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A comparison of the methods used to assess the nutritional status of selected crop species

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Abstract: Various devices and applications are used for the rapid assessment of plant nitrogen nutrition, which give an approximate indication of leaf chlorophyll saturation by giving the relative chlorophyll content or leaf greenness intensity. In this study, chlorophyll content and leaf greenness determined by three devices were compared: SPAD-502 (spectrum technology), Hydro N-Tester, and Samsung smartphone (RGB app). Additionally, laboratory determination of chlorophyll content was compared to soil-plant analysis development (*SPAD*) values. Based on the results obtained, indices characterising the vegetative or direct state were calculated and the values obtained with these devices were compared. The crops tested were soya, potatoes, wheat and sunflower. The results show a close relationship between the size of the *SPAD* index and RGB light sources of colour the intensity of red (R), green (G) and blue (B). The indices I_{PCA} and $R+G-2B$ showed a very high negative correlation with *SPAD* readings (−0.82 and −0.83). Statistical analysis showed that *SPAD* readings obtained from the two chlorophyll meters showed a high correlation regardless of the crop species tested (R^2 = 0.98). The correlation analysis also showed the possibility of substituting equipment and vegetation indices based on readings taken with a smartphone, with an accuracy not much inferior to standard chlorophyll meters. This situation could occur in case of failure or absence of the standard device.

Keywords: chlorophyll meter, N-Tester, soil-plant analysis development, SPAD-502, *SPAD* index, *SPAD* measurement

INTRODUCTION

Chlorophylls (Chl *a* and Chl *b*) are the most important pigments in leaf chloroplasts and are practically essential for the aerobic conversion of light energy into stored chemical energy that feeds the biosphere. From a physiological point of view, the chlorophyll content of leaves is therefore a parameter of great importance and varies both between and within species. Leaf pigmentation is important to both farmers and physiologists for several reasons: (i) the amount of solar radiation absorbed by a leaf is largely a function of the concentrations of photosynthetic pigments in the leaf, so low Chl concentrations can directly limit photosynthetic potential and thus primary production (Curran, Dungan and Gholz, 1990; Filella and Penuelas, 1994; Richardson, Duigan and Berlyn, 2002); (ii) chlorophyll is high in nitrogen, so quantifying leaf Chl content gives an indirect measure of the nutritional status of plants with this macronutrient (Filella and Penuelas, 1994; Moran *et al.*, 2000; Richardson, Duigan and Berlyn, 2002); (iii) pigmentation is directly related to stress physiology, as carotenoid concentration increases and Chl amount decreases under stress and during plant ageing (Peñuelas and Filella, 1998; Richardson, Duigan and Berlyn, 2002).

The amount of Chl in a leaf is usually expressed as concentration (i.e. mg Chl·g^{-1}) or content (i.e. mg Chl·cm^{-2}). Traditional chemical methods for measuring Chl require its extraction in a solvent, followed by spectrophotometric determination of absorbance through the chlorophyll solution and conversion of absorbance to concentration using standard published equations (e.g. Arnon (1949) and their modifications). However, chemical extraction, considered the standard method for Chl determination, requires destructive sampling (which precludes, for example, developmental studies of individual leaves) and is relatively time-consuming.

In recent decades, researchers have emphasised the need for more efficient use and recycling of nitrogen in agro-ecosystems. Wang *et al.* (2018) found that increased use of nitrogen and phosphorus fertilisers can have a strong impact on the diversity, composition and functioning of natural ecosystems through eutrophication of freshwater and marine ecosystems, which can lead to loss of biodiversity and shifts in food chain structures. Increasing the efficiency of nitrogen fertilisation is therefore a priority in crop production. It reduces the leaching of nitrates into groundwater and decreases nitrogen oxide emissions into the atmosphere through better utilisation of fertilisers given to plants at the correct rate. Non-destructive optical methods determining the so-called leaf greenness index, based on the absorption and/or reflection of light by an intact leaf, are increasingly used in agricultural practice to precisely determine the nutritional status of nitrogen and the size of the second and third post-harvest nitrogen application. Optical methods read the value of the "chlorophyll index", which expresses its relative content. It is not the absolute Chl content per unit leaf area or concentration per gram of leaf tissue that can be obtained by destructive methods. Optical methods do not destroy plant material, are very fast and can be applied directly in the field (Markwell, Osterman and Mitchell, 1995; Gamon and Surfus, 1999).

