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A Comparative Study of Chlorophyll Content Estimation in Barley (*Hordeum vulgare* L.) Genotypes Based on RGB (Red, Green, Blue) Image Analysis

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Abstract

Background: Chlorophyll is the most important pigment in plant which absorbs light and subsequently transfers its energy to drive the photochemical reactions of photosynthesis. The numerical image processing techniques have been widely used in the analysis of leaf characteristics.

Methods: The methods based on RGB (Red, Green and Blue) image analysis may emerge as a new and low-cost method for estimation the chlorophyll content. In this work, we use eight RGB vegetation indices as alternative for chlorophyll content estimation.

Result: The student t-test showed that all the RGB indices tested are suitable to estimate the chlorophyll content in barley genotypes. In addition, the results which based on the correlation analysis in combination with the values of root mean squared error (RMSE) demonstrate that the very suitable RGB indices are these with high values of correlation coefficient and lowest values of RMSE. Data collected from barley genotypes leaves indicated that digital image processing technology can be a useful and rapid non-destructive method for assessment of chlorophyll content. Among the RGB indexes tested in this study the 100-(2R-B) and RGRI (R/G) are the most promising index to estimate the chlorophyll content in barley genotypes.

Key words: Barley, Chlorophyll, Leaf image analysis, RGB model, SPAD.

Introduction

The quality and productivity of crop are directly related to the green pigment visible in the leaves which is due to the presence of Chlorophyll. Leaf chlorophyll is mostly used as an index to diagnose diseases and getting the nutrient and nitrogen status in the plants. The chlorophyll content of plants can provide useful information concerning photosynthetic status during plant growth and can be used as a key indicator of the physiological stage of plants. Several methods for estimating chlorophyll content can be found in the current literature and are based on the transmittance or reflectance of the leaf; nevertheless, chloroplast arrangement in the cells is modified by the intensity, color and duration of the incident light, which produces variations in the values obtained with measurement devices (Pérez-Patricio, 2018). Digital cameras can record spectral leaf information in visible color bands, with high resolutions and low costs (Guendouz *et al*., 2012; Guendouz and Hafsi, 2017; Bama *et al*., 2011). In addition, digital color images contain rich information of plant morphology, structure and leaf colors. So, leaf digital images are often exploited to identify changes in leaf color (Zhang *et al*., 2014). The most commonly used color representation for digital color images is the RGB color model. For an RGB color image, three color sensors per pixel can be used to capture the intensity of light in the red, green and blue channels, respectively. 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. Many software tools, such as MATLAB and Mesurim Pro are used to process the obtained digital pictures (Chen *et al*., 2020; Guendouz and Hafsi, 2017). A common non-destructive device is the Minolta SPAD-502 leaf chlorophyll meter. It measures the transmittance of red (650 nm) and infrared (940 nm) radiation through the leaf. The main disadvantage of the SPAD system is that it only estimates the transmittance at one point of the leaf under analysis, calculating the chlorophyll content only within a small spatial location on the leaf. To solve this problem, iterative measurements at different spatial locations must be performed (Pérez-Patricio, 2018). Many studies and researchers suggested a number of RGB-based color features for the determination of chlorophyll levels in potato, rice, wheat, broccoli, cabbage, barley, tomatoes, quinoa and

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amaranth (Adamsen *et al*., 1999; Yadav *et al*., 2010; Guendouz and Hafsi, 2017). Almost all the methods applied so far to determine the amounts of chlorophyll in wheat and Barley from leaf color information are RGB color index based. In addition, Guendouz *et al*. (2013) proved that the declines in chlorophyll content with time affect the final grain yield in durum wheat. Therefore, to overcome this, a new chlorophyll meter (SPAD 502) is an improved model over SPAD 501 was developed by the Minolta Camera Company, Japan and made commercially available (Pushpanathan *et al*., 2014). Kawashima and Nakatani (1998) proposed (R-B)/(R+B) as a good tool to estimate wheat chlorophyll content; Guendouz and Hafsi (2017) described the positive and significant linear relationship between 100-(R+B) and 100-(2R-B) and SPAD reading in durum wheat. The objective of this study is to evaluate the efficiency of using the RGB index to estimate chlorophyll content in some genotypes of Barley (*Hordeum vulgare* L.) growing under semi-arid conditions.

