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Rapid assessing of water and nitrogen status in wheat flag leaves

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Abstract

In semiarid regions, moisture supply is the most important uncontrollable factor influencing the effects of nitrogen (N) applications on wheat yields, protein content and test weight. The major effect of increasing N is to increase protein, but excessive N encourages the crop to outgrow its moisture supply, resulting in depressed crop yields and test weights. In contrast, yield may respond to N applications under abundant moisture, but protein increases occur only at very high rates. This study tested the use of near infrared transmittance (NIRt) and near infrared reflectance (NIRr) spectrometry for monitoring water and N contents in flag leaves at heading of spring wheat growing in a semi-arid region during three growing seasons (2000 to 2002). Four optical devices were compared: Minolta's SPAD-502 that measures transmittance at two wavelengths (650 and 940 nm); Ocean Optics' S2000 that measures transmittance spectra (530-1100 nm); Licor's Li-1800 spectroradiometer equipped with an external integrating sphere, that measures reflectance within the range of 400-1100 nm; and Foss NIR System's model 5000 that measures reflectance within the NIR region (1100-2498 nm). Each set of measurements was analyzed with the Foss WinISI® II software. Leaf water and nitrogen contents were calibrated with the whole spectra, with no restriction to specific vegetation indices. It was found that the SPAD values were dependent on water supply and were not correlated to water content. Reflectance was found to be an accurate indicator of water and nitrogen contents in the flag leaf. Best results for water and nitrogen were obtained by calculating the first and second derivatives, respectively, of the reflectance spectra in the 1100-2498 nm spectral region. Calibration accuracy was high and restricted only to standard error of 4 g kg^{-1} for cross validation of the water content and 1.4 g kg^{-1} for the nitrogen content. Validation procedure resulted standard errors of prediction of 9-11 g kg^{-1} and 2-2.5 g kg^{-1} for the water content and nitrogen content respectively. By providing a new tool for precision management of growing wheat we anticipate that these results will help decision-making regarding early hay harvest or will enable grain protein concentration to be enhanced. Both of these outcomes are important for increasing farmers' income and improving the quality of wheat yields in semi-arid regions.

Key words: Crop monitoring, fertilizing, flag leaf, multivariate analyses, near infrared, nitrogen, precision agriculture, stress, water, wheat.

Introduction

In semi-arid regions, water supply is the most important uncontrollable factor influencing the effect of nitrogen (N) applications on the yields, protein contents and test weights of wheat. The ability to monitor the dynamics of wheat situation during the course of the growing season by means of visible and near infrared (NIR) reflectance could lead to a considerable improvement in the management and economics of the crop. Environmental concerns have been aroused by problems such as contaminated aquifers and soil salinization caused by intensive use of fertilizers, and such improper irrigation management could be reduced by monitoring the precise condition and requirements of the crop. Insufficient water during the grain filling stage may restrict grain yields, and drought often prevents starch accumulation so that the subsequent milling can extract only a small quantity of flour; it can also inhibit protein accumulation in the grains [1]. Early N application, during tillering, mainly affects the total grain yield [2], whereas late application, at heading, mainly increases the grain protein content [3, 4]. The yield responds to base N applications under abundant moisture, but unless N top dressing is also applied, protein increases only occur at very high rates of base application. Under N-deficiency conditions, accumulation of storage proteins (gliadin and glutenin) in the seeds is limited and the resulting low protein level leads to the harvest of low-quality grains [5].

Excessive N applications prior to sowing encourage vegetative growth of the wheat, and therefore the crop water use is greatly enhanced during that growth stage [6]. The main outcome is lack of water during the grain filling stage, with the result that poor quality grain is produced [1, 7]. Furthermore, under wet conditions, excessive N fertilization can lead to leaching of nitrate that could reach the ground-water table later. Therefore, the base application should use less N than would be needed in seasons of heavy rainfall. However, even if the best decision has been taken on base N application, in case of more rain, higher biomass production would dilute the N within the plant, with the result that low-quality grain, containing low protein levels, would be harvested [8]. In this situation late top-dressing can be applied to ensure suitable protein content in the grain.

