DEVELOPMENT OF NEW NON-DESTRUCTIVE IMAGING TECHNIQUES FOR ESTIMATING CROP GROWTH AND NUTRIENT STATUS

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CERTIFICATE OF AUTHORSHIP/ORGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature of Candidate

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Active light source (ALS); lab-based analysed leaf N content (Lab_{Chl}); weeks after treatment (WAT); days after sowing (DAS); dark green colour index (DGCI); red, green and blue (RGB); linear discriminant analysis (LDA); phosphorus (P); Nitrogen (N); chlorophyll (Chl); N-use-efficiency (NUE); genetic algorithm (GA); leaf colour chart (LCC); remote sensing (RS); near infrared (NIR); infrared (IR); visible and near infrared (Vis/NIR); Hydro N Tester (HNt); chlorophyll content index (CCI); joint photographers expert group (JPEG); leaf area index (LAI); ratio vegetation index (RVI); difference vegetation index (DVI); green vegetative index (GVI); land perpendicular vegetation index (PVI); inductively coupled plasma (ICP); normalized difference vegetation index (NDVI); leaf chlorophyll content measured by the modified RGB technique (Chl_{RGB})

ABSTRACT

Leaf dimensions and pigments are the important traits in plants that play a key role in estimating light interception, absorption and food production. In predictive research, these parameters are a useful data source for devising crop management techniques such as cultivation, pruning and fertilisation. Destructive and non-destructive techniques are commonly used for estimating crop growth and nutrient status. Although, destructive methods are more accurate, these are expensive, laborious and impracticable for large fields. In contrast, various non-destructive techniques have been developed for predicting crop N requirements that are relatively fast and less expensive. However, lack of consistency in accurately predicting the true N levels of different crop species under variable environments require further exploration of this area. In the present study, a new and relatively more efficient technique has been proposed for measuring leaf dimensions, chlorophyll, and N and phosphorus (P) content.

In the initial study, leaf images from a range of plant species were collected using a handheld portable digital scanner (Pico Life). Edge detection and filtering algorithms were applied to identify the leaf section of the image against the background. Data of forty leaves that vary in shape and size (from grasses to broad leaf plant species) were collected and processed using a new algorithm as well as the Li-Cor 3100. Data indicated high accuracy of the proposed algorithm for estimating leaf area, length, width and perimeter. It was verified by a strong correlation (R^2=0.999) between leaf area measured by Li-Cor 3100 and by digital scanner.

After successful application of the digital scanner for estimating leaf size and dimensions, the images collected by this scanner were used for predicting chlorophyll, P and N content of tomato, broccoli and lettuce leaves. The plants were grown under controlled conditions using nutrient solution and at early reproductive growth (after 8 weeks of growth) these were exposed to various N levels for seven weeks. Data on leaf chlorophyll and N content were collected through biochemical assays (Lab_{Chl}). In addition, data were collected by the SPAD-502 and portable scanner. Images collected by the portable scanner were processed by averaging the R (red), G (green) and B (blue) values of all the leaf pixels. Based on the RGB values, a new algorithm was developed that estimates leaf chlorophyll content (Chl_{RGB}). Despite slight variations in response to

applied N levels in the three crops, the leaf chlorophyll and N content significantly increased with increasing N levels in nutrient solution in the studied crop species. Under N deficient conditions (N0), tomato and broccoli plants showed significantly lower leaf N content just 2 weeks after treatment (WAT), compared with N-treated plants (any N level) suggesting a rapid response of these crops to N deficiency. However, response to various N levels in lettuce was slower and the difference in N concentrations in the leaves of N-deficient (0 and 0.2 N) and N-treated plants became significant at 5 WAT. Compared with leaf N, reduction in leaf chlorophyll levels in response to N deficiency was slow, and the difference in leaf chlorophyll content of N-deficient and N-sufficient plants was significant at 5 WAT in all the studied crops. The chlorophyll values calculated by SPAD and by the modified RGB technique were plotted against Lab_{Chl} and N content. The correlation coefficient (R^2) between SPAD values and Lab_{Chl} was 0.90, 0.73 and 0.81 for tomato, lettuce and broccoli, respectively. In contrast, the relationship between Chl_{RGB} and Lab_{Chl} was relatively stronger and more consistent for all three crop species that is 0.97, 0.90 and 0.91 for tomato, lettuce and broccoli, respectively. Similarly, highly significant relationships (R^2 values) were recorded between the leaf N content and Chl_{RGB} such as 0.94, 0.93 and 0.72 for broccoli, tomato and lettuce, respectively.

The high accuracy of the modified RGB technique for measuring the crop N and chlorophyll content was further confirmed by field-based studies. This technique again outperformed the SPAD-502 in estimating leaf chlorophyll content. For example, R^2 values for SPAD readings and Lab_{Chl} were 0.90, 0.92 and 0.84 for broccoli, tomato and lettuce, respectively. The efficiency of this modified RGB technique was also tested against dark green colour index (DGCI), a commonly used algorithm for estimating leaf chlorophyll and N. The result indicated that the modified RGB technique outperformed DGCI in the precision of predicting leaf Chl levels. A separate study was conducted to estimate N requirements of field-grown cotton using the modified RGB technique, where the efficiency of this technique was compared with other non-destructive methods. The crop was grown under various N levels, and leaf N concentrations were measured at different growth stages; late vegetative, peak reproductive and late reproductive growth phase. The data showed that the modified RGB technique was more effective and accurate in estimating cotton leaf N status compared with the SPAD-502 as well as other handheld crop sensor.

In the final experiment, the leaf P and anthocyanin levels of different crops such as cotton, tomato and lettuce was estimated using the modified RGB technique. The plants were grown under on different P concentrations. Leaf chlorophyll anthocyanin and P content were measured using laboratory techniques, while leaf images were collected by the handheld crop sensor. Using RGB values of the collected images, leaf area, leaf perimeter and chlorophyll content were calculated. These data were further used to train a linear discriminant analysis (LDA) classifier for estimating leaf anthocyanin and P content. In addition, a decision tree model was used to classify cotton plants into different groups containing variable P levels. Both LDA and decision tree models successfully classified these plants on the basis of leaf P content, indicating that P deficiency in crop plants can be predicted using morphological data. It also suggested that the modified RGB technique is highly efficient in estimating P requirements in different crop species.

CHAPTER ONE

1 General introduction

1.1 Background

Nitrogen (N) is an essential element for plant growth, which often becomes a major growth limiting factor under field conditions. In order to achieve optimum yield potential, N fertilisation is commonly practised for field-grown crops. However, excessively applied N may leach into surface and groundwater causing eutrophication and economic losses. Similar to N, plants require sufficient supply of phosphorus (P) in the soils for their proper growth and development (Batten, 1992). Due to its crucial role in cell division and expansion, P deficiency can inhibit leaf size, light interception and overall carbohydrate assimilation resulting in stunted plant growth (Rodríguez et al., 1998, Lloyd et al., 1995). On the other hand, higher P concentration in plant tissues can cause toxicity leading towards growth inhibition, leaf senescence and development of chlorotic or necrotic regions on leaves (Shane et al., 2004).

Thus, estimating crop N and P requirement is not only important for increasing crop profitability but also for overcoming the environmental pollution. Traditionally, N requirements for a crop are estimated through soil testing and plant tissue analysis. To measure N and P concentration in plant tissues, a destructive leaf sampling method is used, where these samples are analysed in the laboratory. This method is laborious, time consuming and expensive, and is not practicable in large fields. Leaf pigments are an integral part of plant physiological functioning. Green colour pigments containing chlorophylls are probably the most efficient molecules in green plants for absorbing light and transferring energy into the photosynthetic apparatus. In addition, some other pigments such as carotenoids may also contribute part of the energy to the photosynthetic system (Demmig-Adams and Adams III, 1996). Due to their role in growth related processes (photosynthesis), leaf pigments provide a valuable understanding of the physiological performance of plants. Chlorophyll tends to decline more rapidly than carotenoids when plants are under stress or during leaf senescence (Gitelson and Merzlyak, 2004). Plant growth and chlorophyll content in plant leaves are influenced by various environmental factors. Thus, measuring leaf chlorophyll content is an important indicator of senescence and plant growth. Due to a close relationship between leaf greenness, many non-destructive methods such as leaf colour chart (LCC) and chlorophyll meters have been extensively used for estimating crop N status (Daughtry et al., 2000).

The most widely used leaf chlorophyll estimating device is SPAD-502, a handheld absorbance meter. It measures chlorophyll content by estimating the relative greenness of leaves. Since chlorophyll is closely associated with N concentration in leaves (Balasubramanian et al., 1999), SPAD readings have also been used to estimate N levels in plants. These meters measure leaf greenness, which is strongly linked with extractable leaf chlorophyll content in a wide range of crops (Hikosaka, 2004). Despite its simplicity and timesaving nature, leaf chlorophyll meters have some limitations. Since leaf N content of a crop are estimated assuming a linear correlation between leaf N and chlorophyll content, which may vary depending on plant type and environmental conditions (Markwell et al., 1995). It will be even more difficult in larger fields with spatial N variations. For example, Blackmer and Schepers (1995) recorded variable data, using SPAD readings relative to reference areas in each field with sufficient N supply.

More recently, there has been an increasing interest in the use of computer automated digital image analysis of leaf colour for estimating leaf chlorophyll and N content. For example, SPAD-502 and Hydro N-tester can estimate leaf chlorophyll content using specific electromagnetic spectrum wavelengths. These instruments collect digital images and analyse them using software packages. These methods allow collection of large amount of quality images in a short span of time, while the images can be analysed at a later stage with a great deal of automation. The digital images can easily be stored for future use or as a reference as an historical archive of plant nutrient status. This image collection and processing approach has become attractive due to availability of inexpensive computers, cameras, scanners and software packages. Digital colour image analysis based on red, green and blue (RGB) colour models has been used to determine plant Chl and N. Many studies used RGB colour models to find a correlation with Chl and N status of plant (Mercado-Luna et al., 2010, Su et al., 2008, Suzuki et al., 1999, Hu et al., 2010). For example, RGB-based image analysis techniques have been used for weed recognition (Ahmad et al., 2006), weed and crop mapping (Tillet et al., 2001), weed identification (Hemming and Rath, 2000), seed colour test for identification of commercial seed traits (Dana and Ivo, 2008), quantitative analysis of specially variable physiological process across leaf surface (Aldea et al., 2006), quantification of turf grass colour (Karcher and Rechardson, 2003) and weed/crop discrimination (Aitkenhead et al., 2003). However, fewer studies have reported on the use of other colour models with Chl and N status. As the leaf colour largely depends on the quality of images collected and processed using digital cameras, the lighting conditions can influence the image quality and ultimately the process of estimating plant nutrient status. In addition, to accurately estimate the leaf area and perimeter, the camera height and angle should be carefully considered (Murdock, 1998).

To avoid these difficulties, in a series of experiments, we suggested the use of a portable scanner (Pico Life) that is not very sensitive to lighting condition and does not require calibration of angle/distance when collecting leaf images. These images were further processed to get RGB values and develop models for estimating leaf dimensions, chlorophyll, N and P content. The objectives of the experiments described in this thesis, which were conducted on a range of economically important crops varying in leaf morphology and N requirements, are listed in the next section.

1.2 Objectives

The main objectives of this thesis are:

1. To develop a new and easy to use non-destructive technique for accurately measuring leaf dimensions in a range of crop species.
2. To propose non-destructive methods for estimating leaf chlorophyll and N content for a crops growing on variable N levels under greenhouse as well as under field conditions.
3. To compare the efficiency of the newly developed (modified RGB) technique with the commonly used leaf chlorophyll/N estimation methods.
4. To propose methods for classifying crops on phosphorus status, using plant morphological parameters.

List of publications directly associated with this research

Articles published

Ali, M., Al-Ani, A., Eamus, D., Tan, D. (2015) Image-based technique for estimating phosphorus level of crops" has been published in the proceedings of 17th International Conference on Agricultural, Biotechnology, Biological and Biosystems Engineering, Dubai, ICABBBE Dubai, 9(10),03.

Ali, M., Al-Ani, A., Eamus, D., Tan, D., Rochester, I. (2015) Image-based (RBG) technique for estimating phosphorus levels in cotton (*Gossypium hirsutum* L.) published in the proceedings of "3rd International Scientific Events Conference, Bulgaria" in Journal of International Scientific Publications, 3, 234-240.

Ali, M., Al-Ani, A., Eamus, D., Tan, D. (2014). An algorithm based on the RGB Colour model to estimate plant chlorophyll and nitrogen content. *2013 International Conference on Sustainable Environment and Agriculture*, Singapore: International Association of Computer Science and Information Technology Press (IACSIT Press).

Ali, M., Al-Ani, A., Eamus, D., Tan, D. (2012). A new image processing based technique to determine chlorophyll in plants. *American-Eurasian Journal of Agricultural and Environmental Sciences*, 12(10), 1323-1328

Ali, M., Al-Ani, A., Eamus, D., Tan, D. (2012). a new image-processing-based technique for measuring leaf dimensions. *American-Eurasian Journal of Agricultural and Environmental Sciences*, 12(12), 1588-1594.

Ali, M., Al-Ani, A., Eamus, D., Tan, D., Rochester, I. (2014) detection of nitrogen status in cotton using an image processing approach, published in "*25th International Scientific - Experts Congress On Agriculture and Food Industry*" 25-27 September 2014, Çeşme-İzmir/Turkey.

Ali, M., Al-Ani, A., Eamus, D., Tan, D. (2012). Leaf nitrogen determination using handheld meters. *16th Australian Agronomy Conference 2012*, Armidale, NSW: The Regional Institute Ltd.

Articles accepted

Ali, M., Al-Ani, A., Eamus, D., Tan, D. (2015) Leaf nitrogen determination using non-destructive techniques – a review. Accepted for publication in Journal of Plant Nutrition.

Patent

Ali, M., Al-Ani, A., Eamus, D., Tan, D. (2013) An image processing based method to estimate crop requirements for nutrient fertiliser (WO2014100856 A1). https://www.google.com.au/patents/WO2014100856A1?cl=en&dq=mahdi+ali&hl=en&sa=X&ei=Ozs7VZRKpNOYBfHbgPAC&sqi=2&pjf=1&ved=0CDIQ6AEwAw

CHAPTER TWO

2 Review of literature

2.1 Introduction

The measurement of leaf chlorophyll content and nutrient status in crop plants is crucial for agronomic studies of crop growth and yield quality. Various macro and micro nutrients are essential for plant metabolism, and their deficiency can severely limit crop yield. Nitrogen plays an important role in key growth processes such as cell division, protein synthesis and enzyme production. If cell division is inhibited, leaf area expansion is similarly inhibited, and the plant, thereby loses its potential to produce high yields. Despite the importance of N supply to plant growth, excessive fertiliser application increases input costs. In addition, excess N that runs off arable land can negatively impact the environment. Thus, a mismatch between N supply and crop requirement can hamper crop growth and harm the environment, resulting in low N-use-efficiency (NUE) and economic losses. Increasing NUE can reduce additional fertiliser application and protect the environment. However, for the best N application rate, the farmers and advisers must have the most up-to-date information on the crop and soil N status (Li et al., 2010).

There are two commonly used techniques for estimating tissue nutrient concentration; destructive and non-destructive. Ramirez (2010) showed that plant N status can be accurately estimated using a destructive technique in which foliar samples are analysed using laboratory procedures. This technique is generally laborious, time consuming and expensive (Sui et al., 2005). In contrast, non-destructive methods are rapid and cost-effective but are generally less accurate. A number of non-destructive methods with varying complexity and optimality are available. These include handheld methods such as the use of a leaf colour chart (LCC), which relies on visual comparison between leaf colour and a colour chart to assess the N status of certain plants. One of the most widely used digital tools is the chlorophyll meter (SPAD-502). This handheld device estimates chlorophyll content of leaves and hence gives an indication of leaf N content, as leaf chlorophyll level is closely correlated to leaf N concentration.

Recently, digital imaging has been investigated in the agriculture industry for plant colour analysis. Digital cameras or scanners in combination with computers and appropriate software can collect images of leaves and evaluate their colour with relative ease and at a reasonable cost. Handheld devices are quite appropriate for small fields. For large fields, tractor – mounted systems sensors to detect N status are used that save time and effort for large scale N application. The three most commonly used commercial technologies that measure plant N status in real-time are Yara N-Sensor, Crop-Circle, and GreenSeeker (Samborski et al., 2009). Similarly, remote sensing (RS) techniques have also been used for estimating nutrient content in growing crops using a single wavelength or combination of wavelengths (Osborne et al., 2002). Due to their influence on leaf chlorophyll and photosynthesis, a strong relationship between leaf nutrient content and spectral reflectance, particularly in visible absorption is expected. The RS techniques have mainly been used in natural resources management for land cover and biomass estimation, and to note changes in land usage (Sala et al., 2000; Kogan et al., 2004; Henebry et al., 2005). In the last decade, some successful efforts have been made to apply this approach to commercial agriculture. Below is a description of available techniques to estimate N content in plants.

2.2 Destructive techniques

2.2.1 CHN analyser

There are two fundamental destructive techniques for measuring N concentrations in plant tissues. The original Dumas technique, which requires the oxidation of the sample in copper oxide to produce N_2 gas. N contents are measured by the volume of N_2 gas produced. Although in older system incomplete combustion of the sample (70–80%) was a problem resulting in lower levels of converted N_2, the problem is solved by the introduction of a specifically designed for coupled total N and precise 15N analysis.

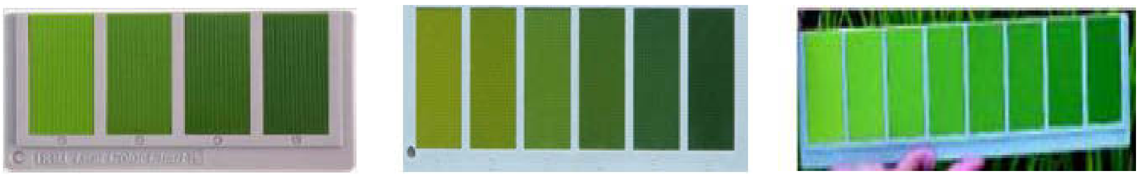
2.2.2 Kjeldahl digestion method

A second approach, 'wet' Kjeldahl digestion method, uses hot and concentrated sulphuric acid to reduce organic and mineral N into NH_4^+ in the presence of a catalyst. The NH_4^+ is recovered by distillation or diffusion and estimated by titration or colorimetrically.

2.3 Spectral techniques

2.3.1 Leaf colour chart (LCC)

Leaf colour is a good indicator of plant health and nutrition. Different types of stress may cause different symptoms, and a comparative analysis can yield information about the type of stress. N deficient leaves turn pale or yellowish green rather than dark green and farmers generally prefer dark green leaves in the crop. The standardised LCC used for estimation leaf nutrient content contains four colours (Figure 2.1A). The six- panel LCC (Figure 2.1B), which was an improved version of the standard LCC (Thind and Gupta, 2010, IRRI, 1996) and the 8 -panel LCC (UCD-LCC), was recently developed by the researchers at the University of California (Figure 2.1C).



A B C

Figure 2.1: Commonly used leaf colour chart (LCC)

The LCC has mainly been applied for rice and despite the fact that it has made some improvements in NUE of rice; it is not an optimal tool to assess foliar N content, as it is largely affected by a number of factors, such as ambient lighting conditions and variations amongst the cultivars in leaf colour. Accordingly, the development of a more reliable tool for more accurately detecting the inception of N stress before it becomes visible to the human eye would be valuable, especially if it can be used across a large range of species. Consequently the development of leaf chlorophyll meters has received much commercial interest (Debaeke et al., 2006).

2.3.2 The SPAD meter

Minolta Co. (Japan) has developed the chlorophyll meter (SPAD-502 Figure 2.2), which can effectively measure the relative greenness or chlorophyll content of leaves (Turner and Jund, 1994). Because leaf chlorophyll content is closely related to leaf N concentration (Balasubramanian et al., 1999), this meter has commonly been used to assess foliar N content. The SPAD meter estimates the relative chlorophyll concentration in leaf tissues by measuring the differential transmittance of light through it. Within a small chamber (2 - 3 mm) in which part of a leaf is held, the meter emits light from two diodes, one producing a peak wavelength near 650 nm (red), which is absorbed by chlorophyll and the other, a peak near 940 nm wavelength (near infra-red, NIR) is transmitted through leaves, and serves as an internal reference to compensate for leaf thickness and moisture content (Shapiro et al., 2006). More the red light is absorbed by the leaves when more chlorophyll is present. Thus, chlorophyll concentrations of leaves are correlated with SPAD meter values. Understanding the basic theory of the electromagnetic spectrum will give a good idea about the basic functional theory of SPAD.

Figure 2.2: Close-up of SPAD Meter

The electromagnetic spectrum covers a wide range of wavelengths and photon energies, and is broken up into a number of different categories, each of which shares certain properties (Figure 2.3). The energy reaching the surface can be either transmitted, absorbed or reflected (Figure 2.4). The physical properties of a body and the precise wavelength of radiation determine the degree of each, thus obtaining profiles of the surface reflectance (Figure 2.5). The spectral response of the plants mostly relies on the plant leaf structure. Layers of diverse types of cells make up the leaf (Figure 2.6). Each cell contains chlorophyll pigment in specialised structures called chloroplasts, which are responsible for the healthy green living vegetation. Every colour in the electromagnetic radiation spectrum is absorbed by chloroplasts except green, which reflected back. Approximately 60% of the NIR radiation from this leaf layer is reflected by the mesophyll cells, resulting in the healthy green vegetation, and give a brighter and higher NIR response than in the spectrum of green. The leaf loses its green pigment upon senescence. The dying leaves have a brown and yellow appearance as red and blue is no longer used, so these reflect back green spectrum light. Also, the NIR wavelengths are completely absorbed by the leaf as they can no longer be reflected, giving a dark or black appearance in the NIR (Gobson, 2000; Havránková, 2007).

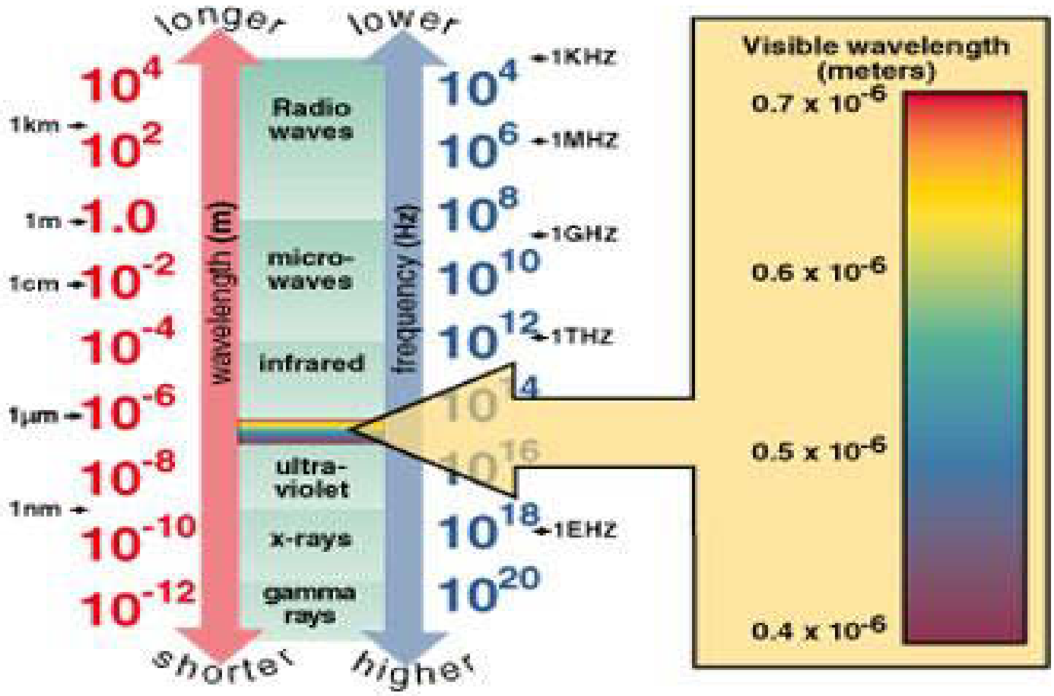


Figure 2.3: Electromagnetic spectrum (Casady and Palm, 2002)

Figure 2.4: Incident (I), reflected (R), absorbed (A) and transmitted (T) energy from a leaf surface (Havránková, 2007)

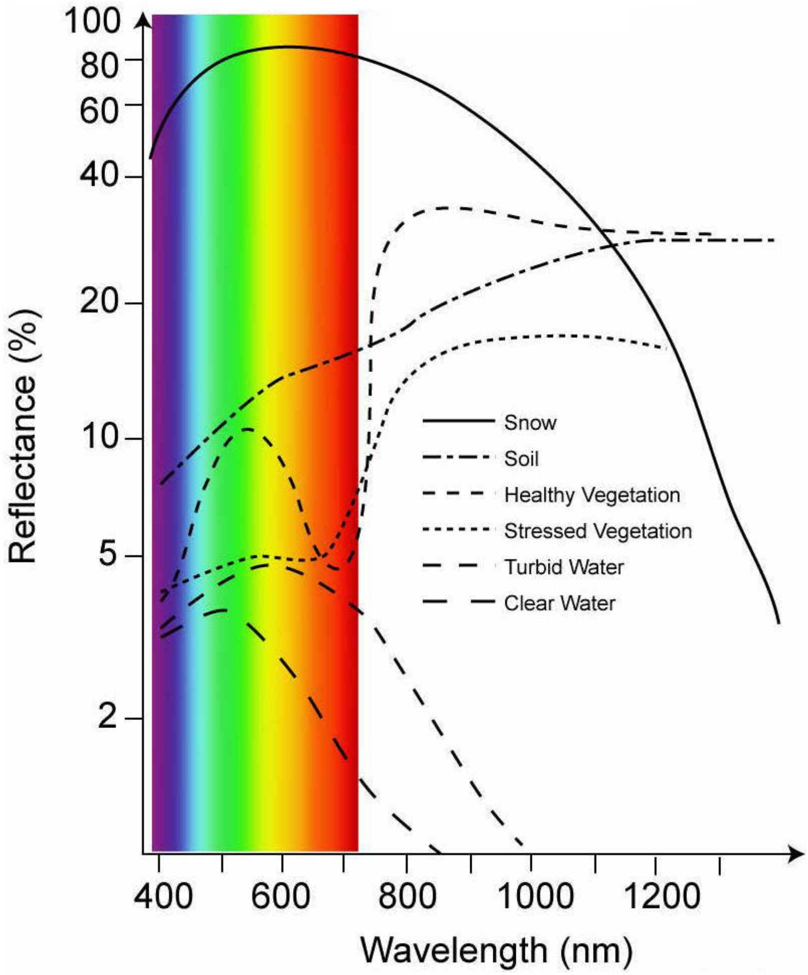


Figure 2.5: Light reflectance curves from different objects (Keiner and Cilman, 2007)

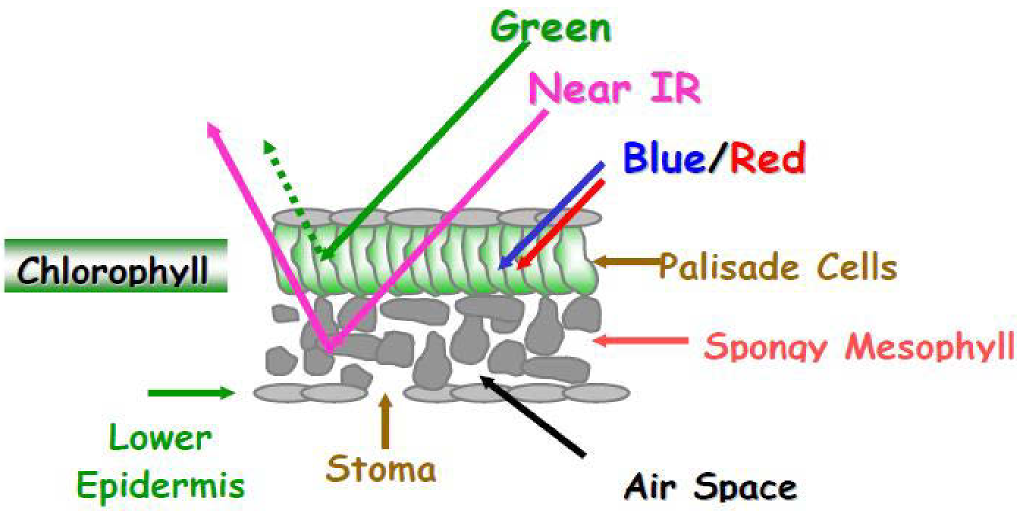


Figure 2.6: Leaf inner structure and light absorbance (Schepers, 2005)

2.3.2.1 **Basic functional mechanism of SPAD**

The SPAD meter determines the relative chlorophyll concentration by measuring the variance of light being transmitted through the leaf. Fairly large nonlinear relationship between leaf chlorophyll content and chlorophyll meter values allows calculation of a unitless “SPAD” value (on an index from 1 to 100). The SPAD values are calculated from the difference between optical density of red and infrared (IR) wavelengths, which is detected by a photodiode situated in the leaf chamber (Minolta Camera Co, 1989) As a result, the values calculated by SPAD meter reflect leaf chlorophyll concentrations. Since leaf chlorophyll concentration is positively correlated with leaf N concentration (as chlorophyll is made up of most of N containing enzymes and organic matter), a regression equation can be used to link the SPAD estimates to the foliar N status, and therefore, it can be used as a rapid diagnostic tool for measuring leaf N concentrations (Chapman and Barreto, 1997). The following equation is used for the SPAD meter;

$$\mathrm{SPAD} = \mathrm{A}\left[\log\left(\frac{\mathrm{RCo}}{\mathrm{RC}}\right) - \log\left(\frac{\mathrm{IRCo}}{\mathrm{IRC}}\right)\right] + \mathrm{B}$$

where:

A and B are Constants

RC and IRC are Currents from red and IR detectors, respectively, that pass through the chamber that contains the leaf sample. RCo and IRCo are the currents from red and IR detectors, respectively, without leaf sample.

