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An Algorithm Based on the RGB Colour Model to Estimate Plant Chlorophyll and Nitrogen Contents

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Abstract. Leaf colour gives a good indication of chlorophyll (Ch) and nitrogen (N)status in plants. In this paper we developed a new, easy to use and non-destructive diagnostic approach to detect plantCh and N levels using an image processing technique developed using the RGB (Red, Green and Blue) colour model. The experiment was conducted on tomato (Tommy Toy) in field with three N treatments (0, 60 and 140 kg N / ha), where leaf images were collected using a handheld scanner. The new algorithm achieves superior correlation with the value of Ch and N, measured in laboratory, compared with the existing non-destructive methods of SPAD 502 and Dark green Colour Index (DGCI).

Keywords: Nitrogen, chlorophyll, SPAD 502, DGCI, image processing

1. Introduction

Nitrogen (N) is a major element for plant growth and is an integral part of chlorophyll (Ch), which is primary absorber of light energy needed for photosynthesis [1]. Ch and N affects the green colour of plants and ultimately determines their biomass yield and quality. Plants sufficiently supplied with N are green and healthy, while plants insufficiently supplied with N are pale green or yellow in colour and remain small and stunted. Hence, leaf colour changes have led researchers to exploit this property by using image processing analyses to detect Ch and N status in plants.

In order to detect Ch and N in plant leaves there are two approaches. Destructive methods based on laboratory procedures are accurate but time-consuming and expensive. In contrast, non-destructive meters are less accurate, but are not time consuming nor expensive. Accordingly, non-destructive methods have been commonly used in estimating foliar chlorophyll or N for many species [2]-[5]. The most widely used device is SPAD-502, a hand-held absorbance meter. It measures the relative greenness and estimates chlorophyll content of leaves [6]-[10]. Since leaf chlorophyll content is closely related to leaf N concentration [11], SPAD has also been used to estimate N levels in plants.

Digital colour image analysis based on Red, Green and Blue (RGB) colour models have been used to determine plant Ch and N. Many studies used RGB colour models to find a correlation with Ch and N status of plant [12]-[15].

There are few studies that use other colour models with Ch and N status. [16] suggested using HSI and L*a*b colour models to diagnose nutrient elements deficiency in faba bean (ViciafabaL.), pea (Pisumsativum L.) and yellow lupine (Lupinusluteus L.). [17] showed a close correlation between the N concentration of broccoli plants and the L*a*b colour model.

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[18] developed the Dark green Colour Index (DGCI) algorithm to measure greenness grades in quantifying turf colour and this algorithm was created from the HSI colour model. [19] and [20] used this algorithm to detect N status in corn (Zea maysL.) and cotton (Gossypiumhirsutum L.) with promising results.

The aim of this paper is to develop a new algorithm based on the RGB colour model to detect Ch and N levels in tomato under field conditions and compare it with existing destructive (Ch and N laboratory methods) and non- destructive methods (SPAD 502 and DGCI values).

2. Materials and Method

2.1. Experiment design

Seeds of tomato (Solanumlycopersicumcultivar Tommy Toe) were sown into plastic trays with 18 mL cells filled with vermiculite on 10 October 2011. The seedlings were first grown under greenhouse conditions and fertilised with standard nutrient solution consisting of 5.4 mM NH4NO3, 1.6 mM K2HPO4, 0.3 mM K2SO4, 4 mM CaCl2.2H2O, 1.4 mM MgSO4.H2O, 5 µM Fe-EDDHA, 2 µM MnSO4.H2O, 1 µM ZnSO4.7H2O, 0.25 uM CuSO4.5H2O,0.3 µM Na2MoO4.2H2O, and 0.5 µM H3BO3. The nutrient solution was maintained at pH 6.0 - 6.1 and renewed every three days for three weeks [21]. On 2November 2011, the seedlings were transplanted in three rows with each row divided into 12 plots; each plot consisted of 8 plants. During the field growing period, overhead sprinkler irrigation system was used. Weeds were kept under control manually. The experimental design was a randomised complete block design with three replications at Lansdowne farm in the Camden campus of the University of Sydney (latitude 34 °01'S, longitude 150 °40'E, elevation 75m). The three nitrogen treatments were N0: without nitrogen (control), N1: 60 kg N/ha and N2: 140 kg N/ha where nitrogen (N) was applied as urea (46% N). The field experiment was applied with the recommended rates of potassium, phosphorus and other micronutrients to ensure that they are not limiting plant growth. Laboratory based Chlorophyll (Ch) and N measurements as well as estimation using SPAD and digital images (our proposed method) were recorded at three different stages of plant development on 16, 29 December 2011 and 13 January 2012.

