Korean J. Crop Sci. 49(1): 41~45(2004)

Nondestructive and Rapid Estimation of Chlorophyll Content in Rye Leaf Using Digital Camera

Hyun Hwoi Ku, Seung Hwan Kim*, Kwan Soo Choi*, Ho-Yong Eom***, Sang-Eun Lee*, Seung-Gil Yun*, and Tae Wan Kim*†**

**Department of Plant Resources Science, Hankyong National University, Anseong 456-749, Korea*
***Institute of Ecological Phytochemistry, Hankyong National University, Anseong 456-749, Korea*
****Environment & Undergroundwater Co. Ltd, Anseong 456-749, Korea*

ABSTRACTS : We have developed and tested a new method for nondestructive estimation of chlorophyll- and nitrogen-contents in rye leaf. It was found that the relationships among nitrogen, chlorophyll content and fresh weight were significantly positive correlated. Nitrogen and chlorophyll content were positively correlated whereas correlation coefficients among R, G, R-B and G-B on the basis of photo-numerical values were negative. We have found that R/(R-B) obtained from data of digital camera is the best criterion to estimate the chlorophyll content of leaves. The regression curves of the relation between R/(R-B) and chlorophyll content were also calculated from the data collected on cloudy days. The coefficients of determination (r^2) were ranged from 0.33 to 0.99. In this study, the accuracy in estimating chlorophyll content from the color data of digital camera image could be improved by correcting with R, G, and B values. It is suggested that, for practical purposes, the image values estimated with sufficient accuracy using a portable digital camera can be applied for determining chlorophyll content and nitrogen status in plant leaves.

***Keywords*:** Nondestructive estimation; Chlorophyll; Nitrogen status; Photo numerical values; Digital camera

The nitrogen content in plant and leaf color reflect the relative amount of chlorophyll in leaf which are closely related to plant nutrient status. Therefore, leaf color dependent on the chlorophyll content can be used as an index to determine the status of plant growth. Various kinds of color standard plates and SPAD-502 chlorophyll meter have been developed to estimate leaf color or the chlorophyll content. Using of these tools is common in the researches for soil and fertilizer management (Chang and Robinson, 2003).

To estimate the chlorophyll content of leaves, many researches have been carried out using optical methods. Nondestructive techniques for determining chlorophyll content have been developed based on the spectral property of chlorophyll, the highly specific reflectance at ca. 675nm included leaf reflectance at about 675 nm (Benedict and Swidler, 1961; Inada, 1964; Takano and Tsunoda, 1970; Wallihan, 1973; Hardwick and Baker, 1973; Macnicol *et al.*, 1976). Chappelle *et al.* (1992) reported remote estimation of chlorophyll, developing an algorithm for the ratio analysis of reflectance spectra (RARS). Besides determining spectral properties, the digital image was used for estimating the number of apple fruits and measuring their diameter in an orchard (Stajnko *et al.*, 2004). Murphy *et al.* (2004) suggested that field-based digital Colour-InfraRed (CIR) photography could be usefully applied for the nondestructive determination of chlorophyll.

In these methods, not only plant nitrogen status but also chlorophyll content could be estimated simultaneously throughout leaf color analysis. Using a portable digital camera, we have developed and tested a new method for nondestructive estimation of chlorophyll content and nitrogen status in rye. In addition, the accuracy of algorithm used in the estimation of the amount of chlorophyll in leaves was discussed. It was also discussed on an algorithm to estimate the amount of chlorophyll in leaves.

MATERIALS AND METHODS

Plant materials and cultivation

This study was carried out with rye cultivars in the experimental field of the Hankyong National University in Korea (36°N, 128°E). Cultivation was carried out from October 2002 to May 2003 according to the conventional cultivation method used in Korea. In brief, three rye cultivars (Olhomil, Paldanghomil and Koolgrazer) were planted by strip seeding with row width of 40 cm. Each plot was twelve and the size of the field was 4×0.4 m. Nitrogen levels treated on the basis of nitrogen input were applied as basal fertilization with the levels of liquid pig manure (LPM) 0, 100, 200, and 300 kg ha^{-1}. Imaging data from the digital camera was obtained at grain filling stage (May 2003) and then plant leaf was collected for chlorophyll and nitrogen analysis.