Farmers often use the crop colour as an indicator, by performing a subjective visual inspection, to determine the time when N fertiliser is required (Wells and Turner, 1984, Furuya, 1987), and they prefer dark green colour of their crop, which leads to the overuse and inefficiency of N fertiliser. Thind and Gupta (2010) found that if the farmers used spectral properties in a more rational manner, they could be guided in a need-based N application. Use of the chlorophyll meter allows early detection of the onset of N stress before it is visually obvious (Schepers et al., 1992) and assist in correcting N deficiencies and minimising yield losses (Shapiro et al., 2006). Thus a chlorophyll meter is more reliable than the simple leaf colour chart visual assessments (Debaeke et al., 2006).

Broad testing has been done with the SPAD in wheat (Reeves, 1993, Fox et al., 1994), rice (Peng et al., 1993; Turner and Jund, 1994), cotton (Wu et al., 1998), tall fescue (Kantety et al., 1996), fruit trees (Li et al., 1998; Peryea and Kammereck, 1997) and maize (Chapman and Barreto, 1997). Use of the SPAD meter in predicting N status or foliar chlorophyll has been documented in over 200 publications (Uddling et al., 2007). A reading using the SPAD meter may be performed in 2 seconds, without destroying the plant tissue sample, and it will save space, resources and time. The two main limitations of SPAD are; (1) high initial cost, which is approximately US$ 1350 per unit, which even farmers in the USA (Turner and Jund, 1994) are reluctant to accept, and (2) relatively small measuring area (12.57 mm^2) which may not reflect the true value of leaf chlorophyll and lead to fluctuating readings (Netto et al., 2002)

2.3.2.2 **Estimation of leaf nutrients using the SPAD meter**

In 1963, the original SPAD meter was designed to diagnose leaf N status of rice (*Orvza sativa* L.) in Japan (Takebe and Yoneyama, 1989), and which now include new models called the SPAD-501 and SPAD-502 (Uddling et al., 2007). When taking N readings from a crop, it is essential to ensure that plant samples are representative of the whole crop. It is also necessary to have an average of approximately thirty readings to get a representation of the whole crop, and each individual reading may be different but anomalies should be recognised. Thirty individual readings are automatically stored, and the average is calculated by the Minolta SPAD-502. To achieve this goal, 30 individual samples must be collected and averaged from both the reference field and the bulk field, and then compared (Note: the first fully expanded leaves from the top of a plant must be used for each reading).

A guide to in-season N fertilisation, using the chlorophyll meter, is based on a sufficiency index, which is calculated as follows (Varel, 1997);

$$\text{Sufficiency index} = \frac{Average\ Chlorophyll\ meter\ readings\ of\ unknown\ area}{Average\ Chlorophyll\ meter\ readings\ of\ well\text{-}fertilised\ area} \times 100\ \%$$

N is applied when the sufficiency index < 95%.

Balasubramanian et al. (1999) determined the SPAD threshold value in SPAD reading, which shows the N deficiency that could cause yield loss if not corrected (the crop is suffering N deficiency). Upon reaching this SPAD level, the farmers are able to take an immediate action (apply N), to limit possible yield reduction (Swain and Sandip, 2010). Nevertheless, Schepers et al. (1992) showed that cultural practices, stage of growth and crop variety being grown all affect the critical SPAD value. Critical SPAD value for different crop species varies, each crop has a specific critical SPAD value under a specific growing environment (Huang et al., 2008). Balasubramanian et al. (1999) recommended SPAD threshold as 35 for dry season transplanting of rice, where 32 SPAD value is suggested for wet-seeded rice transplantations in cloudy weather or low radiation in the Philippines, Maligaya and Nueva Ecija as 32. The critical SPAD value of 37.5 was determined by (Kyaw, 2003) for rice in Pakistan to receive need-based N top-dressing. In West Bangal, (Maiti et al., 2004) the critical value was 37 for rice cultivar IET-4094 for high yields and less fertiliser N application to the tune of 27.5 to 45.5 kg N ha^{-1} rather than a blanket dose of 150 kg N ha^{-1}.

2.3.2.3 **Factors affecting leaf chlorophyll content**

Various environmental and plant growth factors such as temperature, moisture stress and sunlight (Schepers et al., 1992), nutrient deficiencies other than N (Turner and Jund, 1991), varietal variations (Minotti et al., 1994, Hoel, 2003), plant genotype (Villa et al., 2000) and growth stages (Ramesh et al., 2002, Swain and Sandip, 2010), could influence leaf chlorophyll content or greenness. Variation in leaf chlorophyll concentrations in turn affect the SPAD readings and varietal differences are generally the most common reason for variable SPAD meter reading. Some hybrids in crops such as tomato, sorghum and corn have darker green colour compared with varieties. Villa et al. (2000) found that SPAD readings could be easily affected by any factor changing leaf chlorophyll content. Due to such a large range of factors influencing the plant chlorophyll, it is impossible for a meter to accommodate all crops for accurately measuring N sufficiency. In order to make the meter effective, it must be calibrated to the specific variety of the crop being grown as well as other environmental factors. To accomplish this several small areas (strips or spots) are over-fertilised with N, so a calibration may be taken using these spots as reference points for the rest of the field (Murdock et al., 1997).

2.3.2.4 **Limitations**

There are mainly two limitations which restrict the use of the SPAD meter for indirect N measurement in plant the tissue (Rostami et al., 2008). The stress-induced sampling errors may influence chlorophyll content in the plants (Villa et al., 2002), and the varietal/species based difference that may lead to variable results from different plant species using the same SPAD meter (Murdock et al., 1997). The latter problem could be avoided by calibrating the SPAD meter for the specific crop variety being grown. Furthermore, this method is not helpful for detecting luxury N uptake in crops such as maize that may achieve maximum chlorophyll content regardless of the level of over-fertilization (Hawkins et al., 2007).

2.4 Digital techniques

The Hydro N Tester or HNt (Yara International ASA, Oslo, Norway) is another type of chlorophyll meter (Figure 2.7) that uses two electromagnetic spectrum wavelengths (940 nm NIR and red 650 nm) but the digital readout range for the chlorophyll index is from 0 to 800 (HNt). According to Richardson et al. (2001) the chlorophyll content meter (CCM-200, Opti-Sciences, Tyngsboro, Massachusetts, USA) weighs 180 g, 2 measurement areas of 0.71 cm and based on the absorption measurements at 660 and 940 nm calculates a chlorophyll content index (CCI). In fact, both the SPAD and N-Tester have some common limitations.

Figure 2.7: Hydro N-Tester
http://www.arablefarmer.net/uploads/media/mineral_fertil.pdf

Another type of chlorophyll meter is the Spectrum CM1000 or R meter (Spectrum Technologies, Inc. 2009), which is a hand held chlorophyll reflectance meter, working on parallel principles to the SPAD meter. The Spectrum meter works on the fine-leaf turf canopy. This allows larger area assessment, and integrates many leaf surfaces. At 30 cm from the plant leaf, it integrates 1.27 cm diameter area, gives consistent readings, and is simple to use. At the pull of the trigger, chlorophyll measurements may be made from a standing or walking position. The sun angle and heavy overcast conditions restrict the use of the R-meter, as it is dependent on ambient sunlight. Figure 2.8 shows how the sunlight intensity, meter angle to wheat canopy, sun angle and sunlight intensity influence the R-type meter readings.

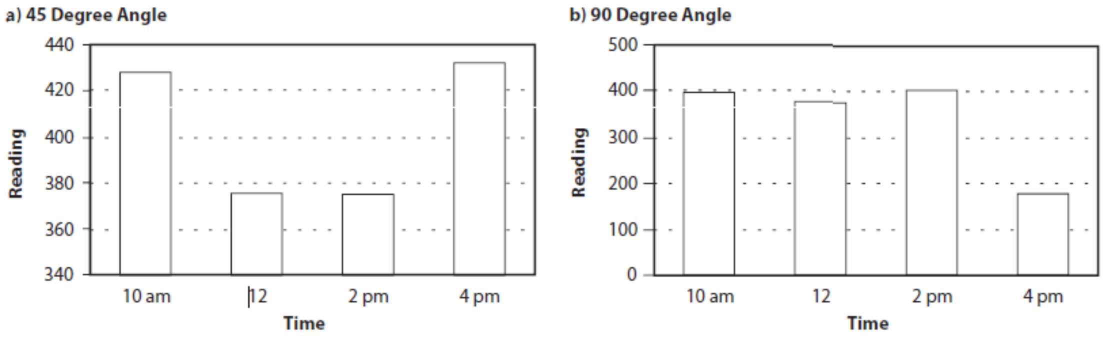


Figure 2.8: Effect of the time of day on CM 1000 readings at 45 and 90 degrees to the canopy surface

Recent work based on the use of transportable NIRs systems has been published (Ecarnot et al., 2013, Serbin et al., 2012). These NIR systems are not affected by genotype or environmental factors, and present a better alternative for developing leaf N content calibration at species scale. Edrees et al. (2013) used the HandHeld 2 Portable Spectroradiometer to detect chlorophyll content from remotely sensed spectral data for optimising N doses without reducing wheat yield. The comparisons between these handheld tools are shown in Table (2-1).

Table 2.1 Comparison among various techniques used for measuring leaf chlorophyll and N levels of crops

Instrument	Wavelength	Measurement area	Reference
SPAD	650 nm in the red and 940 nm in the near-infrared	12.57 mm^2	(Netto, 2002)
N Tester	650 nm in the red and 960 nm in the near-infrared	2 x 3 mm^2	(Richardson et al. 2001; (Goffart and Olivier, 2004)
CCM-200	660 nm in the red and 940 nm in the near-infrared	0.71 cm^2	(Richardson et al., 2001)
R meter (CM 1000)	ambient and reflected light at 700 and 840 nm	1.27 cm^2	(Murdock et al., 1998, Carter and Spiering, 1999)
Handheld 2 Portable Spectroradiometer	Spectral range (wavelength range) 325-1075 nm	Precise data over wide 25° field-of-view	(Edrees et al., 2013) http://www.asdi.com/products/fieldspec-spectroradiometers/handheld-2-portable-spectroradiometer

2.4.1 Digital image and colour analysis in agriculture

Plant nutrition and vigour are closely associated with foliar colour and therefore, changes in leaf colour indicate changes in plant health (Graeff et al., 2008). Some scientists suggested the computer automated digital image analysis is an alternative, unbiased, more precise and consistent method for analysing leaf nutrition (Turner et al., 2004, Mirik et al., 2006). It is a non-invasive and non-destructive method for capturing, processing and analysing information from leaf images (Richardson et al., 2001, Karcher and Rechardson, 2003). This method allows simultaneous collection and analysis of hundreds of images at convenience (Diaz-Lago, 2003). Currently, the equipment for this process is inexpensive, making it an attractive method of data collection and storage. Digital imaging analysis has been effectively used in crop studies for quantifying water deficiency, nutrient status, and disease, insect and stress-induced damage (Karcher and Rechardson, 2003, Richardson et al., 2001, Adamsen et al., 2000).

2.4.2 Use of digital image and colour analysis for plant growth and yield estimation

Various applications have been considered for the RGB (red, green and blue) based image analysis in agriculture such as weed recognition (Ahmad et al., 2006), weed and crop mapping (Tillet et al., 2001), weed identification (Hemming and Rath, 2000), seed colour test for identification of commercial seed traits (Dana and Ivo, 2008), quantitative analysis of specially variable physiological process across leaf surface (Aldea et al., 2006), quantification of turf grass colour (Karcher and Rechardson, 2003) and weed/crop discrimination (Aitkenhead et al., 2003). Various RGB and HSI (H = Hue, which in turn measures toe permeated colour, S = saturation, the colour permeated with white colour and I = light intensity) based methods which have been used for discriminating between plant and soil have been described by Georg and Bockisch (1992) which have guided the development of an automatic seedling transplanting machine (Lin et al., 1994).

These studies suggested higher efficacy of digital imaging analysis for quantifying biophysical plant parameters, especially when dealing with leaf diseases such as rust and tan spot in wheat (*Triticum aestivum* L.). However, the impact of image size, format, quality and sample size on digital image analysis results was determined covering a range of disease intensity (Steddom et al., 2004). They concluded that digital image analyses for disease quantification, even using the low quality JPEG (joint photographer expert group) images, is extremely desirable due to robustness, low-cost and commercial availability of equipment. This technique is emerging as a favourable tool for crop yield management due to its ability to detect crop stress situations before visual symptoms appear and adverse effects established. This technique also allows for the digital images to be archived for future use, therefore, maximum data are available for future users. Colour parameters of the digital images may be easily interpreted by the computer processing system and evaluated using different colour systems. Erickson et al. (1988) reported that the RGB colour values could be effectively used for analysing and describing colour images.

To study the influence of illumination on the quality analysis of apple using image processing, Truppel et al. (1998) used three different colour spaces RBG, HSI and L*a*b (where L = lightness component, a = determines the degree from green to red, and b = determines the degree of blue to yellow). They concluded that both the selected colour space and illumination were very important for developing good quality images. Using various colour spaces [(HIS, Luv, (CIE 1976, L*, u*, v* colour space), Lab, (CIE 1976 L*, a*, b* colour space)], Chapron et al. (1999) developed a system that efficiently recognises the weeds in maize fields if foliar overlapping was less than 5%. To improve the separation of soil and vegetation they tested different colour spaces like HSI and Lab. Similarly aerial images with high resolution IR were used for detecting N stress in maize GopalaPillai et al. (1998). They determined that canopy reflectance in the red channel was a good predictor of maize yield, as the canopy reflectance was closely correlated to the applied N. For assessing N status of bush bean plants, Thai et al. (1998) used spectral video images utilising two handpass filters and distinguished different N application level using two selected bands. Minimum of light deviation, maximum resolution and image distortion are important parameters for developing good quality scanned images (Cometti et al., 2003). The video/image camera is the most practical approach for determining the leaf chlorophyll and nutrient status (Chappelle et al., 1992, Ferns et al., 1984, Curran et al., 1991). Without making any contact with the plants, digital camera images can be acquired from a point in time. This method allows for a more accurate and reliable measurement of plant growth and no plant harvesting is required.

2.4.3 Use of digital imaging for plant N estimation

Using colour imaging technique Luna et al. (2010) successfully examined the tomato leaf polyphenolic and chlorophyll content, and correlated these values with leaf N status. Many other field studies suggested the use of video camera and computer based image analysis as an effective method for estimating chlorophyll content and nutrient status of crop plants. Jia et al. (2004) indicated that the level of N fertilisation can be detected using digital photography, while Yadav et al. (2010) showed that real time predictions of chlorophyll content of plants could be achieved by an image analysis method using the three primary colours, red (R), green (G) and blue (B). A pixel in a digital leaf image is represented as a combination of the three primary colours, which can be used to develop a mathematical formula that reflects a correlation with the chlorophyll content of the plant (Su et al., 2008). Pagola et al. (2009) developed a new technique for measuring leaf greenness using the RGB components of a colour image and established a greenness index, which estimates barley yield and N requirements. They compared the values estimated by the new method with the value given by the SPAD-502 chlorophyll meter and observed that RGB-based greenness index gives an equal or better prediction to that of SPAD.

The normalised variance (red-blue)/(red+blue) estimated by portable video camera was found the most relevant variance for data collection under a variety of meteorological conditions (Kawashima and Nakatani, 1998). Using variable algorithms of RGB values obtained from video images, various plant growth parameters have been estimated for example Iwaya and Yamamoto, (2005) used (R-G)/(R+G) equation for measuring water content of the wheat panicle, Suzuki et al. (1999) used G/(R+G+B) value for measuring chlorophyll content in broccoli, Cai et al. (2006) used R/(R+G+B) and G/R and R/(R+G+B) for estimating leaf chlorophyll and carotenoid content, respectively, of the cucumber leaf. Researching wheat senescence, Adamsen et al. (1999) observed a linear correlation between the G/R and SPAD values, and the G/R efficiently responded to the changes in both leaf chlorophyll concentrations and leaf number. The image colour analysis has also been applied to studies on nutrition deficiency of plants (Xu et al., 2002) and flower number detection (Adamsen et al., 2000).

Despite the fact that all analysis performed by researchers was carried out under different environmental conditions, with different plant materials and selected optimum indices, most of them analysed the RGB image by ratio values, while concentrating on plants colour variances and more particularly on plant canopy chlorophyll evaluation (Cai et al., 2006). The provision of N estimates can be quickly conducted using RGB image analysis. On the contrary, a SPAD chlorophyll meter was unable to estimate real time leaf chlorophyll content of regenerated plants enclosed in a culture vessel (Yadav et al., 2010). Regenerated plants chlorophyll estimates could be much better estimated utilising RGB based image analysis.

Whilst investigating cucumber plants grown under various levels of nutrients, Qin and Zhang (2005) developed a method to employ a special image sampler to take leaf images and determine correlation between the image property and leaf N content. The system involved a platform, eight lamps and a window for fixing the camera. Images were taken by placing the leaves on the platform, around which the lamps were arranged. RGB and HSI modes were used to analyse the correlation between leaf N content and leaf images. The use of the camera proved more efficient and faster for obtaining the highest correlation for cucumber leaf N content. A strong correlation was achieved for N status of broccoli plants when digital images were taken under unchanging light conditions (Graeff et al., 2008). Similarly, by minimising the image taking limitations such as the use of the automatic camera setting, the higher width camera angle and using a 100 W lamp to control light, Luna et al. (2010) developed a method of taking images of tomato leaves (Figure 2.9). Red and blue colour of images was used for estimating and analysing N status of tomato seedlings. With an R^2 above 0.89, it was shown that red and blue colours yielded a good N predication. Thus, this method could quickly and more efficiently estimate the N deficiencies in tomato seedlings.

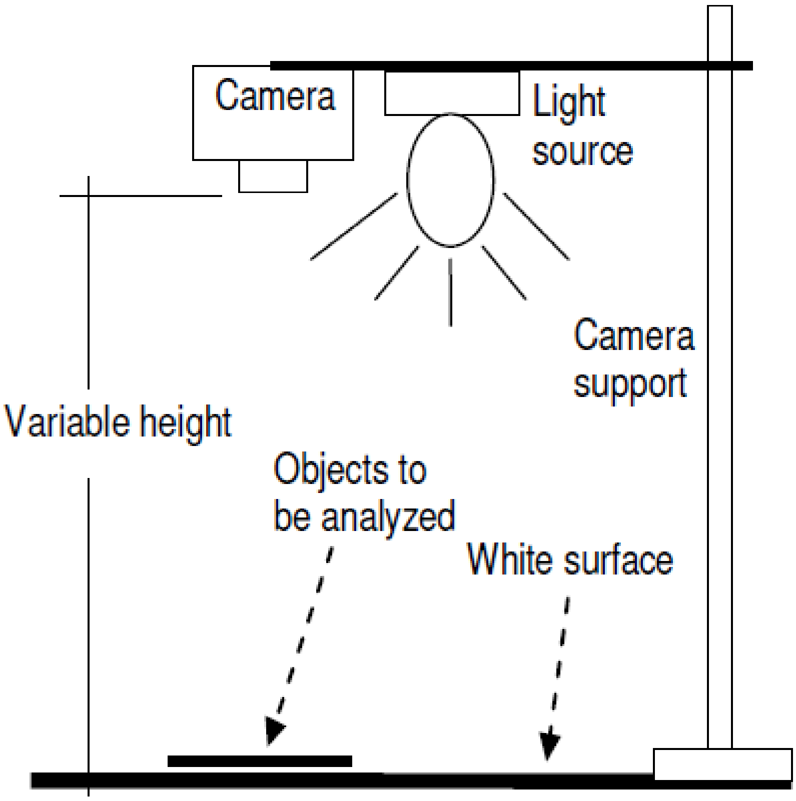


Figure 2.9: Camera set up for estimating leaf N status (Luna et al. 2010)

A recently developed handheld crop fluorescence sensor is Multiplex®, which detects leaf chlorophyll content using three wavelengths such as Blue (450 nm), Green (530 nm), and Red (630 nm). This optical sensor is more efficient compared with the chlorophyll meter as it can distinguish N treatments under any irradiance level such as shade or full sunlight and can be used at any time during the day. However, the short distance (approximately 10 cm to plant) requirement for measuring leaf chlorophyll content and small covering area (50 cm^2) limits its use for larger areas (Muñoz-Huerta et al., 2013).

2.4.4 Use of digital imaging for estimating crop phosphorus status

Phosphorus (P) is an essential macro-nutrient required for various plant functions vital for growth, such as cell division, plant energy relations and photosynthesis. It is the key element involved in root initiation and structural development and P deficiency in soils or in plant tissues can severely interfere with plant metabolic processes. On the other hand, its higher cellular concentration can cause tissue toxicity leading towards growth inhibition, leaf senescence and development of chlorotic or necrotic regions on leaves

(Shane et al. 2004). Soil testing has been widely used for estimating P fertilizer requirements of annual crops. In this technique, results from previous soil analysis along with additional fertiliser applied to the crop are used for recommending P application rates. Another relatively less laborious and accurate method for estimating the P status of the crop is analysis of leaf tissues. Plant leaves are commonly used to estimate P requirement in a standing crop. More recently, instead of colorimetry, inductively coupled plasma (ICP) spectrometry is used for quantifying plant P content (Sikoria et al. 2005).

However, as compared to leaf N, only a few reports are available for estimating P status using non-destructive methods, and most of those studies showed either incomplete or poor estimation of crop P content. For example, Zhang and Li (2008) used a handheld spectroradiometer to measure P content in cucumber leaves. They measured spectral reflectance range from 325 nm to 1075 nm and proposed a non-linear model for estimating leaf P content and observed that the estimated P values were close to the true leaf P content measured through laboratory analysis. Similarly, using a portable spectroradiometer, Özyigit and Bilgen (2013) collected spectral data of a rangeland. The stepwise regression was applied to select wavelengths to investigate relationships between laboratory measured foliar nutrients (N, P and K) and spectral data. The data showed significant relationships between predicted and measured N (R^2=85) and K (R^2=84) but the relationship was relatively weak for P (R^2=43).

Ponzoni et al. (1999) induced N, P and potassium (K) deficiency symptoms in *Eucalyptus saligna* seedlings, and radiometricaly measured the leaves of these seedlings to spectrally characterize the symptoms by a LICOR-linked SPECTRON SE-590, running at 0.4 to 0.09 μm spectral range. However, they could only spectrally detect the K deficiency symptoms in the visible region. Liu et al. (2011) used visible and near infrared (Vis/NIR) spectroscopy and scan imaging to determine the leaf N, P and K status in plants, although prediction was less reliable for P content, they effectively predicted the plant N and K status. Using continuum-removed absorption features of measurements, Mutanga et al. (2004) effectively predicted N and P concentrations in grass pastures. Albayrak (2008) used reflectance measurements for determining nutrient status and quality of sainfoin (*Onobrychis sativa* Lam.) pasture. In a stepwise regression of reflectance with varying wavelengths (460, 550, 650 and 780 nm), the R^2 value of predicted and measured N, P content were approximately 0.85, whereas R^2 value of predicted and measured N, P in stepwise regression of the first derivatives (440, 530, 630 and 760 nm) were 0.87, 0.91, respectively. Osborne et al. (2004) used spectral radiance ranging from 350 to 1000 nm during various growth stages of maize and correlated them with plant N and P concentration. Reflectance in the NIR and blue regions effectively predicted early season P stress, but could not estimate P stress during late growth stages of a maize crop. Reflectance in the red and green regions effectively estimated plant N concentrations.

2.4.5 Use of digital imaging for estimating leaf development

Due to a relatively strong association of chlorophyll and N content and leaf colour, the non-destructive techniques were mainly used for estimating crop N status. However, there have been reports of using non-destructive methods for measuring leaf dimension, estimating crop P and K status and weed discrimination.

Leaf dimensions such as leaf area, height, width, average width and perimeter can play an important role in light harvesting and overall plant growth (Ali and Anjum, 2004, Mohsenin, 1986) and direct agricultural production practice (Xiandong et al., 2006).

Currently, there are two classical methods for measuring leaf dimensions: digital and non-digital. In the most commonly used non-digital methods, the leaf is placed on a grid paper and the number of grid squares is calculated to estimate leaf area according to the following formula (Li et al., 1998):

$LeafArea = GN \times GA$ ……………………………………….Equation 2-1

Where *GN* is the number of grid squares and *GA* is the area of each grid square.

Another way for measuring leaf area is described in (Bai et al., 2005, You-Wen and Xiao-Juan, 2009). This method is based on the weight of graph paper, where the leaf shape is copied on a graph paper, and the copy is carefully cut and weighed. Leaf area is then measured using the following formula:

$LeafArea = \frac{W}{C}$ ………………………………………………Equation 2-2

Where W is the weight of the paper trace of the leaf and C is a coefficient of the paper (weight of unit area). A regression equation was used for this purpose according to the following formula (Eftekhar et al., 2011, Li et al., 2008):

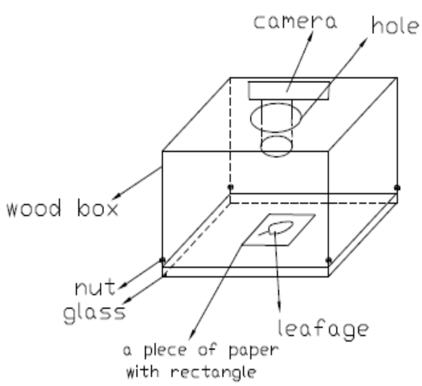
$LeafArea = f(L, W)$ …………………………………..………..Equation 2-3

Where L is leaf length, W is leaf width, and f is a function that can either be linear or polynomial. The parameters of the linear and polynomial model need to be estimated for each plant genotype as shown in Table 2-2.