MATERIALS AND METHODS

Field experiment was conducted during the 2018-2019 cropping season at the experimental field of ITGC, Setif, Algeria (5°20′E, 36°8′N, 958 m above mean sea level). The statistical design employed was based on a complete randomized block design (CRBD) with three replications. Eight Barley (*Hordeum vulgare* L.) genotypes were used in this study. The seeds were sown using an experimental drill in 1.2 m × 2.5 m plots consisting of 6 rows with a 20 cm row space and the seeding rate is about 250 seeds per m^2. During this study, we use the numerical image analysis (NIA) to estimate the reflectance at Red, Green and Blue (RGB). Leaves were photographed between 11:00 and 13:00 solar time with a color digital camera (Canon, Power Shot A460, AiAF, CHINA). Images were stored in a JPEG (Joint Photographic Expert Group) prior to downloading onto a PC computer and analyzed using Mesurim Pro (Version 3.3) software (Fig 1). The RGB indices used for evaluation are listed and defined in Table 1. The SPAD-502 measures the content of chlorophyll (Chl) in the leaf, which is related to leaf greenness, by transmitting light from light emitting diodes (LED) through a leaf at wavelengths of 650 and 940 nm. Simple linear regression was employed to determine the strength of the relationships between the SPAD measurements and the indices. The root mean squared error (RMSE), coefficient of correlation (r), the coefficient of determination (R^2) and the student test (t-test) were used to compare the performance of the RGB indices tested.

RESULTS AND DISCUSSION

We use during this study eight RGB index to estimate flag leaf chlorophyll content in barley genotypes. The models relating between the RGB indices and the measured chlorophyll content by SAPD-502 illustrated in Table 2. In addition, as shown in Table 3, the chlorophyll content measured by SPAD ranged from 33.50 for the genotype Tichedrette to 52.10 for the genotype Rihane 03. The correlation analysis between the chlorophyll measured by SPAD and the chlorophyll estimated by the RGB index models showed no significant correlation between the values estimated based on the Green and Blue models (r = 0.373 and r = 0.12); this results proved that the Green and Blue index are unsuitable to estimate chlorophyll content. Various functions derived from the R, G and B values and the correlation coefficients between the function values and

Table 1: List of RGB indices tested in this study.

RGB index	Definition	Reference
R	Red	Kawashima and Nakatani (1998)
G	Green	
B	Blue	
r	R/(R+G+B)	
2R-B	2R-B	Moghaddam *et al.* (2011)
100-(2R-B)	100-(2R-B)	Guendouz and Hafsi (2017)
GRVI	(G-R)/(G+R)	Pettorelli *et al.* (2005)
RGRI	R/G	Saberioon *et al.* (2014)

r: normalized red; GRVI: Green-red vegetation index; RGRI: Red green ratio index.

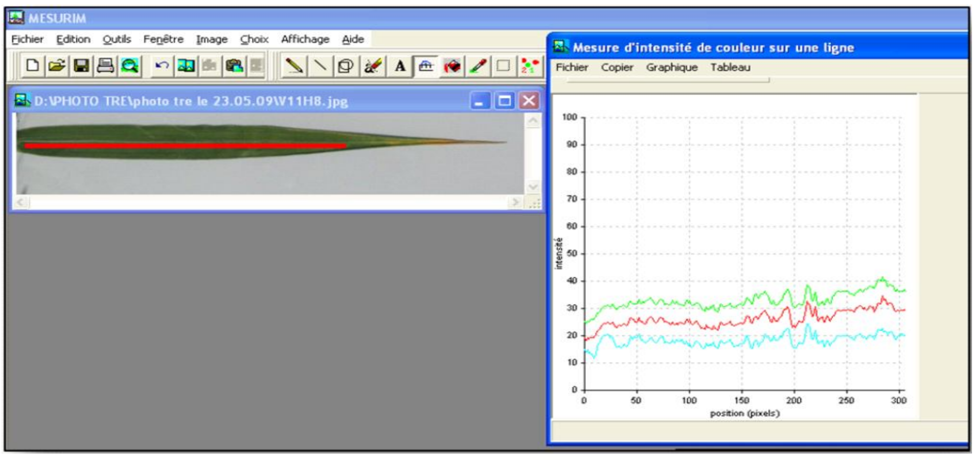

Fig 1: Description of measuring the reflectance at RGB (Red, Green, Blue) using Mesurim Pro software (Guendouz *et al*., 2012).

Table 2: Regression equations, coefficients of determination (R^2) for models relating SPAD-502 index (SPAD) with eight RGB indices.