Chlorophyll content measurements are frequently represented by soil-plant analysis development (*SPAD*) values, which have become essential tools for assessing plant conditions (Zhang *et al.*, 2022). Variables that affect SPAD meters include the surrounding environment, leaf age, species-specific characteristics, and nutrient availability (Swoczyna *et al.*, 2022). A variety of instruments operating on a similar principle (SPAD-501, SPAD-502, SPAD-502Plus by Konica Minolta and commercially available sensor system Hydro N-Tester by Yara International ASA) are used for direct readings. The values presented on these devices are not the same, but comparable, and the difference is due to the different values of the constants taken as a calibration factor. However, these devices do not directly show the Chl *a* and *b* content and their sum, but only their relative content expressed in dimensionless units. These devices work by measuring the transmission of red and infrared light (690 nm and 940 nm) through the leaves. They can be used not only to determine the level of constant and variable post-emergence application rates, but also to calibrate radiometers – devices for the remote sensing determination of variable fertiliser application rates, such as Hydro N-Tester for N sensor ASL. Furthermore, in the scientific literature, the application of the results obtained to yield forecasting is reported. Many researchers have attempted to determine the relationship between the results obtained from different instruments in order to substitute or complement them. However, a disadvantage of hand-held chlorophyll measuring instruments, such as the SPAD-502, in assessing crop condition is the small sampling area (6 mm^2) (Wang *et al.*, 2014). In addition, readings are subject to operator error. Therefore, a large number of repetitions are needed to obtain reliable results (Blackmer and Schepers, 1995; Rorie *et al.*, 2011), and the SPAD meter has difficulty distinguishing chlorophyll levels when plants are close to or above the optimum N rate (Zhang *et al.*, 2008; Lin *et al.*, 2010).

As an alternative to readings with the hand-held instruments described above, images from digital colour cameras that capture spectral information of the visible bands can be used. These cameras have a low price but offer very high image resolution (consumer cameras capture up to 40 megapixels (Mpx) per image, and cameras used in mobile phones capture between a dozen and several dozen of megapixels per image). As reported by Li *et al.* (2010) and Scharf and Lory (2020), at a sensor height of about 1 m above the plants, high spatial resolution images separate the crop from the background soil or other elements. This is important for accurate diagnosis of N status when the vegetation fraction was low. In addition, digital camera images contain a large amount of information about crop structure and leaf colour, such as plant orientation and height, biomass accumulation and leaf ageing (Berger, Parent and Tester, 2010; Golzarian *et al.* 2011; Fanourakis *et al.*, 2014). Digital colour images provide spectral information in the visible bands, which are closely related to leaf N concentration (*LNC*) and *SPAD* readings (Rorie *et al.*, 2011). Colour-related indicators from digital cameras can diagnose crop N status (Pagola *et al.*, 2009; Rorie *et al.*, 2011; Hunt *et al.*, 2013). However, results obtained with controlled light cannot be fully applied to natural light due to variable light conditions (Noh *et al.*, 2005; McCarthy, Hancock and Raine, 2010; Sakamoto *et al.*, 2012). Besides, there are still many uncertainties in the use of digital cameras for N diagnosis under natural light conditions and further validation is needed to ensure field application (Wang *et al.*, 2014).

The aim of the study was firstly to compare Hydro N-Tester and SPAD-502 DL chlorophyll meter readings on four selected plant species and to compare *RGB* colour intensity readings and *SPAD* readings, as well as to determine the relationship between chlorophyll meter readings and Chl content determined spectrophotometrically.

MATERIALS AND METHODS

METHODOLOGICAL ASSUMPTIONS OF THE STUDIES

The study was carried out at the Experimental Station of the Agricultural University in Kraków. Four plant species were included: soybean, maize, wheat and sunflower. The species were sown at optimum dates, using standard agrotechnics, taking into account the average level of fertilisation. Three weeks before flowering stage of each species required measurements were carried out.

The study involved three single experiments in order to compare: 1) soil-plant analysis development (*SPAD*) readings obtained from SPAD-502 and traditional (laboratory) method of chlorophyll (Chl) content, 2) *SPAD* readings and *HNT* readings obtained from Hydro N-Tester, 3) *SPAD* readings and *RGB* colour readings from the mobile application "ON-ColorMeasure", using a smartphone camera under natural conditions. The use of smartphones was examined with access to free apps to measure *RGB* (red, green and blue respectively) colour intensity and determine their correlation with leaf greenness index readings. Furthermore, the Chl content of soybean leaves was determined and the correlation between this trait and *SPAD* readings was calculated. This article attempts to evaluate the non-destructive absorbance method, the reflectance method and chlorophyll extraction under laboratory conditions.

COMPARISON OF *SPAD* READINGS AND CHLOROPHYLL CONTENT IN PLANT LEAVES

First experiment was dedicated to soybean. The aim was to compare the *SPAD* readings obtained from SPAD-502 with the values obtained from traditional methods (Arnon, 1949) of Chl content determination in plant leaves. In soybean plantations, 15 plots (10 m^2 area) were randomly selected. At each plots, readings were taken on soybean plants at 30 leaves. The readings were averaged and the leaves immediately after measurement were picked, weighed and stored at 0°C for further Chl content studies.

Laboratory determination of chlorophyll content. Determination of Chl *a* and *b* content was carried out on stored leaves. Scrapings of all harvested leaves were cut with a surgical scalpel, excluding leaf nerves. After excision of the leaf scrapings, the leaf area was measured using a LI-3100C area meter (LI-COR Biosciences). The pigments were then extracted from all 15 leaf scrapings with 80% acetone by crushing part of the leaves with a small amount of sodium carbonate – the extracts were kept in the dark until spectrophotometric readings. Chlorophyll determination was carried out according to the method of Arnon (1949) based on the Equation (1) and (2):

$$\text{Chl } a = (12.7\text{A}_{663} - 2.69\text{A}_{645}) \cdot (V/1000) \cdot P \tag{1}$$

$$\text{Chl } b = (12.7\text{A}_{645} - 2.69\text{A}_{663}) \cdot (V/1000) \cdot P \tag{2}$$

where: Chl *a* = chlorophyll *a* (mg·cm^2 of leaf), Chl *b* = chlorophyll *b* (mg·cm^2 of leaf), A = optical density at the corresponding wavelength, V = volume of extract in cm^3, P = leaf area (cm^2).