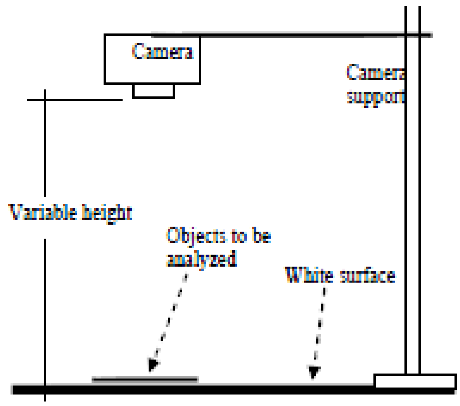
The most widely used digital device for measuring leaf area and dimensions is the LI-3000 that is manufactured by LI-COR. This handheld meter can measure leaf area, average width, maximum width, length and height. Despite its speed and accuracy, this method has two main limitations. Firstly, it cannot handle large leaves. Accordingly, a large leaf has to be cut into smaller pieces and the area for each piece has to be individually measured so that one can later obtain the area of the whole leaf; however, this process can cause measurement errors. Secondly, the device is quite expensive (You-Wen and Xiao-Juan, 2009, Eftekhar et al., 2011).

Recently, there has been an increased interest in the use of image processing techniques for measuring leaf dimensions. Digital cameras have been widely used to acquire images, which can then be analysed using dedicated software. For example, James and Newcombe (2000) used Adobe Photoshop, O'Neal et al. (2002) used a public domain software (Scion Image) and Rico-García et al. (2009) used an algorithm that was written in Matlab and AutoCAD (Computer Aided Design) to measure leaf area. Tian and Wang (2009) developed a software code written in C^{++} for the same purpose. Leaf area measurement using image processing could give high accuracy compared with the estimated leaf area using the grid paper method. Li et al. ((2008) developed an inexpensive system using a camera and personal computer (Figure 2.10A). They used a wooden box and made a hole in the centre of the top face to install the camera and placed it in the hole and fixed the distance between the lens of the camera and bottom at 450 mm. The camera was adjusted to be vertical to the flat surface and the lighting conditions were controlled using natural light directed on to leaf on the bottom of the box under the camera to take photos.

Rico-García et al. (2009) used the same technique (Figure 2.10B) as in the previous study, where a stand was used to control the camera height at 40 cm (from the lens of the camera and the target leaf), and a white surface was suggested for the base. Matlab code was developed to estimate the leaf area. The aim of the setting shown in Figure 2.10 (B) is to control (i) distance between the lens of the camera and the target (leaf), (ii) lighting condition, (iii) angle of camera (it should be vertical), and (iv) leaf position (the leaf should be placed as flat as possible and parallel to a known object) (Patil, 2011). The ability to control all the above factors led to a very high accuracy with very low error of estimation (Li et al., 2008, Rico-García et al., 2009). However, these systems are impractical and are not convenient for field usage. Hence, developing a new system that can control all these factors was the aim in this study.



(A)



(B)

Figure 2.100: (A) Image acquisition system structure (Li et al., 2008), (B) Camera set up (Rico-García et al., 2009)

Table 2-2: Various proposed formulas for calculating leaf area for a number of plant species

Plant species	Proposed leaf area calculation formula $f(L, W)$	Reference
Banana (*Musa* Spp.)	$0.033 + 0.840\ (L \times W)$	(Potdar and Pawar, 1991)
Cucumber (*Cucumis sativus* L.)	$-210.61 + 13.358\ (L \times W)$	(Cho et al., 2007)
Sunflower (*Helianthus annuus* L.)	$6.720 + 0.6494\ W^2$	(Rouphael et al., 2007)
Kiwifruit (*Actinidia deliciosa*)	$0.82\ (L \times W) - 0.28$	(Cristofori et al., 2007)
Ginger (*Zingiber officinale*roscoe)	$0.919 + 0.682\ (L \times W)$	(Peksen, 2007)
Potato (*Solanum tuberosum* L.)	$71.267 + 0.2798\ (L \times W)$	(Silva et al., 2008)
Sugar beet (*Beta vulgaris* L.)	$31.928 + 0.5083\ (L \times W)$	(Tsialtas et al., 2008)
Pistachio (*Pistacia vera* L.)	$-0.0017(L \times W)^3 + 0.1746(L \times W)^2 + 71.786(L \times W) + 79.966$	(Karimi et al., 2009)

2.4.6 Tractor-Mounted digital systems

Handheld meters are more suitable for a limited area and most farmers use them in small farms. For the large farms, tractor mounted systems are more appropriate to detect leaf chlorophyll and N content in plants. These systems are quite fast and labour efficient. The compact size and low weight design allow easy adaptation of these devices to be pole-mounted, and the information produced by sensors is utilized to quantify the crop N deficiency. Currently, there are three main commercially available technologies used on board to assess the plant N status in real-time to drive fertiliser spreaders and apply variable N rates to crops: Yara N-Sensor/FieldScan, Crop-Circle, and GreenSeeker (Samborski et al., 2009). Tractor-mounted systems are based on sensors that measure crop characteristics which saves time and avoids any delay between the assessment of needs and the actual N application time (Kim et al. 2000).

Philippa et al. (2012) conducted a series of experiments to compare the performance of 3 visible/near infrared (VIS/NIR) sensors such as Greenseeker™ from Trimble, Crop Circle ACS-470™ from Holland Scientific and CropSpec™ from Topcon for N fertiliser estimation for crop production. Despite variation in the sensing footprint, the wavelengths used and the indices the data collected from all these sensors had a common management purpose. Even the tractor-mounted systems also have some limitations, as they cannot directly estimate crop N requirement (Mulla, 2013). Thus scientists developed N fertiliser response functions by comparing the sensor readings with readings in reference strips from the crops receiving adequate N supply which helped to overcome this issue (Scharf et al., 2011). Using different active sensors (GreenSeeker RT100, Holland Scientific CropCircle ACS 470, YARA N-Sensor ALS), Kipp et al. (2014) studied how environmental variations such as light intensity and temperature, and measuring distance influence the accuracy of spectral reading. Depending on the type of sensor used, measuring distance (between sensor and target surface) was found to be the main determinant of the accuracy of spectral readings. Optimum measuring distances from crop canopy to sensor were set from 10 to 200 cm which enabled stable sensor outputs. Depending on the sensor and spectral index, the device temperature variably affected the spectral readings but light conditions had little or no effect on the performance of these sensors.

2.4.6.1 **GreenSeeker**

GreenSeeker® is an incorporated system of optical sensor and application system for optimising N application (Figure 2.11). This unit emits light in two wavelengths and the light reflectance from the target (plants in the soil) is measured. The GreenSeeker®TM active lighting optical sensor uses high intensity light emitting diodes (LEDs) that radiate light at 780 mm (NIR) and 600 nm (red) as light sources. The region between 400 nm and 700 nm is what plants use to drive photosynthesis, and is typically referred to as photosynthetically active radiation (PAR). These LEDs are pulsed at high frequencies. The photodiode detector measures the magnitude of light reflected from the leaves (Figure 2.12). Background illumination is eliminated by electronic filters. The magnitude of the filtered signal is measured by a multiplexed A/D converter (convert the signal from analog-to-digital). Measurements are collected and averaged across the treatments and sensing distance of 0.61 m.

The normalised difference vegetation index (NDVI) is calculated from the NIR and red values by the computer. The leaf pigment absorbs visible light (from 0.4 to 0.7 μm) and reflects near-infrared light (from 0.7 to 1.1 μm). Thus variations in the absorbed and reflected light are used for calculating green patch (chlorophyll or leaf health) in a field. The more leaves a plant has, the more these wavelengths of light are affected, respectively. Therefore, using spectral reflectance, variation in vegetative indices is computed to predict plant growth parameters related to photosynthetic activity and plant productivity. The NDVI is successful in predicting photosynthetic activity, as it includes both near infrared and red light. Photosynthetic capacity of plants is measured by chlorophyll content and activity.

GreenSeeker® sensors have a 0.6 m field of view and 0.8 – 1.2 m above the plant is an optimal sensing height. The number of sensors used and their spacing affect the percentage of area covered by the mapping system. The sensor calculates application rates and works out the mix of the three valves required to have this rate applied. The computer then forwards the data to the valve control module computer which controls the valves (Industries, 2005). During the process of photosynthesis, chlorophyll tissues of plant absorb red as an energy source. The plants are considered healthy when they reflect more NIR and absorb more of the red light, whereas, varying light conditions have very little impact on the GreenSeeker® measurements as it is an active sensor (Jones, 2004). GreenSeeker® sensors have been used in several published studies for detecting crop N status (Arnall et al., 2006). Many producers have improved N fertilisation of cereal crops by efficient GreenSeeker® application (Mullen et al., 2003). Raun et al. (2002) observed 15 % improvement in NUE of wheat crop using the GreenSeeker®.

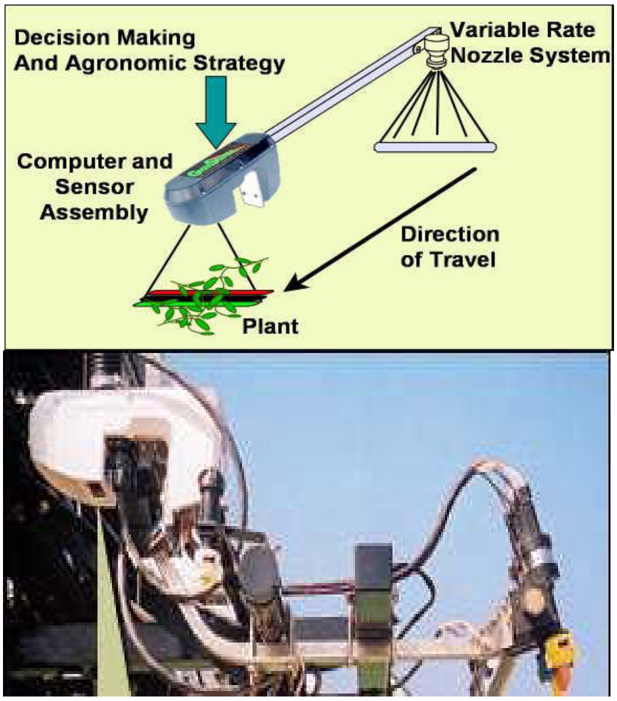


Figure 2.11: Fertilising systems using the GreenSeeker (Havránková 2007)

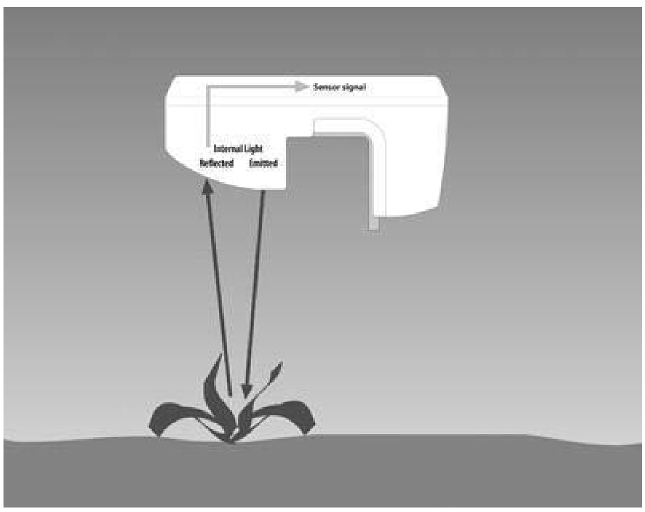


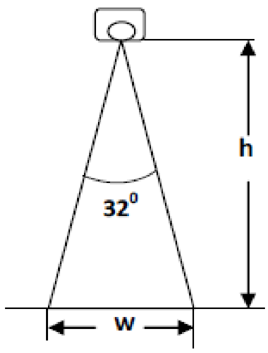
Figure 2.12: Illustration of GreenSeeker's light emission and reflectance measurement system (Image was taken from http://www.lesspub.com).

2.4.6.2 **Crop-Circle**

The Crop-Circle sensor, ACS-210 (Holland Scientific Inc., NE, USA) (Figure 2.13) can measure plant reflectance using an on-the-go light sensor (Havránková, 2007). It can either be held or mounted on any type of vehicle. The ACS-210 uses PolySource® technology containing a single LED light source which emits NIR and visible light simultaneously. There are two sensor models, which use yellow/NR (590 and 880 nm) and red/NIR (650 and 880 nm) sensing capabilities. Spectrally sensitive photo sensors capture the portion of light reflected back to the sensor from the plant canopy. The ACS-210 minimises its dependency on other light sources by distinguishing its own light source which is a modulating source (rapid pulsing many times per second). In this way, regardless of the light conditions (artificial lighting, complete darkness, cloudy skies, full-sun, etc.), the ACS-210 remained efficient and unaffected.

The sensor can run at a height from 25 cm to 213 cm. The width of the projected beam when the sensor is mounted above a target is defined by the following equation;

$$\mathrm{w} = 2.\mathrm{h}.\tan\left(\frac{\theta}{2}\right) \approx 0.57.4$$



Where:

θ = Angular FoV (field of view) in degrees (≈32 degrees for the ACS-210)

w = Projected beam width

h = Height of the sensor above target

Figure 2.13: Crop-Circle sensor (Havránková 2007)

The output capacity of the Crop-Circle sensor ranges between 1 to 20 samples per second. The samples are collected using a standard RS-232 interface with a laptop, Personal Digital Assistant (PDA) or other data acquisition device, and stored using a comma-delimited format. Basic reflectance and the classical vegetative index are provided by the sensor (HollandScientific, 2004). However, recently a new model of Crop-Circle sensor ACS-470® (Holland Scientific Inc., Lincoln, Nebraska), which can be spectrally customised measures 20 samples per second. The sensor measures reflectance from the crop canopy at 0.8 m distance and has a footprint of 0.1 x 0.5m.

The working process of ACS-470® is similar to that of the GreenSeeker system, and it has been used to detect biomass and leaf area of many cereals (Shearman et al., 2005, Trotter et al., 2008). It is a three channel instrument that has a range of interference filters in the VIS and NIR range; the filters are placed in the respective channel, usually one filter is in the NIR range (Channel 2) and normally the other two in the VIS range. Each channel gives an actual value and then computing ratios or indices and it finally gives five outputs. The ACS-470 is an active sensor, which has an optimal sensor-to-canopy distance is 750 to 900 mm. This system has more flexibility in terms of programming and use of sensors in the range of 440 to 800 nm. Each sensor contains six filters as a standard set; these include 450, 550, 650, 670, 730 and 760 nm.

2.4.6.3 **Yara N-sensor**

The Yara passive N-Sensor/Fieldscan (Yara International ASA, Oslo, Norway) is a tractor-roof-mounted multispectral scanner (Zebarth et al., 2003, Berntsen et al., 2006), which utilises a two diode array spectrometer (Figure 2.14). With two spectrometers on either side of the vehicle, the spectrometer utilising four lenses with an oblique view (64° with the zenith, solar azimuth effects are largely avoided by the special viewing geometry), to analyse the light reflectance from the crop. This allows simultaneous measurements from both the left and right side of the tractor if desired. Approximately 25% of the total area is scanned at a 24 m working width. The second spectrometer measures the ambient light for permanent correction of the reflectance signal and guarantees constant measurements under variable irradiance conditions (Reusch et al., 2002, Zillman et al., 2006). The N status of the crop may be gauged by determining crop reflectance characteristics using selected wavebands from 450 to 900 nm, and then using algorithms to calculate optimal N application rates either from NDVI or from other vegetation indices. The data are transmitted to spreader equipment which controls the N fertiliser application rate on-the-go (Reusch et al., 2002). Link and Reusch (2005) presented an N-Sensor ALS (active light source) in 2005 that has a 24 hours per day working capacity due to its own light source and had an on-the-go capacity to vary N application rates irrespective of ambient light conditions. The passive N-Sensor is comparable with effective geometry and spectral channels and has been efficiently used for N management in various crops such as potato, maize, wheat, barley, onion and oilseed rape (Yara, 2006, Havránková, 2007). In order to minimise the errors caused by cloud cover and low light, a new version of Yara-N with active sensor is introduced, which improved overall performance of the sensor (Link and Reusch, 2006).

A dealer of the N sensor in Czech Republic, Company Leading Farmers reported approximately 5% increase in crop yield when N sensors were used (Farmers, 2006). Keeping in mind the optimal yield potential of individual fields, Ebertseder et al. (2005) recommended that the technology should be improved by taking the soil condition into account whilst using the sensor.

Figure 2.14: Yara N sensor (Image was taken from http://www.agricon.com)

2.4.7 Crop Reflectance

Crop reflectance is the ratio of the amount of radiation reflected by an individual leaf or leaf canopy to the amount of incident radiation (Schröder et al., 2000). Chappelle et al. (1992) found that dark green plants exhibit relatively low light reflectance and transmittance into the visible spectrum due to pigmentation and photosynthetic tissue. Visible light is selectively absorbed by the pigments involved in photosynthesis (chlorophyll a and b). Also, the green wavelength is reflected, whilst the red and blue wavelengths of the visible spectrum are absorbed. Hence a good indication of how green the crop is can be measured at these wavelengths using crop reflectance.

Chlorophyll content in plants is the most important feature that influences leaf reflectance in certain parts of the spectrum, and is the most advantageous pigment in leaves containing sufficient N content (Gausman, 1974). For example, sunflower leaves suffering N stress were found to have higher reflectance in the visible part of the electromagnetic spectrum and a lower reflectance in the NIR (Filella et al., 1995). The NDVI and the total N update were found to be highly correlated (Stone et al., 1996). Using an assortment of spectral indices and optical techniques Filella et al. (1995) determined N status of wheat crop, which looked promising. Similarly Zhao et al. (2005) found foliar N deficiency significantly reduced the leaf chlorophyll content, which subsequently reduced the leaf reflectance of approximately 550 and/or 710 nm spectrum (Carter and Estep, 2002).

On the basis of multispectral images, various ways have been investigated for assessing crop N stress (Yang and Anderson, 2000, Noh and Zhang, 2003). Leaf chlorophyll content are tested in various ways to increase the sensitivity; some NDVI are defined by a ratio, variance, or difference/ratio combination of the measured reflectance at different wavelengths (Gitelson et al., 1996). Chapman and Barreto (1997) obtained low chlorophyll content data, and confirmed it through field testing that the data suggested by NDVI approach were more sensitive than the direct reflectance approach. Multispectral reflectance analysis is now used on RS images to give a synoptic view of crop N status to producers with spatial variability (Moran et al., 1997, Lee et al., 1999, Thai et al., 1998). Further investigation has shown that whilst RS can provide producers with a comprehensive overview of crops and greatly assist with variable rate N management, the technical difficulties requiring professional suppliers and associated costs such as accessibility to satellite/aerial images, calibration and registration of images are considerable (Zhang et al., 2002, Noh et al., 2005).

Reflectance and NIR area has been related to crop N status in various ways. For example, leaves with stronger spectral reflectance in particular NIR and blue wavebands have higher N content. Alternatively, leaves containing lower chlorophyll and N content reflect more wavelength in the red region and less in the NIR region (Serrano et al., 2000). N deficiencies in plant tissues have been detected using various reflectance indices and ratios (Plant et al., 2000, Lukina et al., 2000).

2.4.8 Vegetation indices

Vegetation indices are the values generated using reflectance measurements from two or more spectral wavelengths. Reflectance is the ratio of the total amount of radiation (energy) reflected by a surface to the total amount of radiation incident on the surface. The correlation of vegetation indices with biomass, leaf area index (LAI), N status or with yield depends on the index used (Thorp and Tian, 2004). Some of the most commonly used vegetation indices are, ratio vegetation index (RVI), difference vegetation index (DVI), green vegetative index (GVI), and land perpendicular vegetation index (PVI) (Ramirez, 2010). NDVI is one of the most widely used vegetation indices that can provide a simple answer for vegetation health. It can be calculated by the following formula;

$NDVI = \frac{(NIR-RED)}{(NIR+RED)}$ ………………………………………..Equation 2-4

Where NIR is the reflectance in the near infrared region (770 ± 15 nm) and RED is the reflectance in the red region (650 ± 10 nm) of the electromagnetic spectrum. Reflectance in the NIR region is positively correlated with the total area of all leaves of a plant, while reflectance in the red region is negatively correlated with total chlorophyll contents of plant leaves (green leaf area) (Knipling, 1970). Likewise, vegetation reflectance is governed by the contribution of stems and leaf orientation to canopy reflectance (Carter and Estep, 2002). The NDVI value shows a discrepancy between -1.0 and +1.0. NDVI values for vegetation typically range between 0.2 and 0.7. Practically, a value less than 0.2 usually mean unhealthy vegetation and a value close to +1.0 (more than 0.6) means very healthy green leaves or very dense vegetation, whereas, zero or negative values means no vegetation. Nevertheless, real wavelengths and bandwidths for NDVI calculations vary with different sensors and applications.

A good relationship has been found among the SPAD readings, sap nitrate concentration, aboveground biomass and total tissue N concentration, and the spectral indices DVI, GNDVI, NDVI and RVI (Flowers et al., 2003, Lina et al., 2007). A typical reflectance spectrum was suggested by Kumar and Silva (1973), which identifies healthy and stressed plants using the reflectance ratio of the energy reflected from an object to the energy incident on an object (Figure 2.15). The spectral reflectance of a crop significantly varies within the electromagnetic spectrum in the NIR range (λ = 700-1300 nm), and in the visible red range (λ = 550-700 nm). Plants are identified as green due to low reflectance in the red and blue wavelength range, high chlorophyll absorption, and a high reflectance rate of green wavelength. In the case of NIR radiant energy, various leaf attributes such as cellular structure and the air-cell wall-protoplasm-chloroplast interfaces determine the level of reflectance from plant surface (Kumar and Silva, 1973). Considering the difference between soil and vegetation in the NIR and red regions as maximum, Adamsen et al. (1999) suggested that the spectral reflectance data could be effectively used to calculate a diverse range of vegetative indices, which are well-correlated with biophysical and agronomic parameters connected to plant productivity. Since the vegetative indices utilise both red light and NIR, Carlson et al. (1990) stated that NDVI is effective in forecasting photosynthetic activity. Chlorophyll content determines the level of photosynthetic activity of a plant, and is associated with leaf N (Chapman and Barreto, 1997). Thus estimating leaf chlorophyll content could help to determine vegetation nutrient level.

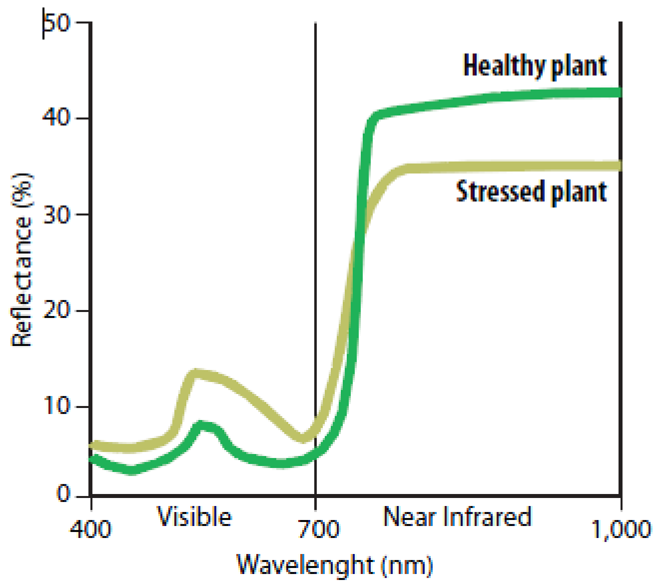


Figure 2.15: Typical reflectance spectrum of a healthy and a stressed plant (Govaerts and Nele, 2010)

2.4.9 Remote sensing technology

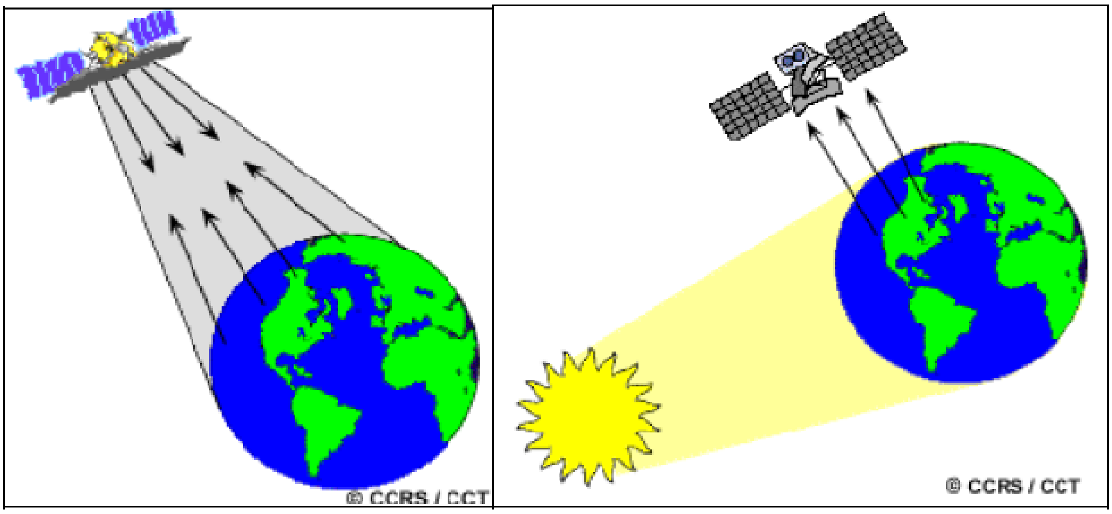
Near-real-time and real-time in field nutrient management can be enhanced with the use of Remote Sensing (RS) technology. Negative environmental impact and costs can be minimised by monitoring the changes in field conditions which can provide an opportunity to the producers of adjusting fertilisers and/or chemicals application rates and times accordingly (Godwin et al., 2003). Depending on the platform and type of RS system, the use of RS is widespread in precision farming technology and it can help to determine crop yield (Freeman et al., 2003, Aparicio et al., 2000), weed detection, water stress, and crop N deficiency (Scotford and Miller, 2005, Yang et al., 2005, Goel et al., 2003). Nusz (2009) showed that the RS is the science and art of collecting information about an object, area, or phenomenon through analysing the data acquired by a device that is not directly in contact with that specific object, area or phenomenon (Lillesand and Keifer, 2000). Henebry et al. (2005) found that natural resources management in the area, where RS was extensively used has exhibited significant improvements in land use, biomass estimation and land cover estimates. Some success has been seen in the last decade with implementing this system into commercial agriculture.

Several studies have confirmed a close relationship among crop N status, chlorophyll content and spectral reflectance and propose a high potential of RS application in precision agriculture (Barnes et al., 2003, Pinter et al., 2003). However, the adoption of RS by farmers is limited due to lack of training, high costs and time consumption in imagery (Robert, 2002). Within precision agriculture, there is a great capacity of RS applications for improving crop production and quality (Barnes et al., 2003, Pinter et al., 2003). RS has long been known as a non-destructive method, and subsequently after its first introduction to agriculture in the early 1920s, when aerial photography was used to assist the soil mapping, it has been successfully implemented for various agricultural operations such as rangeland surveys, yield forecasting, crop identification and monitoring for disease or insect pressures in crops (Riedell et al., 2004).

Many field studies have witnessed the RS and vegetation indices application as a successful technique for detecting various stresses in crop plants. The first use of aerial photography for detecting plant viruses in crops was conducted by Bawden (1933) in potato (*Solanum tuberosum* L.) and tobacco (*Nicotiana tabacum* L.). Following this, stress detection using spectral data have been used for wheat (Jones, 2004), barley (*Hordeum vulgare* L.) (Nilsson, 1995), soybean (*Glycine max* L.) (Adamsen et al., 2000), maize (*Zea mays* L.) (Kim et al., 2000), sugar beet (*Beta vulgaris* L.) (Laudien et al., 2004), alfalfa (*Medicago sativa* L.) (Guan and Nutter, 2002), watermelon (*Citrullus lanatus* Schrad.) (Blazquez and Edwards, 1986) and tomato (*Lycopersicon esculentum* L.) (Zhang et al., 2003). Moreover, Alchanatis and Schmilovitch (2005) mentioned its successful application for crops, predominantly the grain crops like barley and winter wheat. The use of RS in precision farming technology is wide; it depends on the platform and system of RS used. RS systems are used for assessing crop yield N deficit, weeds, water stress (Yang et al., 2005). Use of remote sensing technology has been enormously increased in many fields. However, its acceptance in agricultural industry remained slow due to distrust of farming community in RS technology, low profit margins, limited spatial resolutions and timeliness of data (Robert, 2002).