†Corresponding author: (Phone) +82-31-670-5081 (E-mail) taewkim@hnu.hankyong.ac.kr
<Received February 28, 2004>

Numeric evaluation of digital image

A portable digital camera (Digimax 210SE, SAMSUNG, Korea) was used to record images of plant. The distance between the digital camera and plants was 0.4-0.5 m. We have collected the digital data on cloudy day to reduce the excessive reflectance. Images were transferred to a personal computer, and then converted to full color images. The full color images consisted of red (R), green (G) and blue (B) channels; each color had 256 grades. One leaf, perpendicular to the lens surface of camera and without glossy reflectance, was selected from a captured image. Thus, images from unsuitable leaf were excluded from the analysis. The selected images were extracted by image processing software (Photoshop ver.7.0, Adobe systems, USA), and the mean values of R, G and B of the images recorded as arbitrary 'digital numbers' with 0 to 255 were obtained in 10 replicates.

Immediately after the image was recorded in the field, the amount of chlorophyll per unit area of the leaves measured by a spectrophotometric method (Arnon, 1949) was compared with the data of chlorophyll meter (SPAD-502, Minolta, Japan). The chlorophyll meter was calibrated using 30 leaves of rye differing in chlorophyll content.

Chlorophyll and nitrogen analysis

After measuring with chlorophyll meter, 9 cm^2 of leaves were sampled and homogenized in 80% acetone. After centrifugation, the residue was extracted again with 80% acetone. The supernatants were combined and their chlorophyll concentrations determined by the method of Arnon (1969), with a UV/Vis spectrophotometer (Beckman DU 650). Nitrogen contents were measured by Kjeldahl method (RDA, 1988).

RESULTS AND DISCUSSION

Effect of nitrogen application on biomass production

With increasing nitrogen level, fresh weight in Koolgrazer was proportionally increased whereas the highest biomass productions of aerial part were obtained at N 200 kg ha^{-1} (Fig. 1) in both Olhomil and Paldanghomil. However, it appeared that the fresh weight of Olhomil was higher than other cultivars (Paldanghomil and Koolgrazer) at any N application rate. Also, leaf chlorophyll contents in Olhomil extracted from shoot grown at high level of nitrogen were also higher than other cultivars (see Fig. 2). It seemed that the relationships between nitrogen and fresh weight was highly positive (Table 1).

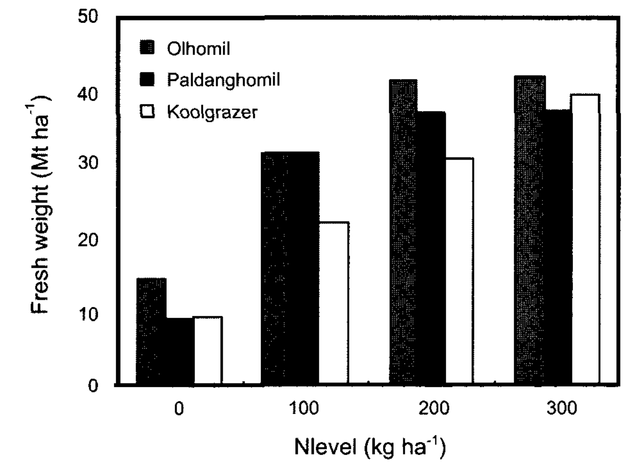

Fig. 1. Effect of nitrogen application on biomass production (fresh weight) in rye.

Table 1. Correlation matrices among nitrogen level, chlorophyll content, and some photo-numeric values.

	N level	SPAD502	T-Chl	Chl-a	Chl-b	R	G	B	R-B	G-R
SAPD 502	0.87**	-								
T-Chl	0.72*	0.62*	-							
Chl-a	0.67*	0.59	0.97**	-						
Chl-b	0.71*	0.65*	0.94**	0.97**	-					
R	-0.83**	-0.69*	-0.57	-0.53	-0.54	-				
G	-0.83**	-0.68*	-0.64**	-0.59*	-0.60	0.98**	-			
B	0.30	0.41	0.04	0.01	0.05	-0.03	0.04	-		
R-B	-0.87**	-0.79**	-0.54*	-0.49	-0.51	0.92**	0.88**	-0.42	-	
G-R	0.69*	0.60*	0.29	0.27	0.29	-0.87**	-0.77*	0.19	-0.87**	-
G-B	-0.88**	-0.81**	-0.59*	-0.53	-0.55	0.88**	0.87**	-0.47	0.99**	-0.78*

T-Chl; total chlorophyll content, R; red numeric value, B; blue numeric value, G; green numeric value.
*,**; significant level of 0.05 and 0.01, respectively.