There are known to be major differences between rain-fed and irrigated wheat. In the case of irrigated wheat an N top dressing can be applied, regardless of the wheat growth stage, and application efficiency is relatively high [9-11]. However, N application to rain-fed wheat depends on rain as the carrier, especially if the N was applied as a solid formulation, in order to prevent the damage to flag leaves that can occur after liquid N application. In the test area, the amount of rain that fell later than the wheat heading stage varies between zero and more than 100 mm, therefore it could serve as a carrier for late N application. However, the N deficiency must be determined. Several procedures, based on different devices, are already in use for assessing the N top dressing requirement; they include stem nitrate detection [12], the SPAD chlorophyll meter [13], the Hydro-N-Sensor system [14] and the Green-Seeker (http://www.greenseeker.com). However, the results of these procedures and their interpretation depend on many factors (e.g., water content and cultivar). Moreover, water rather than N is the dominant factor affecting dryland crop yields [15]. There is a good correlation between SPAD values and N content, but a clear difference can be noticed between the values obtained in irrigated and non-irrigated plots [16]. Consequently, these devices need a within-field reference, and the farmer must decide which part of the field provides the best reference for specific growth conditions. Moreover, the devices supply only N data, and ignore water status, which is very important for late application decisions. In the light of the above complications and limitations, the development of new methods is worthwhile.

Traditional analytical methods for detecting ecological abuse, such as wet chemistry, are very time consuming and expensive. A technology that would characterize the nutritional status of growing plants in a timelier manner (preferably in real-time as an applicator moves through the field) is needed to control the volume of amendments and to ensure precise crop management. A developing technology for site specific management farming involves multi-spectral remote-sensing systems that produce visible and near infrared images, carried by aircraft or satellites. These systems are used to monitor spatial and temporal changes in growing crops [17-23]. The data collected can be processed, geo-referenced, mapped and analyzed with the help of image processing and geographic information systems software, to provide additional data to analysts and decision makers. Vegetation indices, which are commonly used in remote sensing for assessing the state of crops, are numerical measures generated by reducing data from multispectral observations to a single value. They are designed to take advantage of the spectral signature of live green vegetation and thus to enhance the vegetation signal in remotely sensed data. Many studies have shown correlations between the normalized difference vegetation index (NDVI) or the Green NDVI, and the crop biomass and crop yield [23-25]. Remote sensing can inexpensively provide large-area estimates of N status and can be used to monitor the N status. Other studies have determined correlations between vegetation indices and crop N status and have used these data in building devices that apply topdressing N [26-29], and others estimated wheat water status [19, 30]. Only few data are available on the combined effect of N and water stresses on reflectance spectra [31-34].

Nevertheless, several questions may arise when independent devices and data retrieval procedures at the wheat heading stage are being considered. Should we apply nitrogen at heading? Can wheat utilize this late N? Alternatively, could hay/silage be a better end use? In order to achieve further improvements in grain yields and quality, and in adaptation to a range of dryland systems, wheat production requires a simple precise monitoring device for both crop water and crop N. The present study extends the exploration of the potential of NIR spectrometry for measuring water and nitrogen in flag leaf. The focus was on a new improved method for predicting water, N and their stress-interaction status in wheat plants at heading, under various environmental conditions.

Materials and Methods

The research was based on long-term fixed experimental plots located in the Gilat Research Center, Israel[7]. Hard red spring wheat (*T. aestivum*) cv. Galil (Hazera Genetics) was sown in all experiments. This study examined wheat growth under various crop management systems: rainfed plots as well as plots that received supplemental irrigation; soil tillage and mulch; rotation; and fertilization with nitrogen (at 0, 50, 100 and 150 kg ha^{-1}) and phosphorus (at 0 and 10 kg ha^{-1}). The fertilization treatments were established at 1975 as base applications. During this study (over three seasons, 2000 to 2002) we maintained four N application rates but modified them to 0, 50, 50 + 50 and 100 + 50 kg ha^{-1}, with the 3rd and 4th application rates divided between base and top dressing at heading, respectively. These plots comprise many sub-plots that vary every year in wheat production, protein yield, and test weight. In 2002 another experimental field that had six N treatments – N at 0/0; 0/50; 50/0; 50/50; 100/0; and 100/50 kg ha^{-1} for base/early top dressing, respectively – was tested as well. Several subplots in all treatments received an additional N 50 kg ha^{-1} at heading. From both experiments 344 sub-plots were analyzed.