2.4.9.1 **Types of remote sensing systems**

Havránková (2007) indicated two basic types of RS systems, active or passive (Figure 2.16). The passive system measures naturally emitted energy from the sun or earth. According to Gobson (2000), the process of the solar radiation interacting with the surface (for example reflection) and the sensing equipment enable the measurement of the energy that is reflected. The electromagnetic radiation source in active RS equipment is carried on-board. The electromagnetic radiation is reflected off the surface and then a record is made of that scattered back from the surface (Gobson, 2000).



(A) (B)

Figure 2.16: Active (A) and passive (B) remote sensing system (CCRS 2006)

2.4.9.2 **Satellite platform**

A satellite has the capacity to collect images covering a much greater area than that of a plane. However, the quality of obtained images could be affected by various factors such as nutrient disorders, which are not related to N status of the crop, soil water content, weather conditions and soil background reflectance (Gerard et al., 1997, Shou et al., 2007). Multi-spectral images are now commercially available from satellites, and the future might bring higher spatial and spectral resolution images. Satellite based radar systems (SAR) have effectively been used for studying soil (Leconte et al., 2004) and crop moisture content (Griffiths and Wooding, 1996).

New opportunities for distinguishing spatial details have been offered by the successful launch of high-resolution satellite sensors such as QuickBird and IKONOS. The QuickBird's spatial resolution imagery data are 0.61 m for panchromatic and 2.44 m for multispectral images at nadir (The point on the ground, which is directly below the

QuickBird spacecraft) (DigitalGlobe, 2002). Yoder and Pettigrew-Crosby (1995) observed that it was imperative to discriminate between field N deficiency, and differences between nutrient and water stress. Therefore, the QuickBird's imagery, which uses high spatial resolutions, and NIR (760-900 nm), red (630-690 nm) and green (520- 600 nm) wavelength bands, provide an essential support for this. However, making timely management decisions using QuickBird technology may not necessarily be possible due to slow turnaround of image processing, clear sky conditions, high cost of commercial satellite images and potential cloud interference during image acquisition (Weiss et al., 2000, Wu et al., 2007).

2.4.9.3 **Airborne platform**

This technique provides high spatial resolution and possibly short revisit time, but its application is limited by high operational complexity, costs and inconvenience to control in windy conditions. The potential use of airborne data for cereal production has already been demonstrated (Wood et al., 2003). Digital cameras or radiometers could obtain images, while an electrical signal proportional to the light energy exposed is produced by radiometers. Hence, the radiometer is exposed only to the specific wavelengths required by filtering the light. Scotford and Miller (2005) observed that an optical band pass filter may be fitted onto a digital camera, whereas Morris (2006) evaluated the case for aerial images being used;

- The adaptability of flight schedules, for example as required by husbandry considerations.
- Images have a high resolution.
- Economical and relatively rapid capacity to map large areas.
- Turnaround time is feasible, a few days.
- The producer can be involved with the system.
- If repeating the imaging is required, turnaround time is short.

Negative aspects of aerial image use are:

- Clear weather conditions are required for image collection
- It is uneconomical for only a small area due to the cost of running the aircraft
- Images need to be ground calibrated.

A substantially large area can be surveyed in a short time using remote sensing from aerial platforms (Shou et al., 2007). Aerial photography has successfully been used to vary in-season N application rate, reproductive growth phases of crop, prediction of side-dressing N rates for assisting with growth by utilising green or blue colour spectrum photography (Scharf and Lory, 2002). Aerial photographs taken from a helium filled balloon were used to monitor growth status of winter wheat (Jia et al., 2004). According to Quilter and Anderson, (2000), aerial photography from radio controlled model aircraft could deliver high-resolution imagery at a relatively low cost. Hunt et al. (2002) advised that model-aircraft photographic imagery has considerably lower costs spatial resolution associated compared with satellite and airborne sensors. Model aircraft NDVI by colour IR film with a low-cost automatic camera was found equivalent to NDVI from the advanced sensors. Hunt et al. (2003) used an Olympus D40 4.1-megapixel colour digital camera (Olympus, Inc., Melville, NY, USA), and found it useful for precision agriculture and obtained high spatial resolution at relatively low cost that was comparable with the NDVI from advanced sensors. However, they experienced various complications such as overexposure of the film, lack of radiometric and spectral calibration, and the requirement of long landing and take-off areas during the initial work.

The above three approaches are time consuming and prone to measurement error, which may not be very practical for large scale measurements. Moreover, no one can claim that manual approaches are highly accurate. Accordingly, a number of digital methods have been proposed to overcome these limitations.

2.5 Evaluation of LCC, SPAD and image processing-based N estimation meters

Various factors influence the suitability and applicability of different N estimation meters. LCCs are the cheapest, and have been widely used in rice, maize and sugarcane. However, this approach is based on visual inspection of leaf colour and the accuracy is not guaranteed, especially for different lighting conditions. SPAD on the other hand, is less sensitive to lighting conditions, but has not shown consistent performance across all species. The fluctuation of SPAD performance is influenced by its small measuring area (around 12.57 mm^2). The relatively high cost of SPAD makes it less appealing to small farmers, especially from developing countries. Similar to LCC, image processing techniques are also affected by the environmental conditions, as these have to be set appropriately to produce reliable results across the different species. Despite being only applied to a limited number of species, image processing showed good potential compared to SPAD and LCC. The image processing technique may require calibration, but its cost is generally less than that of SPAD meters. In general, each of these methods may have their own advantages and disadvantages. However, the image processing technique is yet to reach its full potential. A comparison among LCC, SPAD and image processing based N estimators is presented in Table 2-3.

Table 2-3 Effect of different factors on LCC, SPAD and image processing-based nitrogen estimators

Factors	LCC	SPAD	Image processing technique	Reference
Applicability to wide range of species	More relevant for cereals such as rice, maize and sugarcane	Has been successfully applied to a number of species, but did not produce accurate results for others. For example, not suitable for leaves of regenerated plants.	As this is a relatively new technique for N estimation, it has been applied to a limited number of species. However, it has the potential to be applied to a wide range of species.	(Nagappa et al., 2002, Yadav et al., 2010)
Effect of environment on readings	As direct sunlight affects leaf colour readings, it is recommended to take the reading in the shade.	Environment has no or very little effect on readings.	Similar to LCC, sunlight can affect the colour of an acquired image.	(IRRI, 1996)
Accuracy	Accuracy is not guaranteed, as reading depend on visual assessment.	Can be quite accurate. However, due to the small measuring area of the device (around 12.57 mm^2), constancy in performance is not guaranteed	Has the potential to produce accurate reading when images are collected in optimal environment/setting.	(IRRI, 1996, Netto et al., 2002)
Potential for improvement	Limited improvement only, through increasing the number of panels (green shades)	Improvement in the underlying technology. The latest version is SPAD-502	Has the potential for further improvement by enhancing the image processing technique.	(IRRI, 1996, Yang et al., 2003)
Calibration	Does not require calibration	Does not require calibration	Digital cameras may require calibration	
Unit Price	USD $1	USD $1300-1400	Depends on the type of camera, but costs usually lower than SPAD	(Balasubramanian et al., 2000)

2.6 Summary

Estimating crop requirement of nutrients such as N and P is crucial for higher productivity and cost-effectiveness. Out of two techniques used for N estimation, the non-destructive techniques are relatively fast and less expensive. A range of non-destructive methods with varying complexity and optimality are used in the agriculture sector. Digital meter devices, such as SPAD and N-Tester are usually more accurate than simple leaf colour charts but are more expensive as well. Image processing techniques have started to attract the agricultural scientists due to their promising results and possibly moderate cost. However, they are still to be commercially available. For detecting nutrient requirements of the crops on large farms, tractor-mounted systems are more appropriate as these are less labour-intensive, and their compact size and low weight design allow for easy adaptation to being pole-mounted. Since its development 40 years ago, RS has been successfully applied in a large number of fields due to its efficiency and wide coverage but still have limitations like clear weather conditions are required for image collection, specialised software for data analysis and professional operators are needed.

Despite the recent success of the use of data on spectral variation in various fields, there has been a little interest in the use of these techniques in the agriculture sector. Sensitivity to the light intensity is the major limitation in the quality of images collected from the field. Thus, developing an image collection technique that is not influenced by the light variation can facilitate the field scale data collection. A number of leaf scanners are commonly used for collecting leaf images. As the quality of these images is not influenced by light variation, a handheld scanner may offer an alternative method of image collection. The images can be processed to get RGB value, which can be used for calculating leaf dimension and nutrient status using appropriate programs.

CHAPTER THREE

3 General material and methods

3.1 Introduction

In order to develop a method for estimating leaf area, chlorophyll content, crop N and P status, a series of greenhouse and field experiments were conducted at different locations. This chapter describes crop species, cultivars, climate descriptions and experimental design of field and greenhouse experiments. The data collection method is also discussed, although experiment-specific material and methods are described in the relevant chapter.

3.2 Crop species and genotypes

To validate the performance of the methods proposed in this study over a wide range of crop species different economically important crops were selected varying in phenotypic characteristics, fertiliser requirement and leaf growth. Four crop species, tomato, lettuce, broccoli and cotton, were used in these experiments. In addition, these are among the most important economic crops in Australia. The tomato (*Lycopersicon lycopersicum*) cultivar Tommy Toe exhibits indeterminate growth habitat, matures in approximately 70 days and produces round to slightly elongate large red cherry tomatoes (Grassbaugh et al., 2000). The semi-head lettuce (*Lactuca sativa* L.) cultivar green mignonette is a heat-tolerant, slow to bolt, and tip-burn resistant cultivar widely cultivated in high temperature zones (Kratky, 1996). The Chinese Broccoli (*Brassica oleracea* var. alboglabra) Kailaan express (hybrid), which is widely cultivated in Australia (Lee and Lee, 1995), requires well-drained fertile soils for optimal growth. For cotton (*Gossypium hirsutum* L.) a commonly cultivated and high yielding commercial cotton cultivar Sicot 71BRF was selected ([Bollgard II® Roundup Ready Flex®], CSIRO Australia) (Stiller, 2008).

3.3 Greenhouse experiments

3.3.1 Experimental design

Greenhouse experiments were conducted at the Faculty of Science, University of Technology Sydney, Australia. Seeds of three crop species, tomato, broccoli and lettuce were planted in plastic pots (12 cm in diameter, 12 cm deep) filled with vermiculate. Three plants per pot were germinated and then thinned to one plant per pot after 2 weeks of growth. The experimental design was a randomised complete block design with five replicates of each treatment that is 25 pots for each species that were divided into five groups; each group received a pre-specified N treatment.

3.3.2 Mineral nutrition and processing

For the first eight weeks, all plants received a complete nutrient solution composed 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 $CaCl_2.2H_2O$, 1.4 mM 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 µM of $CuSO_4.5H_2O$, 0.3 µM of $Na_2MoO_4.2H_2O$, and 0.5 µM of H_3BO_3. The pH of nutrient solution was maintained at 6.0-6.1 (Sanchez et al., 2002), and the solution was renewed every week. This basic nutrient solution provides an equivalent N concentration of 0.43 g N L^{-1}. After week 8 of growth (at crop maturity), five different N treatments (in the form of NH_4NO_3) were applied for a period of seven weeks, (N0: without N, 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}).

For the phosphorus experiment, four crops, tomato, broccoli, lettuce and cotton were grown in a greenhouse condition on a nutrient solution (as described in the above) containing 2.5/10 L of P. After seven days, three different P treatments in the form of NaH_2PO_4 were applied for a period of seven weeks, (P0 = no P, L; P1 = 2.5 mL/10 L of P and P2 = 5 mL/10 L of P). The P levels applied to each pot represent three different levels of recommended P application, P0 = 0 %, P1 = 50 % and P2=100 % of recommended P concentration.

3.4 Field experiments

3.4.1 Experimental set up

A field experiment was conducted at the Lansdowne farm, Camden campus of the University of Sydney (latitude 34°01'S, longitude 150°40'E, elevation 75m). The area is located in the southwest of Sydney, NSW. The region experiences a hot summer (average high 29.5°C to average low 16.8°C) and a mild winter (average high 17.3°C to average low 3.0°C) with an average rainfall of 762 mm (BOM 2014). The soil is classified as Tenosol soil (formerly known as Siliceous Sands) according to the Australian Soil Classification (Isbell, 2002).

Three crops, namely tomato, lettuce and broccoli were used in the study. Seeds were initially germinated in plastic trays of vermiculite using a standard nutrient solution (details are provided in the above section). After three weeks of germination, healthy seedlings were transplanted into the field. Each crop was transplanted on a separate block consisting of three rows bed (12×2 m, length×width), which was further subdivided, into 12 plots (8 plants per plot).

An overhead sprinkler system was used for irrigating the field crops. Weeds were kept under control by manual removal. The experimental design was a randomised complete block design with three replications. Three N treatments including N0: without nitrogen (control), N1: 60 kg N ha^{-1} and N2: 140 kg N ha^{-1} were applied to the soil before seed sowing. N was applied in the form of urea (46% N). The field was fertilised with the recommended rates of potassium, phosphorus and other micronutrients to ensure that these are not limiting the plant growth. Data on leaf chlorophyll and N content were recorded at different growth stages of plant, 68 days after sowing (DAS), 83 DAS and 98 DAS.

3.4.2 Cotton experiment

The experiment was conducted at the Australian Cotton Research Institute, Narrabri, NSW, Australia (150^{o}E, 30^{o}S). The soil of the area is self-mulching medium grey clay overlying brown clay, and is classified as a fine, thermic, montmorillonitic Typic Haplustert (SoilSurveyStaff, 1996). A commercial cotton cultivar Sicot 71BRF was grown in a split plot design, where main plots were 8 m × 200 m and the N rates (subplots) were 8 m × 16 m. The design had four replicates. Cotton crop was grown in the field, where cotton-wheat-fallow-cotton system was practised during previous seasons. Various rates of N (urea) were applied at 30 cm depth below plant line before sowing as N0: without N (control), N1: 80 kg N ha^{-1}, N2: 160 kg N ha^{-1}, N3: 240 kg N ha^{-1} and N4: 320 kg N ha^{-1}. Cotton crop was irrigated when soil water deficit approached the commercial thresholds (50–80 mm), and insects were controlled when they exceeded commercial thresholds (Deutscher et al., 2005). Weeds were controlled with mechanical cultivation and herbicides.

3.5 Data Collection

3.5.1 Biochemical assay

3.5.1.1 **Chlorophyll measurements**

Leaf chlorophyll content of tomato and lettuce were collected four times during different growth phases, 2, 5, 7 and 10 weeks after treatment, whereas data for leaf chlorophyll content of broccoli were collected five times at 2, 5, 7, 10 and 13 weeks after treatment.

A leaf punch was used to cut 1.2 cm diameter leaf disks from a fully expanded leaf from each of the 5 replicate plants, and then the leaf disks were 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 was determined at 646.6, and 663.6 nm using a Varian DMS-70 Spectrophotometer. Chlorophyll a, b and total chlorophyll content were calculated using the equations of Porra et al. (1989):

Chl a = 12.25 $A^{663.6}$ - 2.55 $A^{646.6}$ ……………………….…………..Equation 3-1

Chl b = 20.31 $A^{646.6}$ - 4.91 $A^{663.6}$ ………………………………Equation 3-2

Chl a + b = 17.76 $A^{646.6}$ + 7.34 $A^{663.6}$ …………………………Equation 3-3

A refers to absorbance at a specific wavelength (663.6 or 646.6). The chlorophyll contents were expressed in terms of nmol/mL.

3.5.1.2 **Leaf N concentration**

The remaining part of leaf samples (after removing 1.2 cm leaf disk) was oven-dried at 75°C for at least 24 hours, ground using a ball mill grinder and sieved through a 1 mm screen. The ground samples were oven-dried again at 75°C for another 24 hours before measuring leaf N content (%) using a LecoTruSpec CHN analyser (Leco, 2006).

3.5.1.3 **Leaf P concentration**

The oven-dried samples of the youngest fully expanded leaves were used for tissue nutrient analysis using an inductively coupled plasma mass spectrometer (ICPMS). The samples (1 mg) were placed in 50 mL tubes and digested by adding 2.0 mL HNO_3, and 0.5 mL H_2O_2. The tubes were vortexed to completely mix the samples and then allowed for an overnight pre-digestion at room temperature (20-22^{o}C). P concentrations in leaf tissues were determined using a standard technique (Wheal et al. 2011).

3.5.1.4 **Leaf anthocyanin**

The process of measuring leaf anthocyanin content was started by collecting 10 mm diameter discs from the youngest (uppermost) fully expanded leaves of each tomato, lettuce and cotton plants. Fresh leaf samples were grounded in 7% acetic acid or 3 M HCl-H_2O-$MeOH$ (1:3:16), filtered, centrifuged and dried at 35°C. The dried samples were re-dissolved in 1 mL solution of acetic acid (7%) and anthocyanin was purified by a reverse-phase chromatography using Isolute C18 500 mg/3 mL columns (IST Ltd., Hengoed, U.K.), and rinsed with 3 M HCl-H_2O-$MeOH$ (1:3:16). Absorbance of the purified extracts was taken at 530 and 653A for estimating leaf anthocyanin (Murray and Hackett, 1991) as:

Anthocyanin level = $A_{530} - 0.24\ A_{653}$ ....Equation 3-4

A refers to absorbance at a specific wavelength (530 or 653).

3.5.2 Non-destructive measurements

3.5.2.1 **Leaf greenness**

The SPAD (Minolta SPAD-502, Konica Minolta Sensing, Inc., Tokyo, Japan) chlorophyll meter was used to determine total leaf chlorophyll in the same leaves that were used in the acetone extraction method described above. SPAD readings were taken from the youngest fully expanded leaves of 12 plants from each treatment plot and averaged. This device emits two different light intensities from two diodes: peak wavelength 650 nm (red) absorbed by the leaf tissues, which estimates the chlorophyll content (greenness) a second peak 940 nm (infrared LED) is emitted simultaneously with red LED to compensate for leaf thickness (Shapiro et al., 2006).

3.5.2.2 **Leaf scanning for image collection**

A simple handheld crop sensor (Trimble) was used for scanning plant leaves. This is an affordable, easy-to-use device that can assess leaf dimensions, colour and consequently nutrient status. The sensor emits brief bursts of red and infrared light, and then measures the amount of each type of light that is reflected back from the plant. The sensor continues to sample the scanned area as long as the trigger remains engaged. The sensor displays the measured value in terms of a normalized difference vegetation index (NDVI) reading (ranging from 0.00 to 0.99) on its LCD display screen. The strength of the detected light is a direct indicator of the crop health; the higher the reading, the healthier the plant would be. The price of a handheld crop sensor (GreenSeeker) starts from US$495 (Trimble, 2012), and hence it is cheaper than the SPAD-502, which costs to US$ 1350 per unit (Turner and Jund, 1994).

We used a handheld portable scanner (Pico Life) with (40 × 22) cm reference plate. Images collected with this instrument were analysed in Matlab. 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 records 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. Based on RGB values, various algorithms that estimate leaf chlorophyll and N content of plants growing under greenhouse and field conditions were developed (Detail of the each method is given in the respective chapter).

CHAPTER FOUR

4 Estimating leaf chlorophyll and nitrogen content in different crop species growing on variable N levels

4.1 Introduction

In predictive research, leaf parameters (area, height, width, average width and perimeter) represent a key data source for making decisions regarding cultivation pattern, trimming, pruning and managing fertilisation programs (Ali and Anjum, 2004, Mohsenin, 1986). Moreover, leaf parameters are highly useful for studying plant biological characteristics, and in guiding agricultural production practice (Xiandong et al., 2006). Currently, there are two classical methods for measuring leaf dimensions: digital and non-digital. In the most commonly used non-digital methods; the leaf is placed on a grid paper and the number of grid squares is calculated to estimate leaf area according to the different formulas.

Compared with the non-digital methods, the digital methods are faster, more reliable and suitable for large scale measurement. The most widely used digital device for measuring leaf area and dimensions is the LI-3000, which can measure leaf area, average width, maximum width, length and height. Recently, image processing techniques have been used for measuring leaf dimensions. The images are collected using digital cameras and are analysed by dedicated software. As the image quality is influenced by camera angle and light conditions, these systems are not convenient for field usage. Hence, developing a new image-based system that is unaffected by these factors can help in collecting field scale data.

In addition to leaf area, leaf colour also provides a good indication of chlorophyll content of leaves and crop vigour (Gaddanakeri et al., 2007, Islam et al., 2007). Hence, farmers usually prefer to keep leaves of their crops dark green, and have routinely used leaf colour as a gauge for plant health (Kawashima and Nakatani, 1998, Singh et al., 2010). Leaf chlorophyll concentrations are generally estimated through destructive and non-destructive techniques. Destructive method is a laboratory based technique that measures foliar chlorophyll concentration by organic extraction and spectrophotometric analysis (Arnon, 1949, Porra et al., 1989). This approach is accurate and is 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 (Minolta, 1989).

A more recent progress in non-destructive leaf chlorophyll determination is based on the utilisation of leaf pigment analysis. In the past two decades, many image processing techniques have been developed to monitor plant health using mainly the RGB colour model (Erickson et al., 1988). According to Yadav et al. (2010) 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 could be more effective for chlorophyll estimation in regenerated plants. Vollmann et al. (2011) used a Sony digital camera to capture leaf images and Sigma Scan Pro image analysis software to obtain the averaged G value of leaves. The obtained value was then used to estimate the chlorophyll concentration. When comparing the calculated chlorophyll values with the values computed by a SPAD meter, they found very similar results. Kawashima and Nakatani (1998) used a portable colour video camera and a personal computer to estimate the chlorophyll content in wheat and rye leaves. In almost all studies, digital cameras were used to acquire leaf images, which were then analysed to examine the relationship between R, G, and B values. On the basis of these relationships, chlorophyll and nitrogen (N) content of plants are calculated (Mercado-Luna et al., 2010).

Various formulas have been proposed for calculating leaf chlorophyll and N content of vegetation; however, none of these is universally accepted for all crops. Kawashima and Nakatani (1998) showed that (R-B)/(R+B) is a good formula to determine foliar chlorophyll status in wheat. In contrast Yuzhu et al. (2011) formulated G/(R+G+B) for the estimation of N status in pepper leaves. Suzuki, Murase and Honamin (1999) used G/(R+G+B) equation to estimate leaf chlorophyll content in broccoli. Cai et al. (2006) found that R/(R+G+B) is a good formula for estimating leaf chlorophyll content in cabbage, whilst Adamsen et al. (1999) 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 total number of wheat leaves. Finally, Hu et al. (2010)

showed that the RGB colour indices of *R*, *G* and *R*+*G*+*B*, *R*-*B*, *R*+*B* and *R*+G had a significantly strong relationship with leaf chlorophyll content.

In order to improve the image quality and get consistent data, Mercado-Luna et al. (2010), developed a new method. They installed a camera inside a box, and controlled the light environment 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. (2009) used a normalized difference vegetation index (NDVI) as an indicator to estimate leaf N content in tomato plants and obtained reasonable correlation between NDVI values and N concentration. 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 (Kawashima and Nakatani, 1998), especially light flux density, light spectral quality and angle of incidence. In order to make the image acquisition system acceptable for the farming community, there is a need to develop a technique that is both practicable and efficient for most of the cultivated crops.

Here I proposed new techniques for estimating leaf dimensions, leaf chlorophyll and N content. The efficiency of each of the modified RGB technique was verified by comparing the estimated leaf characteristics (leaf dimensions, leaf chlorophyll and N content) with their true values that were calculated using the recommended techniques, which are destructive for the cases of chlorophyll and N. A series of greenhouse and field experiment with collected leaf images were conducted using a portable scanner.

4.2 Image processing-based technique for measuring leaf dimension

4.2.1 Experimental procedure

4.2.1.1 Leaf scanning and image processing

We used a handheld portable scanner manufactured by Pico Life with 40 × 22 cm reference plate. For measuring leaf dimensions such as area, width, height, average width and perimeter, a new algorithm in Matlab was developed. The key benefit of using a portable scanner over a digital camera is to reduce the effect of the environment (lighting condition as well as angle/distance) where leaf dimensions and RGB values for each photo are highly affected by these factors. The scanning process involves placing a leaf on a white sheet while it is still attached to the plant. Below is a brief description of the algorithm (Figure 4.1 and 4.2).

Forty leaf images with different shapes and sizes (appendix) were used to validate the modified RGB technique and compare its performance with that of Li-Cor 3100. The digital vernier was used to measure the true length and width of the leaves. Moreover, the correlation between the estimated area of the modified RGB technique and Li-Cor 3100 was also calculated.

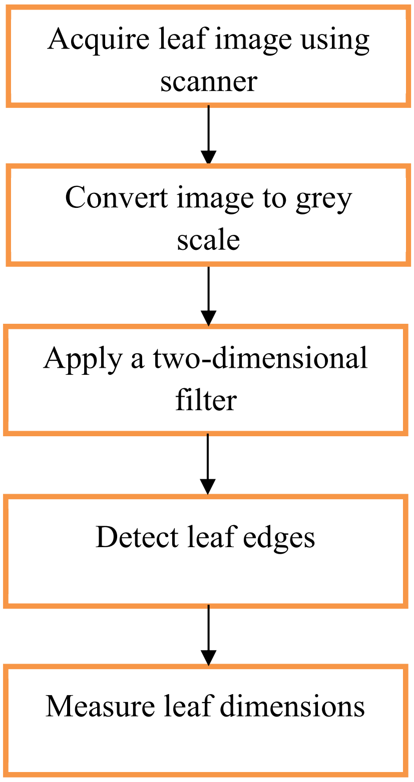


Figure 4.1: Block diagram of the modified RGB technique

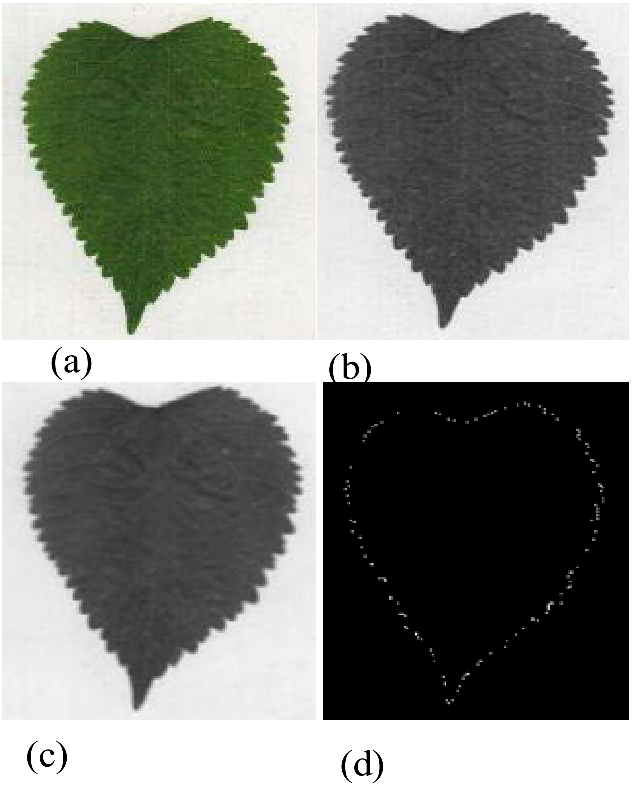


Figure 4.2: Processing steps of an acquired image. (a) an original leaf image, (b) image in greyscale, (c) after applying a 2-dimensional filter, (d) after edge detection

The image is converted to grey scale, and then a two-dimensional filter is used to remove any outlier, which assisted in identifying the leaf boundaries using an edge detection algorithm. Since a leaf is scanned from top to bottom, the highest and lowest points were noted and used to calculate the length. The extreme points on the left and right were used to measure the leaf width. The average width, perimeter and area are estimated after identifying the leaf contour. The leaf is placed between the light background and a transparent sheet to hold the leaf flat during scanning.

The use of a conventional handheld scanner gave maximum flexibility, and ease of use compared with conventional systems, which generally use specifically designed apparatus. In order to validate this modified RGB technique, it was decided to compare its readings with the Li-Cor 3100.

