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A New Image Processing Based Technique to Determine Chlorophyll in Plants

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Abstract: Leaf colour is usually used as a guide for assessments of nutrient status and plant health. We propose a new inexpensive, hand-held and easy-to-use technique for the detection of chlorophyll content and foliar nitrogen content in plants based on leaf colour. This method provides a rapid analysis and data storage at minimal cost and does not require any technical or laboratory skills. Most of the existing methods that examined relationships between chlorophyll status and leaf colour were developed for particular species. These methods acquire leaf images using digital cameras, which can be sensitive to lighting conditions (colour, angle, flux density) and hence, require proper calibration. Our method analyses leaf colour images obtained from a digital scanner that requires minimal calibration compared as it has its one light source and the angle and distance between light and leaf are constant. Our new algorithm produced superior correlations with the true value of foliar chlorophyll content measured in the laboratory compared with existing non-destructive methods when applied to three different species (lettuce, broccoli and tomato).

Key words: Image analysis · Chlorophyll content · SPAD · RGB · Optileaf

INTRODUCTION

Leaf colour gives a good indication of chlorophyll content of leaves [1, 2] Hence, farmers usually prefer to keep leaves of their crops dark green and have routinely used leaf colour as a gauge for plant health [3, 4]. 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 [5, 6]. 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. 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 [7]. In the last decade, the use of the SPAD chlorophyll meter for agricultural and research purposes has increased and presently there are more than 200 published studies using SPAD [8].

In the past two decades, many image processing techniques have been developed to monitor plant health using mainly the RGB (Red Green Blue) colour model space needed here [9]. 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 [10]. Kawashima and Nakatani [3] showed that (R-B)/(R+B) is a good formula to determine foliar chlorophyll status in wheat. In contrast, Yuzhu *et al*. [11] observed that G/(R+G+B) gave good results for the estimation of nitrogen status in pepper. Moreover, Suzuki *et al*. [12] used G/(R+G+B) to estimate chlorophyll content in broccoli. Cai *et al*. [13] found that R/(R+G+B)

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is a good formula for estimating leaf chlorophyll content in cabbage, whilst Adamsen *et al*. [14] stated that the relationships between G/R and SPAD were linear over most of the range of G/R and this ratio responded to both chlorophyll concentrations and the number of wheat leaves. Su *et al*. [15] developed a linear RGB model to estimate chlorophyll content in algae and demonstrated that RGB features can be extrapolated to detect chlorophyll content. Finally, Hu *et al*. [16] 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. Vollmann *et al*. [17] used a Sony digital camera to capture leaf images and Segma Scan Pro image analysis software to obtain the averaged G value of leaves. The obtained value was then used to estimate the chlorophyll concentration and used SPAD values as a reference.

Yadav *et al*. [18] showed that real time estimation of leaf chlorophyll content in regenerated plants enclosed in a culture vessel is not possible with the SPAD meter and they indicated that RGB based image analysis was a useful tool for chlorophyll estimation in regenerated plants. Kawashima and Nakatani [3] used a portable colour video camera and a personal computer to estimate the chlorophyll content in wheat and rye leaves.

The image acquisition process is inexpensive and easy to use, but the two main problems that are yet to be solved for leaf colour-based chlorophyll estimation are: (i) maintaining high accuracy across different species and (ii) limitations imposed by observational conditions [3], especially light flux density, light spectral quality and angle of incidence.

In order to take more consistent photos, Mercado-Luna *et al*. [10] developed a new method where they installed a camera inside a box and controlled the light environment by using a 100 W lamp. Although this method was impractical and complex, they succeeded in controlling the factors limiting camera usage.

On the other hand, Cui *et al*. [19] used a normalized difference vegetation index (NDVI) as an indicator to estimate leaf nitrogen content in tomato plants. They obtained reasonable correlation between NDVI values and Nitrogen concentration.

In this paper, we present a new leaf colour based algorithm to estimate foliar chlorophyll contents. The algorithm will be compared with the SPAD-502 leaf chlorophyll meter and other image processing based methods in terms of their relationships with the true chlorophyll status that was measured using organic extraction followed by spectrophotometric measurement.

MATERIALS AND METHODS

Experimental Design: Seeds of tomato (Cultivar Tommy Toe), lettuce (cultivar Green Mignonette) and broccoli (cultivar Kailaan Express F1) were planted in pots (12 cm in diameter, 12 cm depth) filled with vermiculate for a period of three months starting from the 2nd of February 2011 in the greenhouse facility of the Faculty of Science at UTS. The experimental design was a randomized complete design with five N nutrient-supply. Each treatment was replicated five times.