Analysis of the extracted chlorophyll was performed using a JASCO V-730 spectrophotometer.

COMPARISON OF READINGS FROM SPAD-502 AND HYDRO N-TESTER

The second experiment aimed to compare the *SPAD* readings obtained from SPAD-502 with values *HNT* readings obtained from Hydro N-Tester. Four crop species: soybean, maize, wheat and sunflower where analysed to determine leaf greenness index. During the readings, the top side of the leaf was always directed towards the emission window of the instrument. In order to get value from Hydro N-Tester device 30 records should be done to obtain single results. On each species, readings were taken on 30 leaves per species. A total of 30 leaves were analysed per species. The values that were obtained by the chlorophyll meters have no units and express the relative chlorophyll content per unit leaf area or concentration per gram of leaf tissue (Samborski, Kozak and Rozbicki, 2006). The SPAD-502 has a measurement area of 0.06 cm^2 and calculates the index in "SPAD units" based on absorbance at 650 nm and 940 nm. The declared accuracy of the SPAD-502 is ±1.0 SPAD units (Richardson, Duigan and Berlyn, 2002).

Readings were taken on 10 samples of each species (each sample of 30 leaves) at the post-flowering stage on the following dates: wheat – first decade of June, soybean – second decade of July, sunflower and maize – first decade of August.

The SPAD-502 meter calculates the output *SPAD* value (M) according to the Equation (3) (Uddling *et al.*, 2007; Süß *et al.*, 2015):

$$M = k\log_{10}\frac{I_{0(650)}I_{(940)}}{I_{(650)}I_{0(940)}} \tag{3}$$

where: I_0 = intensity of incident monochromatic light at 650 nm and 940 nm, I = intensity of transmitted light at 650 nm and 940 nm, k = confidential proportionality coefficient (Udding *et al.*, 2007; Süß *et al.*, 2015).

The SPAD-502 and Hydro N-Tester chlorophyll meters exploits Beer's Law and the efficient absorbance of red light (650 nm) by chlorophyll. The Hydro N-Tester is equipped with two light-emitting diodes and one silicon photodiode to measure light transmission through green plant tissues at red (650 nm) and near-infrared (960 nm) wavelengths in a 6 mm^2 area (Hughes *et al.*, 2016).

The Hydro N-Tester is technically based on the SPAD-502, but the k-values differ between the instruments, one measurement is considered the average of 30 individual readings representing a leaf area of 2×3 mm, whereas with the SPAD-502, individual readings can be collected separately and averaged later (Goffart, Olivier and Frankinet, 2008; Uddling *et al.*, 2007).

This is the reason why these devices present the values in different range. The *HNT* index (dimensionless) for plant leaves ranges from 300 to 800 while *SPAD* values range between 0.0 and 100.0.

COLOUR READINGS FROM THE MOBILE APPLICATION ON-COLORMEASURE USING A SMARTPHONE CAMERA

The third experiment aimed to compare the *SPAD* readings with the values obtained from the calculation of colour indices from the ON-ColorMeasure mobile application (version 7.0 by PotatotreeSoft) installed on a Samsung Galaxy S4 I9506 phone with a 4128×3096 pixel, 13 Mpx, 30 fps camera, which detects or recognises colour using the device's camera. In the present study, the use of smartphones was examined with access to free apps to measure reed, green, blue (RGB) colour intensity and determine their correlation with leaf greenness index readings. Furthermore, the Chl content of soybean leaves was determined and the correlation between this trait and *SPAD* readings was calculated. This article attempts to evaluate the non-destructive absorbance method, the reflectance method and Chl extraction under laboratory conditions.

Previous studies on leaf colour analysis have mainly been undertaken under controlled light conditions (Tab. 1).

In order to capture the images, the camera's autofocus was set at the point where the *RGB* measurement was desired, so that the camera triggered focus at the appropriate part of the image.

Table 1. Selected examples of applications of leaf greenness measurement with the SPAD-502, Hydro N-Tester; RGB and chlorophyll content

