000-seed weight and protein content, and lower oil content as compared to non-nodulating lines. Moreover, the development of leaf chlorophyll content over time was also depending on the nodulation type (Fig. 3): it was higher and had a later peak in nodulating than in non-nodulating lines. These findings underline the dynamics and close relationship between N and C accumulation in soybean (Sinclair, 2004; Zhou et al., 2006). As a consequence, leaf chlorophyll content of lines as well as S (saturation, HSB colour model) or G (green, RGB colour model) values at full flowering stage were highly correlated to seed protein content at maturity (Fig. 4). This is in correspondence with the peak and time course of nitrogen uptake in nodulating soybeans as reported by Zapata et al. (1987) which suggests that seed protein content or other N-related traits could be predicted from SPAD values or image analysis data of leaf images taken at the full flowering stage.

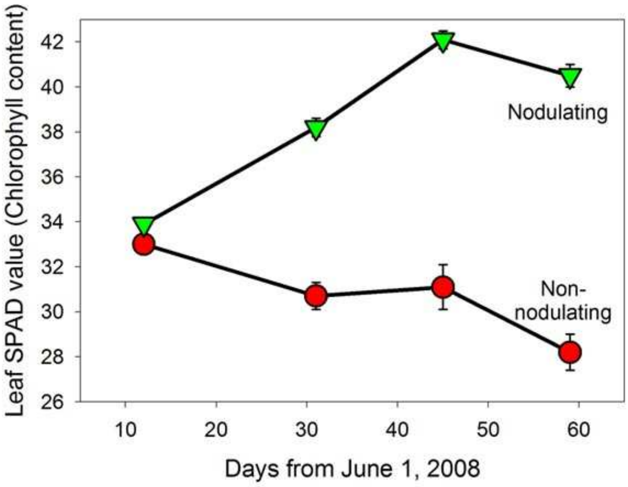

Fig. 3 Development of soybean leaf chlorophyll content (SPAD-value) of nodulating vs. non-nodulating lines over a period of seven weeks from the late vegetative growing stage through flowering and up to the beginning of seed filling (bars: standard error of means)

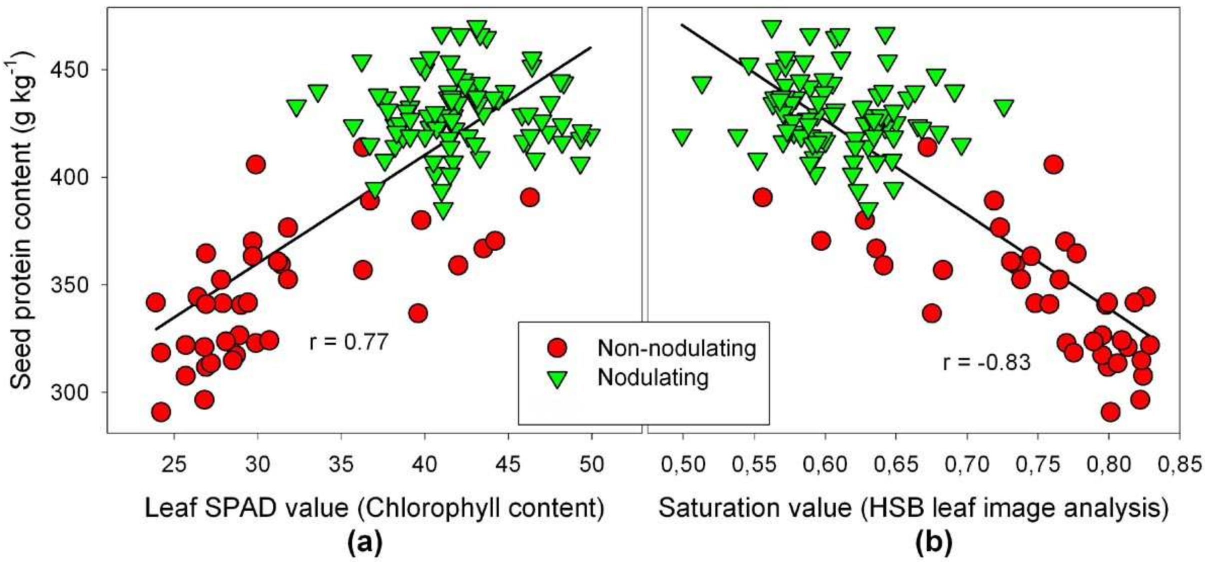

Fig. 4 Relationship between soybean leaf chlorophyll content (SPAD-value) or saturation value at full flowering stage and seed protein content at harvest

