ISSN (Online) 2321 – 2004
ISSN (Print) 2321 – 5526

INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH IN ELECTRICAL, ELECTRONICS, INSTRUMENTATION AND CONTROL ENGINEERING
Vol. 3, Issue 7, July 2015

Evaluation of leaf chlorophyll content by a non-invasive approach

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Abstract: Leaf chlorophyll is the most photochemically active compound present in the plant cells, which plays an important role in the process of photosynthesis by capturing light energy and converts it into chemical energy. Health status and nitrogen content in the plant can be determined by chlorophyll concentration, so a rapid image processing method has been proposed to measure the chlorophyll. The spectral properties of an image like Hue, Saturation and Intensity are modeled as linear correlation functions for chlorophyll content. A significant correlation was observed between the predicted chlorophyll by model and chlorophyll content measured by atLEAF meter. The value of R^2 and Root Mean Square Error (RMSE) between the observed and estimated chlorophyll content was 0.95 and 2.045 respectively.

Keywords: Image analysis, RGB model, HSI model, hsi model, chlorophyll content, atLEAF meter, RMSE.

I. INTRODUCTION

Chlorophyll is a green molecule present in plant cells responsible for greenness of leaves that indicates the nitrogen status in the plant [1]. Recent studies have indicated that the cultural conditions especially glucose are the growth regulators and can influence the photosynthetic ability of plants. The evaluation of photosynthetic properties is essential to know the growth rate of plant for efficient productivity.

The determination of chlorophyll concentration of plants would provide useful information regarding the photosynthetic capability and nitrogen status. Conventional methods to measure chlorophyll contents are spectroscopic and chromatographic techniques [2, 3]. The methods are destructive, too laborious, and time consuming. Also such methods are not applicable for real time measurement of chlorophyll concentration. On the other hand nondestructive methods like chlorophyll meters are costly [4, 5]. So for real time estimation of chlorophyll content and for cost effectiveness proposed image processing is a better alternative.

Image analysis has been used in many research areas and agriculture is one of them. RGB based analysis was primarily done by Kawashima and Nakatani using video camera [6]. Later on image analysis has been employed for chlorophyll measurement in various crops [7, 8]. RGB analysis is also used to determine nitrogen status in plants [9, 10].

Any color is composed of three primary colors that are red (R), green (G) and blue (B). The intensity of each primary color can be represented by an image histogram i.e. the brightness level of each primary color against the number of pixels. So it would be possible to establish a mathematical correlation between the chlorophyll content of plant and the brightness values of three primary colors [11]. Recently RGB based image analysis has been done by Su et al. and they developed a mathematical relation to find the leaf chlorophyll content. For that they extracted RGB values from leaf image and computed the chlorophyll content [12]. Later on many research papers have been published that used other color spaces into consideration like HSB, HSI and HSL etc.

The present work describes an image analysis method based on intrinsic features hue, saturation and intensity of an image for rapid and non-invasive estimation of chlorophyll content of betel leaves.

II. MATERIALS AND METHODS

A. Plant material and Climatic conditions

The betel leaf is also known as paan in India. It grows best under the rainfall of about 2250-4750mm, relative humidity and temperature required is 40-80% and 15-40°C respectively. A sandy clay soil with pH range of 5.6 –8.2 is suitable for its cultivation. It requires about 400-600 Kg N/ha, 200-300 Kg P_2O_5/ha and 200-250 Kg K_2O/ha for good production [13].

B. Determination of chlorophyll content

Hundred betel leaves (area 81mm^2) were excised randomly for analysis. Then the chlorophyll content of the leaves was measured using atLEAF chlorophyll meter. This meter is a powerful, easy to use device to measure the relative chlorophyll content of green leaf plants and has a good correlation with the values observed by SPAD-502 chlorophyll meter [14]. The atLEAF+ sensor was introduced in 2013 by FT Green LLC. (Wilmington, USA). The atLEAF meter measures transmission of red light (660 nm), at which chlorophyll absorbs light, as well as transmission of infrared light at 940 nm, where no absorption occurs [15].

