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A comparative study of chlorophyll content estimation techniques through image analysis

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ABSTRACT

Leaves are treated as photosynthetic engines of the plant. Chlorophyll content is considered as most important agronomic parameter for studying physiological features related to plant growth, photosynthetic and transpiration process etc. Manual Chlorophyll Extraction procedure using DMSO (Dimethyl sulfoxide) solution and Spectrophotometer are accurate and considered as benchmark for the chlorophyll measurements. But, it is destructive, laborious, time consuming and relatively expensive. In this context, high-throughput colour image analysis is a fast and automated alternative of non-destructive phenotyping.In this paper, we have studied and compared existing techniques of chlorophyll estimation through image processingon a real experimental data and the technique given by Adamsen et al. (1999) was found as best in our experiment with RMSE 0.67.

***Keywords*: Chlorophyll, DMSO, image analysis, non-destructive phenotyping, RGB, rice and RMSE**

Leaves are considered as photosynthetic engines of the plant. Chlorophyll content is the most important agronomic parameter for studying physiological features of the plant (Ali and Anjum, 2004). Recently, many image processing techniques have been developed to monitor plant health using mainly the RGB (Red Green Blue) colour model (Erickson *et al.,* 1988). In almost all studies, digital cameras were used to acquire leaf images, which were then analysed to examine the relationship between the R, G and B values and chlorophyll and nitrogen content of plants (Mercado-Luna *et al.,* 2010). Kawashima and Nakatani(1998)has developed a low-cost diagnostic method to assess the nutrient status of plants, based on the estimation of chlorophyll content of leaves using a portable colour video camera and a personal computer. They examined the relationship between chlorophyll content and various functions derived from red, green and blue wavelengths. They showed that (R-B)/(R+B) is good formula to determine foliar chlorophyll status in wheat.Yuzhu *et al.*(2011) determined the chlorophyll index to induce nitrogen status in pepper plants by color image analysis. The chlorophyll index was determined using a soil plant analysis development (SPAD)-502 chlorophyll meter. Also, color images were taken with a digital camera and images were processed in order to determine the averages of the red (R), green (G) and blue (B) colors. They observed that G/(R+G+B) gave good results for the estimation of nitrogen status in pepper plants. Suzuki *et al*. (1999) used this formula i.e. G/(R+G+B) to estimate chlorophyll content in broccoli. Adamsen*et al*. (1999) identified a linear relationship between G/R and measurements based on SPAD chlorophyll meter. Hu *et al*.(2010) also considered leaf colour as an index for crop stress status diagnosis. They also developed a low-cost and non-destructive method that is easy to use to assess the health status of plants, based on the estimation of chlorophyll content of leaves using a portable digital camera. They showed that the RGB colour indices of *R*, *G* and *R*+*G*+*B*, *R*-*B*, *R*+*B*, *R*+G had significant relationship with chlorophyll content. Mahdi *et al.*(2012) proposed a technique for the detection of chlorophyll content and foliar nitrogen content in plants based on leaf colour. Theyproposed an algorithm that non-linearly maps the normalized value of G, with respect to R and B, using a logarithmic sigmoid transfer function *logsig* (G-R/3-B/3)/255.

In our research, the above discussed methodologies had been studied and applied in a real experimental data and finally, a comparative study of these techniques had been done for estimation of chlorophyll content through image analysis. Rice crop was selected in this experiment because of its importance as a major food crop and availability of diverse germplasm resources.