The results reported above were largely confirmed by the findings in the 2009 and 2010 experiments carried out with nodulating breeding lines exclusively. In both of these experiments, SPAD values were positively (r = 0.29 to r = 0.41) and leaf G (green) values were negatively (r = -0.34 to r = -0.42) correlated to seed protein content (see Fig. 5 as an example) across genotypes from different crosses. While correlations were of the same direction as in the 2008 experiment, they were of lower magnitude in 2009 and 2010. This is probably due to the fact that in nodulating genotypes differences in leaf chlorophyll content are due to quantitative variation in nitrogen fixation as well as to other quantitative differences between genotypes while the relationship was much more pronounced due to qualitative variation (nodulation vs. non-nodulation) in N fixation for the 2008 experiment.

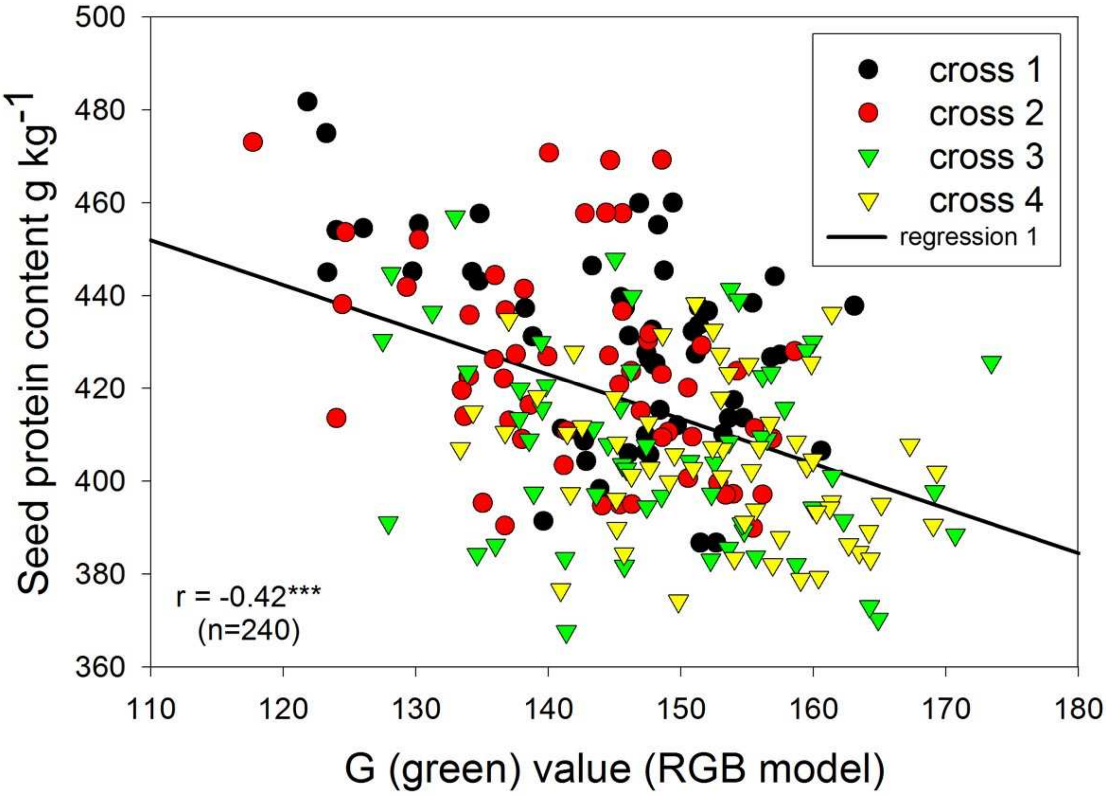

Fig. 5 Relationship between soybean leaf G (green) value (image analysis) at full flowering and seed protein content in lines derived from four different crosses (2010 experiment)

CONCLUSIONS

Soybean biological nitrogen fixation and photosynthesis parameters such as chlorophyll content are closely related. Consequently, leaf chlorophyll content at the full flowering stage can serve as an indicator for the quality of nitrogen fixation. Therefore, chlorophyll content could be utilized for predicting seed quality parameters such as seed protein content, which is of particular interest to food-grade soybean production. Moreover, these findings could be utilized for optimizing biological nitrogen fixation for high quality crop production as well as in selection of soybean genotypes with high efficiency of nitrogen fixation.

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