4.2.1.2 **Li-Cor 3100 leaf area meter**

A digital vernier was used to measure the actual dimensions of leaves and validate the readings of the modified RGB technique and Li-Cor 3100. The leaf dimensions were also estimated using the most commonly used devices Li-Cor 3100 leaf area meter. As mentioned earlier, this device is relatively accurate, but had some limitations such as it is not applicable to larger leaves and it is expensive to buy.

4.2.1.3 **Benchmark images**

Five regular shapes were also used to compare the output with known areas and perimeters of these shapes. Two rectangles and three circles were drawn on white papers and filled with a black colour were used for the purpose. A digital vernier was used to measure the actual area. The dimensions of these shapes were then estimated using the modified RGB technique and the Li-Cor 3100.

4.2.2 Results

The absolute average error and standard deviation for the area of benchmark images produced by the Li-Cor 3100 and the modified RGB technique with respect to digital vernier actual values are shown in Table 4-1.

The actual lengths and widths, measured by a digital vernier, and the estimated lengths and widths obtained using Li-Cor 3100 and using the modified RGB technique were examined. The data showed that Li-Cor 3100 measurements achieved high levels of accuracy with actual measurements. The modified RGB technique achieved slightly higher levels of accuracy for all leaves with minimum absolute average error.

The error formula as presented by You-Wen and Xiao-Juan (2009) and Rico-García et al. (2009), which is:

$$Error = \left(\frac{A_1 - A_2}{A_2}\right) \times 100 \quad \text{..Equation 4-1}$$

Where, A_1 is the estimated area by either Li-Cor 3100 or the modified RGB technique, A_2 is the actual Area. The data indicated that the absolute average error was around 3.53% for Li-Cor 3100 with a standard deviation of 1.57. On the other hand, the absolute average error was 1.8% with a standard deviation of 0.99 for the modified

RGB technique. This technique was more accurate, and in most cases, the absolute average error was noticeably lower than that of Li-Cor 3100.

One of the limitations of Li-Cor 3100 is that it does not have the capacity to measure leaf perimeter. In contrast, this new method is capable of such measurements with an absolute average error of around 4.07% and a standard deviation of 1.76.

Table 4.1: Actual and estimated areas of the benchmark shapes using Li-Cor3100 and leaf scanning technique

Figures	Actual area (cm^2)	Li-Cor 3100	Error %	Modified RGB technique	Error %	Actual perimeter (cm)	Li-Cor 3100	Modified RGB technique	Error %
R1	32	32.7	2.18	31.52	-1.49	26.4	NA	25.76	-2.4242
C1	10.17	10.66	4.78	10.15	-0.22	11.3		10.71	-5.2212
C2	78.5	80.12	2.06	76.73	-2.25	31.4		29.37	-6.465
C3	3.4	3.59	5.58	3.30	-2.76	6.28		6.04	-3.8217
R1	27.84	28.69	3.05	27.20	-2.26	23.54		22.96	-2.4639
Average error			3.53		1.80				4.07
SD			1.57		0.98				1.76

D= standard deviation; NA= not available

The comparison of values for leaf width and length estimated by the Li-Cor 3100, the new method and digital vernier (true leaf dimensions) are presented in Table 4-2. (Appendix) The average length error of the Li-Cor 3100 was 12.83% with a standard deviation of 14.66, while the average width error was 2.21 with a standard deviation of 7.61. In contrast, the modified RGB technique exhibited an average height and width errors of 5.39% and 0.88%, respectively. Similarly, the modified RGB technique showed relatively lower standard deviation for average height and width 2.36 and 4.88, respectively. Thus, the modified RGB technique provided extremely good estimates of leaf length and width, which were quite similar to the true values (calculated by vernier) and were relatively better than those calculated by Li-Cor 3100 (with a lower error standard deviation).

The correlation between the area estimates of Li-Cor 3100 and the modified RGB technique of forty leaf images (Figure 4.3) were also calculated and a very strong correlation was observed between the estimated value of the two methods ($R^2 = 0.999$).

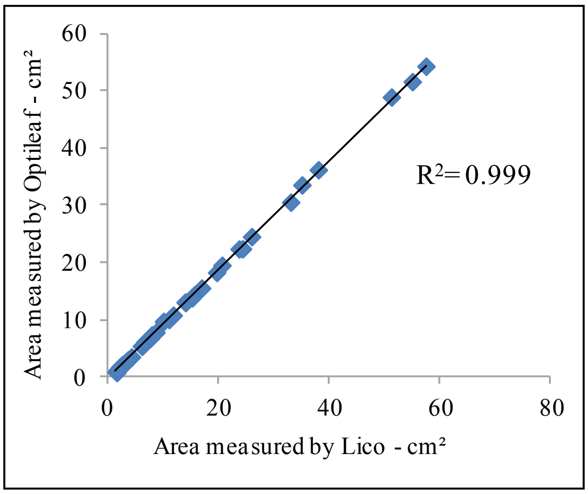


Figure 4.3: **Correlation between leaf area estimations of Li-Cor 3100 and the modified RGB technique**

4.3 Image processing-based techniques for determining leaf chlorophyll and N content of greenhouse-grown plants

4.3.1 Experimental procedure

Seeds of tomato (cultivar, Tommy Toe), lettuce (cultivar, Green Mignonette) and broccoli (Kailaan Express F1) were planted in pots (12 cm in diameter, 12 cm deep) containing vermiculite. For the first eight weeks, all plants received a nutrient solution providing N concentration of 0.43 g N L^{-1} (details of nutrient solution are given in Chapter 2). After week 8 of growth, five different N treatments (in the form of NH_4NO_3) were applied for a period of seven weeks, (N0: without N, 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}).

Leaf chlorophyll content of tomato and lettuce were collected four times during different growth phases, 2, 5, 7 and 10 weeks after treatment, whereas data for leaf chlorophyll content of broccoli were collected five times as 2, 5, 7, 10 and 13 weeks after sowing.

4.3.1.1 **Destructive chlorophyll measurement**

A leaf punch was used to cut 1.2 cm diameter leaf disks from a fully expanded leaf from each of the 5 replicate plants, and then the leaf disks were homogenised using a ten Broeck tissue grinder in 5 mL chilled aqueous 80% acetone (for further detail, please see Chapter 3, Section 3.7.1.1).

4.3.1.2 **Non-destructive estimation of leaf chlorophyll**

Leaf chlorophyll data were measured with the SPAD-502 chlorophyll meter using the same leaves as those which were used in the acetone extraction method described above.

Leaf images were also collected with a handheld portable scanner (Pico Life) using the same leaves. The RGB (Red Green Blue) values were analysed to achieve maximum correlation with the true chlorophyll status of the plants. 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 was then processed by averaging the R, G and B values of all the leaf pixels.

4.3.1.3 **Lab-based leaf nitrogen measurement**

The remaining part of the leaf samples (after removing 1.2 cm leaf disk) were oven-dried at 75^{o}C for at least 24 hours, ground using a ball mill grinder and sieved through a 1 mm screen(Details are provided in Chapter 3, Section 3.7.1.2).

4.3.1.4 **The proposed algorithm to measure chlorophyll**

A logsig nonlinear mapping in Eq. (4-5) has been utilised as the experimental results indicated that the total leaf chlorophyll content estimated by the modified RGB technique was more linearly correlated to the ground true leaf chlorophyll content in the nonlinear domain rather than the original domain. A comparison will be presented in section 4.2.5 between the proposed formula and other formulas proposed in the literature to validate the significance of the proposed measure.

$$\mathrm{Ch}_{\mathrm{OL}} = \mathrm{logsig}\left(\frac{\mathrm{G}-\frac{\mathrm{R}}{3}-\frac{\mathrm{B}}{3}}{255}\right) \text{..Equation 4-2}$$

Where, $\mathrm{Chl}_{\mathrm{oL}}$ = total leaf chlorophyll content estimated by the proposed for greenhouse-grown plants this experiment.

G= Green Colour, R= Red Colour, B= 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. The data were transformed 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 adopted so that the data points have zero mean and unit variance.

4.3.2 Results and discussion

4.3.2.1 **Effect of N fertilisation on leaf N status**

The N deficient tomato plants (0 N) showed significantly lower leaf N content just 2 weeks after treatment (WAT). Once the N application rates increased the leaf N content also increased. Thus, it is suggested that the tomato plants might suffer an immediate N deficiency if no N fertiliser is applied at this growth stage. The gap between leaf N content of plants treated with higher (1.05 N) and lower N rates (0 – 0.2 N) grew wider with plant age, indicating higher plant N requirements with reproductive growth (Figure 4.4). The leaf N content of broccoli also responded to applied N fertiliser in a similar manner to that of tomato (Figure 4.5).

In the lettuce, difference among leaf N content under various N application rates was not high at 2 WAT, especially for the N deficient to optimum level (0 – 0.43 N). This might be due to limited N requirements at this growth phase. In addition, no symptoms of N deficiency in the upper leaves may be the result of re-mobilisation of N from the lower plant parts, masking N deficiency. With the plant growth, leaf N content gradually declined, especially under low N application rates (0 and 0.2 N). No significant change in leaf N content was observed under highest N application rate (1.05 N) with plant growth, which indicated plants were unable to fully utilise additional N fertiliser (Figure 4.6).

N uptake and assimilation are essential growth-promoting processes in plants. The relationship between N application and assimilation depends on the inter-regulation of multiple crop physiological processes such as N uptake, crop C assimilation and C and N allocation between organs. Variable response towards applied N fertilisers in different plant species indicated that N assimilation process in these species is associated with their growth rates and N utilisation efficiency (Mazzoncini et al., 2011). It also suggested that a species/cultivar specific N fertilisation strategy should be recommended for crops.

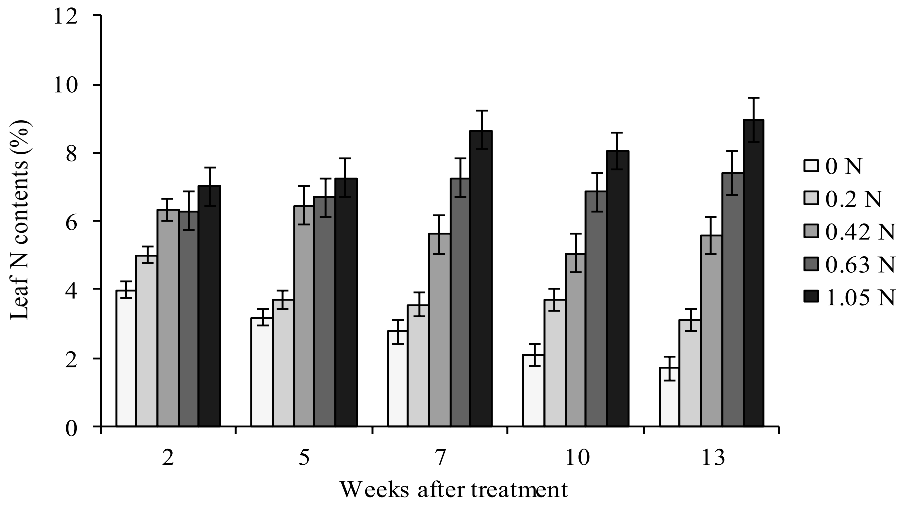


Figure 4.4: Changes in tissue N concentration of uppermost fully expanded leaves of tomato plants under variable N application rates (data collected during different growth stages).

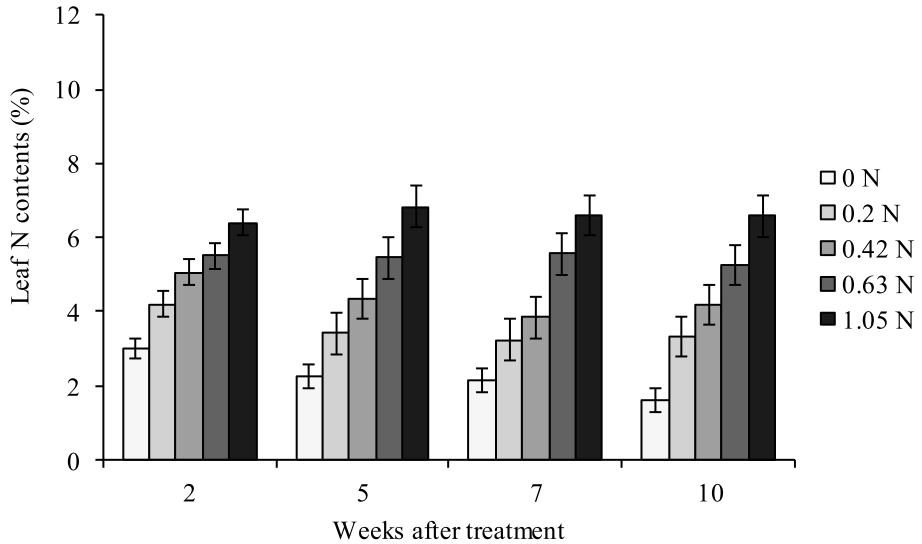


Figure 4.5: Changes in tissue N concentration of uppermost fully expanded leaves of broccoli under variable N application rates (data collected during different growth stages).

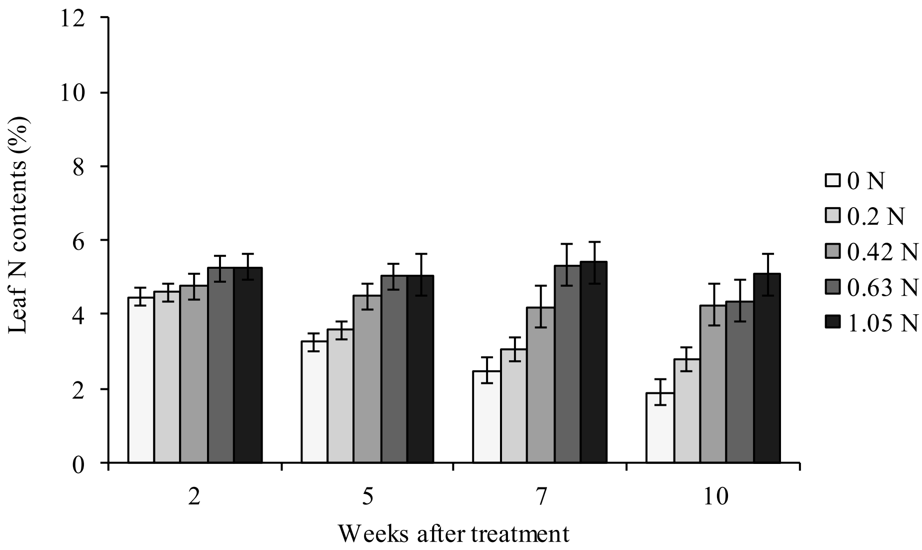


Figure 4.6: Changes in tissue N concentration of uppermost fully expanded leaves of lettuce under variable N application rates (data collected during different growth stages).

4.3.2.2 **Effect of N fertilisation on leaf chlorophyll content**

The increasing rate of N application significantly increased the total leaf chlorophyll content of all the three crop species. The broccoli plants grown under various N rates showed no significant change in total leaf chlorophyll content at 2 WAT except at the highest concentration (1.05 N), which significantly increased chlorophyll content. It indicates that, despite the variation in rates of applied N (Figure 4.7), the broccoli sustained leaf chlorophyll content, presumably by re-mobilising N from lower plants. The difference between the chlorophyll content of N deficient (N0) and N sufficient (1.05 N) in broccoli grew wider with the plant growth. The plants treated with no or lower than recommended N had significantly lower chlorophyll content compared with plants receiving abundant N supply, indicating that plant N consumption increases with the increased reproductive growth. Thus, plants may require additional N fertiliser to maintain leaf chlorophyll level and proper growth. Similar findings have been reported by Ouda and Mahadeen (2008) who reported that increasing N application rate up to 60 kg ha^{-1} not only contributed to plant growth but also to increase in broccoli yield.

Compared with broccoli, leaf chlorophyll content of tomato plants responded immediately to N fertilisation at 2 WAT (Figure 4.8 and 4.9). With the increasing plant growth phase the amount of leaf chlorophyll content significantly fell and N deficient plants contained only 2 mg cm^{-2} leaf chlorophyll at 10 WAT. Tomato plants may suffer severe growth and yield losses, if adequate N is not supplied during reproductive growth. In a 4-year field study, Clark et al. (1999) found that N application rate and its availability in soil is the most important growth limiting factor for tomato crop. Lower N supply can impair leaf chlorophyll and subsequent plant growth (Scholberg et al., 2000). Leaf chlorophyll content of lettuce plants also responded to N application at 2 WAT; however, there was no significant decline in leaf chlorophyll content of N deficient plants with plant growth. It is suggested that lettuce might have some compensation mechanism to meet N deficiency.

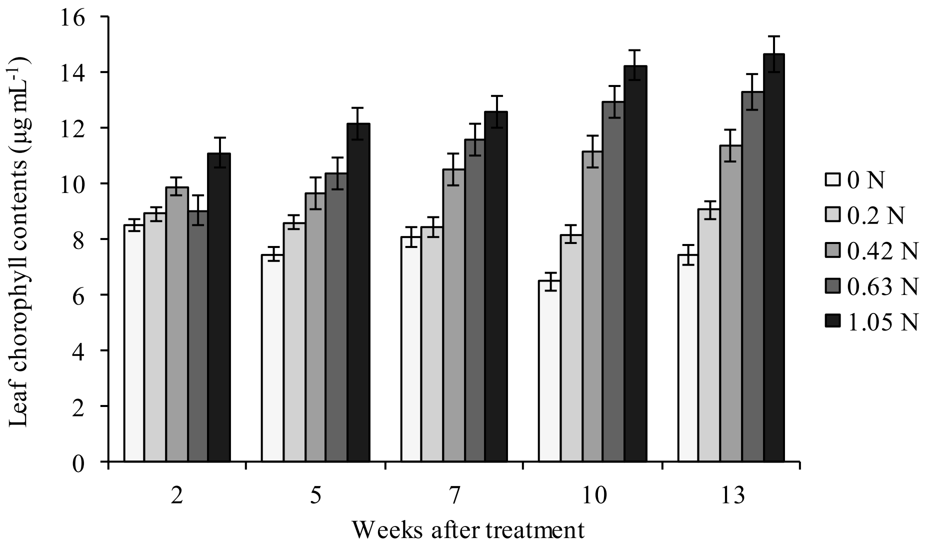


Figure 4.7: Changes in leaf chlorophyll content of broccoli under various N concentrations.

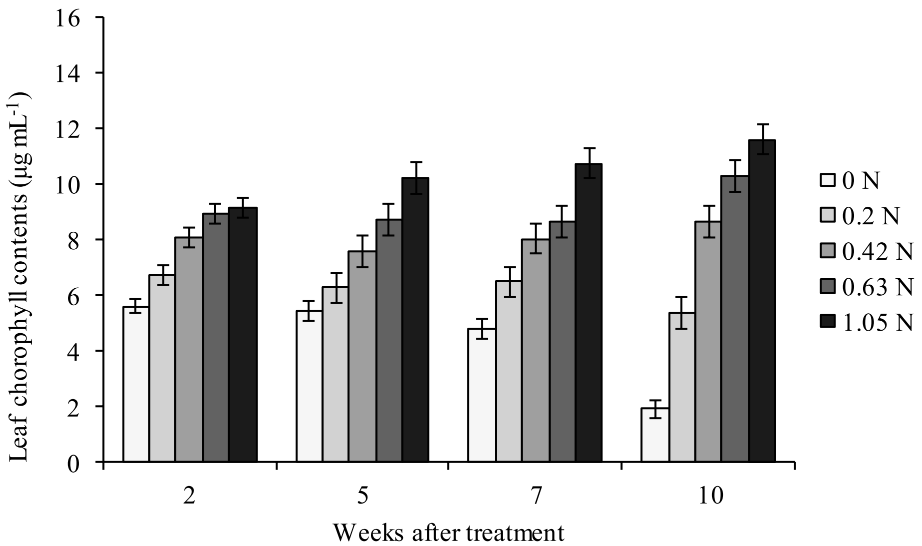


Figure 4.8: Changes in tomato leaf chlorophyll content under various N concentrations.

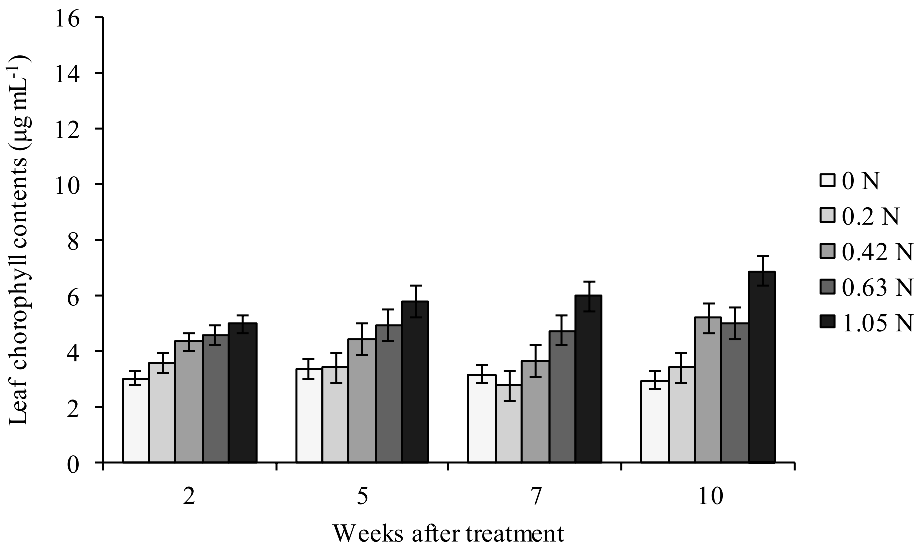


Figure 4.9: Changes in leaf chlorophyll content of lettuce under various N concentrations.

4.3.2.3 **Comparison of modified RGB technique and SPAD-502 for measuring leaf chlorophyll content in three plant species**

Leaf chlorophyll contents were estimated by a spectrophotometer (Lab_{Chl}) from 100 tomato leaves using destructive sampling during various growth stages. Non-destructive estimation of leaf chlorophyll was conducted using the proposed algorithm (Chl_{RGB}) and the SPAD-502. The correlation coefficient (R^2) was determined amongst measurements taken by those two devices and Lab_{Chl}.

The correlation between SPAD-502 readings and Lab_{Chl} is presented in Figure (4.10A), which shows a good consistency ($R^2 = 0.91$) between the SPAD-502 readings and the actual leaf chlorophyll values. The correlation between the Chl_{RGB} and Lab_{Chl} was even stronger ($R^2 = 0.97$), which indicated relatively better performance of the modified RGB technique over the SPAD-502 method (Figure 4.10B).

Similar to the tomato, values of chlorophyll content recorded from 100 leaf samples during different stages of plant growth (10, 13, 15 and 18 weeks after sowing) both by Chl_{RGB} and SPAD-502 were correlated against Lab_{Chl} measurements. The data showed a reasonably good correlation ($R^2 = 0.73$) between the chlorophyll content calculated by SPAD-502 and Lab_{Chl} (Figure 4.10C), while the correlation was higher ($R^2 = 0.90$) between Chl_{RGB} and Lab_{Chl} (Figure 4.10D).

In this experiment the readings for Lab_{Chl}, Chl_{RGB} and SPAD-502 were taken five times during various growth phases (10, 13, 15, 18 and 21 weeks after sowing). The correlation coefficient (R^2) value for the chlorophyll content calculated by Lab_{Chl} and SPAD-502 was 0.81 (Figure 4.10E), whereas the correlation between Lab_{Chl} and Chl_{RGB} readings was ($R^2 = 0.91$) and thus consistent with the previous experiments (Figure 4.10F).

The above data indicated that determination of chlorophyll content by the modified RGB technique (Chl_{RGB}) achieved a significantly better performance than the SPAD-502 in chlorophyll measurements for all the three species examined.

The SPAD-502 was designed to detect the chlorophyll content of leaves (Balasubramanian et al., 1999) and it is the most commonly used technique for field crops. In the last decade, there has been great interest in the use of the SPAD chlorophyll meter for agricultural and research purposes, where more than 200 studies have been published on successful application of SPAD in estimating leaf chlorophyll content of crops (Uddling et al., 2007). The correlation coefficient values calculated for SPAD-502 and original plant chlorophyll in the present study are very close to many previous studies (Mercado-Luna et al., 2010). Although there was a good correlation between SPAD-502 readings and spectrophotometric chlorophyll content of leaves, chlorophyll content calculated by SPAD could be affected by many factors such as plant genotype, nutrient concentration, leaf thickness or abiotic and biotic stresses. In order for SPAD-502 readings to be accurate, the instrument must be calibrated for the variety of plant species examined and other environmental factors. This calibration can be accomplished by over-fertilising three or more areas in the field with N, and then taking reference measurements from these areas to compare with the rest of the field. Moreover, it is recommended not to rely on one reading to detect chlorophyll status, but to use the mean of several readings (Murdock et al., 1997). However, this is obviously a time and labour consuming process.

A relatively stronger association was achieved by the modified RGB technique with the actual leaf chlorophyll content for all three studies, which suggest that this method provides better estimation of crop chlorophyll content. In addition, this newly modified RGB technique can be more effectively used for a range of crop species under variable environmental conditions.

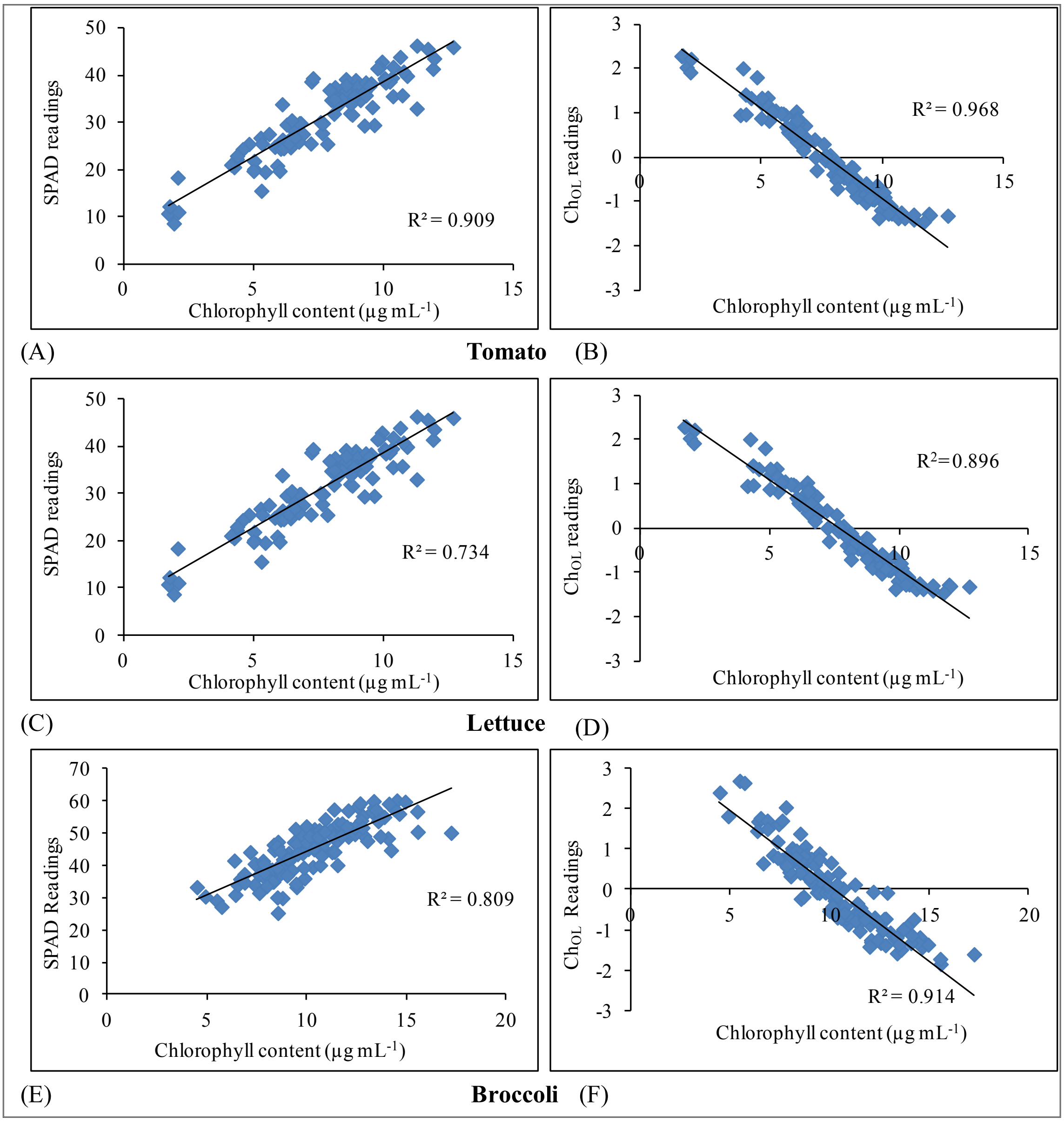


Figure 4.10: Correlation between Lab_{Chl} and the estimated chlorophyll readings using SAPD (A, C and E) and Chl_{RGB} (B, D and F) for tomato, lettuce and broccoli

4.3.2.4 Comparison between Chl_{RGB} and other image processing based algorithms

In order to perform a thorough analysis of the proposed Chl_{RGB} technique, the results of the modified RGB technique were compared with the existing image processing based chlorophyll estimation methods that are described below and listed in Table 4-2.