In this study, rice plant of two genotypes (IR-64 and N22) were planted in each of 15 pots (32 cm and 23 cm diameter, 29.5 cm depth) both in control (with soil moisture) and stress (without soil moisture) condition in the Phenomics facility of Division of Plant Physiology Department of Indian Agricultural Research Institute (IARI), New Delhi. Protocol of Porra *et al.* (1989) was used for chlorophyll content estimation in lab (ground truth). According to the protocol, DMSO (Dimethyl sulfoxide) solution was used and absorbance was taken at different wavelength (A) of 470 nm, 645 nm and 663 nm using spectrophotometer and chlorophyll content was measured using the following formula: Total chlorophyll (μg ml^{-1}) = 20.2 (A_{645}) + 8.02 (A_{663})

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Table1: Laboratory data of estimated chlorophyll by DMSO method (mg g^{-1} dry weight)

	Day 1	Day 2	Day 3	Day 4	Day 5
Stress IR 64	1.2	1.2	1.6	1.3	1.7
Stress IR 64	1.3	1.3	1.5	1.6	1.7
Stress IR 64	1.1	1.3	1.5	1.7	1.6
Stress N22	1.1	1.6	1.5	1.8	1.5
Stress N22	1.1	1.7	1.7	1.5	1.7
Stress N22	1.8	1.7	1.7	1.6	1.5
Ctrl IR 64	1.2	1.4	-	1.7	1.9
Ctrl IR 64	1.7	1.2	-	2.1	2.0
Ctrl IR 64	1.0	0.9	-	2.0	1.7
Ctrl N22	1.3	1.2	-	1.8	2.0
Ctrl N22	1.1	1.3	-	1.7	1.9
Ctrl N22	1.5	1.4	-	1.9	2.1

Note: The sign "-" in the above table indicates missing observation (not available).

Fig. 1: Images taken from four direction of a single plant

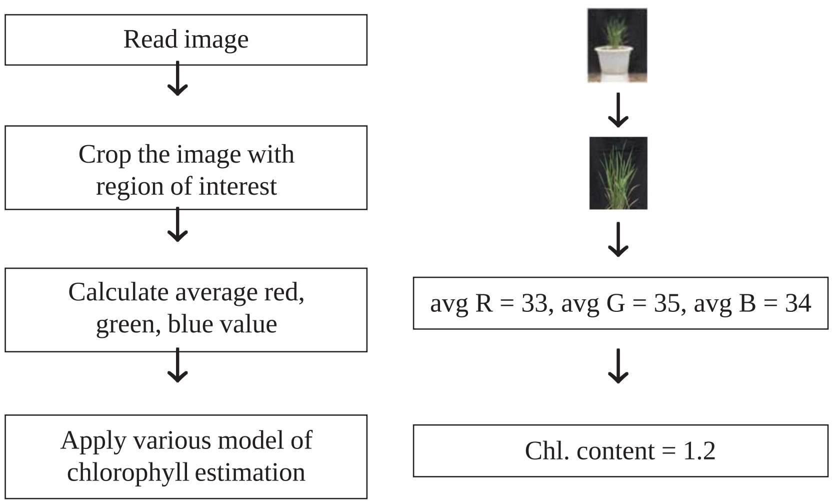

Fig. 2: Steps of chlorophyll content measurement through image analysis with an example

Plant images were captured from four sides (0^o, 90^o, 180^o, 270^o) with respect to the initial position of the camera [Nikon D7000 DSLR (16 megapixel)] by maintaining 1 meter distance from the experimented plant (Patil and Bodhe, 2011). A clean black cloth was used as background for better image processing. Plant images were captured for 5 days from 15th May, 2013 to 19th May, 2013 on 1 day's interval. On each day, 4 images were taken for the 3 experimented plants of each variety (IR 64 and N22) in both control (with moisture) and stress (without moisture) condition at 10.30 am to 11.00 am in the morning. After taking images, leaves were plucked for actual measurement of chlorophyll content. Fig.1 shows the images of the plant taken from various directions.

Average red, green and blue values of the cropped image were feed as input of the following mentioned models developed by various authors given in the table 2.