NIR spectrometry (transmittance and reflectance) methods were evaluated for monitoring the water and N contents of spring wheat flag leaves during heading. Four optical instruments were used: Minolta's SPAD-502, that measures transmittance at two wavelengths (650 and 940 nm); Ocean Optics' S2000 that measures transmittance spectra in the range 530-1100 nm; Li-COR's LI-1800 high spectral-resolution spectroradiometer, equipped with an external integrating sphere (1800-12S), that measures reflectance within the range 400-1100 nm; and Foss NIR System's Model 5000, that measures reflectance within the NIR region (1100-2498 nm). From each sub-plot we brought four tillers that had been cut between leaves minus one and minus two, to the laboratory. Since the nitrogen content varies along leaf sheet, the samples used were 4 cm long and were taken from the centers of the blades of the four flag leaves. All SPAD, spectra, water and nitrogen content data were collected from these samples. The average spectra of four leaves from each plot were used in the analysis. All devices, except the SPAD, were operated at a spectral resolution of 2 nm. With the first three devices spectra were taken with the leaves held in the leaf holder; with the Foss-NIRS instrument the leaf was located on a centering device with a round hole (scanning area ~6.6 cm^2) and covered with a gold reflector (usually used for liquid test). In parallel, water (calculated from the fresh weight and dry weight, after 48 h at 70°C) and N (micro-Kjeldahl, digestion of the whole sample (~100 mg) without milling) contents of the same leaves were determined in the laboratory [35].

All samples were used for calibration of the SPAD and Ocean Optic data. The LiCOR spectroradiometer was used only during the last two years, and analysis with it was based on 257 samples. Because of the experiment conditions, the first-season samples lost water prior to measurement in the Foss-NIRS instrument; therefore they were excluded from the water calibration, and the possibility of storing the leaf samples so that they would retain their water and remain straight and flat was tested. It was found that storing the leaf samples at 4°C in a simple small plastic bag that was folded several times was an adequate procedure. However, in spite of that procedure, 30 samples were removed from the analysis in the last season, since they lost some water during the measurements. There were also a few samples that lost nitrogen data. Thus, the calibration was based on totals of 245 and 341 measurements for water and N, respectively. Descriptive statistical analysis and simple correlations were performed with the SAS statistical package. Spectra data analysis was performed with a modified partial least squares (MPLS) regression by means of the Foss WinISI® II software, on the absorbance log(1/R) or log(1/T), wavelength interval of 2 nm, for each of the three tested instruments. All spectral data points were used for calibration with the leave-one-out and random subsets, cross-validation procedure, and the first or second derivative of log (1/R) was used with a gap of four data points. For random subset analysis, calibration data included 1/2 or 2/3 of the comprehensive data, and the left parts were used for validation set. In all calibration analysis, outliers were rejected using the software threshold default values.

Results and Discussion

Table 1 presents the values of the water and nitrogen contents of the flag leaves, which were calibrated by spectral measurements; it also presents the SPAD values. Overall, wide ranges of water and N contents were available, providing a considerable amount of data for analysis. These wide ranges were mainly associated with the long-term fixed experiment plot, whereas in the field added in 2002 there were only relatively small differences in leaf water and N contents between treatments. Considerable correlation was found between the SPAD values and leaf nitrogen contents (Fig. 1a). However, a clear difference can be seen between the correlations for the irrigated and the non-irrigated plots. In agreement with previous findings [16], our results show that the SPAD values were dependent on other parameters than on N content. Therefore, SPAD can be used for nitrogen estimation only by using a within-field reference. Moreover, SPAD data have no correlation with water content (Fig. 1b), which emphasizes the need for other sources of data rather than the SPAD.

Spectral analysis can be used to evaluate plant water and nitrogen contents as well as bio-physiological parameters. The reflectance spectra had significant peaks in the green (550 nm) and NIR (around 800 nm) regions, and a dip in the red (680 nm). The transmittance spectra showed a similar pattern except for the presence of a dip in the NIR region, probably caused by screening or saturation in particular sensor. Reflectance spectra in the NIR region exhibited two main dips (around 1400 and 1900 nm) that are related to absorption by water. Although the spectral patterns were similar in all leaves, part of the variation can be related to differences between years or between plots, that significantly affect the water content.

Several procedures were used to calibrate spectra data against laboratory data. Best results for water and nitrogen were obtained by using the first and second derivatives, respectively, of the reflectance spectra in the 1100-2498 nm spectral region (Table 2). Calibration accuracy was high and was limited only by the standard errors of 4 and 1.4 g kg^{-1}, respectively, for cross-validation of the water and nitrogen contents.

