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New developed models to estimate leaf chlorophyll content in durum wheat (*Triticum durum* Desf.) under semi-arid conditions

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Abstract

Leaf colors of a plant can be used to identify stress level due to its adaptation to environmental change. The objective of this study is to develop a new functions based on the RGB color model to estimate Chlorophyll levels in durum wheat under stressed conditions and compare it with non-destructive methods (SPAD 502 values). The results showed a significant correlation between Chlorophyll content estimated by RGB color models proposed [100-(R+B) and 100-(2R-B)] and Chlorophyll content measured by SPAD instrument (r = +0.72 and r = +0.77, respectively). In addition, chlorophyll content estimated by RED color model registered a significant correlation with Chlorophyll content measured by SPAD instrument (r = +0.72). The present results shown that, for practical purposes, the chlorophyll content of leaves can be estimated with sufficient accuracy using a portable video camera.

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Introduction

Chlorophyll is a green photosynthetic pigment which helps plants to get energy from light. The plants use the energy to combine carbon dioxide and water into carbohydrate to sustain their life process. There may be many factors that affect the photosynthesis; the main factors are light intensity, carbon dioxide concentration, and temperature (Jolliffe and Tregunna, 1968). The chlorophyll content could depend on seasonal and environmental changes. There are two approaches to measure leaf chlorophyll concentration: destructive and non-destructive.

The destructive method is a laboratory based technique that measures foliar chlorophyll concentration by organic extraction and spectrophotometric analysis (Arnon, 1949).The destructive approach is accurate and considered as a benchmark for the estimation of chlorophyll content. However, it is relatively expensive, time consuming and requires specialist equipment. In contrast, non-destructive methods are easy to use and rapid but not as accurate as the destructive method (Mahdi *et al.*, 2012).A common non-destructive device is the MinoltaSPAD-502 leaf chlorophyll meter. It measures the transmittance of red (650 nm) and infrared (940 nm)radiation through the leaf. Recently, digital imagery has become a new trend in plant color analysis. Digital cameras or scanners in combination with computers and appropriate software can be used to photograph, scan, and evaluate leaves for color with relative ease and at an affordable cost (Guendouz *et al.*, 2012).

In agriculture, digital technology has been used to characterize color in apples (Schrevens and Raeymaeckers, 1992), identify storage-associated color change in chickory (Zhang *et al.*, 2003), and evaluate senescence rates in spring wheat (Adamsen *et al.*, 1999) and durum wheat (Hafsi *et al.*, 2000; Guendouz and Maamari, 2011).Kawashima and Nakatani (1998) showed that (R-B)/(R+B) is a good formula to determine foliar chlorophyll status in wheat. The aim of this paper is to develop a new functions based on the RGB color model to estimate Chlorophyll levels in durum wheat under field conditions and compare it with non-destructive methods (SPAD 502 values).

Materials and methods

Experiment design and Biological material

Field experiment was done during the 2014-2015 growing seasons at the experimental field of INRAA, Setif, Algeria (5°20'E, 36°8'N, 958m above mean sea level). A set of 10 genotypes of durum wheat (*Triticum durum* Desf.) (Table 1) were planted on a clay-silt.

SPAD-502 chlorophyll meter

The Chlorophyll SPAD meter (Minolta SPAD-502, Konica Minolta Sensing, Inc., Tokyo, Japan) was used to determine total leaf chlorophyll in the field. This device emits two different light intensities from two diodes: peak wavelength 650 nm (red) absorbed by the leave tissue, which estimates the chlorophyll content (greenness). A second peak 940 nm (infrared LED) is emitted simultaneously with red LED to compensate for leaf thickness.

The proposed models to estimate chlorophyll

A portable video camera for home use (Canon, Power Shot A460, AiAF, CHINA) was used to record images of plants. A number of images were taken in the study field to obtain a wide variation in chlorophyll concentration. The distance between the video camera and plants was 0.5-0.8m. Images were stored in a JPEG (Joint Photographic Expert Group) prior to be downloading onto a PC computer and analyzed using Mesurim Pro (Version 3.3) software to identified the leaf contour and then averaged red (R), green (G)and blue (B) reflectance values of the leaf pixels, the proposed functions are:

1- Chl_{est} = 0,1368 (100-(R+B))+ 49,851

2- Chl_{est} = 0,2306 (100-(2R-B)) + 40,263

Moreover, linear regression models were also analyzed and the obtained results were compared with the neural network model. In general, the goal of linear regression is to find a line that best predicts Y (chlorophyll) from X (red, green, and blue components).