Mineral Nutrition and Processing: Seeds of the three species were planted in 75 pots (25 pots for each species). For each of the three species, the 25 pots were divided into five groups; each group received a pre-specified nitrogen treatment. For the first eight weeks, they all received a complete nutrient solution compose of 5.4 mm of NH_4NO_3, 1.6 mm of K_2HPO_4, 0.3 mm of K_2SO_4, 4 mm of $CaCl_2$. $2H_2O$, 1.4mm of $MgSO_4$. H_2O, 5 μm of Fe-EDDHA, 2 μm of $MnSO_4$. H_2O, 1 μm of $ZnSO_4$.$7H_2O$, 0.25 um of $CuSO_4$. $5H_2O$, 0.3μmof $Na_2MoO_4.2H_2O$ and 0.5 μm of H_3BO_3. The nutrient solution was maintained at pH 6.0-6.1 and renewed every three days [20]. This is equivalent to an N supply of 0.43 g N L^{-1}

After week 8, five different nitrogen treatments in the form of NH4NO3 were applied for a period of seven weeks, (N0: without nitrogen, N1: 0.2 g L^{-1}, N2: 0.43 g L^{-1}, N3: 0.63 g L^{-1} and N4: 1.05 g L^{-1}) which corresponds to 0, 25, 50, 75 and 100% (control treatment) of the recommended nitrogen supply.

Chlorophyll Measurements

Chlorophyll Extraction: A leaf punch was used to cut 1.2 cm diameter leaf disks from a fully expanded leaf from each of 5 replicate plants and then homogenised using a ten broeck tissue grinder in 5 mL chilled aqueous 80% acetone. The extract was centrifuged for 5 min at 3000 rpm and the absorbance determined at 646.6 and 663.6 nm using a Varian DMS-70 Spectrophotometer. Total chlorophyll was measured using the equations below [6]:

Chl a = 12.25 A 663.6 - 2.55 A 646.6

Chl b = 20.31 /1646.6 -4.91 A 663.6

Chl a + b = 17.76 A 646.6 + 7.34/1663.6

Foliar chlorophyll was measured on weeks X, Y, Z.

SPAD-502 Chlorophyll Meter: The SPAD-502 Chlorophyll meter was used to determine total chlorophyll in same leaf as was used in the acetone extraction method described above using absorptions of 650 and 940 nm wavelengths. One reading was taken for each leaf. Although this device is a good example of a non-destructive method to estimate total chlorophyll in plants, it still has some limitations. For example, a small measuring area of 12.57 mm^2 means that it needs more than 30 measurements to average and get a high and accurate result and it has limited memory (SPAD 502 catalogue).

Optileaf (Our Proposed Image Processing Technique): A hand-held portable scanner (Pico Life) was used with (40 × 22) cm reference plate. I Images collected with this instrument were analysed using a Matlab code. The RGB (Red Green Blue) values were analysed to achieve maximum correlation with the true chlorophyll status of plants. The advantage of using a portable scanner over a digital camera is the reduced effect of variation in lighting conditions on the images.

The scanning process involves placing a leaf on a white sheet of paper, while it is still attached to the plant. The scanner recorded the leaf image as it scans the leaf from top to bottom. The recorded image is then processed by averaging the R, G and B values of all the leaf pixels.

The Proposed Algorithm to Measure Chlorophyll: The algorithm we propose non-linearly maps the normalised value of G, with respect to R and B, using a logarithmic sigmoid transfer functions as follows:

$$Ch_{OL} = logsig\left(\frac{G - \frac{R}{3} - \frac{B}{3}}{255}\right)$$

where:

Chl_{ol} : Chlorophyll estimation by Optileaf
G : Green Colour: Red Colour: Blue Colour

In many real-world applications, the data analyst will have to deal with raw data that are not in the most convenient form. The data might need to be re-expressed to produce effective visualisation or an easier, more informative analysis. In this paper, we transform the data by applying a single mathematical function to all of the observations for which the power transformation (logarithmic operator) was chosen to change the shape of the data distribution. Additionally, a standardisation process was also utilised so that the data points have zero mean and unit variance.

RESULTS AND DISCUSSION

Tomato: One hundred measurements of foliar chlorophyll content determined spectrophotometrically (Lab_{Ch}), with concomitant estimates of foliar chlorophyll using our Optileaf algorithm (Ch_{OL}) and estimates with the SPAD 502 were used to determine the correlation coefficient (R) amongst these three values. These measurements were taken in weeks 10, 13, 15 and 18 (25 readings in each week).