Author	Species	Device	Interdependence
Bullock and Anderson (1998)	maize	SPAD-502	*SPAD* readings and leaf N concentration to N fertiliser rate were low but significant. The correlation coefficients for leaf N concentration with N fertiliser rate at plant stages: V7, R1 and R4 were 0.23, 0.21 and 0.20, respectively
Goffart, Olivier and Frankinet (2008)	potato	SPAD-502, Hydro N-Tester	– accuracy/precision: favourable but relatively low, medium, respectively; – sensitivity: medium but restricted to comparison of non-fertilised vs. fertilised plots; – specificity: medium and highly unfavourable; – feasibility: medium favourable
Hughes *et al.* (2016)	three tropical grass species: – *Brachiaria decumbens* hybrid: cv. 'Mulato II' (*B. ruziziensis* 9 *B. decumbens* 9 *B. brizantha*); – *Panicum maximum* cv. 'Mombasa'; – *Paspalum atratum* cv. 'Ubon'	Hydro N-Tester; solvent extractable chlorophyll analysis	the relationship between *HNT* readings and total acetone-extracted chlorophyll concentration was best described by an exponential regression model represented as: $Y = 0.119e^{0.0046x}$ (R^2 of 0.87); *HNT* readings were a strong predictor of total acetone-extracted chlorophyll concentration in all three grasses
Pacewicz and Gregorczyk (2009)	oats, barley, triticale, wheat, rye	SPAD-502, Hydro N-Tester	high efficiency of linear regression
Udlling *et al.* (2007)	wheat, potato, birch	SPAD-502, Hydro N-Tester; laboratory chlorophyll extraction	a curvilinear relationship between leaf chlorophyll concentration and *SPAD* values
Wang *et al.* (2014)	rice	SPAD-502, leaf N concentration, digital still colour camera	significant correlations were observed between *SPAD* readings, leaf N concentration (*LNC*) and 13 image colour indices calculated from digital camera images using three colour models: RGB; colour image analysis could be a simple method of assessing rice N status under natural light conditions

Source: own elaboration based on literature.

The distance between the camera and the leaves was from 0.5 to 1.0 m. No camera flash was used to avoid altering the natural lighting. Care was also taken to avoid the photographer casting a shadow on the leaves, which allowed the photo to be lit as naturally as possible. The images were taken and saved with all the detailed colour information, including red (R), green (G) and blue (B) channels, each with 256 gradations, HSV values, colour names and hexadecimal code. The average *R*, *G* and *B* values for the acquired images were calculated.

First, 30 soybean leaves were selected. On each leaf, 30 *SPAD* readings were taken at different leaf blade locations. The values were averaged for each leaf separately. The same leaves were then used to take *RGB* readings with the ON-ColorMeasure mobile app: 30 readings on each leaf to obtain variation in Chl concentration. The resulting readings were averaged. The relationship between *SPAD* readings and the presence of red (R), green (G) and blue (B) colours was then analysed in MS Excel.

RESULTS

COMPARISON OF READINGS FROM SPAD-502 AND HYDRO N-TESTER

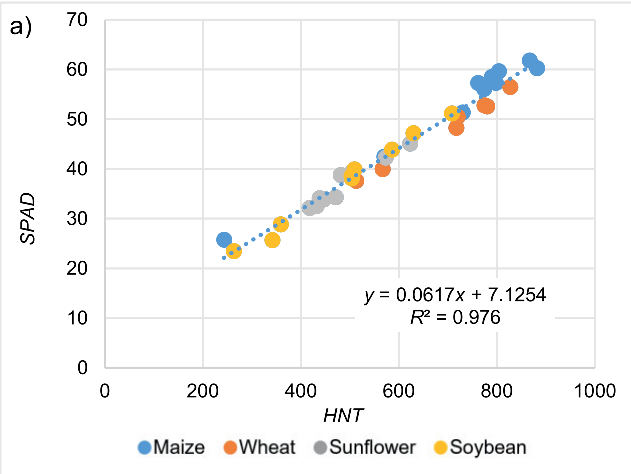
The magnitude of the leaf greenness index readings varied by species for the *SPAD* obtained from SPAD-502: for maize 25.8–61.8, for wheat 37.6–56.5, for sunflower 32.2–45.1, and for soybean 23.5–51.2, while the HNT readings obtained from Hydro N-Tester were 243–882, 513–827, 418–623 and 263–708, respectively (Tab. 2). The median and arithmetic mean were similar, with the median most often being greater than the arithmetic mean (except for sunflower). The coefficient of variation (*CV*%) of the leaf greenness index across species varied from 12.9 to 26.2, and in each case its value was greater when measured with the Hydro N-Tester device. These values were 20.9% for maize, 14.5% for wheat, 12.9% for sunflower and 25.7% for soybean, respectively (*SPAD*). The results of readings with the Hydro N-Test device for these species were 26.2, 16.6, 15.3 and 23.5, respectively. The measurement range in the experiment studied was 243–882 for the *HNT* measurement, while for *SPAD* it was 23.5–61.8 (Tab. 2). The results proved low variability for measurements taken with the SPAD-502 meter compared to Hydro N-Tester. Among the species, the lowest variability of chlorophyll (Chl) content measurement was recorded for wheat and sunflower, while it was high for soybean. This observation proved, that the reliability of measurements on soybean should be verified by other suitable devices such as the Hydro N-Tester.

Figure 1 shows the correlation between the measurement values obtained using SPAD-502 and Hydro N-Tester. The rectilinear regression equations determined by regression analysis show a high coefficient of determination and demonstrate that for the four species studied, the response of the devices is similar and proportionally they show a similar response for a change in leaf greenness.