C. Histogram analysis

Betel leaves were scanned using HP scan jet scanner with 300 pixels per inch (ppi) resolution and 24 bit color depth. As the measuring area of atLEAF meter is (9mm × 9mm), a fixed number of pixels (12 × 12) were selected from the digital image of leaves. The images were stored in .jpg format. Histogram of leaf image was obtained using MATLAB 2010a. The histogram obtained gives the information regarding red, blue and green components. Also R, G and B values were calculated from Photoshop ver. 7 an image processing software. Hue (H), saturation

DOI 10.17148/IJIREEICE.2015.3702

(S) and intensity (I) values are obtained from RGB values and corresponding h, s, i values were calculated as h = H/(H+S+I), s = S/(H+S+I), i = I/(H+S+I) respectively for the three color components. The correlation between observed and estimated chlorophyll content was determined and square of the correlation coefficient (R^2) was evaluated using MS-Excel. Histogram of the three primary colors of scanned betel leaf has been shown in Fig.1.

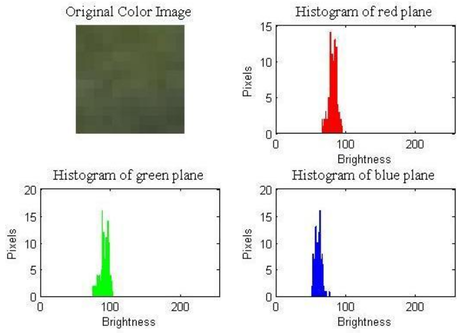

Figure 1: Histogram of the brightness values of the three primary colors

D. Determination of chlorophyll content by proposed hsi model

The image characteristics like hue, saturation and intensity are obtained from the three primary colors that are R, G and B. These brightness values are linearly correlated to get a linear equation as:

$$f(x, y) = p00 + p10*x + p01*y \quad (1)$$

where f(x, y) is the predicted chlorophyll by proposed model. Here p00, p10, p01 are model parameters, x and y corresponds to h and s respectively that is given as:

$$h = H / (H+S+I)$$
$$s = S / (H+S+I)$$

Also the chlorophyll was measured using atLEAF chlorophyll meter. Then the correlation between the predicted chlorophyll and actual chlorophyll content was determined with the help of Microsoft Excel. By putting the values obtained for model parameters as -109.1, 341.8 and -3.609 in eq. (1) we get squared correlation R^2 as 0.95 with RMSE 4.32. That shows that the obtained result has less error for that particular data set. The complete process of chlorophyll measurement has been shown in Fig. 2.

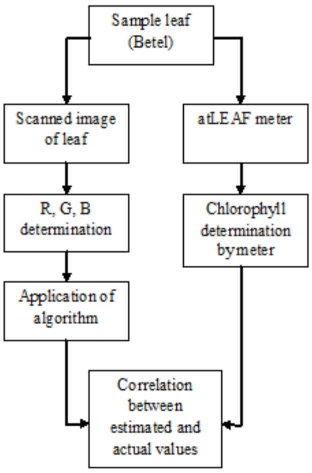

Figure 2: Flow scheme of chlorophyll measurement by proposed methodology

III. RESULTS AND DISCUSSION

A. Correlation of mean brightness values (RGB) and spectral information (HSI) with chlorophyll content

The primary colors R, G and B shown a very good correlation with the chlorophyll measured by atLEAF meter. R and G have shown good correlation with the chlorophyll content obtained with the help of atLEAF meter whereas B color exhibits a poor correlation. The value of R^2 obtained for red and green colors was 0.80 and 0.76 respectively but was 0.14 for the blue color as shown in Fig. 3.

The primary colors are not capable of reproducing each of the pixel information correctly [16]. So the red, green and blue colors are converted into spectral information hue (H), saturation (S) and intensity (I). Results show that H and S show strong correlation with the chlorophyll content whereas I shows moderate correlation. The squared correlation coefficient (R^2) obtained as 0.82, 0.87 and 0.52 respectively shown in Fig. 4.