The SPAD 502 was designed to detect the chlorophyll content of leaves [21]. Although there was a good correlation between SPAD 502 readings and spectrophotometric chlorophyll content of leaves, chlorophyll content in leaf is affected by many factors such as plant genotype, nutrient concentration, leaf thickness or biotic stresses like disease. In order for SPAD-502 readings to be accurate, it must be calibrated for the variety and species of plant examined and other environmental factors. This calibration can be accomplished by over-fertilising three or more areas in the field with nitrogen and then taking reference measurements from these areas to compare with the rest of the field. Moreover, it is recommended not to depend on one reading to detect chlorophyll status but take the mean of several readings [22]. However, this is obviously a time and labour consuming process.

Figure (1- A) shows the correlation between SPAD-502 readings and Lab_{Ch} with correlation coefficient of 0.90. This result is similar to many studies using SPAD to estimate chlorophyll content [10].

Correlation between Ch_{OL} and LabCh was higher (Fig. 1-B), where correlation coefficient was around 0.97

Lettuce: Lab_{Ch}, Ch_{OL} and SPAD 502 measurements for 100 samples during different weeks of plant age (10, 13, 15 and 18) were used to obtain correlation coefficient between Lab_{Ch} and both Ch_{OL} and SPAD. (Figure1- C) shows that the value of correlation coefficient for SPAD was 0.74 and for Ch_{OL} were 0.90.

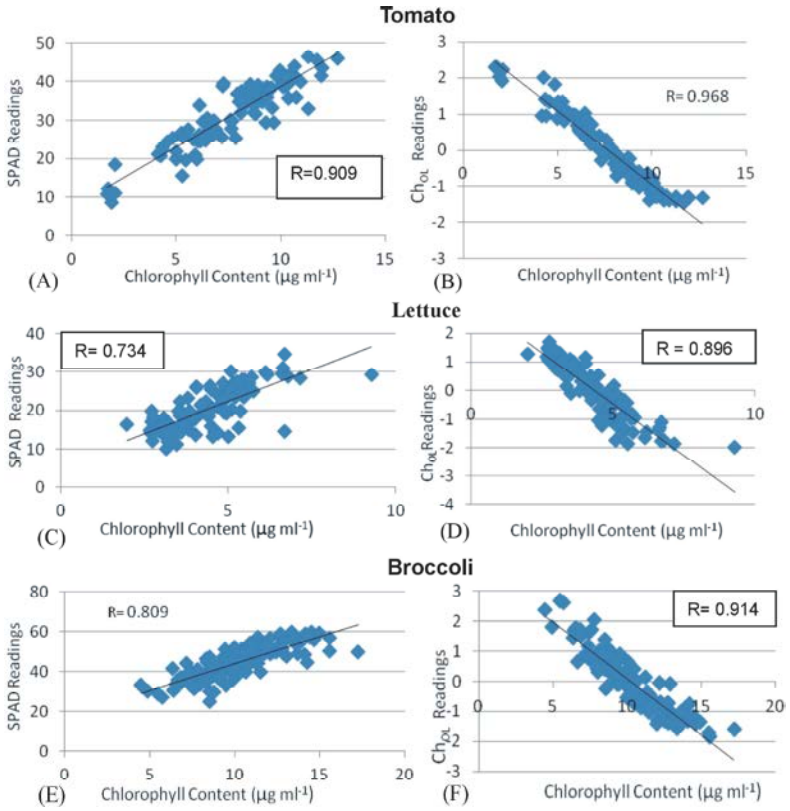

Fig. 1: Correlation between Lab_{Ch} and the estimated chlorophyll readings using SAPD (A, C and E) and Ch_{OL} (B, D and F) for tomato, lettuce and broccoli

Broccoli: In this experiment the readings for LabCh, Ch_{OL} and SPAD 502 were taken for five weeks (10, 13, 15, 18 and 21). The correlation between Lab*Ch* and the SPAD readings produced correlation coefficient of 0.81 (Fig. 1-E). The correlation between Lab_{Ch} and the Ch_{OL} readings produced correlation coefficient was 0.91 and (Fig. 1-F).

The above results indicate that Ch_{OL} achieved better performance than SPAD in chlorophyll measurements for the three species examined.