Estimated leaf chlorophyll contents = $(R-B)/(R+B)$………………. Equation 4-3

This formula was developed by Kawashima and Nakatani (1998) to measure leaf chlorophyll content of wheat. They used a portable digital 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 as ($R^2 = 0.81$). In fact, Kawashima and Nakatani (1998) have examined a number of other formulas and recommended the above one as it outperformed the other formulas.

Estimated leaf chlorophyll contents = $G/(R+G+B)$ …….…………Equation 4-4

Suzuki et al. (1999) applied this formula to detect chlorophyll content in broccoli using a digital camera under artificial light conditions. This formula has also been applied by Jia et al. (2004) to detect N status in winter wheat. Recently, Yuzhu et al. (2011) proposed a formula that gives a good correlation with N status in pepper plants.

Estimated leaf chlorophyll contents = R …………………..…………Equation 4-5

Mercado-Luna et al. (2010) developed a new method to take leaf images of tomato in a green house. They fixed the camera height and angle to control the light by using a standard lamp of 100 watts, and they used an automatic camera setting. They suggested that the colour image analysis can be applied to estimate the N status of tomato seedlings using R and B colours. From the colour image analysis, R is the most accurate predictor of N status of plants with a linear coefficient of around 0.91.

Estimated leaf chlorophyll contents = $R/(R+G+B)$ …………..…………..Equation 4-6

This formula was developed by Cai et al. (2006) to estimate the chlorophyll content of cucumber leaves. This formula can be considered as a normalized version of the previous one.

Estimated leaf chlorophyll contents = G/R ……………………………Equation 4-2

Adamsen et al. (1999) applied this formula to estimate chlorophyll concentration in wheat leaves. Cai et al. (2006) suggested the same formula to estimate chlorophyll content of cucumber leaves. In both studies, they used digital cameras to obtain images.

Estimated leaf chlorophyll contents = $R+G$ ………………………….Equation 4-8

Hu et al. (2010) 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 (2009 Adobe Systems Inc., USA).

Table 4-2 shows correlation levels between the various RGB ratios and the true chlorophyll content, as is 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 with only one or two of the three species, such as $(R-B)/(R+B)$ (a) and G/R (e). The $R+G$ formula proposed by Hu et al. (2010) was relatively more consistent as it outperformed the other five formulas. The new algorithm, on the other hand, was not only found consistent, but it achieved a better performance than all the existing methods, including $R+G$ formula.

Table 4.2: Correlation coefficient (R^2) values for chlorophyll contents estimated by image processing (IP) based algorithms and actual leaf chlorophyll contents of tomato, lettuce and broccoli

IP based Chl method	Developed model	Correlation coefficient (R^2)		
		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
Chl_{RGB}	Chl_{RGB}	0.968	0.896	0.914

4.3.2.5 **Leaf N estimation by SPAD and the modified RGB technique**

In the present study, the effectiveness of three methods for estimating leaf N content of broccoli, tomato and lettuce was compared under various levels of N treatment. Leaf N contents were determined by destructive method and were regressed against the SPAD value, total leaf chlorophyll content (destructive measurement) and the modified RGB technique. All the three methods used for estimating leaf N such as leaf chlorophyll content (Lab_{Chl}), SPAD readings and the modified RGB technique showed a good relationship with leaf N content estimated by the destructive method (Figure 4.11A-I). A significant relationship was recorded between the leaf N content and the modified RGB technique for broccoli, tomato and lettuce under various N treatment levels, where the correlation coefficient (R^2) value was 0.94, 0.93 and 0.72 for broccoli, tomato and lettuce, respectively. The correlation values were slightly lower for SPAD as well as Lab_{Chl}.

The data showed that SPAD achieves lower correlation for broccoli and tomato, while it achieved similar correlation for lettuce, which make it less consistent than our method. The SPAD values can fluctuate according to plant age or growth status (Shapiro et al., 2006). Despite achieving good correlation with N, Lab_{Chl} was slightly outperformed by the modified RGB technique for broccoli and tomato.

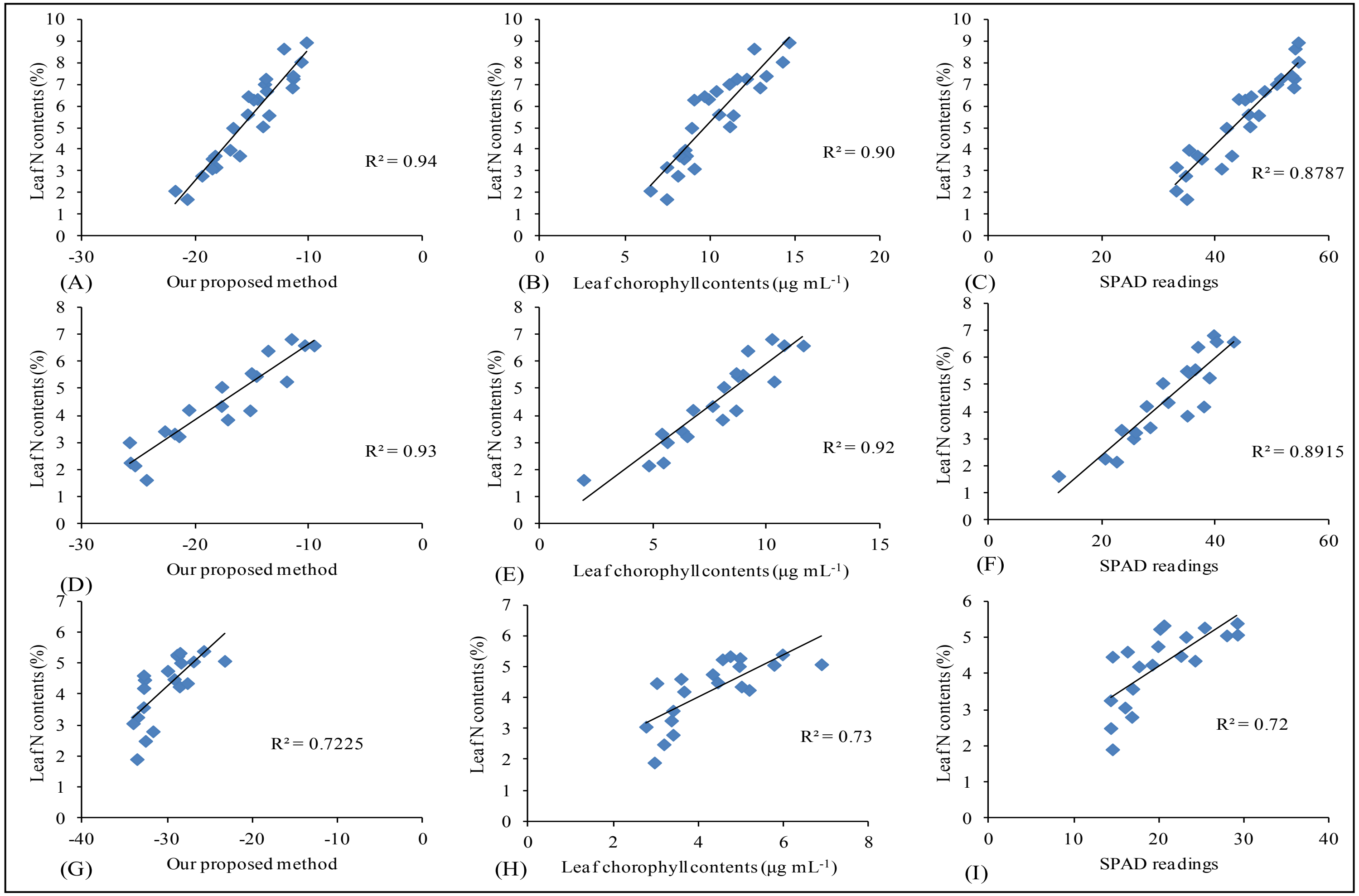


Figure 4.11 Comparison of various leaf N estimation methods in three different plant species i.e. broccoli (A, B and C), Tomato (D, E and F) and lettuce (G, H and I)

4.4 Image processing-based technique for estimating chlorophyll and N content of field-grown crop

4.4.1 Experimental procedure

Seedlings of tomato, lettuce and broccoli were initially germinated under greenhouse conditions using the nutrient solution (as previously explained in material and methods of section 3.2). Three weeks old seedlings were then transplanted into the field at Lansdowne farm, Camden campus of the University of Sydney (latitude 34°01'S, longitude 150°40'E, elevation 75m). The experimental design was a randomised complete block design with three replications. Three N treatments including N0: without nitrogen (control), N1: 60 kg N ha^{-1} and N2: 140 kg N ha^{-1} were applied before seed sowing in the form of urea (46% N).

The youngest fully expanded leaves of each species were used for collecting data on leaf chlorophyll or N content at three different stages of plant development on 68 days after sowing (DAS), 83 DAS and 98 DAS. Data on leaf chlorophyll and N content was collected using destructive laboratory techniques as well as using SPAD-502. Digital images of the leaves were collected and used for estimating leaf chlorophyll and N content. The detail of these methods is provided below.

4.4.1.1 **Dark green colour index**

Karcher and Richardson (2003) studied the quality of turf grass in response to N fertiliser 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 the equation given below:

$\mathrm{DGCI} = [(\mathrm{H} - 60)/60 + (1 - \mathrm{S}) + (1 - \mathrm{I})]/3$ ……………..Equation 4-9

Recently, Rorie et al. (2011) used DGCI as an in-season N status measurement tool, and they found a strong correlation between the DGCI values and N concentration in corn. Moreover, Raper et al. (2012) used this equation to estimate N status in cotton, and mentioned that the DGCI could possibly replace measurements using a chlorophyll meter for detecting plant N status. Both of them used a digital camera for taking photos, and the images were processed using the SigmaScan Pro (v. 5.0, Chicago, IL) software to calculate DGCI. It is important to mention that there were many factors that could influence the DGCI values such as difference in lighting conditions, camera quality and setting.

4.4.1.2 **The modified image-based nitrogen/chlorophyll estimation method**

Leaf images were collected using a portable scanner (Pico – Australian made) from different species as previously described. The RGB values of leaf pixels were averaged to obtain three values (one for each colour). On the basis of RGB values a formula was proposed that estimates leaf N content of three crop species (eq. 4-10):

$\text{Leaf N}_{\text{RGB}} = \text{G} - \text{R}/2 - \text{B}/2$ ……………………………………..…Equation 4-3

R and B are included for a normalisation purpose.

In addition, a single formula was developed for estimating leaf chlorophyll content of all three crop species under greenhouse as well as under field condition (eq. 4.10).

$Chl_{RGB} = \frac{R+G+B}{3} - G$ ……………………………………..…….Equation 4-4

The main difference between Eq. (4.10) and Eq. (4.11) is that chlorophyll content of plants has a higher dependency on the green colour (G) of the plant than on the nitrogen, and hence different weighting schemes were applied in the normalization terms in the two equations which reflect a better association between the resulting estimations and their ground truth.

4.4.2 Results and discussion

4.4.2.1 **Crop response to N fertilisation**

This study verified the results of the greenhouse experiments, where leaf Chl and N content of three crop species, tomato, broccoli and lettuce were positively correlated with N fertilisation. All three crop species used in the present study variably responded to the increasing N fertilisation rates, although the leaf N concentration (%) under 0 N remained almost same (Figure 4.12A). In broccoli, addition of 60 kg N ha^{-1} significantly increased the leaf N content (%), while further increasing the N application to 140 kg N ha^{-1} had no significant effect on leaf N content suggesting that applying additional N fertiliser would not further enhance the health of those plants. A similar response to applied N fertiliser was recorded in leaf N content in lettuce but N fertilisation-induced increase in leaf N content was significantly lower compared with that of broccoli. In contrast, application of 60 kg N ha^{-1} had no significant effect on leaf N content of tomato but 140 kg N ha^{-1} significantly increased leaf N content. No response of tomato crop to lower rate of N fertiliser could be due to higher N consumption by the plants. Thus, the estimation of appropriate N application rate is of utmost importance for those three species and this helps to reduce input cost for crop production and to minimise N leaching problem (Di and Cameron, 2002).

Changes in leaf Chl content of all three crop species in response to applied N fertiliser were also dissimilar (Figure 4.12B). In broccoli and lettuce, there was a significant increase in leaf chlorophyll content by applying any rate of fertiliser (60 N or 140 N), while leaf chlorophyll content of tomato were increased only by the higher rate of N (140 kg N ha^{-1}). A similar response of both leaf N and Chl content to applied N fertilisers indicated a close association between crop N status and chlorophyll content. It also suggests that leaf green colour (chlorophyll content) can be used for estimating crop fertiliser requirements.

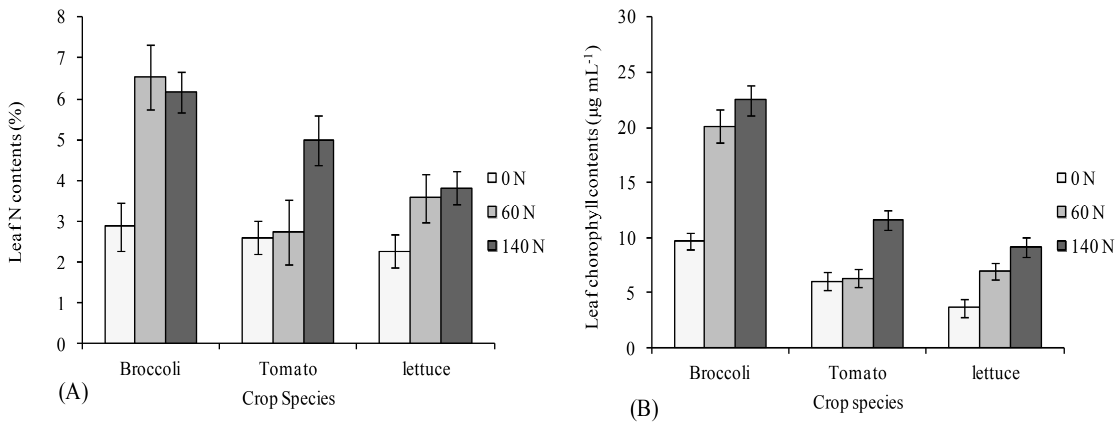


Figure 4.12: Changes in leaf N (A) and chlorophyll content under various N treatments.

0 N= no N fertiliser applied; 60N = N fertiliser applied @ 60 kg N ha^{-1}; 140 N= N fertiliser applied @ 140 kg N ha^{-1}

4.4.2.2 **Comparing efficiency of various crops N estimating techniques**

In order to evaluate the performance of the modified RGB technique and SPAD-502 in terms of their ability to estimate leaf Chl and N content, correlations were calculated between the Chl values estimated by these non-destructive methods and Chl values calculated by lab-based techniques. The SPAD readings showed a strong correlation (R^2 = 0.90) with the leaf Chl content of broccoli (Figure 4.13A) but did not show a strong correlation (R^2 = 0.72) with leaf N content (Figure 4.13C). In comparison, modified RGB technique achieved a relatively stronger correlation both for leaf Chl and N content, 0.94 and 0.87, respectively (Figure 4.13B and D).

For tomato leaves, the SPAD-502 performed slightly better at estimating leaf Chl and N content, where the R^2 values were 0.92 and 0.80 for leaf Chl and N content, respectively (Figure 4.14A and C). As with the broccoli, readings obtained by the modified RGB technique showed relatively stronger association with both the leaf Chl and N content compared to SPAD readings [R^2 = 0.96 and 0.85 for leaf Chl and N content, respectively (Figure 4.14B and D)].

The relationship between SPAD readings and Chl content of lettuce leaves was relatively weaker (R^2 = 0.86) than those for broccoli and tomato (Figure 4.15A and B) but the correlation between SPAD readings and leaf N content (R^2 = 0.78) was slightly better than that for broccoli (Figure 4.15C and D). The modified RGB technique again provided a stronger correlation both for leaf Chl (R^2 = 0.87) and N (R^2 = 0.81) content.

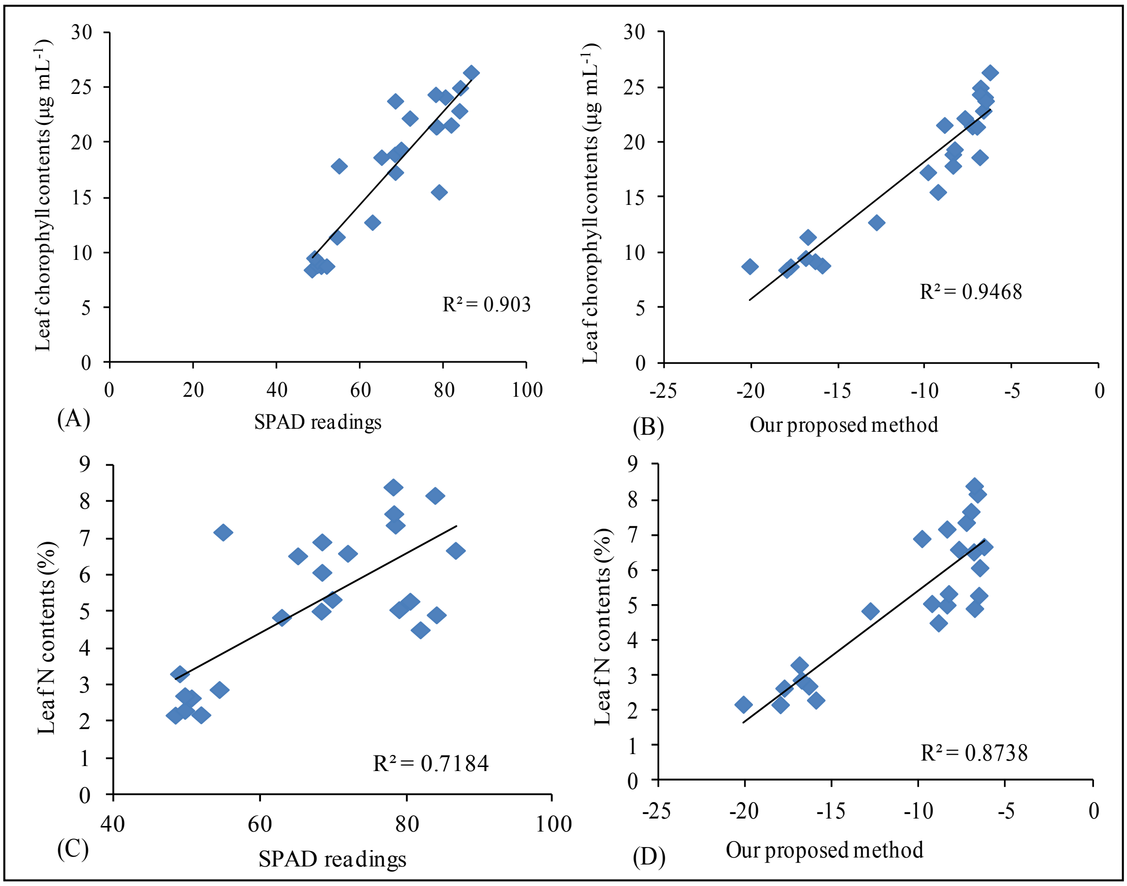


Figure 4.13: Relationships between leaf chlorophyll content and SPAD readings

(A), leaf chlorophyll content and modified RGB technique (B), leaf N content and SPAD readings (C), and leaf N content and the modified RGB technique (D) in broccoli leaves.

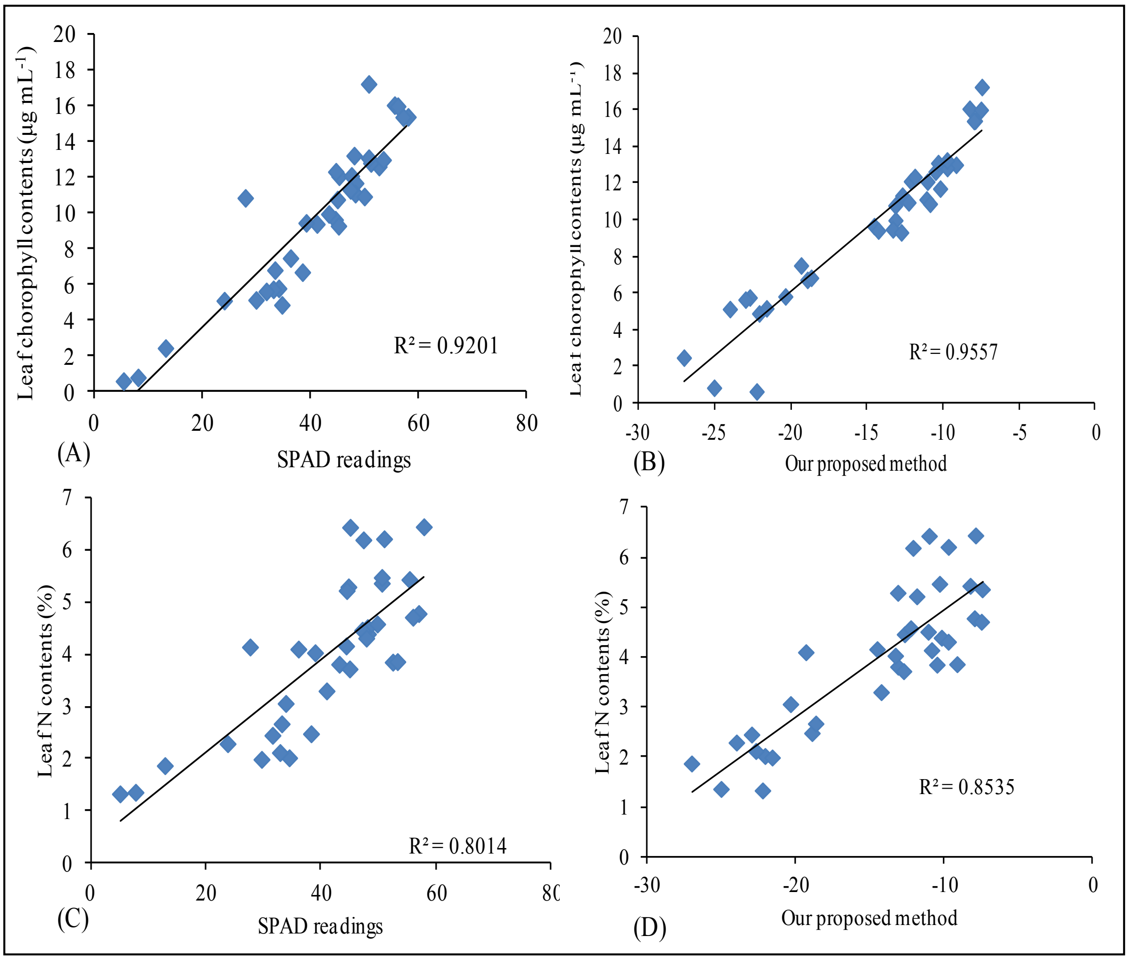


Figure 4.14: Relationships between leaf chlorophyll content and SPAD readings

(A), leaf chlorophyll content and modified RGB technique (B), leaf N content and SPAD readings (C), and leaf N content and modified RGB technique (D) in tomato leaves.

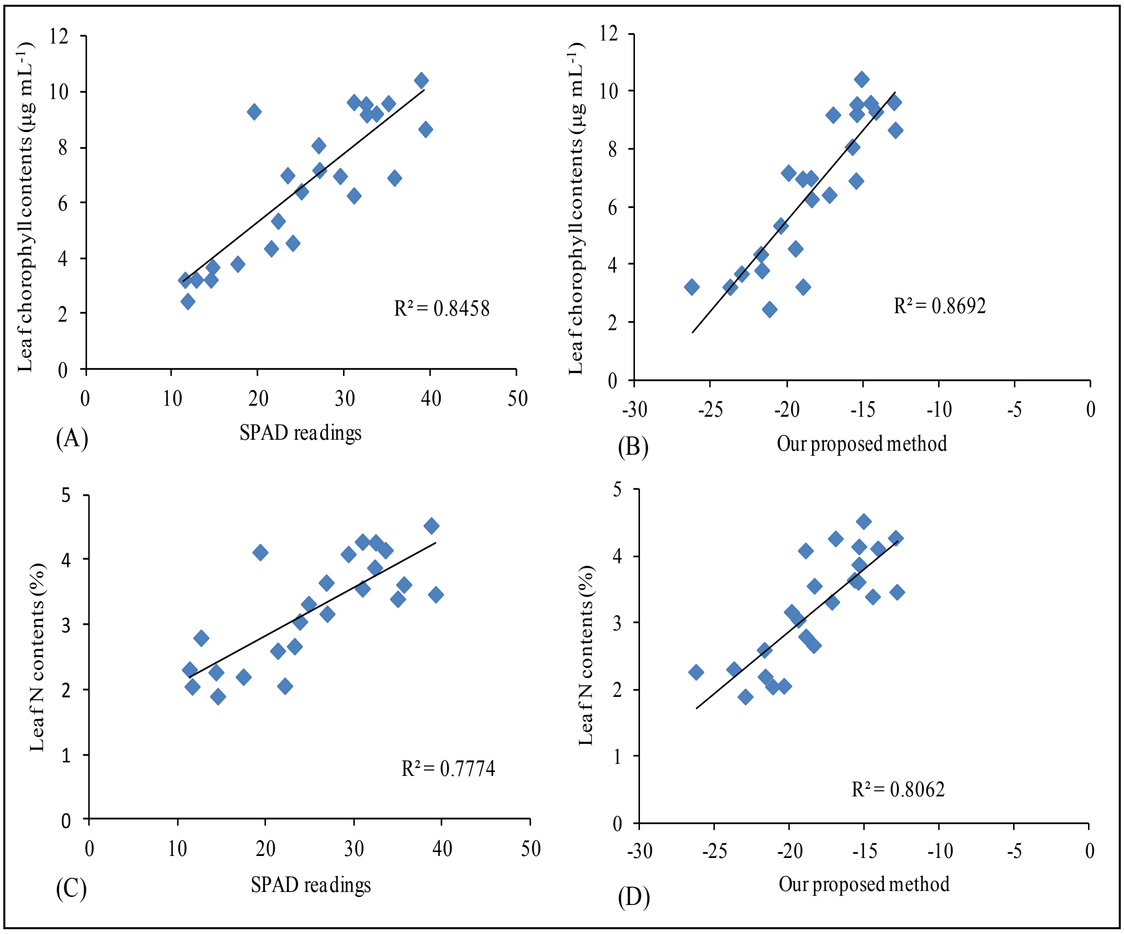


Figure 4.15: Relationships between leaf chlorophyll content and SPAD readings

(A), leaf chlorophyll content and modified RGB technique (B), leaf N content and SPAD readings (C), and leaf N content and the modified RGB technique (D) in lettuce leaves.

4.4.2.3 A single formula for estimation of leaf Chl and N content of greenhouse and field grown crops

The consistency of the modified RGB technique was further verified using a single formula for crops growing under greenhouse and field conditions. Leaf Chl and N content calculated using the modified RGB technique showed a strong correlation with laboratory-based estimates of leaf Chl and N content (Table 4-3). Except for the leaf N content of greenhouse grown lettuce, the correlation coefficients (R^2) between Chl or N content estimated by the modified RGB technique and laboratory-techniques were significant (0.80-0.95) for the three studied species grown under greenhouse or field conditions. It indicates that compared with other non-destructive methods the modified

RGB technique of estimating leaf Chl and N content is not influenced by environmental factors.