Table 2. Descriptive statistics for leaf greenness readings using SPAD-502 (SPAD) and Hydro N-Tester (HNT) for selected four crop species (n = 10 readings × 30 leaves)

Statistics	Maize		Wheat		Sunflower		Soya		Average values	
	SPAD	HNT	SPAD	HNT	SPAD	HNT	SPAD	HNT	SPAD	HNT
Average	53.07	722.00	48.33	699.86	36.73	481.45	37.44	489.33	43.97	597.46
Minimum	25.80	243.00	37.60	513.00	32.20	418.00	23.50	263.00	23.50	243.00
Maximum	61.80	882.00	56.50	827.00	45.10	623.00	51.20	708.00	61.80	882.00
Scope	36.00	639.00	18.90	314.00	14.60	254.00	27.70	445.00	38.30	639.00
Median	57.30	782.00	50.50	720.00	34.30	471.00	38.60	504.00	45.18	619.25
SE	3.50	59.69	2.65	44.05	1.43	22.28	3.20	48.26	2.70	43.57
SD	11.08	188.76	7.00	116.55	4.75	73.88	9.61	144.77	8.11	130.99
CV%	20.9	26.2	14.5	16.6	12.9	15.3	25.7	23.5	18.5	20.4

Explanations: *SE* = standard error, *SD* = standard deviation, *CV%* = coefficient of variation.
Source: own study.



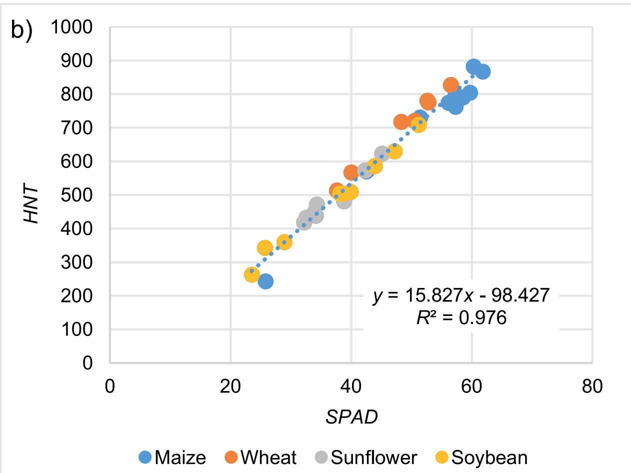


Fig. 1. Interdependence of leaf greenness index readings: a) *SPAD* vs. *HNT*; b) *HNT* vs. *SPAD*; *SPAD* = soil-plant analysis development, R^2 = coefficient of determination; source: own study

The equation in Figure 1a can be used to convert *HNT* readings to *SPAD*, and the one in Figure 1b the other way round.

Table S1 provides comparative tables in the range of 30–60 *SPAD* units and 375–852 HNT-determined units respectively. There are 8 *HNT* units per half unit indicated by *SPAD*.

COMPARISON OF SPAD-502 DL MEASUREMENT VALUES AND CHLOROPHYLL CONTENT IN SOYBEAN LEAVES

In this part of the experiment, the value of the *SPAD* parameter was determined, followed by the Chl *a* and *b* content by spectrometry. The determinations were carried out on 15 samples of soybean leaves. The average *SPAD* measurement value was 29.6 units, and the average Chl *a* and *b* contents were respectively: 0.647 and 0.228, and their sum was 0.875 mg·g^{-2} fresh leaf weight. The coefficient of variation value for the *SPAD* assay was 18.5 (Tab. 2). The range of this variability was for Chl *a* from 0.03178 to 1.38584 mg·g^{-2} fresh leaf weight and from 0.02366 to 0.49723 mg·g^{-2} for Chl *b*, respectively. The correlation between the determination of Chl content by the Arnon (1949) method and *SPAD* readings is shown in Figure 2. The relationship for Chl *a*, Chl *b* and total Chl (*a*+*b*) shows a polynomial character (rank 2) and the coefficient of determination is approximately 0.93, 0.93 and 0.89, respectively (Fig. 2).

The Chl *a* to *b* ratio has a logarithmic relationship with a coefficient of determination (R^2) of 0.51 (Fig. 3). The high coefficients of determination show that the Chl *a* and *b* content as well as the *SPAD* readings are highly correlated with each other.

The ratio of Chl *a* and *b* contents is considered generally as a ratio of 3:1 (Katayama and Shida, 1970). It was proved that the contents of Chl *a* and *b* change in their absolute amount and also in their ratio *a*/*b* under environmental conditions.

The effects of drought stress on chlorophyll and carotenoid content have been investigated in cereals. Sayed (2003) proved that drought stress inhibits Chl *a*/*b* synthesis and decreases the content of Chl *a*/*b* binding proteins, leading to reduction of the light-harvesting pigment protein associated with photosystem II.

In Figure 4, the scatter plot relating *HNT* index readings to the measured Chl *a*, Chl *b* and total Chl concentrations are presented. The plot indicates a close positive correlation between measured Chl and *HNT* indices. The derived corresponding calibration equations are presented in Figure 4. The *HNT* values used to obtain the equations were developed using the conversion values from Table 3. In addition, the Chl content was measured for leaves of soybean, and they may not accurately predict the Chl content of leaves of other species.