Models evaluation

In the present study, root mean squared error (RMSE), minimum prediction accuracy (MPA), and coefficient of correlation (r) were considered to evaluate the performance ofthe linear regression models. These criteria were calculated using the following equations:

$$MPA = \left[1 - \frac{CCspad - CCest}{CCspad}\right] * 100$$

$$RMSE = \sqrt{\frac{1}{n} * \sum (CCest - CCspad)^2}$$

$$r = \frac{\Sigma[(CCspad - \overline{CCspad}) * (CCest - \overline{CCest})]}{\sqrt{\Sigma(CCspad - \overline{CCspad})^2 * \Sigma(CCest - \overline{CCest})^2}}$$

CCspad: Chlorophyll content measured by SPAD instrument; CCest: Chlorophyll content estimated by models based on RGB reflectance.

Statistical analysis

The test of correlation was used to evaluate the significant and no significant correlations between Chlorophyll content measured by SPAD instrument and Chlorophyll content estimated by models based on RGB reflectance, by using STATISTICA software.

Results and discussion

Methods to estimate the chlorophyll content from R, G and B values of leaf images were examined. Various functions derived from the R, G and B values are shown in Table 2.Correlation coefficients between the function values and chlorophyll content are given in Table 2. The functions: 100-(2R-B), 100-(R+B), RGB, R+B and Red show a significant correlation coefficients ranging from-0.66 to 0.78 with chlorophyll content, but the function (R-G)/(R+G) and (R-B)/(R+B) are not significantly correlated with chlorophyll content. Kawashima and Nakatani (1998) to measure chlorophyll in wheat plants developed (R-B)/(R+B) formula.

Table 1. Name and origin of the ten genotypes used in the study.

Cultivar	Name	Origin	Cultivar	Name	Origin
1	Bousselem	ICARDA/CIMMYT	6	$Altar_{84}$	CIMMYT
2	Hoggar	Espagne	7	Dukem	CIMMYT
3	Oued Zenati	Algeria	8	Kucuk	CIMMYT
4	Polonicum	Algeria	9	$Mexicali_{75}$	CIMMYT
5	Waha	ICARDA/CIMMYT	10	Sooty	CIMMYT

They used a portable digital video camera and the acquired images were transferred to a personal computer, which were then analysed using Photoshop (ver.1.0.7, Adobe systems, USA) to obtain R, G and B values of the images.

Correlation between the true chlorophyll level and chlorophyll estimation based on the above formula was reported to be around -0.81. In contrast with the last results we register a no significant correlation with chlorophyll content measured by SPAD instrument (r = 0.56ns) and the chlorophyll estimation based on the formula (R-B)/(R+B).

The root mean squared error (RMSE) between Chlorophyll content measured by SPAD instrument and Chlorophyll content estimated by model [(R-B)/(R+B)] is 2.76, RMSE: Close to zero indicates better model performance (Anjum Iqbal *et al*., 2014).

Significant and positive correlation between Chlorophyll content measured and Chlorophyll content estimated by model RGB values suggested the efficiency of using this model to estimate Chlorophyll content in durum wheat. Hu *et al*. (2010) showed that the RGB colors indices such as R, G and RGB, R-B, R+B, R+G had significant relationship with chlorophyll content. Hu *et al*. (2010) showed that the RGB colors indices of R+B had significant relationship with chlorophyll content.

Table 2. Correlation coefficient and Models evaluation between Chlorophyll content measured by SPAD instrument and Chlorophyll content estimated by RGB reflectance.

Genotype	Chlorophyll content	Chlorophyll content estimated						
	measured by SPAD	100-(2R-B)	100-(R+B)	R,G,B	R+B	Red	(R-G)/(R+G)	(R-B)/(R+B)
Oued Zenati	51,48	51,45	51,31	51,70	51,32	51,06	56,10	56,36
Altar	56,22	55,10	55,19	54,73	55,19	55,08	54,92	56,33
Sooty	60,24	57,39	55,75	56,68	55,76	56,37	55,23	56,78
Polonucum	50,96	54,32	54,66	53,62	54,66	54,42	53,48	56,27
Waha	58,65	61,38	60,25	61,43	60,25	60,94	59,45	57,12
Dukem	60,70	59,55	58,54	59,31	58,55	59,08	57,14	56,79
Mexicali	57,94	56,11	56,95	56,49	56,95	56,66	56,90	56,11
Kucuk	58,85	56,43	59,22	57,64	59,22	58,29	58,43	55,10
Hoggar	55,37	56,50	55,47	57,09	55,48	55,82	58,03	56,61
Bousselem	53,18	55,35	56,24	54,89	56,24	55,88	53,90	56,11
RMSE	---	2,11	2,29	2,03	2,29	2,14	2,76	3,30
MPA%	---	93,40	92,55	94,11	92,56	93,21	91,02	89,58
R^2	---	0,60	0,53	0,63	0,53	0,59	0,31	0,02
R	---	0,77*	0,72*	0,79*	-0,72*	0,76*	$0,56^{NS}$	$0,16^{NS}$

Root Mean Squared Error (RMSE), Minimum Prediction Accuracy (MPA%), Coefficient of determination (R^2) and Coefficient of correlation (R). * and ^{NS} significant and no significant correlation between Chlorophyll content measured and estimated at 5%.