Table 4.3: Correlation of leaf chlorophyll and N contents estimated by RGB algorithm (a single formula) with actual leaf chlorophyll and N contents of three crops growth under greenhouse and field conditions

Species	Greenhouse		Field	
	R^2 value for leaf chlorophyll	R^2 value for leaf nitrogen	R^2 value for leaf chlorophyll	R^2 value for leaf nitrogen
Broccoli	0.89	0.94	0.94	0.87
Tomato	0.92	0.93	0.95	0.85
Lettuce	0.87	0.72	0.86	0.80

4.4.2.4 **Correlation between SPAD, DGCI and the modified RGB technique with respect to lab N and Chl**

The change in N application rates led to visible differences in leaf colour; the control (0 N) plants appeared to have pale green or yellow leaves with stunted growth. In contrast, leaves of plants treated with the highest N level (140 kg N ha^{-1}) turned dark green.

On plotting Lab_{Chl} content against SPAD-502 readings, a strong correlation was observed between Lab_{Chl} and SPAD readings (R^2 = 0.92) (Figure 4.16A). The obtained results are similar to those reported in many previous studies (Mercado-Luna et al., 2010, Yuzhu et al., 2011). Similar data were observed using DGCI (Figure 4.16B) with a correlation coefficient of 0.91 for Lab_{Chl} content. On the other hand, the modified RGB technique achieved a relatively stronger correlation (R^2 = 0.96) and has outperformed both SPAD and DGCI in the precision of predicting Chl content (Figure 4.16C).

For tomato leaves, the correlation coefficient (R^2) between SPAD readings and N content was 0.80 (Figure 4.16D), while DGCI gave a slightly better N detection result compared with SPAD (R^2 = 0.83) (Figure 4.16E). Similar results were obtained by Rorie et al. (2011), who suggested a close relationship between SPAD and DGCI with leaf N concentration in corn. As with Chl, the modified RGB technique outperformed both SPAD and DGCI for the detecting N concentrations in tomato leaves, where it achieved a higher correlation coefficient (R^2 = 0.85) (Figure 4.16F).

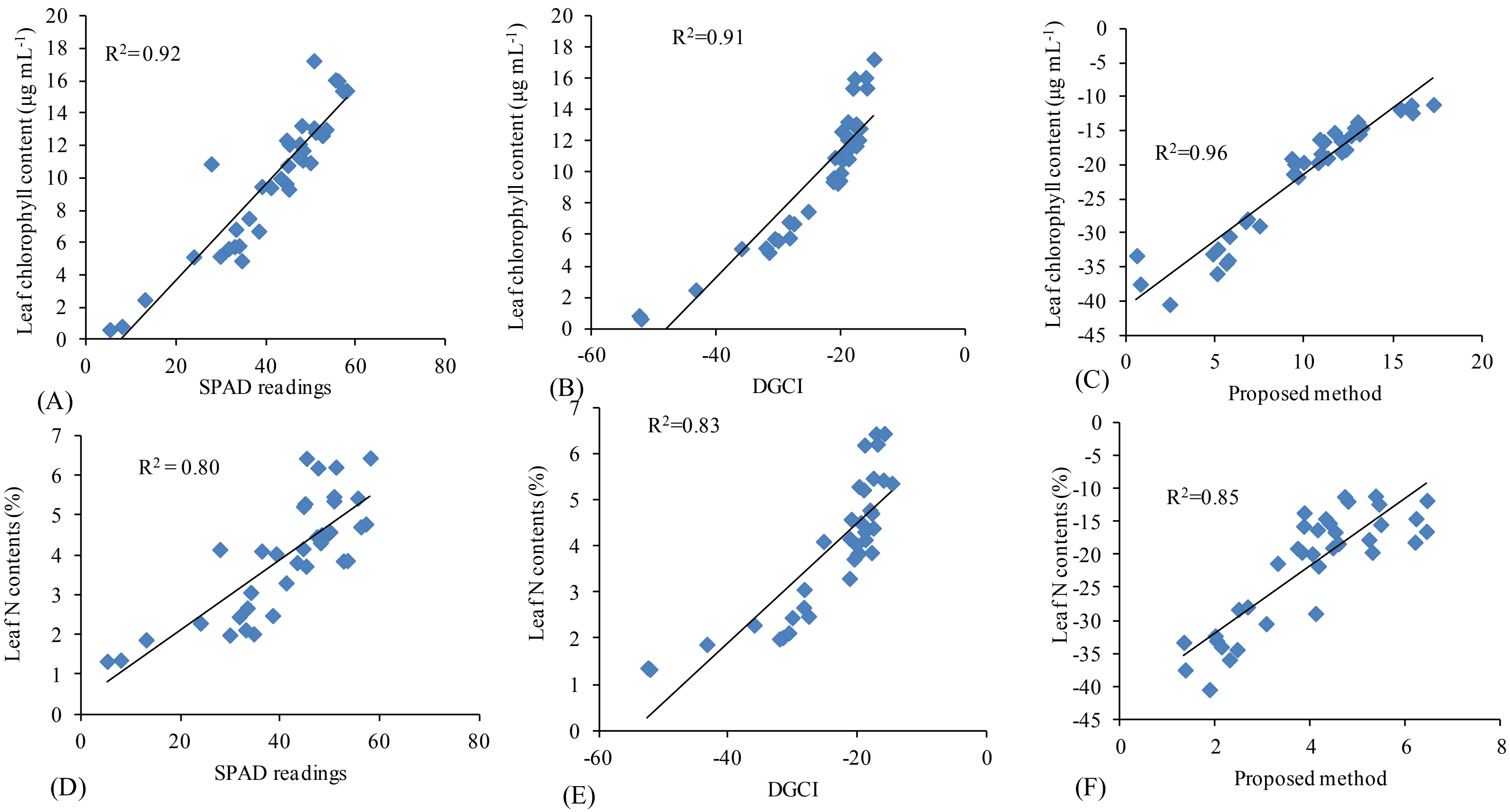


Figure 4.16: Correlation between SPAD, DGCI and the modified RGB technique with respect to lab N and Chl

4.5 Image processing-based technique for detecting the N status of cotton crops

4.5.1 Experimental procedure

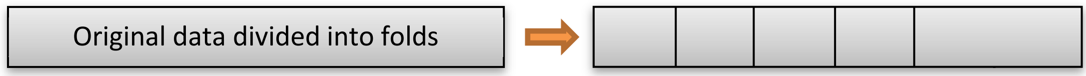
The experiment was conducted at the Australian Cotton Research Institute, Narrabri, NSW, Australia (150^o E, 30^o S). A commercial cotton (*Gossypium hirsutum* L.) cultivar Sicot 71BRF was grown in a split plot design, with 8 m × 200 m main plots and the N rates were the subplots (8 m × 16 m). The design had four replicates. Cotton crop was grown in the field, where cotton-wheat-fallow-cotton system was practised during previous seasons. Various N application rates that is N0: without N (control), N1: 80 kg N ha^{-1}, N2: 160 kg N ha^{-1}, N3: 240 kg N ha^{-1} and N4: 320 kg N ha^{-1}N, were applied at a 30 cm depth below the plant line before sowing.

Leaf N contents were measured using destructive as well as non-destructive techniques such as SPAD, handheld crop sensor (Trimble) and modified RGB technique at three different stages of plant growth, at 75 days after sowing (early reproductive phase), 118 days after sowing (peak reproductive phase) and 161 days after sowing (late reproductive phase).

4.5.1.1 Image processing based nitrogen estimation in cotton (IPNC)

In order to construct a formula that relates the R, G and B values to the N estimations, it was decided to use a cross-validation approach due to the limited number of leaf samples. Cross-validation is a statistical method used for evaluating and comparing learning algorithms by dividing data into two segments; one segment is used to learn or train a model, and the other is used to validate the model. The K-fold cross validation method works by splitting the data into K groups. A loop that is run K times is used, wherein each run, K-1 groups are used to identify the model parameters, while the remaining group is used to validate the outcome. Based on this approach, each of the K groups is used once for validation. The parameters obtained in each fold are then averaged to obtain the final model parameters. The detailed steps of the cross validation technique are given below:

- The data were divided into K roughly equal parts: assuming below that the total chunk of data are the rectangle that is divided into K pieces



- For each k= 1,2,3…., K: we started with the current value of k, which is initially set to 1, and used the data represented by the first small box for testing, while using the data represented by the rest of the small boxes, such as 2,3,4…..,K for training.
- Fitted a model with the parameters α, β, γ to the other K-1 parts, and computed its error in predicting the k^{th} part as per their equation below, and repeat for all values of k.

$\mathrm{E}_k(\alpha, \beta, \gamma) = \mathrm{Nitrogen} - \mathrm{IPNC} = \mathrm{Nitrogen} - (\alpha\mathrm{G} + \beta\mathrm{R} + \gamma\mathrm{B})$ ….Equation 4-5

Where G= Green colour B = Blue colour R = Red colour

- This gives the cross validation error

$CV(\alpha, \beta, \gamma) = \sum_{k=1}^{K} \mathrm{E}_k(\alpha, \beta, \gamma)$ …………………………………..Equation 4-6

- Made the above combinations of α, β, γ, and choose the values of α, β, γ that make the *CV* value the smallest

After optimising the above formula, the best values were selected as α = 0.01, β = 0.6 and γ = 0.8. Thus, According to this approach, the image processing based nitrogen estimation in cotton (IPNC) is formulated as follows:

$\mathrm{IPNC} = 0.01\ \mathrm{G} - 0.6\ \mathrm{R} + 0.8\ \mathrm{B}$ …………………………………..Equation 4-7

Where R, G and B are the red, green and blue colour average of the leaf area

4.5.2 Results and discussion

4.5.2.1 **Correlation with the actual nitrogen readings**

One hundred and eighty measurements of laboratory-based analysed nitrogen content (%), handheld crop sensor, SPAD-502 and IPNC readings were used to find the correlation coefficient (R) between the actual N levels and the readings of each of the three non-destructive methods. These measurements have been taken in three different growth stages of crop; early reproductive phase, the peak reproductive phase and the last one during late reproductive phase. At least 60 readings were taken for each growth stage.

Figure 4.17A shows the correlation between laboratory-analysed N values and SPAD readings, where R^2 value was approximately 0.63. A similar correlation value for SPAD based N content has been reported in cotton (Johnson and Saunders, 2003). The correlation between leaf N content calculated by the destructive laboratory method and estimated by the handheld crop sensor was quite low (R^2 = 0.51), as shown in Figure 4.17B. These results confirmed the findings of Johnson and Saunders (2003), who claimed that Minolta SPAD-502 chlorophyll fluorescence meter was the most reliable hand held meter when applied to infield readouts for monitoring the N status of the cotton crop. The proposed IPNC method, on the other hand, achieved a relatively stronger correlation (approximately 0.71, Figure 4.17C) compared with the SPAD-502 and crop sensor indicating that IPNC can be considered a more reliable tool for estimating N content in cotton leaves.

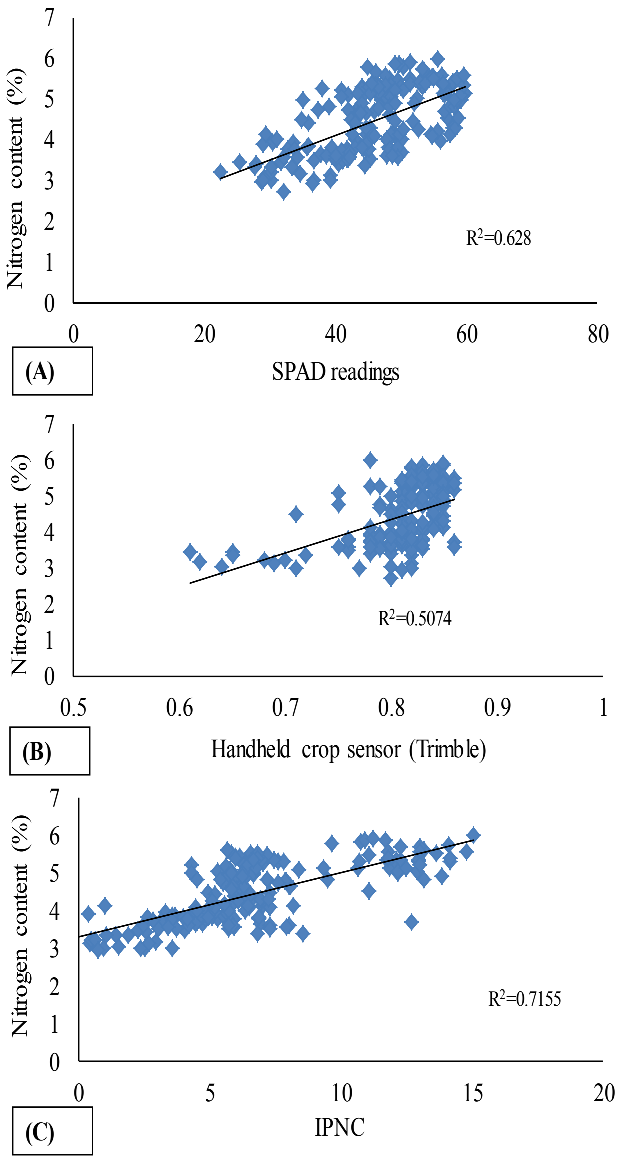
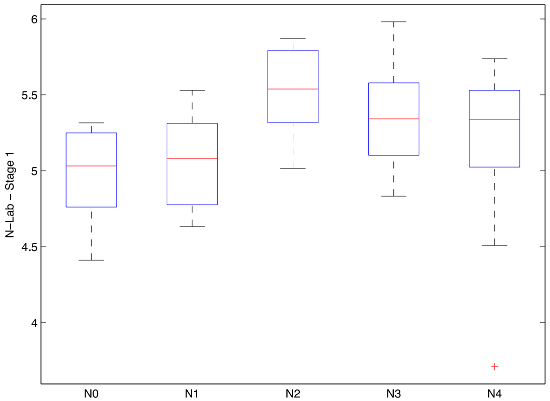


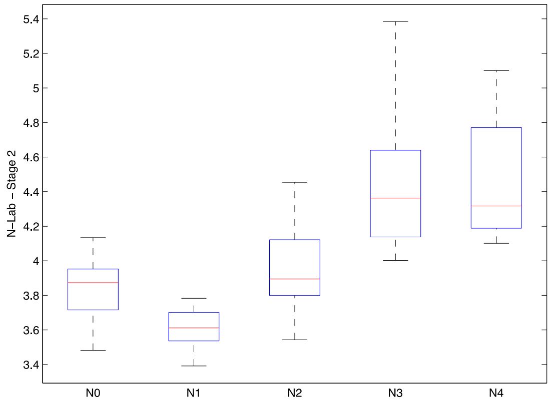
Figure 4.17: Correlation between leaf N content and (A) SPAD, (B) Handheld crop sensor (Trimble) crop sensor, and (C) our proposed image processing based N estimation in cotton (IPNC) algorithm

4.5.2.2 Detection of leaf N treatment levels

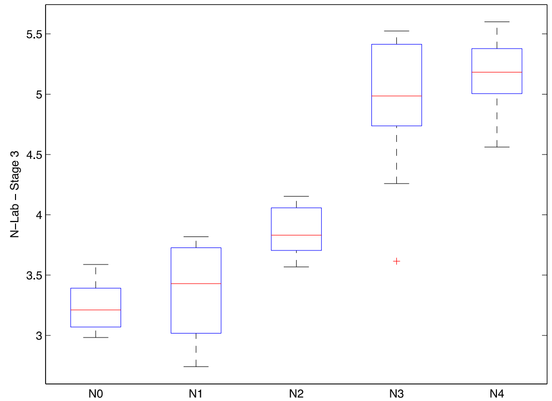
In the second experiment, the ability of the measured N values (from the CHN analyser) was investigated as well as the readings obtained by SPAD, handheld crop sensor (Trimble) and IPNC for detecting the five levels of N treatments. Figure 4.18 (A-L) shows a boxplot for the three stages of plant growth. The plots indicated that in the first stage, there is a relatively high degree of overlap between the five treatments for all four methods. This is expected, as the field soil could have some residual N, and since the plants are still in their early reproductive growth stage, the amount of N consumed may not be much higher than what already existed in the soil. The overlap is relatively reduced in the second growth phase with few exceptions where N1 is not properly aligned by both the lab-nitrogen and IPNC. In growth stage 3, lab-nitrogen, SPAD and IPNC provide a good estimation of the N treatments, while the handheld crop sensor (Trimble) still showed some degree of overlap.


N–Lab – Stage 1
6
5.5
5
4.5
4
N0
N1
N2
N3
N4

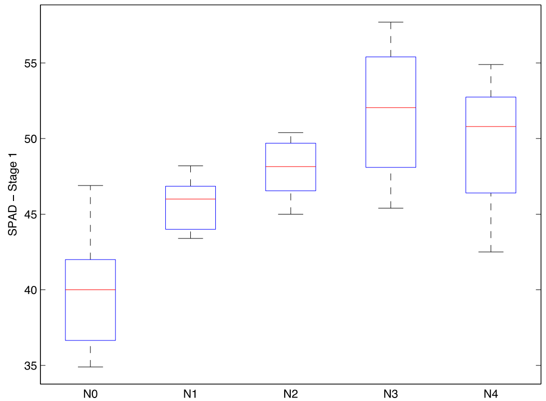
(A)


N–Lab – Stage 2
5.4
5.2
5
4.8
4.6
4.4
4.2
4
3.8
3.6
3.4
N0
N1
N2
N3
N4

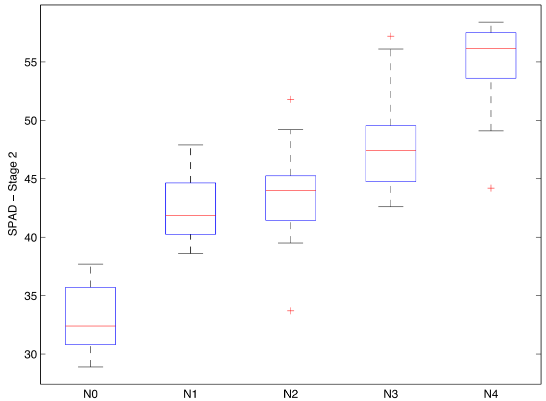
(B)


N–Lab – Stage 3
5.5
5
4.5
4
3.5
3
N0
N1
N2
N3
N4

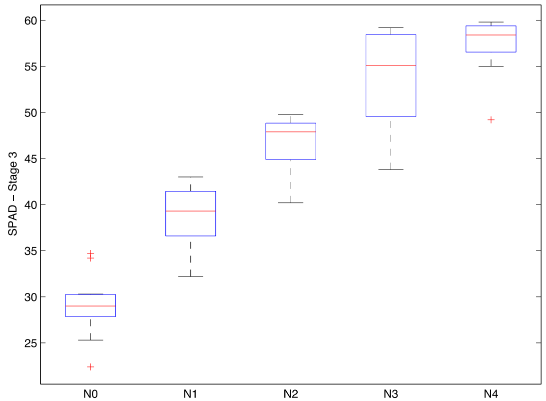
(C)


SPAD – Stage 1
55
50
45
40
35
N0
N1
N2
N3
N4

(D)


SPAD – Stage 2
55
50
45
40
35
30
N0
N1
N2
N3
N4

(E)


SPAD – Stage 3
60
55
50
45
40
35
30
25
N0
N1
N2
N3
N4

(F)

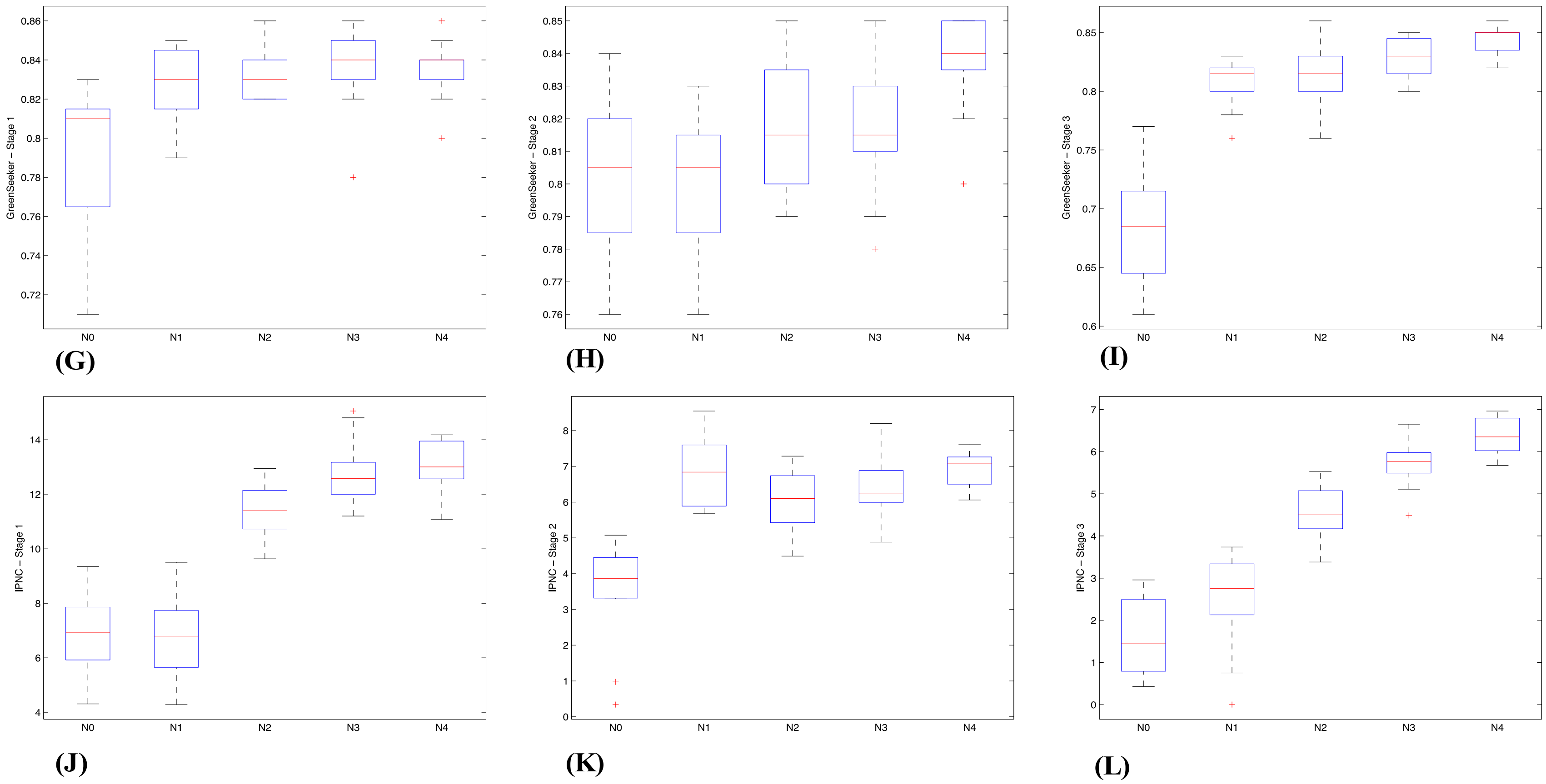


Figure 4.18: Column 1: first growth stage (73 days after sowing), column 2: second growth stage (118 days after sowing), and column 3: third growth stage (163 days after sowing).

Each plot represents the five treatments vs. one of the methods. Lab nitrogen (A, B and C), SPAD (D, E and F), Handheld crop sensor (Trimble) (G, H and I), and IPNC (J, K and L)

4.5.2.3 **Yield increment under increasing nitrogen application rate**

There was a significant increase in cotton lint yield under the incremental N application rate up to 160 kg ha^{-1}; however, no further yield improvement was recorded by the additional N applied (Figure 4.19). Compared with N0 (control), addition of 80 kg ha^{-1} N caused approximately 40% lint yield increase in cotton and lint yield was further improved by 14% under N application rate of 160 kg ha^{-1}. The two higher treatments (above 160 kg ha^{-1}) showed no noticeable improvement in crop yield, which indicate the importance of optimised N application rate for cotton crop to avoid unneeded addition. It was obvious that under higher N application rates cotton leaves contained significantly higher N content at the late reproductive phase, but this high tissue N was not utilised for higher boll formation. In previous studies, cotton yield has been found positively correlated with N application rate and timings (Boquet and Breitenbeck, 2000). Constable et al. (1992) optimised N fertiliser rates to145, 189, and 210 kg ha^{-1} for cotton crop cultivated under crop rotation, minimum tillage and maximum tillage conditions, respectively. Thus, extra concentration N in soil has no benefit on plant yield; instead N leaching into soil can lead to economic loss and environmental contamination.

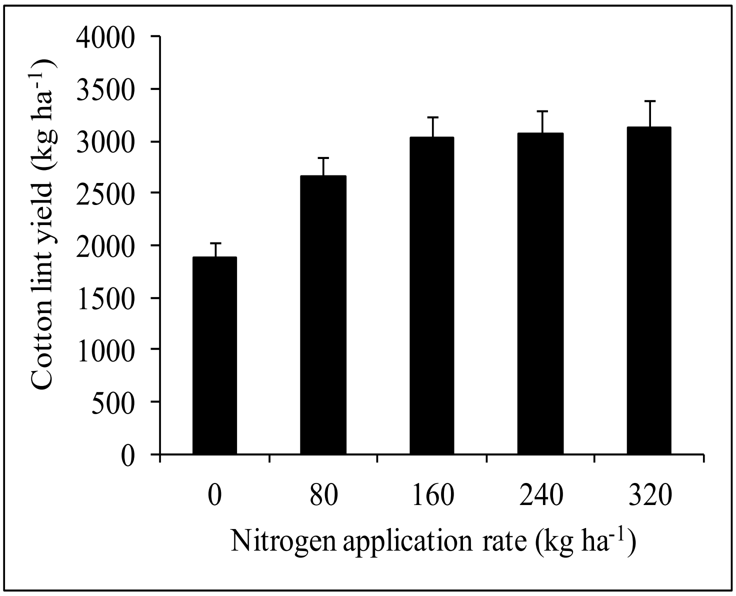


Figure 4.19: Effect of increasing nitrogen application rate on cotton lint yield

4.6 Summary

This chapter presented an in-depth investigation of the use of leaf images in the estimation of leaf dimensions, chlorophyll and N content. A number of new simple and non-destructive techniques were developed. For the estimation of leaf dimensions, images of leaves with varying size were collected using a commercially available handheld page scanner. This is a simple scanning machine which does not require any sophisticated settings and is easy to use in the field. The images were processed to estimate total area, width, length, average width and perimeter of these leaves. The data collected by scanner showed a strong correlation with the values obtained by Li-Cor 3100 using destructive sampling technique. Validation of benchmark images and real leaf images showed that the modified RGB technique effectively estimated the various leaf metrics that were significantly similar to the true values. Thus, this new method provides plant scientists more flexibility in estimating crop growth rate and crop management options.

The laboratory techniques used in the estimation of N content (Kjeldahl and Leco CHN analysis) proved to be quite accurate, but these techniques cannot be applied for large fields. Thus, non-destructive techniques such as the SPAD-502 are commonly used for estimating N levels in the fields. After the initial success in data collection from leaf images through estimating leaf dimension, leaf images were used for estimating leaf chlorophyll and N content. Three different crops, tomato, lettuce and broccoli, were grown on variable N application rates under greenhouse and field conditions. The leaf images were collected using a handheld scanner and processed to get RGB values. An algorithm was then proposed for estimating leaf chlorophyll and N content for each crop. Leaf chlorophyll and N content produced by the modified RGB technique showed a strong correlation with original leaf chlorophyll and N content measured by destructive laboratory-based techniques, demonstrating the accuracy of this technique for estimating the N requirement of a range of plant species. Further, the performance of the modified RGB technique was compared with other image processing-based methods as well as with the SPAD-502 chlorophyll meter. The data showed that the modified RGB technique is relatively more accurate and cheaper than the existing leaf chlorophyll estimating non-destructive techniques.