Table 3. Descriptive statistics of *SPAD* (soil-plant analysis development) and *RGB*

Description	Mean	*SD*	Median	Minimum	Maximum
SPAD	40.61	1.88	43.60	3.50	50.70
R (red)	19.43	1.65	17.67	11.63	62.09
G (green)	20.58	0.91	20.63	12.89	32.39
B (blue)	2.80	0.17	2.74	1.06	4.84
R + G + B	42.81	2.49	40.15	28.72	98.59
r = R/(R + G + B)	0.44	0.01	0.44	0.39	0.63
g = G/(R + G + B)	0.49	0.01	0.49	0.33	0.57
b = B/(R + G + B)	0.07	0.00	0.07	0.03	0.13
R – G	–1.16	1.13	–1.89	–7.04	29.69
R – B	16.63	1.58	14.80	8.30	57.99
G – B	17.79	0.87	18.16	9.11	28.29
R/G	0.93	0.04	0.92	0.68	1.92
VARI = (G – R)/(G + R – B)	0.04	0.02	0.04	–0.30	0.18
I_{PCA} = 0.994|R – B| + 0.961|G – B| + 0.914|G – R|	48.76	3.65	46.73	24.56	137.59

Explanations: *SD* = standard deviation, *VARI* = visible atmospherically resistant index, I_{PCA} = principal component analysis index.
Source: own study.

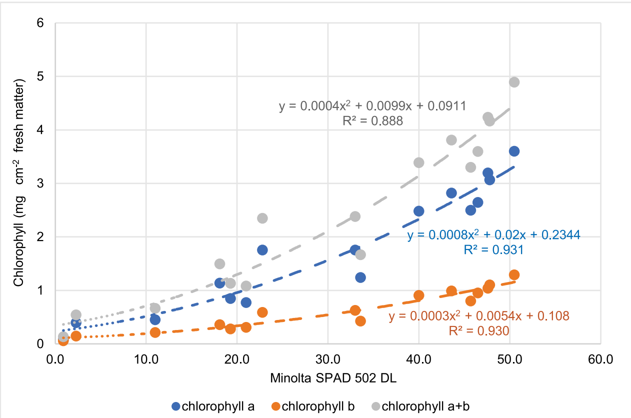


Fig. 2. Correlation between chlorophyll *a*, *b* and *a* + *b* content and *SPAD* (soil-plant analysis development) readings; R^2 = coefficient of determination; source: own study

USING THE CAMERA TO DETERMINE RGB COLOUR AND SPAD SIZE

The soybean leaf greenness index readings ranged from 3.5 to 50.7 for the *SPAD* (Tab. 3). The coefficient of variation for this trait was 25.4%. *RGB* (red, green and blue colours respectively) values ranged 11.6–62.1 for red, 12.9–32.4 for green and 1.06–4.84 for blue. The coefficient of variation was 46.5, 24.1 and 34.2, respectively (Tab. 3).

The *RGB* colour readings obtained with the application were correlated with the *SPAD* readings of the leaves and the determination coefficients (R^2). From these, the correlation coefficients (r) were calculated, which were for the *RGB* colours respectively: 0.651 (moderate correlation), 0.760 (strong correlation) and 0.106 (low correlation) (Tabs. 4, 5). Thus, image analysis with the ON-Colour Measure application shows a moderate correlation with the actual *SPAD* readings for red, a strong correlation for green and no correlation for blue readings. Figure 5 shows a linear decrease in chlorophyll concentration with increasing R and G value.

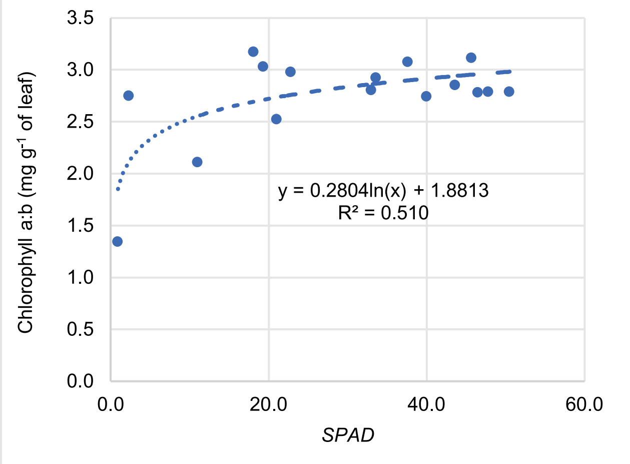


Fig. 3. Correlation between chlorophyll *a* and *b* ratios and *SPAD* (soil-plant analysis development) readings; R^2 = coefficient of determination; source: own study

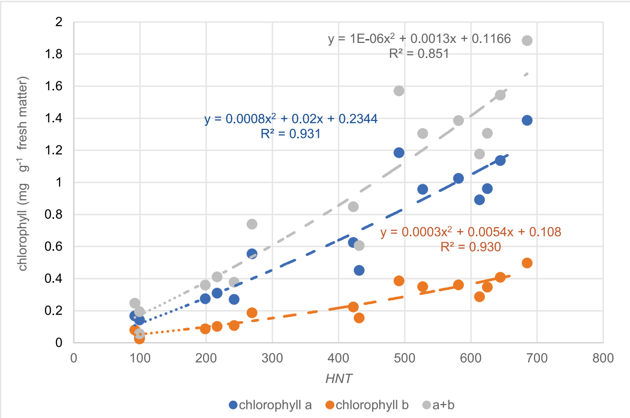


Fig. 4. Scatter plots indicating the relationship between *HNT* and chlorophyll *a*, chlorophyll *b* and total chlorophyll (*HNT* calculated by Hydro N-Tester); source: own study

Table 4. Coefficient of determination (R^2) of *RGB* colours and *SPAD* (soil-plant analysis development) readings

Parameter	*y* (*SPAD*)	R^2
R (red)	$73.403e^{-0.03x}$	0.4242
G (green)	$79.811e^{-0.031x}$	0.5769
B (blue)	$39.538e^{0.0242x}$	0.0113
R + G	$79.168e^{-0.016x}$	0.5280
R + G – B	$79.949e^{-0.018x}$	0.5872
R + G + B	$77.561e^{-0.015x}$	0.4690

Source: own study.