Table 2: Various techniques of chlorophyll estimationthrough image analysis

Sl. No.	Models of chlorophyll estimation
(a)	(R-B)/(R+B)(Kawashima *et al.*, 1998)
(b)	G/(R+G+B) (Yuzhu *et al.*, 2011)
(c)	G/R (Adamsen *et al.*, 1999)
(d)	Ch_{ol}(Mahdi *et al.*, 2012)

Table 3: Statistical parameters of total Chlorophyll content for the two varieties

Parameter	IR 64	N 22
Mean	1.50	1.58
Median	1.50	1.60
Mode	1.70	1.70
Standard Deviation	0.32	0.27
Kurtosis	-0.71	-0.53
Skewness	0.084	-0.25
Minimum	0.9	1.1
Maximum	2.1	2.1

To evaluate and compare the performance of each model RMSE (Root Mean Square Error) was calculated by using the formula :

$$\text{RMSE} = \frac{1}{n}\sum_{i=1}^{n}\left(y_i - \hat{y}_i\right)^2 \quad (1)$$

where, y_i is the ith observation and dependent variable and $\hat{y}_i$ is the predicted value for the ith observation.

Initially, the descriptive component of chlorophyll content was analysed (Table 3) andmean component of the varieties namely IR64 and N22 were1.50 and 1.58 respectively. It was also seen that the median value was very close to each other. For both the varieties, Skewness, kurtosis value was also considered to look the distribution of the chlorophyll content. The distributional properties of colour component (R-G-B) were also analyzed and the mean value of colour component was ranges from 42.35 to 46.21. It could be concluded that, for R and G, the median is very close to each other but for Rand B, some deviation was seen between the two components.

The above discussed methods were considered and for each method, the estimated chlorophyll content was calculated. It was seen that R, G and B were most sensitive to chlorophyll content. In this study, the same sets of images were used and the obtained colour components of different ratios were used to estimate the chlorophyll content.

Table 4: Statistical parameters of R, G and B values of two varieties

Parameter	R	G	B
Mean	42.35	42.36	46.21
Median	42.00	42.00	46.00
Mode	41.00	38.00	42.00
Standard deviation	7.66	7.31	7.91
Kurtosis	6.18	7.24	5.68
Skewness	1.45	1.59	0.98
Minimum	25.00	25.00	23.00
Maximum	80.00	79.00	84.00

Till now, various methodologies were developed to estimate the chlorophyll content and among them the discussed methods perform very well.Hence, the above discussed procedures for estimating the chlorophyll content were taken under consideration. Using the colour component, the estimated chlorophyll content was calculated and compared with the observed chlorophyll content. For comparison purpose, we have taken RMSE values(Equation 1). Table 5 shows the RMSE result for some of the popular technique of chlorophyll content estimation through image analysis and found that among all the techniques, the method developed by Kawashima *et al.*, (1998) performs very poor with corresponding RMSE value 1.67 and G/R (Adamsen *et al.*, 1999) outperformsother methods with RMSE value 0.67.

Table 5: RMSE of several developed techniques for chlorophyll estimation through image analysis

Sl. No.	Other Developed Models	RMSE
(a)	(R-B)/(R+B)(Kawashima *et al.*, 1998)	1.67
(b)	G/(R+G+B) (Yuzhu *et al.*, 2011)	1.31
(c)	G/R (Adamsen *et al.*, 1999)	0.67
(d)	Ch_{ol}(Mahdi *et al.*, 2012)	1.13

Leaves enable the photosynthesis and chlorophyll content is the key component of the procedure which is basis for all types of physiological and biochemical features in plants. Hence, chlorophyll estimation is very important in agronomic point of view and need to extract chlorophyll from study material is also crucial. Manual Chlorophyll Extraction proceduresare destructive, laborious, time consuming and relatively expensive. Hence, high-throughput colour image analysis is taken under consideration and discussed in the present study. Different popular methods of estimation of chlorophyll content were discussed.From the derived colour component values, different parameters were shown to observe the distributional properties. In this study, we have analyzed and compared existing techniques of chlorophyll estimation through image processing on a real experimental data and the technique given by Adamsen*et al*. (1999) was found as best in our experiment with RMSE 0.67.

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