Correlation coefficients among nitrogen status, chlorophyll contents and numeric values from image analysis by Photoshop

Correlation coefficients among chlorophyll contents and nitrogen level, and photo-numerical numbers were presented in Table 1. Chlorophyll contents measured by chlorophyll meter (SPAD 502) and those from extracts with 80% acetone were positively correlated. Values given by the chlorophyll meter and nitrogen contents were also linearly correlated. Matsuzaki *et al.* (1972) had reported that leaf color plates were practical tool for nutrient diagnosis of crops. Furthermore, it was also suggested that green color was proportional to the nitrogen content (%) in leaves. We used the color plates as a tool for measuring chlorophyll concentration in leaves. Images of the leaf color plates were obtained from the portable digital camera and the characteristics of the color components were also investigated. The contents estimated using color image analysis were significantly correlated to the content determined with spectrophotometric method except for the data based of the B value. Correlation coefficients between the component values and chlorophyll content were also given in Table 1. G-B value (-0.81) and R-B (-0.79) showed high negative correlation coefficients with chlorophyll contents measured by chlorophyll meter (SPAD 502). The correlation between chlorophyll content analyzed by acetone extraction and other numeric values from leaf digital image was relatively poor (see also Fig. 5). Furthermore, it was deduced that nitrogen status had also positive correlation with G-R value (Table 1). On the other hand, R, G, R-B and G-B values obtained from the images on Photoshop had negative correlation with N level. In addition, it was observed that chlorophyll contents analyzed by SPAD 502 and spectrophotometric method were quiet positive-correlated significantly.

Estimation of chlorophyll contents and Nitrogen status from R, G and B values

At grain filling stage, the relationships between chlorophyll content and nitrogen level in the leaves of Olhomil, Paldanghomil, and Koolgrazer were presented in Fig. 2. Total chlorophyll contents in leaves were increased with increasing application amount of nitrogen. They were also highly related to the applied nitrogen level. The chlorophyll content of Olhomil was higher than those in Paldanghomil and Koolgrazer. As shown in Fig. 2, it was explained that correlation coefficient between total chlorophyll content and supplied nitrogen was positive as r^2=0.99 and 0.82 in Olhomil and Paldanghomil respectively (Fig. 2).

The values from SPAD-502 were also significantly correlated with total chlorophyll contents and their r^2 values in three cultivars were ranged from 0.69 to 0.88 (Fig. 3).

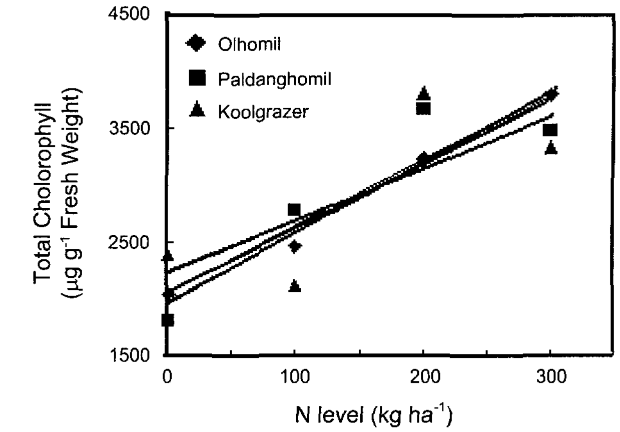

Fig. 2. The relationships between chlorophyll content and nitrogen level. Olhomil; T-Chl=60.65×N-level+1972.0 (r^2=0.99**), Paldanghomil; T-Chl=59.29×N-level+2041.4 (r^2=0.82**), Koolgrazer; T-Chl = 45.45 × N-level + 2235.1 (r^2=0.54*).

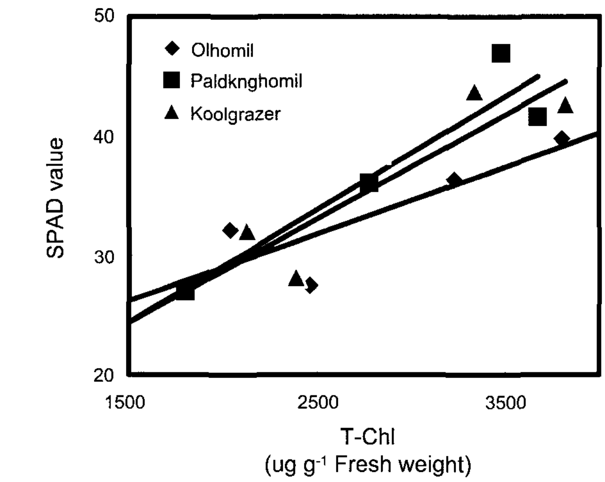

Fig. 3. The relationships between chlorophyll content and SPAD value. Olhomil; T-Chl=178.6×SPAD value 3169.6 (r^2= 0.69**), Paldanghomil; T-Chl=105.3×SPAD value 1071.6 (r^2=0.88**), Koolgrazer; T-Chl=114.9×SPAD value 1300.0 (r^2=0.80*).