Table 5. Correlation coefficient of *RGB* colours and *SPAD* (soil-plant analysis development) readings

Parameter	*SPAD*	R	G	B	R+G+B	r	g	b	R–G	R–B	G–B	R/G	*VARI*	I_{PCA}
R (red)	−0.74													
G (green)	−0.72	0.75												
B (blue)	0.05	0.43	0.33											
R + G + B	−0.75	0.97	0.89	0.47										
r	−0.46	0.83	0.32	0.31	0.69									
g	0.14	−0.64	−0.08	−0.61	−0.49	−0.87								
b	0.58	−0.26	−0.44	0.67	−0.29	−0.10	−0.39							
R–G	−0.51	0.85	0.30	0.36	0.70	0.95	−0.86	−0.03						
R–B	−0.78	0.99	0.75	0.34	0.96	0.83	−0.60	−0.35	0.85					
G–B	−0.76	0.70	0.98	0.14	0.83	0.27	0.04	−0.59	0.24	0.72				
R/G	−0.44	0.83	0.28	0.42	0.69	0.98	−0.92	0.04	0.99	0.82	0.21			
VARI	0.30	−0.75	−0.20	−0.48	−0.61	−0.97	0.97	−0.16	−0.93	−0.73	−0.11	−0.98		
I_{PCA}	−0.82	0.98	0.84	0.31	0.98	0.74	−0.48	−0.42	0.75	0.99	0.82	0.72	−0.63	
R+G–2B	−0.83	0.96	0.89	0.29	0.98	0.68	−0.40	−0.47	0.68	0.96	0.88	0.65	−0.55	0.99

Explanations: r = R/(R + G + B), g = G/(R + G + B), b = B/(R + G + B), *VARI*, I_{PCA} as in Tab. 4.
Source: own study.