The calibration coefficients show that there are a few wavelengths at which reflectance is correlated with water content, in addition to the known reflectance regions from water, i.e. 1460 and 1910 nm (Fig. 2). As expected, there were some outliers, but in negligible numbers. The calibration coefficients for nitrogen show a similar picture (Fig. 2). Again, the spectral range covered includes reflectance regions in addition to those whose properties are known to be determined by the nitrogen bond. The correlation between predicted and observed data is slightly less strong than that for water (Table 2).

Representative results for the best method found (NIRr 1100-2498) of random subset spectral analysis is presented in Table 3. In this case, similar R^2, SEC and SECV were obtained for leave one out method (Table 2) and random subset (Table 3). Figs 3 and 4 show the correlation between predicted and lab measured water and N content data, respectively. Looking at the calibration correlation coefficient, SECV and SEP of random validation subsets shows similar values in comparison with the leave one out method (Table 2 and 3). Only 2 to 3 outliers could not be correctly predicted, and these outliers have been identified by the software. This finding emphasizes that the leave one out method based on the comprehensive data is adequate, and can be applied for future use.

Calibration with the other spectra regions yielded lower correlations, but in some cases they were acceptable (Table 2). However, in spite of the high correlations, their standard errors (SEC and SECV) were much higher than those obtained in the initially used regions, which resulted in inferior predictions by

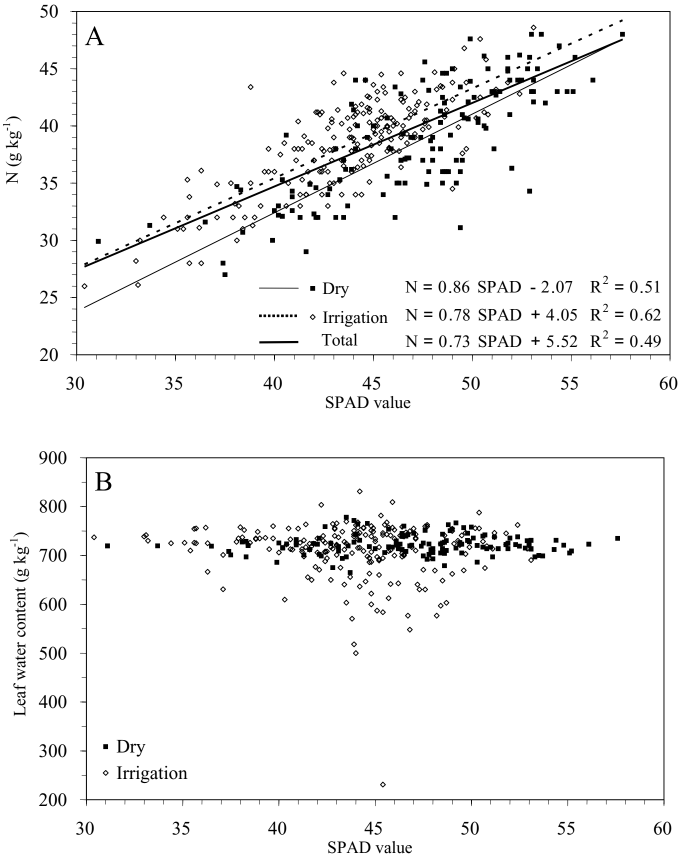

Figure 1. Correlations between SPAD values and nitrogen (A) and water (B) contents in the central part of spring wheat flag leaf at heading. Black squares represent rain-fed wheat plants; open diamonds represent plants that received supplemental irrigation.

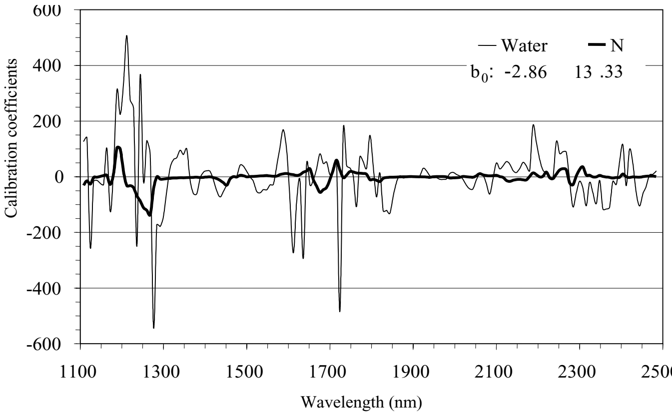

Figure 2. Calibration equation coefficients calculated for water and nitrogen contents in wheat flag leaf, that were estimated from their reflectance spectra (Foss NIR System model 5000). Calculations were conducted by 1stD log(1/R) of 1100-2498 nm, using the MPLS procedure.

these calibrations. It could be that extending the data set used for calibration would improve this calibration. Since equipment that measures reflectance of this region is less expensive – including remote sensing of data via satellites – it is important to note that this route for receiving spectral data is also open. There is a strong correlation between water content and leaf reflectance in the NIR region, which is explained by the effect of water content on the cell wall-air interaction [36]. Possible explanations for the lower ability of NIR reflectance spectroscopy to determine N content than water content are: screening by the high absorption by water [37] and/or low contents of various nitrogen forms that lie below the detection threshold.