Both R and G had clearly negative correlation with total chlorophyll content (Fig. 4 and 5). Kawashima and Nakatan (1998) reported that the values of R-B and G-B are the corrected values of R and G using B as a base value. As this operation reduces the bias noise in R, G and B, the correlation of R-B and G-B with chlorophyll content is higher than those of R and G. These tendencies were observed in our results. In this work, we would suggest that applied N-levels are to be easily estimated only from R and G values rather than R-B and G-B values. These estimations of N-level and chlorophyll content were more exact than previous reports using reflectance analysis (Wallihan, 1973; Takebe *et al.*,

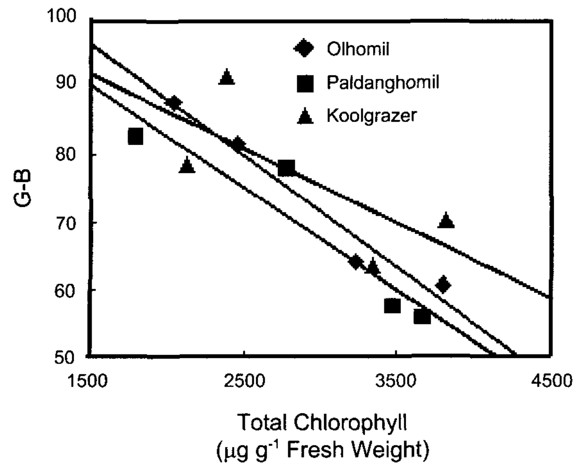

Fig. 4. The correlation between color numeric values (G-B) analyzed by Photoshop and total chlorophyll content. Olhomil; T-Chl=-58.8×(G-B)+7127.1 (r^2=0.96**), Paldang homil; T-Chl=-66.7×G-B+7556.0 (r^2=0.88**), Koolgrazer; T-Chl=-90.9× G-B+9858.2 (r^2=0.52*).

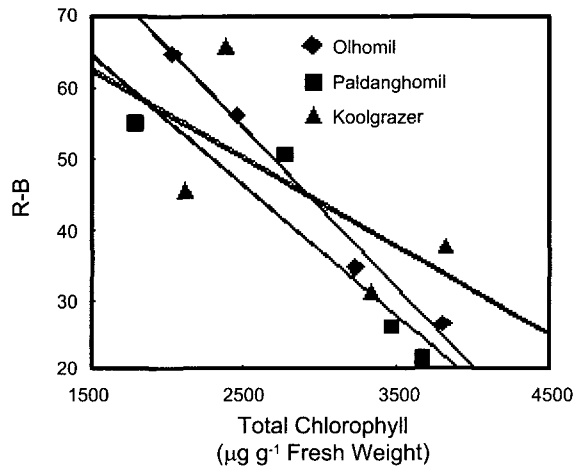

Fig. 5. The correlation between color numeric values analyzed by Photoshop (R-B) and total chlorophyll content. Olhomil; T-Chl=-43.5×R-B+4820.9 (r^2=0.99**), Paldanghomil; T-Chl =-52.6×(R-B)+4868.9 (r^2=0.86**), Koolgrazer; T-Chl=-76.9×R-B+6301.5 (r^2=0.44*).

1990; Yoder & Pettigrew-Crosby, 1995).