Comparison Between Ch_{OL} and Other Image Processing Based Algorithms: In order to perform a thorough analysis of our proposed Ch_{OL} technique, we compared our results with those derived from application of some of the most popular image processing based chlorophyll estimation methods. The same set of images were used in these comparisons and six combinations of various ratios of RGB were used (Table 1).

$$(R-B)/(R+B) \quad \text{(a)}$$

Table 1: Correlation of image processing (IP) based algorithms with LabCh for tomato, lettuce and broccoli

IP based Ch method	Developed model	Correlation Coefficient		
		Tomato	Lettuce	Broccoli
(a)	(R-B)/(R+B)	-0.906	-0.576	-0.724
(b)	G/(R+G+B)	-0.277	0.562	-0.489
(c)	R	-0.874	-0.868	-0.815
(d)	R/(R+G+B)	-0.765	-0.795	-0.692
(e)	G/R	0.498	0.768	0.116
(f)	R+G	-0.926	-0.878	-0.849
Ch_{OL}	Ch_{OL}	0.968	0.896	0.914

This formula was developed by Kawashima & Nakatani [3] to measure chlorophyll in wheat plants. 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 fact, Kawashima and Nakatani [3] examined a number of other formulas and recommended the above one as it outperformed the other formulas.

$$G/(R+G+B) \quad \text{(b)}$$

Suzuki *et al*. [12] applied this formula to detect chlorophyll content in broccoli using a digital video camera under artificial light conditions. This formula has also been applied by Jia *et al*. [23] to detect nitrogen status in winter wheat. Recently, Yuzhu *et al*. [11] showed that this formula gives a good correlation with N status in pepper plants.

$$R \quad \text{(c)}$$

Mercado-Luna *et al*. [10] developed a new method to take leaf images of tomato in a green house. They fixed the camera height and angel controlled the light by using a standard lamp of 100 watts and they used the automatic camera setting. They suggested that the colour image analysis can be applied to estimate the N status on tomato seedlings using red and blue colours. From the colour image analysis, red colour (R) is the most accurate predictors of N status on plants with liner coefficient around 0.91.

$$R/(R+G+B) \quad \text{(d)}$$

This formula was developed by Cai *et al*. [13] to estimate the chlorophyll content of cucumber leaves. This formula can be considered as a normalized version of the previous one.

$$G/R \quad \text{(e)}$$

Adamsen *et al*. [14] applied this formula to estimate chlorophyll concentration in wheat leaves. Cai *et al*. [13] suggested the same formula to estimate chlorophyll content of cucumber leaves. In both studies they used digital cameras to obtain images.

$$R+G \quad \text{(f)}$$

Hu *et al*. [16] used this formula to estimate the chlorophyll content of barley leaves. They used a digital camera and analysed the acquired images using Adobe Photoshop CS3 Extended 10.0 software (2009 Adobe Systems Inc., USA).

Table 1 shows correlation levels between the various RGB ratios and the true chlorophyll content as derived from spectrophotometric analyses of acetone extracts. Some of the ratios performed quite poorly on all species, such as *G/(R+G+B)* (b), while others achieved good performance on only one or two of the three species, such as *(R-B)/ (R+B)* (a) and G/R (e). Other ratios were found to be more consistent, with R+G (f) outperforming all of others In contrast, our new algorithm was not only found to be consistent, but it achieved better performance than all existing methods, including (f).

CONCLUSION

Several studies have recently pioneered image processing techniques as a method to detect chlorophyll contents in leaves. A controlled light environment is required for most of these methods to achieve higher correlation with the true chlorophyll readings. These measures help achieve more consistent readings. Despite the promising results, controlling the environment in such approach may not be practical to the farmer. In this study we applied a new technique based upon a commercially available hand-held scanner which overcomes these problems. The algorithm that was developed and validated using three different species achieved a consistently better performance than other image processing-based methods as well as the well-known SPAD chlorophyll meter.

REFERENCES

1. Gaddanakeri, S.A., D.P. Biradar, N.S. Kambar and V.B. Nyamgouda, 2007. Productivity and Economics of Sugarcane as Influenced by Leaf Colour Chart Based Nitrogen Management. Karnataka J. Agric. Sci., 20(3): 466-8.
2. Islam, Z., B. Bagchi and M. Hossain, 2007. Adoption of leaf color chart for nitrogen use efficiency in rice: Impact assessment of a farmer-participatory experiment in West Bengal, India. Field Crops Research, 103: 70-5.
3. Kawashima, S. and M. Nakatani, 1998. An algorithm for estimating chlorophyll content in leaves using a video camera. Annals of Botany, 81: 49-54.
4. Singh, V., B. Singh, Y. Singh, H.S. Thind and R.K. Gupta, 2010. Need based nitrogen management using the chlorophyll meter and leaf colour chart in rice and wheat in South Asia.areview. NutrCycl Agroecosyst, 56: 250-62.
5. Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts; polyphenol oxidases in Beta vulgaris, Plant Physiol., 24: 1-15.