Again the spectral information Hue, Saturation and Intensity (HSI) are converted into spectral ratios h, s and i. All the three parameters h, s and i show excellent correlation with the chlorophyll measured by atLEAF meter. The value of R-squared (R^2) obtained is 0.98, 0.88 and 0.98 for h, s and i respectively shown in Fig. 5. That means the response of spectral ratios was much more positive as compared to other approaches.

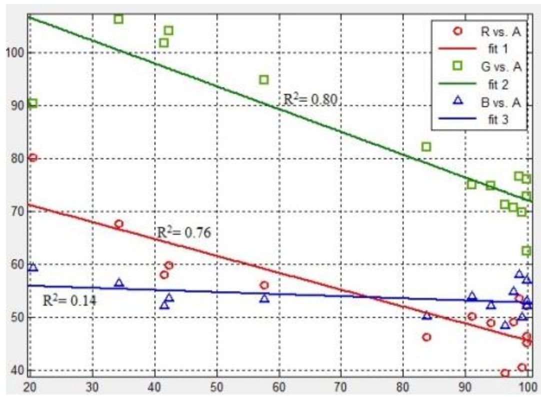

Figure 3: Correlation of R, G and B with chlorophyll content

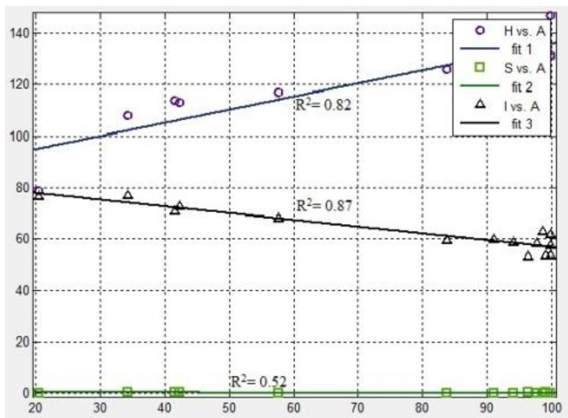

Figure 4: Correlation of H, S and I with chlorophyll content

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B. hsi model for estimation of chlorophyll content

The chlorophyll content of betel leaves were found with RGB, HSI and hsi models. Modal parameters in eq. (1) p00, p10 and p01 were obtained by MATLAB 2010a. Where x and y are variables that represent R and G in RGB model, H and S in HSI model and h and s in hsi model simultaneously. The squared correlation R^2 obtained for RGB model was 0.914 and 0.912 for HSI model. Correlation obtained by RGB and HSI models is better but error rate is quiet high. To overcome this hsi model has been proposed. This hsi model gives less error with high squared correlation coefficient of 0.95.

Using eq. (1) chlorophyll content can be estimated using hsi model. For hsi model the modal parameters p00, p10 and p01 obtained as -109.1, 341.8 and -0.00036 respectively. By putting the values of h and s in eq. (1) in the place of x and y, the value of R^2 obtained as 0.95 with RMSE 4.315. It is clear that with hsi model error has been reduced to a minimal value and also correlation between estimated and observed chlorophyll content got increased.

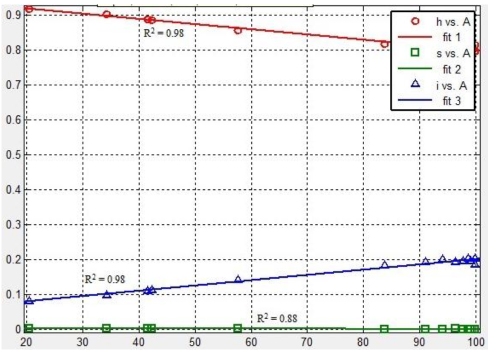

Figure 5: Correlation of h, s and i with chlorophyll content

C. Root Mean Square Error (RMSE) calculation using RGB HSI and hsi models

The chlorophyll content was estimated using eq. (1) with different models that are RGB, HSI and hsi. For the models discussed RMSE has been calculated using curve fitting tool in MATLAB 2010a. As RMSE is the error between estimated and observed values. The results show that error between estimated and observed chlorophyll content was maximum for HSI and RGB models i.e. 9.109 and 8.515 respectively, whereas the error for proposed hsi model was 2.045. The corresponding graphs of RMSE have been shown in Fig. 6, Fig. 7 and Fig. 8 simultaneously. Where A is the observed chlorophyll content by atLEAF meter and ARGB, AHSI and Ahsi is the estimated chlorophyll by RGB, HSI and hsi models respectively.