On the other hand, we have tried to find better function in various transformations of numeric values (data not shown). In final, we have found that R/(R-B) is the best parameter to estimate the chlorophyll content of leaves using only the data from the digital camera (Fig. 6). The regression curves between R/(R-B) and chlorophyll content were calculated for the data collected on cloudy days. Coefficients of determination were from 0.33 to 0.99 (Fig. 6). The correlation between chlorophyll content and R/(R-B), which showed the best result, did not give the best together. At least in the two cultivars, Olhomil and Paldanghomil, however, the suggested functions were quite appropriate for the estimation of

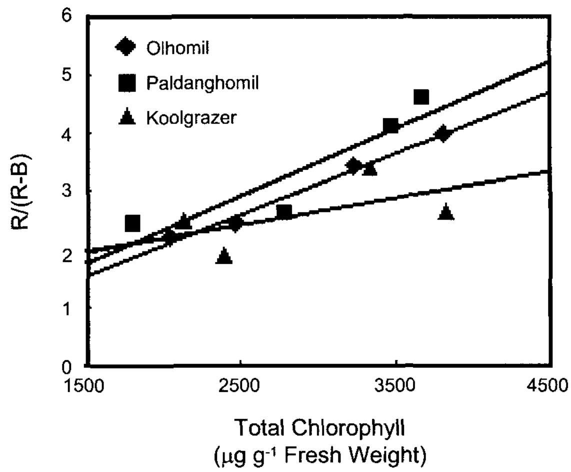

Fig. 6. The correlation between R/(R-B) and total chlorophyll content. Olhomil; T-Chl=909.1×R/(R-B)+79.1 (r^2=0.99**), Paldanghomil; T-Chl=909.1×R/(R-B)-61.7 (r^2=0.83**), Koolgrazer; T-Chl=2500.0× R/(R-B)-3323 (r^2=0.33*).

chlorophyll contents in leaves.

In conclusion, we suggest that image data from digital camera can quickly and inexpensively inform farmers and researchers of crop foliar N status and chlorophyll content in the field.

ACKNOWLEDGEMENT

This work was financially supported by the research project from KRRC, Hankyong National University, and Environment & Undergroundwater Co. Limited.

REFERENCES

Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenol-oxidase in vulgaris. Plant physiol. 24 : 1-15.

Benedict, H. M. and R. Swidler. 1961. Nondestructive method for estimating chlorophyll content of leaves. Science 133 : 2015-2016.

Chang, S. X. and D. J. Robinson. 2003. Nondestructive and rapid estimation of hardwood foliar nitrogen status using the SPAD-502 chlorophyll meter. Forest Ecol. Manag. 181 : 331-338.

Chappelle, E. W., M. S. Kim, and J. E. McMurtrey. 1992. Ratio analysis of reflectance spectra (RARS): An algorithm for the remote estimation of the concentrations of chlorophyll A, chorophyll B, and carotenoids in soybean leaves. Remote Sens. Environ. 39 : 239-247.

Hardwick, K. and N. R. Baker. 1973. In vivo measurement of chlorophyll content of leaves. New phytol. 72 : 51-54.

Inada, K. 1964. Studies on a method for determining the deepness of green color and chlorophyll content of intact crop leaves and its practical application. 1. Principal for estimating the deepness of green color and chlorophyll content of whole leaves. Proceed. crop Sci. Soc. Japan 32 : 301-308.

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

Macnicol, P. K., M. L. Dudzinski, and B. N. Condon. 1976. Estimation of chlorophyll in tobacco leaves by direct photometry. Annals Bot. 40 : 143-152.

Murphy R. J., T. J. Tolhurst, M. G. Chapman, and A. J. Underwood. 2004. Estimation of surface chlorophyll on an exposed mudflat using digital colour-infrared (CIR) photogradhy. Estuar. Coast. ad Shelf Sci., in press.

RDA. 1988. Methods of soil chemical analysis, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon, Korea.

Stajnko, D., M. Lakota, and M. Hocevar. 2004. Estimation of number and diameter of apple fruits in an orchard during the growing season by thermal imaging. Comput. Electron. Agri. 42 : 31-42.

Takano, Y. and S. Tsunoda. 1970. Light reflection, transmission, and absorption rates of rice leaves in relation to their chlorophyll and nitrogen content. Tohoku J. of Agri. Res. 21 : 111-117.

Takebe, M., T. Yoneyama, K. Inada, and T. Murakami. 1990. Spectral reflectance ratio of rice canopy for estimating crop nitrogen status. Plant Soil 122 : 295-297.

Wallihan, E. F. 1973. Portable reflectance meter for estimating chlorophyll concentrations in leaves. Agron. J. 65 : 659-662.

Yoder, B. J. and R. E. Pettigrew-Crosby. 1995. Predicting nitrogen and chlorophyll content and concentrations from reflectance spectra (400-2500 nm) at leaf and canopy scales. Remote Sens. Environ. 53 : 199-211.