2.2. Lab chlorophyll measurement

The first fully expanded leaves were sampled from the field and placed into an ice box and chlorophyll was measured using a Varian DMS-70 Spectrophotometer on the same day. Leaf disks (1.2 cm diameter) were taken and grounded in 5 mL aqueous 80 % acetone using the Ten Broek Tissue Homogeniser; and then the extracts were centrifuged for 5 min at 3000 rpm and the absorbance determined at 646.6, and 663.6 nm using the Varian DMS-70 Spectrophotometer. Total chlorophyll was measured using the equation below [22]:

$$\text{Chl a} + \text{b} = 17.76\, A^{646.6} + 7.34\, A^{663.6}$$

2.3. Lab nitrogen measurement

The remaining leaf (after removing the 1.2 cm leaf disk) were used to determine plant nitrogen where the samples were oven-dried at 75 ˚C for 24 hours, grounded using a ball mill grinder and sieved through a 1 mm screen; and then oven-dried again at 75 ˚C for 24 hours. The nitrogen concentrations (%) for these samples were measure dusing a LecoTruSpecCHN analyser [23].

2.4. SPAD-502 chlorophyll meter

The Chlorophyll SPAD meter (Minolta SPAD-502, Konica Minolta Sensing, Inc., Tokyo, Japan) was used to determine total leaf chlorophyll in the field. This device emits two different light intensities from two diodes: peak wavelength 650 nm (red) absorbed by the leave tissue, which estimates the chlorophyll content (greenness). A second peak 940 nm (infrared LED) is emitted simultaneously with red LED to compensate for leaf thickness [24].

2.5. Dark green colour index

[18] studied the quality of turf grass in response to nitrogen fertilizer where they suggested dark green colour index (DGCI) which covers dark green colour on a scale of zero to one with values closer to one representing a darker green. They used the HIS colour model (hue, saturation, and light intensity) and suggested thee equations below:

$$\mathrm{DGCI} = [(\mathrm{H} - 60)/60 + (1 - \mathrm{S}) + (1 - \mathrm{I})]/3$$

Recently, [19] used DGCI as an in-season N status measurement tool and they found strong correlation between it and nitrogen concentration in corn. Moreover, [20] used it to estimate nitrogen status in cotton and mentioned that the DGCI could possibly replace measurements using a chlorophyll meter to detect nitrogen status. Both of them used a camera to take photos and the images were processed using SigmaScan Pro (v. 5.0, Chicago, IL) software to calculate DGCI. However, there were many factors that affect DGCI values such as difference in lighting conditions, camera quality and setting.

2.6. The proposed image processing technique

Unlike most of the existing image processing methods that use digital cameras to capture image, we decided to capture leaf images using a hand-held portable scanner (Pico – Australian made) with (40 × 22) cm reference plate. This aims at reducing variability in terms of angle, distance and lighting conditions.The acquired images were processed using Matlab, where we identified the leaf contour and then averaged the green (G), red (R) and blue (B) values of the leaf pixles. Due to the importance of green for both Ch and N estimation, we propose the following formula:

$$\mathrm{ChN}_{\mathrm{RGB}} = \mathrm{G} - \mathrm{R}/2 - \mathrm{B}/2$$

R and B are included for a normalisation purpose.

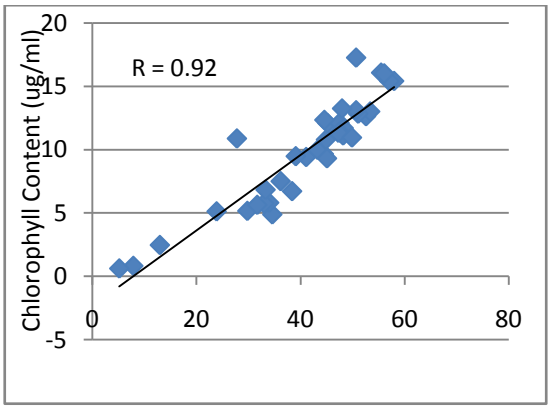
The scanning process involves placing a leaf on a white sheet. The scanner would then record the leaf image as it goes from top to bottom. Even though the hand-held scanner is less influenced by the lighting conditions than cameras, we found that it is better to scan leaves in a shaded location to reduce the effect of sunlight and glare. The images are then transferred to a personal computer for analyses.

3. Results and Discussion

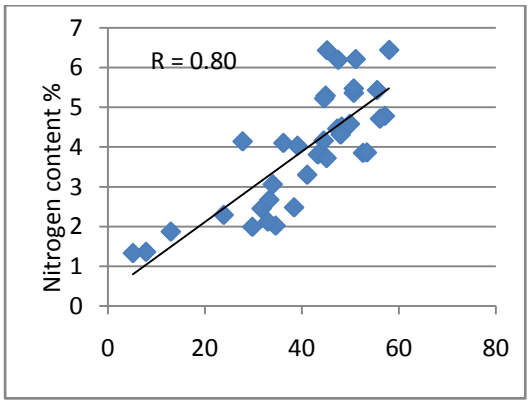
The changes in N treatments led to visible differences in leaf colour; the zero N control appeared pale green or yellow leaves with stunted growth. In contrast, leaves turned dark green in highest N treatment (140 kg N / ha).