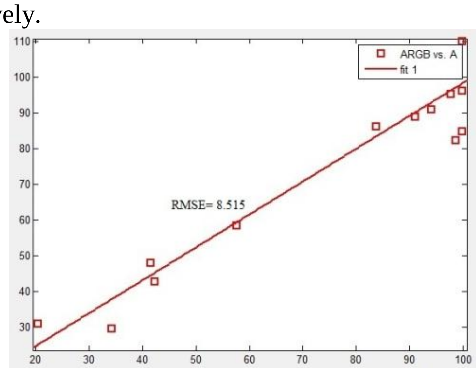

Figure 6: RMSE between A and ARGB using RGB model

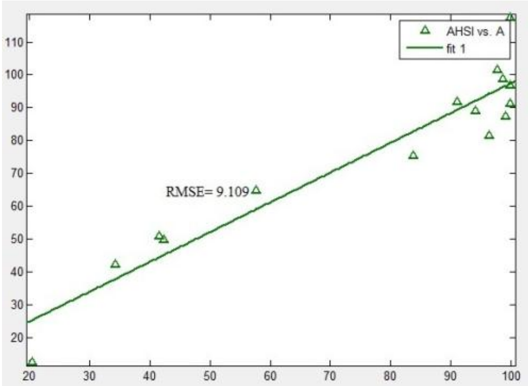

Figure 7: RMSE between A and AHSI using HSI model

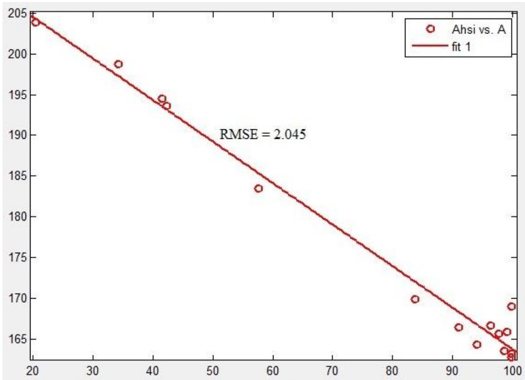

Figure 8: RMSE between A and Ahsi using hsi model

IV. CONCLUSION

In conclusion, the present work describes an image analysis system for rapid estimation of chlorophyll content of betel leaves using spectral information like hue, saturation and intensity. A linear mathematical relation is established to correlate with the chlorophyll content apart from the simple correlation analysis. A good agreement between the predicted and actual chlorophyll content is observed using his model. The findings indicate that the proposed analysis is reliable and is efficient for the estimation of chlorophyll content. The atLEAF chlorophyll meter has been used in the present study to determine the chlorophyll content and is a useful tool for quantitative estimation of chlorophyll content.

To our knowledge, the present work has been demonstrated for the first time a rapid and nondestructive method for estimation of chlorophyll content in betel leaves. The developed image analysis system has the potential for real time estimation of chlorophyll content and to analyze the photosynthetic properties. However, the image acquisition is done by scanning the leaves make the system liable for real time estimation of chlorophyll content. Image acquisition by scanning betel leaves reduces the lightening effects and errors introduced due to camera angle issues [16, 17].

ACKNOWLEDGMENT

The work has been supported by **Dr. A. P. Agrawal** (Project scientist, Plant and breeding department, TCB college of Agriculture and Research station, Bilaspur). We are thankful to him for availing us the necessary equipments to accomplish the research work.

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