Various vegetation indices are known to be potentially applicable in remote sensing. They include the well known Normalized Difference Vegetation Index (NDVI, R_{NIR}-R_{red}/R_{NIR}+R_{red}); the Green-NDVI ($R_{NIR-}$$R_{green}$/$R_{NIR+}$$R_{green}$) that replaces the red with the green region of the spectrum in the NDVI equation; the Normalized Different Greenness Index (NDGI, $R_{green-}$$R_{red}$/$R_{green+}$$R_{red}$), the Water Index (WI, R_{970}/R_{900}) and others. As all the indices are based on only a few wavelengths, the data they yield are limited and they do not extract all the information contained in the whole spectrum. Loss of those extra data reduces calibration efficiency (not shown).

The present study was based on several experiments, but all of them used the same wheat cultivar. Thus, a possible global calibration that would enable all cultivars to be predicted must include leaves of all varieties. In the next study we would like to attempt to obtain spectra of several cultivars by means of remote sensing instead of in the laboratory, as well as to improve the calibration of VIS-NIR spectra.

The satisfactory results of the present study indicate that NIR reflectance spectroscopy can be a fast alternative to other methods of water and N determination. The significance of this finding is that within two to three minutes after starting to analyze the flag leaf, reasonable estimates of its water and N status can be obtained, and these, of course, correlate with their contents in the whole plant. Moreover, our calibration is independent of the field in which it is made; therefore it could be used for every field or plant, without needing a within-field reference.

Table 1. Water and nitrogen composition of wheat leaves used in NIR transmittance and reflectance studies, and their SPAD values. Each sample represents an average of 4 different flag leaves (4 cm long taken from the central leaf blade).

	Year	Samples	Minimum	Maximum	Mean	Std. dev
Water (g kg^{-1})	2000	69	230.8	831.1	662.2	84.4
	2001	143	665.0	778.0	718.5	16.5
	2002	132	675.1	771.7	739.0	17.7
	total	344	230.8	831.1	715.1	49.3
Nitrogen (g kg^{-1})	2000	69	34.9	48.6	40.4	2.8
	2001	142	26.0	48.0	37.4	4.8
	2002	130	26.1	47.6	39.0	4.3
	total	341	26.0	48.6	38.6	4.4
SPAD value	2000	69	36.3	53.1	45.1	2.9
	2001	143	30.4	57.6	45.1	5.4
	2002	132	31.1	53.1	45.4	4.6
	total	344	30.4	57.6	45.2	4.7

Table 2. Calibration results from leave-one-out cross validation with the MPLS procedure by WinISI® II software.

	Spectra	Derivative	SEC g kg^{-1}	R^2	SECV g kg^{-1}	Terms No.
Water	NIRt 530-1100	1stD	8.07	0.673	8.47	4
		2ndD	8.64	0.632	9.00	3
	NIRr 400-1000	1stD	11.80	0.942	13.26	6
		2ndD	12.06	0.938	14.56	6
	NIRr 1100-2498	1stD	3.30	0.956	3.95	11
		2ndD	3.90	0.946	4.39	7
Nitrogen	NIRt 530-1100	1stD	3.29	0.395	3.32	4
		2ndD	3.16	0.402	3.21	4
	NIRr 400-1000	1stD	2.79	0.697	3.03	4
		2ndD	2.66	0.711	2.94	4
	NIRr 1100-2498	1stD	1.32	0.891	1.39	7
		2ndD	1.26	0.892	1.40	6

Conclusions

In summary, the ability to monitor changes in the growth conditions of wheat plants by observing changes in their reflectance in the visible and NIR spectral ranges, could lead to a considerable improvement in crop management. In the present study we demonstrated that leaf water and N contents affect the spectra of the leaves, and these could be calibrated and predicted. This could be done with both Licor's Li-1800 spectroradiometer and Foss NIR System's model 5000, however, our results show that the Minolta's SPAD-502 and Ocean Optics' S2000 (transmittance) are significantly inferior and might not be appropriate for further application. Reflectance spectra can give accurate indications, in real time, of water and nitrogen contents in the flag leaf. Once these flag leaf data are available they can be used to support agronomic decision-making.