In addition, Moghaddam *et al.* (2011) showed acceptable correlation between 2R-B function and Chlorophyll content measured by SPAD. The both proposed functions [(100-(R+B) and 100-(2R-B)] showed a significant correlation (r = +0.72 and r = +0.77) with Chlorophyll content measured by SPAD instrument, our proposed function based on the deviation between the total reflectance and the function proposed (R+B) and (2R-B) (Figure 1).

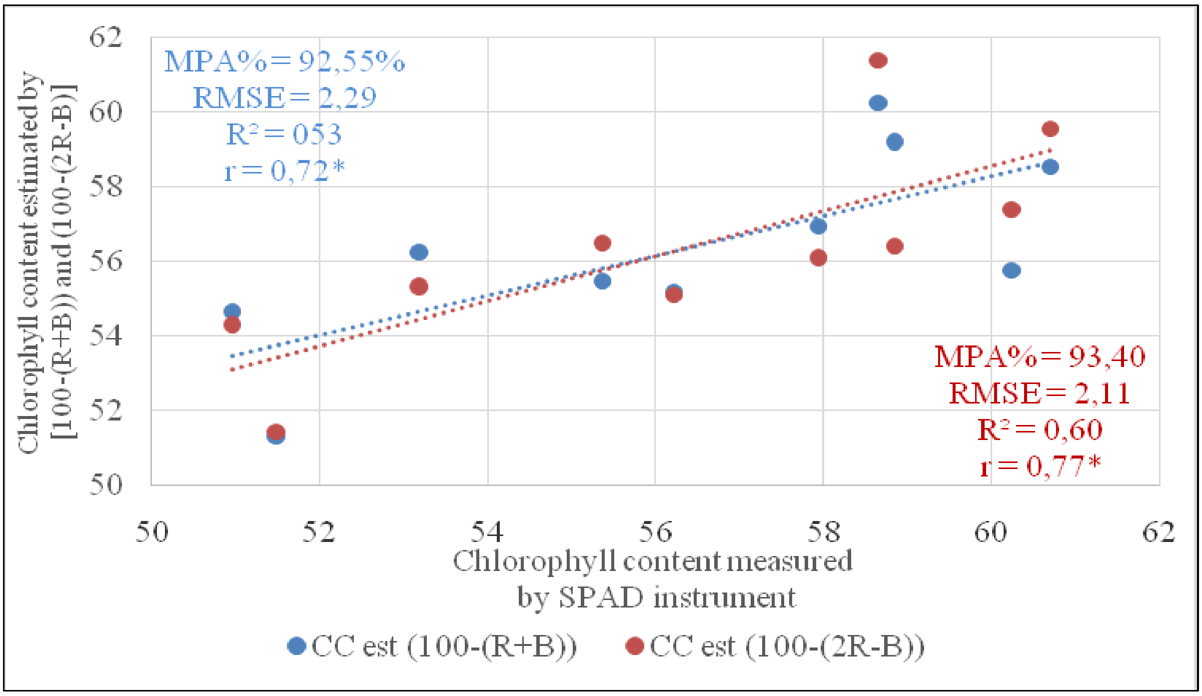

Fig. 1. Relationships between Chlorophyll content measured by SPAD instrument and Chlorophyll content estimated by Models proposed [(100-(R+B) and 100-(2R-B)].

The linear models give a good confidence for prediction of SPAD reading based on RED spectral reflectance values, and R/(R+G+B) values (Teoh *et al*., 2012), our results showed a significant correlation between chlorophyll content measured by SPAD and chlorophyll content estimated by RED spectral reflectance values (r = 0.76).

Conclusions

Relationships between SPAD readings and100-(2R-B), 100-(R+B), RGB, R+B, Red, (R-G)/(R+G) and (R-B)/(R+B) values have been analyzed. The present study demonstrated that these simple methods can be used to estimate the chlorophyll level of durum wheat by analyzing the leaf image color. These methods, compared to the other remote sensing approaches, are low in cost and easy to perform. The both proposed functions [(100-(R+B) and 100-(2R-B)] showed a significant correlation (r = +0.72 and r = +0.77) with Chlorophyll content measured by SPAD instrument. This proposed method is simple and user friendly hence useful tool in laboratory as well as in the fields to measure chlorophyll non-destructively. This methodology is also useful to measure the chlorophyll content of the leaf of other plants.

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