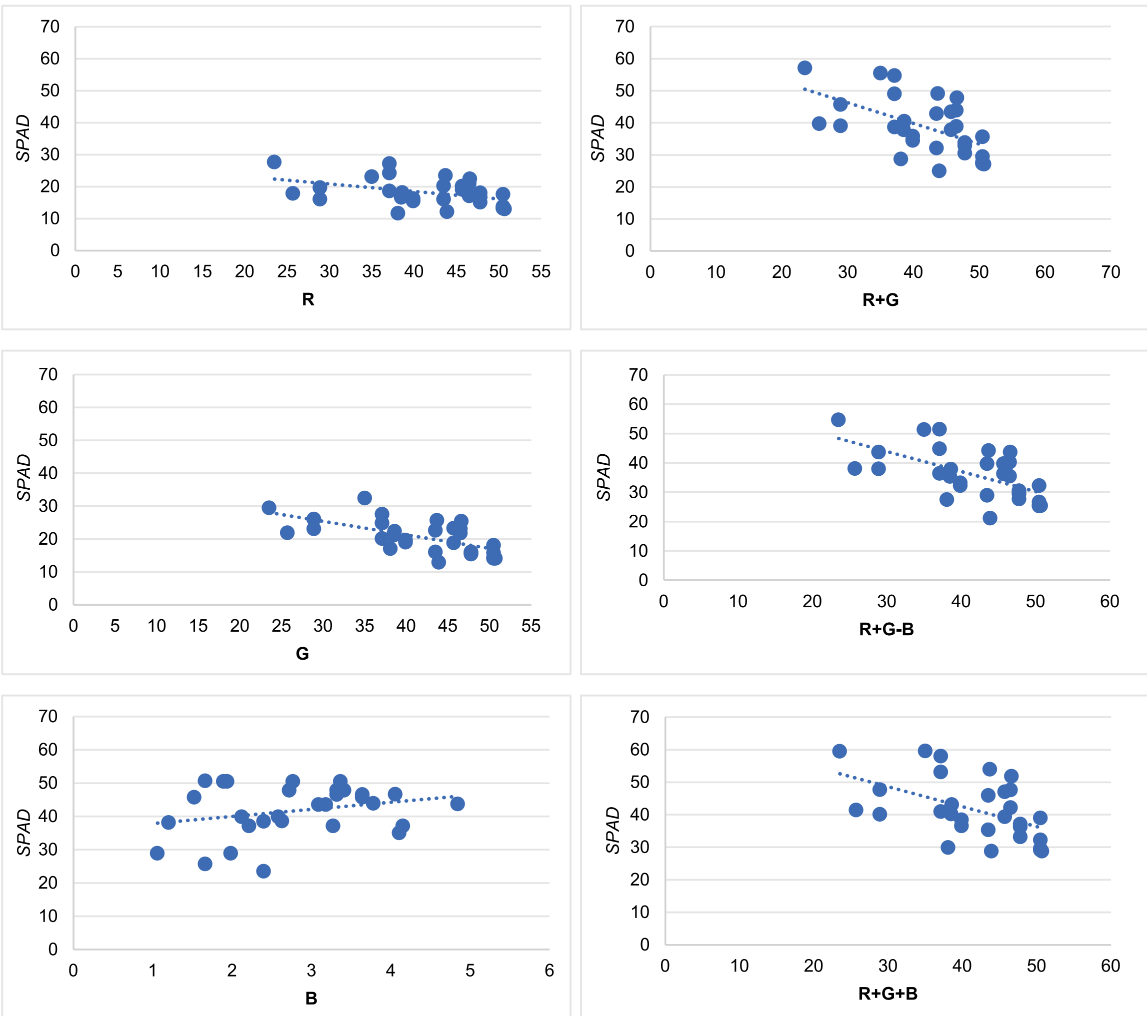


Fig. 5. Scatter plots indicating the relationship between R, G, B, R + G, R + G – B, R + G + B values and *SPAD* (soil-plant analysis development) index calculated by the SPAD-502; R, G, B = red, green, blue colour respectively; source: own study

DISCUSSION

COMPARISON OF READINGS FROM SPAD-502 AND HYDRO N-TESTER DEVICES

The study showed a very high correlation of results from SPAD-502 and Hydro N-Tester readings. The coefficient of determination was 0.976. Similar reports can be found in other scientific papers, such as Uddling *et al.* (2007), who emphasise that the operation of the Hydro N-Tester software is based on the SPAD-502 software. Therefore, readings taken with these devices should indicate correlated results. Earlier studies by Levey and Wingler (2005) and Uddling *et al.* (2007) confirmed the existence of a linear relationship between the readings of the SPAD-502 meter used and other popular chlorophyll meters, including the Hydro N-Tester. Due to the high convergence of results (correlations), both devices are recommended for using in field conditions for plant assessment.

SPAD MEASUREMENT AND CHLOROPHYLL CONTENT

The Chl content of soybean leaves was strongly correlated with the SPAD-502 chlorophyll meter readings. For Chl *a* and for Chl *b,* the correlation coefficient was 0.93, and for the sum of Chl *a* and *b* it was 0.88. The relationship for Chl *a*, Chl *b* and total Chl ($a + b$) showed a polynomial character (degree 2). Similar results were obtained by Monje and Bugbee (1992), who showed a correlation for wheat, rice and soybean leaves between SPAD-502 chlorophyll meter values and leaf Chl content. Also, this relationship was non-linear, with a polynomial 2-step trend line showing the highest coefficient of determination. A study by Markwell, Osterman and Mitchell (1995) conducted on soybean and maize leaves showed a correlation between *SPAD* (soil-plant analysis development) readings and Chl content. In addition to the polynomial relationship, an exponential trend line (exponential) was fitted to the data. Due to the high convergence of results (correlations), this method is recommended for using in field conditions for plant assessment.

USING A CAMERA TO DETERMINE RGB COLOUR AND *SPAD* SIZE

A camera installed on a mobile phone has been used to determine RGB colours using the ON-ColorMeasure application only once so far: in the work of Safarik *et al.* (2019) where the magnetic extraction of textile solid-phase colour compounds was evaluated. The researchers showed that the use of a smartphone image analysis app can be a useful tool, faithfully measurement real colours. The saturation (S) values of the HSV colour space were shown to be directly proportional to the concentration of the dye being analysed.

In our own study, where leaf greenness *SPAD* readings and R, G and B colour readings were compared using the ON-ColorMeasure application, a medium correlation between image analysis and actual *SPAD* readings was shown for red, but strong for green and no correlation for blue readings. Similar results were obtained by Hu *et al.* (2010), who found that R and G values decreased linearly (correlation coefficient −0.852 and −0.922, respectively), while B values did not change linearly with changing Chl content values. In addition, they found that G values were greater than R and B values in all measured images. Due to the large discrepancy in results (correlations), RGB method is not recommended for assessing plant greenness under field conditions, as it may result in an incorrect estimation of the nitrogen dose needed for arable crops.

CONCLUSIONS

The comparative analysis showed a high correlation of the values obtained from the selected leaf chlorophyll content meters – due to the use of similar software. However, no equally high correlation was obtained when using a phone app.

Modern leaf greenness measurement technologies using the ON-ColorMeasure app show a medium level of correlation with readings extracted from the SPAD device, indicating a technology gap to be filled.

It is expected that in the near future there will be a phone app for predicting the nutritional status of plants in real time, with a measurement error of less than 5%.

The tested method can be a valuable addition to standard methods useful for assessing the state of the environment by determining vegetation indices with an accuracy not much inferior to costly standard testing equipment, e.g. SPAD.

It is recommended that both chlorophyll meters (SPAD-502 and Hydro N-Tester) can be used to assess leaf greenness in field conditions due to the high correlation rates with laboratory method.

SUPPLEMENTARY MATERIAL

Supplementary material to this article can be found online at https://www.jwld.pl/files/Supplementary_material_Kulig.pdf.

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CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

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