Acknowledgments

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Table 3. Calibration results from for random subset analysis, calibration data included 1/2 or 2/3 of the comprehensive data, and the left parts were use for validation set.

	Calibration subset size	Derivative	Calibration SEC g kg^{-1}	R^2	SECV g kg^{-1}	Terms No.	Validation SEP g kg^{-1}	Bias g kg^{-1}	R^2	R^2 outlier*	SEP outlier*
Water	1/2	1stD	3.36	0.963	3.74	6	10.95	54.52	0.673	0.912 (3/172)	5.29
		2ndD	3.07	0.962	3.86	7	10.57	51.79	0.695	0.915 (3/172)	5.20
	2/3	1stD	3.32	0.944	4.22	10	9.30	84.43	0.792	0.932 (2/129)	5.10
		2ndD	3.76	0.920	5.10	7	9.20	98.19	0.796	0.940 (2/129)	4.76
Nitrogen	1/2	1stD	1.25	0.887	1.38	6	2.01	-0.65	0.795	0.877 (3/172)	1.63
		2ndD	1.46	0.854	1.57	5	2.13	0.89	0.799	0.845 (3/172)	1.82
	2/3	1stD	1.30	0.906	1.35	7	2.40	9.91	0.642	0.856 (6/129)	1.53
		2ndD	1.28	0.911	1.32	5	2.45	8.41	0.627	0.826 (6/129)	1.69

* Correlation coefficient was calculated on the validation set after deleting (2-6) outliers samples.

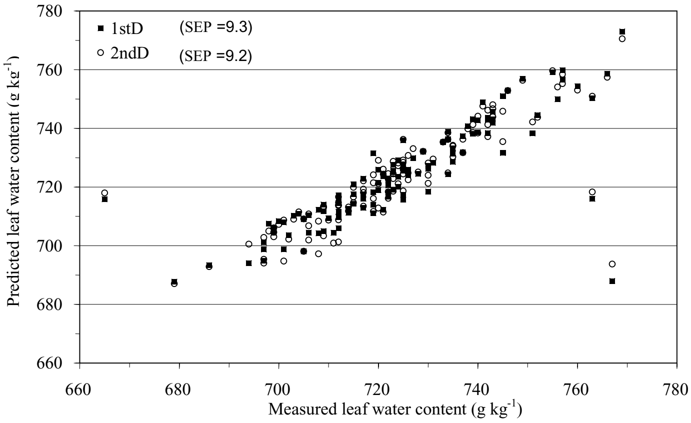

Figure 3. Validation results for flag leaf water content, based on 50% of the comprehensive data, that were left for validation set, (after rejection of samples that had dried prior to spectra collection). Calibrations were conducted by 1stD and 2ndD log (1/R) of 1100-2498 nm, using the MPLS procedure.

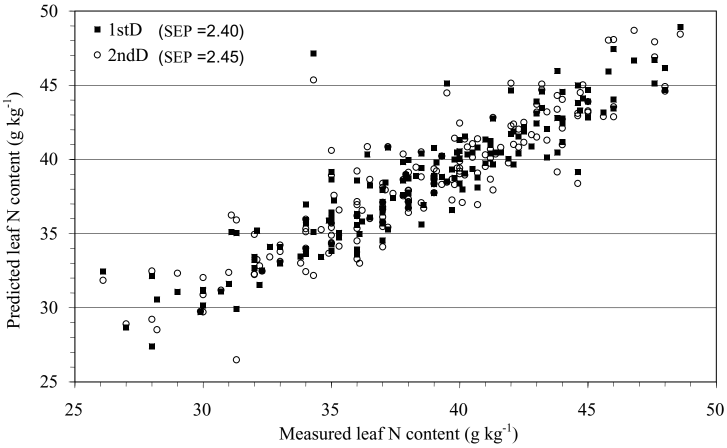

Figure 4. Validation results for flag leaf nitrogen content, based on 50% of the comprehensive data that were left for validation set. Calibrations were conducted by 1stD and 2ndD log (1/R) of 1100-2498 nm, using the MPLS procedure.

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