Figure 1(a) shows a significant relationship between the SPAD-502 and Lab Ch(R = 0.92). In fact, the obtained results are similar to those reported in many studies [12], [21], [25]. DGCI is found to achieve relatively similar performance, as shown in Figure 1(e) with R = 0.91. On the other hand, the proposed method achieved R = 0.96 to outperform both SPAD and DGCI in precision of predicting Ch measurements (see Figure 1(c)).

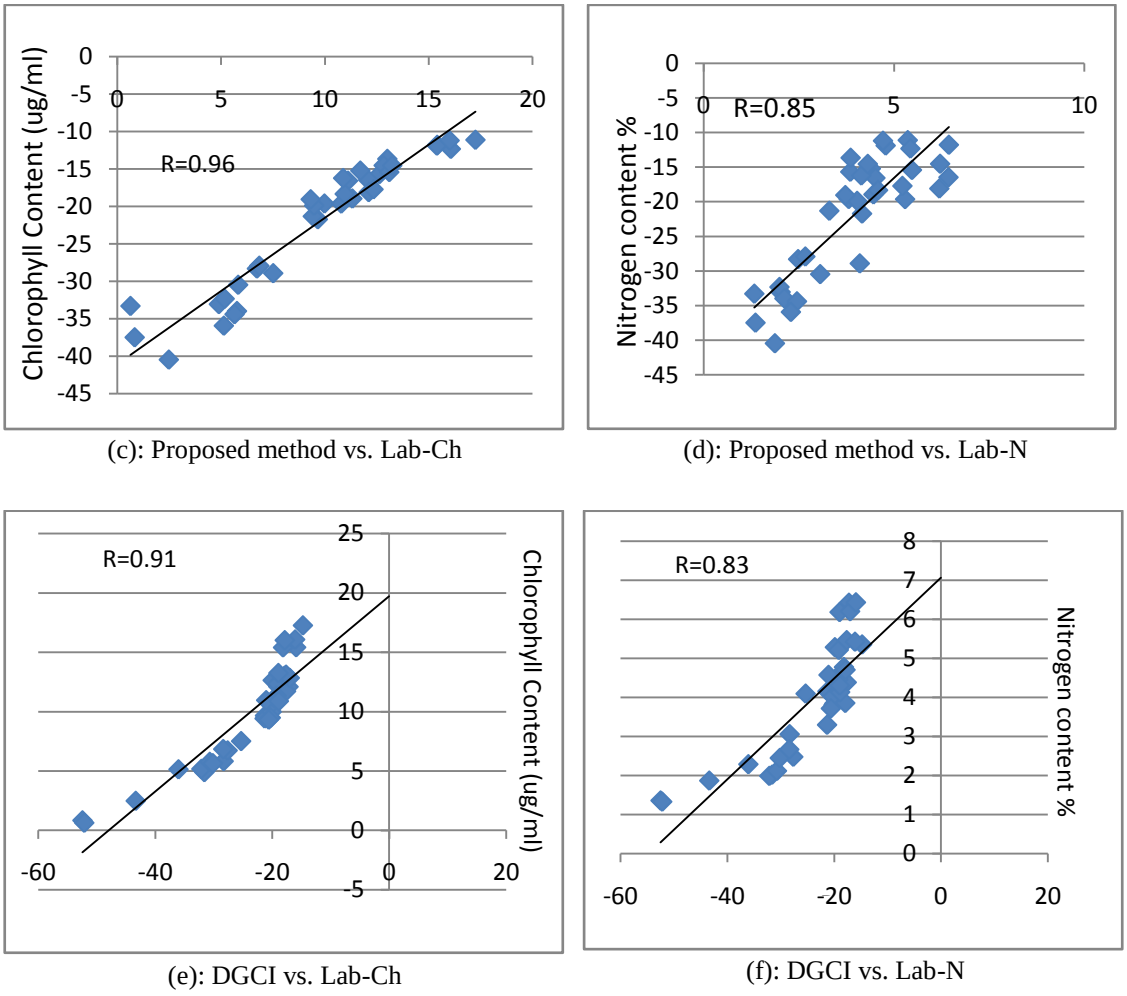
For N estimation in plant, Figure 1(b) shows the correlation between SPAD readings and N content in tomato plants(R = 0.80).DGCI gave a slightly better N detection results compared to SPAD with R = 0.83 (see Figure 1(f)). (2011) mentioned that there was a close relationship between SPAD and DGCI with leaf N concentration).As with Ch, the proposed method outperformed both SPAD and DGCI for the detection of N, where it achieved R = 0.85 (see Figure 1(d)).



(a): SPAD vs. Lab-Ch



(b): SPAD vs. Lab-N



(c): Proposed method vs. Lab-Ch

(d): Proposed method vs. Lab-N

(e): DGCI vs. Lab-Ch

(f): DGCI vs. Lab-N

Fig. 1: Correlation between SPAD, DGCI and the proposed method with respect to lab N and Ch

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