RGB index	Models	R^2	P
R	Chl = 110.978 - (3.413 * Red)	0.5180	0.044
G	Chl = 92.700 - (1.400 * Green)	0.1390	0.362
B	Chl = 59.901 - (0.790 * Blue)	0.0143	0.778
r	Chl = 225.246 - (733.131 * r)	0.7600	0.005
2R-B	Chl = 77.539 - (2.154 * 2R-B)	0.6150	0.021
100-(2R-B)	Chl = -137.889 + (2.154 * 100-(2R-B))	0.6150	0.021
GRVI	Chl = -33.736 + (260.390 * GRVI)	0.5670	0.031
RGRI	Chl = 159.295 - (213.410 * RGRI)	0.5590	0.033

r: Normalized red; GRVI: Green-red vegetation index; RGRI: Red green ratio index.

Table 3: Correlation coefficient and models evaluation between chlorophyll content measured by SPAD instrument and chlorophyll content estimated by RGB indices.

Genotypes	Chlorophyll content measured by SPAD	Chlorophyll content estimated by RGB index models							
		Red %	Green %	Bleu %	r	2R-B	100-(2R-B)	GRVI	RGRI
G1	36.43	36.78	38.94	40.62	36.74	36.46	36.43	38.40	38.47
G2	50.70	48.45	43.90	40.65	52.06	51.11	51.08	47.25	47.14
G3	41.23	43.57	43.34	40.93	42.03	44.20	44.17	39.68	39.76
G4	38.20	43.03	44.54	41.53	37.07	41.87	41.84	35.74	35.78
Fouarra	35.50	32.00	38.00	39.34	32.99	33.92	33.89	32.94	32.90
Saida 183	39.43	41.35	39.60	42.24	39.87	37.80	37.77	44.52	44.52
Tichedrette	33.50	38.90	39.12	40.87	39.77	38.44	38.42	41.46	41.52
Rihane 03	52.10	43.03	39.72	40.99	46.56	43.33	43.31	47.11	47.01
RMSE	/	4.491	3.119	2.283	5.527	2.398	0.028	4.273	0.072
R^2	/	0.518	0.139	0.014	0.760	0.615	0.615	0.567	0.560
t-test	/	0,00050ns	0.0032ns	0,0033ns	0.00011ns	0.0016ns	0.0073ns	0.000048ns	0.0001ns
R	/	0.72*	0.373ns	0.12 ns	0.872**	0.784*	0.784*	0.753*	0.748*

r: Normalized red; GRVI: Green-red vegetation index; RGRI: Red green ratio index; *: Significant correlation at 5%; **: at 1%.
ns: No significant correlation and R: Correlation coefficient.

chlorophyll content are shown in Table 3; the estimated chlorophyll content based on the Kawashima and Nakatani (1998) index [R/(R+G+B)] showed a significant and positive correlation with the measured chlorophyll by SPAD (r = 0.872**) but with highest values of RMSE (5.52). The best RGB indexes were selected based on the highest r values and lower values of RMSE. The results obtained for this indices were consistent with the findings of Kawashima and Nakatani (1998). In contrary of our finding, Hu *et al*. (2010), obtained highest R^2 values for regressions between R and G with the total chlorophyll content in leaves of three barley cultivars, but low Pearson correlation coefficients (r) when the indexes R/(R + B + G) is tested. The chlorophyll content estimated based on the RGB index 2R-B and the index proposed by Guendouz and Hafsi (2017) [100-(2R-B)] showed significant and positive correlation between the SPAD reading chlorophyll content with moderate value of RMSE (2.40) for the index 2R-B, but with lowest value of RMSE for the RGB index 100-(2R-B), this results is in agreement with the finding of Guendouz and hafsi (2017). In addition, Moghaddam *et al*. (2011) showed acceptable correlation between 2R-B function and Chlorophyll content measured by SPAD. The estimated chlorophyll based on the proposed RGB index by Pettorelli *et al*. (2005) (GRVI) showed significant and positive correlation with the SPAD reading (r = 0.753*) with moderate value of RMSE (4.27). The results show a best correlation between the SPAD reading and the estimated chlorophyll based on the R/G with lowest RMSE (0.072) which suggests that this index it's very suitable to estimate chlorophyll content. Saberioon *et al*. (2014) registered no significant correlation between the chlorophyll estimated by R/G (RGRI) index and the chlorophyll measured by SPAD. Overall, based on the t-test (student test) all the RGB indices tested showed no significant difference between the chlorophyll estimated and the SPAD reading (Table 3) which indicate the efficiency of these indices to estimate chlorophyll content.