6. Porra, R.J., W.A. Thompson and P.E. Kreidemann, 1989. Determination of accurate extinctions coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy Biochim Biophys Acta, 975: 384-94.
7. Minolta. 1989. Chlorophyll meter SPAD-502, Instruction manual.Minolta Co., Ltd., Radiometric Instruments Operations, Osaka, Japan.
8. Uddling, J., J. Gelang-Alfredsson, K. Piikki and H. Pleijel, 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynth Res., 91: 37-46.
9. Erickson, J.E., M.D. Keziah, L.A. Nelson and C.T. Young, 1988. Variation of colour of oil cooked Virginia type peanuts, Proceedings of the American Peanut Research and Education Society, 20: 45.
10. Mercado-Luna, A., E. Rico-García, A. Lara-Herrera, G. Soto-Zarazúa, R. Ocampo-Velázquez, R. Guevara-González, R. Herrera-Ruiz and I. Torres-Pacheco, 2010. Nitrogen determination on tomato (*Lycopersicon esculentum* Mill.) seedlings by colour image analysis (RGB). African Journal of Biotechnology, 33: 5326-32.
11. Yuzhu, H., W. Xiaomeil and S. Shuyao, 2011. 'Nitrogen determination in pepper (Capsicum frutescens L.) Plants by colour image analysis (RGB). African Journal of Biotechnology, 77: 17737-41.
12. Suzuki, T., H. Murase and N. Honamin, 1999. Non-destructive growth measurement cabbage pug seedlings population by image information. Journal of Agriculture Mechanical Association, 61: 45-51.
13. Cai, H., C. Haixin, S. Weitang and G. Lihong, 2006. 'Preliminary study on photosynthetic pigment content and colour feature of cucumber initial blooms, Transactions of the CSAE, 22: 34-8.
14. Adamsen, F.J., P.J. Pinter, E.M. Barnes *et al*. 1999, 'Measuring wheat senescence with a digital camera', Crop Science, 39: 719-24.
15. Su, C.H., C.C. Fu, Y.C. Chang, G.R. Nair, J.L. Ye, L.M. Chu and W.T. Wu, 2008. Simultaneous estimation of chlorophyll a and lipid contents in microalgae by three colour analysis. Biotechnology Bioeng, 99: 1034-9.
16. Hu, H., H.Q. Liu, H. Zhang, Jing-huanzhu, Xu-guoyao, Xiao-bin and Kee-zheng, 2010. Assessment of chlorophyll content based on image colour analysis, comparison with SPAd-502', paper presented to the, The 2nd International Conference on Information Engineering and Computer Science Proceedings, Wuhan, China, 25-26 December 2010
17. Vollmann, J., H. Walter, T. Sato and P. Schweiger, 2011. 'Digital image analysis and chlorophyll metering for phenotyping the effects of nodulation in soybean. Computers and Electronics in Agriculture, 75: 190-5.
18. Yadav, S.P., Y. Ibaraki and S.D. Gupta, 2010. Estimation of the chlorophyll content of micro propagated potato plants using RGB based image analysis. Plant Cell Tiss Organ Cult, 100: 183-8.
19. Cui, D., L. Minzan and Q. Zhang, 2009. Development of an optical sensor for crop leaf chlorophyll content detection, Computers and Electronics in Agriculture, 69: 171-6.
20. Sanchez, E., J.M. Ruiz and L. Romero, 2002. Poline metabolis in response to nitrogen toxicity in fruit of French bean plants (*Phaseolu vulgaris* L.cv strike). Scientia Horticulture, 93: 225-33.
21. Balasubramanian, V., A.C. Morales, R.T. Cruz and S. Abdulrahman, 1999, On-farm adaptation of knowledge-intensive nitrogen management technologies for rice systems, Nutrient Cycling in Agroecosystems, 53: 59-69.
22. Murdock, L., S. Jones, C. Bowley, P. Needham, J. James and P. Howe, 1997. Using a chlorophyll meter to make nitrogen recommendationson wheat. Cooperative Extension Service, University of Kentucky. AGR, pp: 170.
23. Jia, L., X. Chen, F. Zhang, A. Buerkert and V. Romheld, 2004. Use of digital camera to assess nitrogen status of winter wheat in the Northern China plain. J. Plant Nutr., 27(3): 441-50.