CONCLUSION

Eight RGB vegetation indices were evaluated for chlorophyll content estimation in barley leaves at the heading stage of the cultivation period. Based on correlations analysis of the RGB index determined chlorophyll contents and SPAD reading the more suitable indices are Red, r [R/(R+G+B)], 2R-B, 100-(2R-B), GRVI [(G-R)/(G+R)] and RGRI (R/G) with significant and positive correlation coefficient. In addition, the student test (t-test) proved that the all RGB indices tested are suitable to estimate the chlorophyll content. In combination between the correlation coefficient and root-mean-square error (RMSE) the very suitable indices are these which have the highest r values and lower values of RMSE; in this case the index 100-(2R-B) and RGRI (R/G) are the very suitable index.

REFERENCES

Adamsen, F.J., Pinter, P.J., Barnes, E.M., La Morte, R.L., Wall, G.W., Leavitt, S.W. and Kimball, B.A. (1999). Measuring wheat senescence with a digital camera. Crop Sci. 39: 719-724.

Bama, B.S., Valli, S.M., Raju, S. and Kumar, V.A. (2011). Content based leaf image retrieval (CBLIR) using shape, color and texture features. Indian J. Eng Mater S. 1: 202-211.

Chen, Z. Wang, F., Zhang, P., Ke, C., Zhu, Y., Cao, W. and Jiang, H. (2020). Skewed distribution of leaf color RGB model and application of skewed parameters in leaf color description model. Plant Methods. 16(23): 1-8.

Guendouz, A. and Hafsi, M. (2017). New developed models to estimate leaf chlorophyll content in durum wheat (*Triticum durum* Desf.) under semi-arid conditions. International Journal of Biosciences. 10(3): 118-123.

Guendouz, A., Guessoum, S., Maamri, K., Benidir, M. and Hafsi, M. (2013). Performance of ten durum wheat (*Triticum Durum* Desf.) cultivars under semi arid conditions (North Africa-Algeria). Indian Journal of Agricultural Research. 47(4): 317-322.

Guendouz, A. Guessoum, S. Maamari, K. and Hafsi, M. (2012). Predicting the efficiency of using the RGB (Red, Green and Blue) reflectance for estimating leaf chlorophyll content of Durum wheat (*Triticum durum* Desf.) genotypes under semi arid conditions. American-Eurasian Journal of Sustainable Agriculture. 6(2): 102-106.

Hu, H., Liu, H., Zhang, H., Zhu, J., Yao, X., Zhang, X. and Zheng, K. (2010). Assessment of Chlorophyll Content based on Image Color Analysis, Comparison with SPAD-502. In: 2010 2nd International Conference on Information Engineering and Computer Science. pp. 1-3.

Kawashima, S. and Nakatani, M. (1998). An algorithm for estimating chlorophyll content in leaves using a video camera. Annals of Botany. 81(1): 49-54.

Moghaddam, P.A., Derafshi, M.A.H. and Shirzad, V. (2011). Estimation of single leaf chlorophyll content in sugar beet using machine vision. Turkish Journal of Agriculture and Forestry. 35: 563-568.

Pushpanathan, K.R., Vijaya Kumar, M. and Siddeswaran, K. (2004). Chlorophyll meter as a tool to determine the time of application of N in rice (*Oryza sativa* L.). Agricultural Reviews. 25(4): 309-312.

Pettorelli, N., Vik, J.O., Mysterud, A., Gaillard, J.M., Tucker, C.J. and Stenseth, N.C. (2005). Using the satellite-derived NDVI to assess ecological responses to environmental change. Trends Ecol. Evol. 20: 503-510.

Pérez-Patricio, M., Camas-Anzueto, J.L., Sanchez-Alegría, A., *et al.* (2018). Optical method for estimating the Chlorophyll Contents in Plant Leaves. Sensors. 18: 650.

Saberioon, M.M., Amin, M.S.M., Anuar, A.R., Gholizadeh, A., Wayayok, A. and Khairunniza-Bejo, S. (2014). Assessment of rice leaf chlorophyll content using visible bands at different growth stages at both the leaf and canopy scale. Int. J. Appl. Earth Obs. Geoinf. 32: 35-45.

Yadav, S.P., Ibaraki, Y. and Dutta Gupta, S. (2010). Estimation of the chlorophyll content of micropropagated potato plants using RGB based image analysis. Plant cell Tiss Org. 100: 183-188.

Zhang, Y.H, Tang, L., Liu, X.J., Liu, L.L., Cao, W.X. and Zhu, Y. (2014). Modeling dynamics of leaf color based on RGB value in Rice. J. Integr. Agr. 13: 749-59.