An image processing based N estimation algorithm for cotton (IPNC) was proposed. Using this technique, relatively better correlation was achieved with the true leaf N levels compared with SPAD and dark green colour index (DGCI). The estimation of N treatment level was quite challenging for all methods in the early stages of plant growth. However, in the later growth stages, the lab-nitrogen, SPAD and IPNC produced quite a good estimation of the N treatment, while the handheld crop sensor was less accurate. More experiments should be conducted to validate the IPNC method that can offer a new system inexpensive, yet reliable in estimating nitrogen levels to the cotton community.

CHAPTER FIVE

5 IMAGE-BASED (RBG) TECHNIQUE FOR ESTIMATING PHOSPHORUS LEVEL IN DIFFERENT CROPS

5.1 Introduction

Crop plants require sufficient phosphorus (P) supply in the soils for their proper growth and development (Batten, 1992). Due to its crucial role in cellular division and expansion in plants, P deficiency can inhibit leaf size, light interception and overall carbohydrate assimilation resulting in stunted plant growth (Lloyd et al., 1995; Rodríguez et al., 1998). On the other hand, higher P concentration in plant tissues can cause toxicity leading towards growth inhibition, senescence and development of chlorotic or necrotic regions on leaves (Shane et al., 2004).

Early vegetative growth phase of plants is relatively more sensitive to P deficiency (Hearn, 1981), which impairs carbohydrate accumulation, protein biosynthesis and consequently inhibits development of new nodes (Sawan et al., 2001). Cotton growth has been strongly associated with the total number of fruiting nodes produced, thus growth reduction during early reproductive growth stages of cotton ultimately reduces cotton yield. Radin and Eidenbock (1984) suggested that P deficiency reduced leaf expansion in cotton by limiting root hydraulic conductivity and cell turgor in the leaf cell. The leaf size reduction in P-deficient cotton was linked with the lower rate of leaf expansion, while photosynthetic reduction was a secondary response mainly influenced by reduced radiation interception. Similar to cotton, P deficiency can cause growth and yield reduction in tomato (Bonser et al., 1996) and lettuce (Asher and Loneragan, 1967). Thus yield penalties can be avoided by estimating crop P deficiency and timely fertilisation at early reproductive crop growth phases.

P requirements for crops are commonly estimated through soil and tissue sampling and subsequent laboratory analysis (destructive techniques). These techniques are generally accurate but time consuming and expensive (Sui et al., 2005). Various non-destructive methods have been suggested for estimating leaf size, chlorophyll (Cai et al., 2006), anthocyanin (Gitelson and Merzlyak, 2004) and N content but little information is available on use of these models for estimating leaf P content. However, unlike N, where various non-destructive techniques are available for estimating crop N requirements, there is hardly any information available on the use of non-destructive techniques for estimating crop P requirements. The P availability and application rates of P can significantly influence crop growth and vegetation index, and estimating these changes by non-destructive techniques (Gérard et al., 2001), can provide a source for classifying crops on the basis of crop P status. Although some studies used canopy reflectance data for estimating N and P content of pastures. For example, Albayrak (2008) and Mutanga et al., (2004), have estimated leaf P content of sainfoin pasture and grass pastures respectively, limited information is available on estimating leaf P content of crops (Christensen and Jørgensen, 2004).

A new approach that utilizes leaf dimensions and area in addition to leaf colour is proposed here. This was motivated by the strong influence of P supply on leaf development (leaf area) (Radin and Eidenbock, 1984; Sawan et al., 2001; Shane et al., 2004). In addition, P supply also influences leaf colour of certain crops (Bouma and Dowling, 1982; Lopez-Cantarero et al., 1994). For this purpose, linear discriminant analysis (LDA) and the related Fisher's linear discriminant methods were used, which can separate two or more classes of objects or events. The resulting combinations can be used as a linear classifier.

5.2 Material and methods

Three crop species; cotton, tomato and lettuce were grown in the greenhouse at the Faculty of Science, University of Technology Sydney, Australia (details of the growing conditions are provided in Chapter 3). Seeds of cotton cultivar Sicot 71BRF were planted in 33 plastic pots (pot size in mm diameter and/or L volume) (11 replicates for each treatment) on 2nd June 2012, while lettuce (*cv*. green mignonette) and tomato (*cv*. Tommy Toe) were sown on 25th December 2012 (6 replicates for each treatment). All pots were filled with vermiculite, and irrigated using a nutrient solution (details of the nutrient solution used are provided in Chapter 3). For the first seven days, P level in the nutrient solution was kept constant for all plants (2.5 mL/10 L), and the nutrient solution was renewed every three days. After seven days, three different P treatments in the form of NaH_2PO_4 were applied for seven weeks, (P0 = no P, L; P1 = 2.5 mL/10 L of P and P2 = 5 mL/10 L of P). The P levels applied to each pot represent three different levels of recommended P application, e.g. P0 = 0 %, P1 = 50 % and P2=100 % of the recommended P concentration.

5.2.1 Data collection

Data from the leaves of three studied crops were collected after 8 weeks of treatment. The leaf area (LA) and leaf perimeter (LP) of the youngest (uppermost) fully expanded leaves of individual plants of each crop were measured using a portable leaf scanner (Pico Life). The RGB values of leaf images were also collected.

5.2.1.1 Determining leaf anthocyanin and P content (destructive method)

The process of measuring leaf anthocyanin content was started by collecting 10 mm diameter discs from the youngest (uppermost) fully expanded leaves of each tomato, lettuce and cotton plants. Fresh leaf samples were ground for estimating leaf anthocyanin using Murray and Hackett (1991) technique (for further detail, please see Chapter 3, Section 3.5.1.4).

After collecting samples for anthocyanin analysis, the remaining portion of the leaves was oven dried at 80^{o}C for 24 h, and ground into powder. The P content was then measured from these samples using an inductively coupled plasma mass spectrometer (for further detail, please see Chapter 3, Section 3.5.1.3).

5.2.1.2 Classifying plants on the basis of anthocyanin and P content (non-destructive method)

RGB, leaf area and leaf perimeter values of the three studied crops were used for classifying plants based on anthocyanin and P levels using a Linear Discriminant Analysis (LDA) classifier. Three distinct groups of tissue anthocyanin (A0, A1 and A2) and P (P0, P1 and P2) concentration were used. The LDA classifier detected the effect of P treatment on leaf and plant dimensions using a cross validation scheme. In this testing scheme, one sample is used for testing at a time, while all other samples, excluding the testing sample, are utilised for training. The error rate is then computed by observing the ability of the classifier to correctly classify all the testing samples.

For the cotton plants, in addition to the RGB, LA and LP, plant height (shoot length), and total shoot dry biomass were also recorded. These attributes were used by a decision tree model (DT) to classify cotton plants based on the P application rates.

Decision tree (DT) is easy to interpret and is considered a "White box" model in the sense that the acquired knowledge can be expressed in a readable form compared with LDA which is more difficult to explain, "Black box", and it is more difficult to read the acquired knowledge in a comprehensible way.

5.2.1.3 **The proposed algorithm for estimating leaf P content**

A genetic algorithm (GA) was used to identify the weight that needs to be assigned to each one of the variables. GA is a random population–based search methodology inspired by evolution theories that imply the survival of the fittest. GA mimics this idea of creating and evolving a collection of possible solution (population) through mating and manipulation operations. The evaluation process starts with generating a random population. Each population member (chromosome) is represented by a collection of variables (genes) that relate to the solution. The best members of the population (parents) will have a higher chance to produce a new generation (children) by combining two parents at a time through mixing operation (crossover). Additionally, there is also a small chance to obtain a new mutant member through (mutation) operator. This mutant member is created by introducing small modifications to one of the parents.

In summary, the GA can be implemented as follows:

- Initialise a random population of N members, which in the first iteration is considered as the current population.
- Evaluate fitness function (accuracy) of each member and rank them according to their performance.
- Copy a subset of best members to the next population (elite children).
- Randomly crossover two members at a time according to their fitness function to produce a number of members for the next population.
- Randomly mutate a number of members in the current population one at time to produce a number of members for the next population.

- Evaluate the fitness of the new population.
- Repeat creating new populations until a stopping criterion is met.

Crossover and mutation operators of a binary string are illustrated in Figures (5.1 and 5.2).

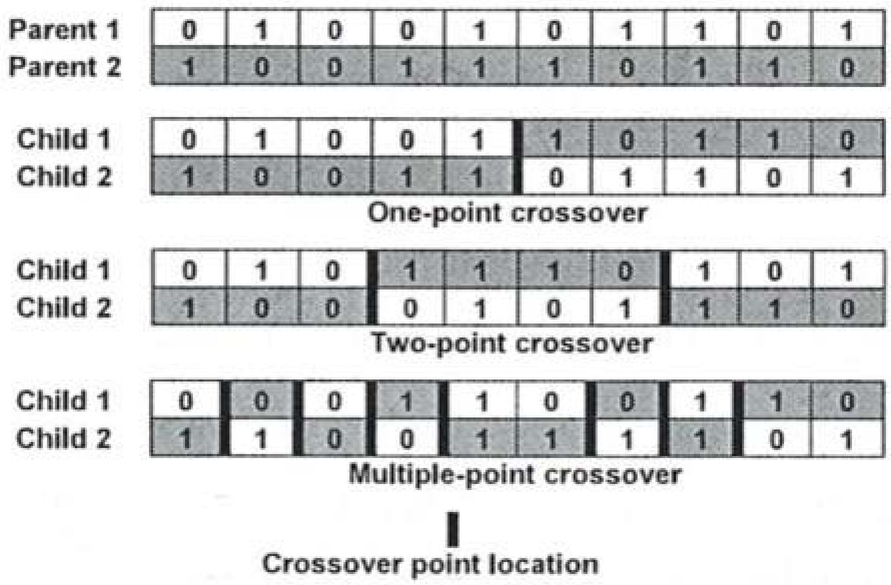


Figure 5.1: Crossover

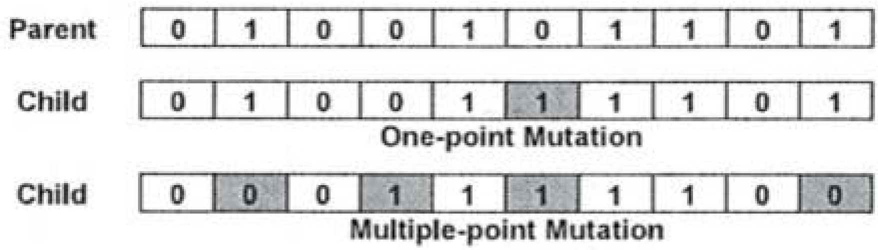


Figure 5.2: Mutation

Since its introduction many variants of genetic algorithms have been developed and applied to a wide range of optimization problems such as graph colouring, pattern recognition, financial market, and multi-objective engineering optimization (AlSukker 2012).

In this work, continuous chromosome representation was used to search for the optimal weight of each variable. The obtained equations for the three crops are listed below:

Data on LA and RGB were used to propose three different formulas that estimate leaf P content of cotton (eq. 5-2), tomato (eq. 5-3) and lettuce (eq. 5-4).

Leaf P (cotton) = R×(-0.6466) - G×0.0203 + B×1.4837 - LA×0.3758…...Equation 5-1

Leaf P (tomato) = R1×1.1236 - G1×0.6644 - B1×0.5851 + LA1×0.0249....Equation 5-2

Leaf P (lettuce) = R2×1.1236 - G2×0.6644 - B2×0.5851 + LA2×0.0249 .Equation 5-3

The leaf P content of all the three studied crops is given in the equation below (eq. 5-5)

Leaf P (all three crops) = 1.0811×R - 0.6518×G - 0.3780×B + 0.2248×LA ……………………………………………………………………….Equation 5-4

5.2.1.4. Linear discriminant analysis (LDA)

In the present experiment, LDA and the related Fisher's linear discriminant method has been used to find the linear combination of features, which best separate two or more classes of objects or events. These combinations were then used as a linear classifier. Considering that two classes or categories, which can be related to a certain plant condition (P sufficient vs. P deficient), as shown in Figure 5.3), DA was used to find a projection matrix; multiplying this matrix by the original data matrix would increase the distance between the two classes and minimise the distance between samples of the same class. For this, the LDA constructs two scatter matrices denoted as: within-class scatter (*Sw*) and between-class scatter (*Sb*):

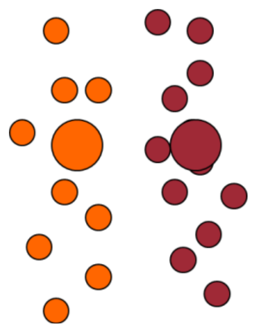


Figure 5.3: A set of reading or measurements that belong to two classes, orange and brown.

When considering the between-class scatter (S_b), the main task was to maximise the distance between the different classes' centers or simply maximise the distance between different classes' centers and the mean (center) of the whole data as per the equation below:

$$S_B = \sum_{i=1}^{c} (v_i - \bar{x})\,(v_i - \bar{x})^T \text{ ....Equation 5-5}$$

where v_i represents the center of each of the classes and $\bar{x}$ the mean of the whole data set. An example of what S_b attempts to achieve is shown in Figure 5.4, as the main task here was to push the centers of the two classes far away from each other.

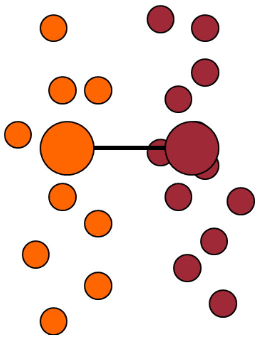


Figure 5.4: An example of what Sb attempts to achieve by pushing away the centres of the two classes far away from each other.

On the other hand, considering the between-class scatter (S_w), the main task is to minimise the distance between the samples of each class and their corresponding centre as per the equation below:

$$S_W = \sum_{i=1}^{c} \sum_{k=1}^{li} (xk - vi)(xk - vi)^T \text{ ....Equation 5-6}$$

Where x_k represents the samples of each of the classes and v_i the mean or the centre of the same class. An example of what S_w attempts to achieve is shown in Figure 5.5; LDA reduces the distance between the samples of the same colour simply by pushing the points toward their centres.

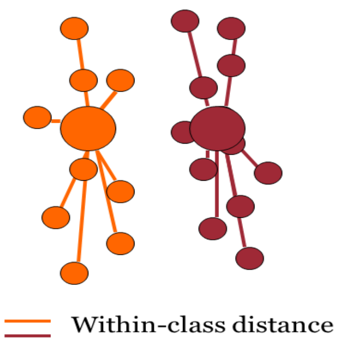


Figure 5.5: An example of what Sw attempts to achieve by bringing the samples of each class closer together.

After constructing these scatter matrices, LDA attempts to project the data in a manner that maximises between-class distance and minimises within-class distance the discriminant criterion in mathematical formulation is given as:

$$Within\ class\ distanc = agr_G max \frac{Trace(G^T S_b G)}{Trace(G^T S_w G)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 5-7}$$

Where, the optimal transformation "*G*" is given by solving a generalized eigenvalue problem$S_W^{-1} S_B$. Once the matrix "*G*" is found, then the next step is to multiply the original data by *G* and submit the result for classification.

5.2.1.5 **Categorisation using a decision tree model**

Decision tree models have one important property that distinguishes them from other types of classifiers, which is the clustering of samples using a pre-defined set of logical rules. These rules are usually built to support human reasoning and decision making. Thus, a decision tree can be utilized as a predictive model (so-called classification tree). It is a non-parametric modelling approach, which is used to separate independent variables into groups (Vayssières et al. 2000). The results are presented in the form of a decision tree with branches and leaves (binary hierarchy structure) that contains the rules to predict the new cases (Dunham 2006).

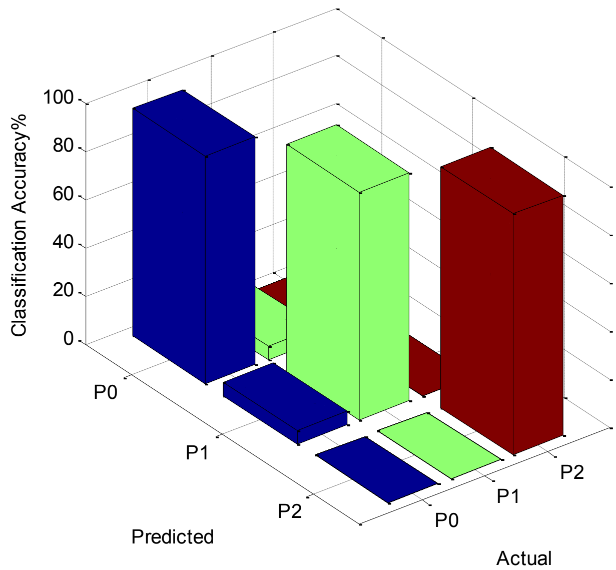
A tree represents each input feature as a non-leaf node. The structure of the decision tree allows representation of the problem with various solutions and presents it in an easy way to understand and demonstrate the relation between features and decisions. In this tree, the arcs coming from a node labelled with a feature are labelled with each of the possible values of the feature. Each leaf of the tree represents a category (decision).

5.3 Results and Discussion

5.3.1 LDA-based classification of plants varying in leaf anthocyanin content

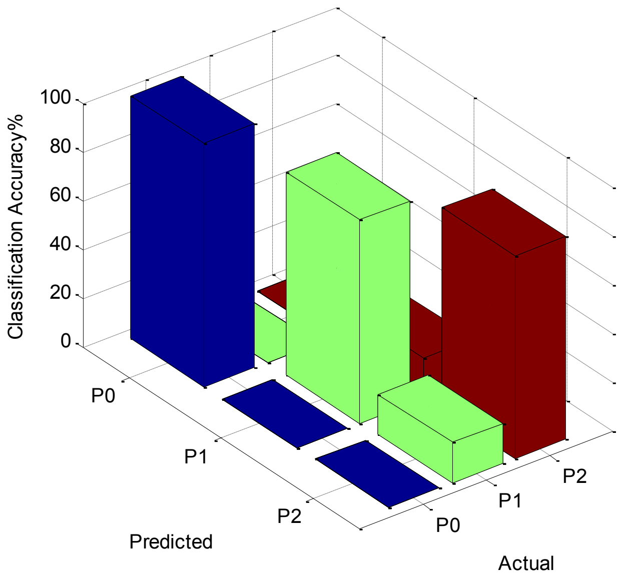
To classify lettuce and tomato plants on the basis of leaf anthocyanin (A) content, LDA classifier was used, which classifies these plants into 3 distinct classes, A0, A1 and A2. Five features are provided to this classifier, which are R, G, B, LP, and LA to classify plants on the basis of a leave-one-out (LOO) testing scheme that guarantees each sample would be tested once. The available data samples were looped in the LOO testing scheme by selecting one sample each time for testing, while the remaining samples were used for training. The final error rate was calculated from the number of samples misclassified under the LOO scheme (where misclassified here means, for example a sample that belongs to A0 being classified as A1). The LDA classifier showed a high rate of accuracy for classifying the plants cultivated on different anthocyanin levels. For example, the accuracy of classifying lettuce plants was 94%, with an error rate of 5.55% (Figure 5.6).

Similarly, LDA classified the tomato plants into three different groups varying in leaf A content. The resulting error rate was 16.67% indicating a high success rate of the LDA classifier to group tomato plants on the basis of leaf A content using R, G, B, LP, and LA features (Figure 5.7).



	Actual		
Predicted	A0	A1	A2
A0	94.44	5.55	0
A1	5.55	94.44	0
A2	0	0	100

Figure 5.6: Confusion matrix for the classification of lettuce plants on the basis of leaf anthocyanin content (A)



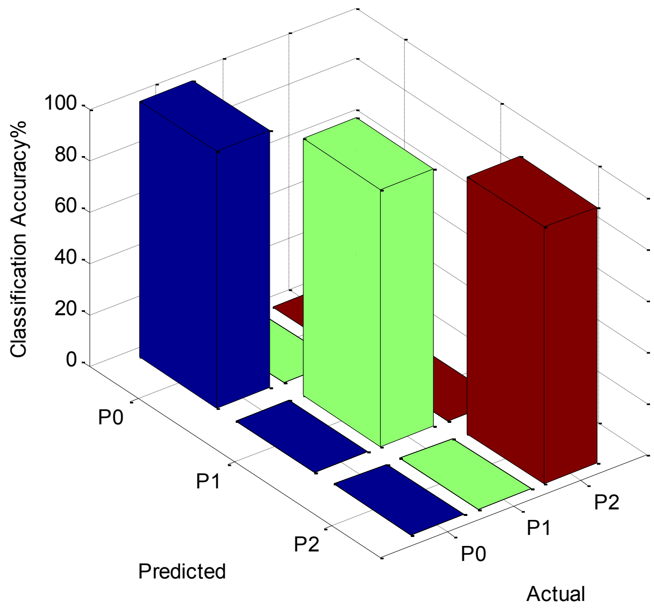
	Actual		
Predicted	A0	A1	A2
A0	100.00	0	0
A1	0	83.33	16.67
A2	0	16.67	83.33

Figure 5.7: Confusion matrix for the classification of tomato plant on the basis of leaf anthocyanin content (A)

5.3.2 LDA-based classification of plants varying in leaf P content

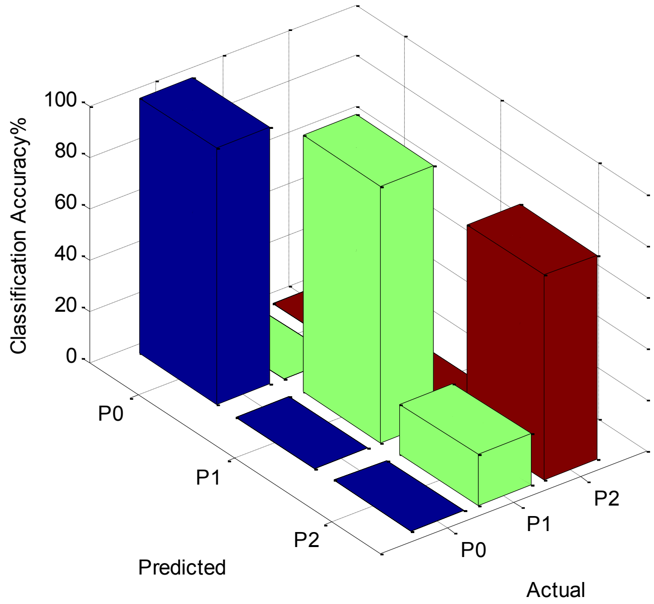
On the other hand, when R, G, B, LP, and LA features are used by that LDA classifier for classifying plants into three groups based on the leaf P levels (P0, P1 and P2), the achieved error rates were 0%, 20% and 12.12%, respectively, for lettuce (Figure 5.8), tomato (Figure 5.9) and cotton (Figure 5.10). The leaf growth features in combination with the LDA classifier could be used to estimate crop P deficiently.

Since P is required for division and expansion of plant cells, P supply can directly influence leaf size and indirectly its colour (Lloyd et al., 1995; Rodríguez et al., 1998). Changes in leaf size and colour are detected by spectral images. LDA uses these variations to classify the plants into different groups. A high accuracy in grouping plants on the basis of leaf anthocyanin and P content indicated the potential of LDA for estimating crop growth and nutrient (P) status. Zhang and Lei (2011) also suggested potential of LDA for classifying vegetation on the basis of leaf sizes. Similarly, Casanova et al. (2009) suggested a plant classification model (LDA) using leaf characteristics such as shape, contour and colour.



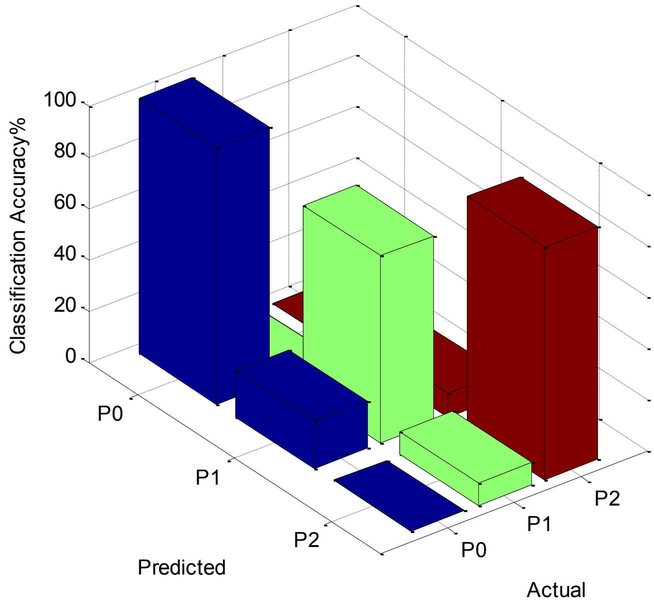
	Actual		
Predicted	P0	P1	P2
P0	100.00	0	0
P1	0	100	0
P2	0	0	100

Figure 5.8: Confusion matrix for the classification of lettuce plants on the basis of leaf P content



	Actual		
Predicted	P0	P1	P2
P0	100.00	0	0
P1	0	100	0
P2	0	20	80

Figure 5.9: Confusion matrix for the classification of tomato plants on the basis of leaf P content



	Actual		
Predicted	P0	P1	P2
P0	100.00	0	0
P1	18.18	72.72	9.09
P2	0	9.29	90.90

Figure 5.10: Confusion matrix for the classification of cotton plants on the basis of leaf P content

5.3.3 Cotton growth under limited P supply

In this experiment, features representing leaf dry weight, leaf perimeter and leaf area extracted from cotton plants were used for classifying plants into three distinct groups, P0, P1 and P2. These three groups appropriately correspond to the true P concentrations in cotton leaf tissues. No overlap was observed between the plants in P0 and P1 in all the considered leaf features (Table 5-1). However, there was some overlap between P1 and P2 for the leaf dry weight and leaf area, but no overlap was observed in the leaf perimeter suggesting that this feature could be a good indicator of plant P status (Table 5-1). Since the data were collected before the start of reproductive growth phase of cotton, biomass production may not be significantly influenced by limited P supply.

Table 5.1 Classification of cotton plants into different group on the basis of leaf P concentration estimated by linear discriminant analysis

	Leaf dry weight (g)	Leaf perimeter (cm)	Leaf area (cm^2)	Leaf P concentration (g/kg)	P class
Average	0.17	23.1	31.1	0.9	
Min values	0.14	18.5	21.9	0.8	P0
Max Values	0.19	26.5	37.9	1.1	
Average	0.33	31.9	54.9	1.9	
Min values	0.21	26.8	37.3	1.4	P1
Max Values	0.52	37.1	70.6	2.6	
Average	0.28	37.2	67.7	4.5	
Min values	0.25	33.8	59.1	3.4	P2
Max Values	0.35	39.4	79.7	5.6	

5.3.4 Decision tree model for estimating P requirements of cotton crop

A decision tree model was used for classifying cotton into different groups on the basis of P levels (Figure 5.11). The clustering in the decision tree model was regulated by leaf area, leaf dry weight and leaf perimeter as key features for separating plants into three distinct groups (P0, P1 and P2), which correspond to leaf P content of these plants (Table 5-1). The first cluster mainly separated the plants into two groups on the basis of leaf perimeter. For example, plants with leaf perimeters ≤ 26.6 were classified as P0 or P deficient plants, whereas, the plants with a leaf perimeter > 26.6 were classified as P sufficient plants. These P sufficient plants were further grouped into two distinct classes using leaf area and leaf dry weight as the basic features. The plants containing an average leaf area < 59 (cm^2) were classified into group P1, whereas the plants with leaf area > 71 (cm^2) were classified into group P2. Further clustering of plants containing leaf area in the range of 59-71 (cm^2) indicated that any of these cotton plants with an average leaf dry weight < 0.33 (g), can be classified as P1, while the plant with leaf dry weight ≥ 0.33 (g) is grouped into P2.

This study indicated that plant with variable P levels can be classified using the decision tree model. Similar results has been reported by Goel et al. (2003) who used spectral data and a decision tree model to classify corn crop under variable weed and N fertiliser management with an 22% and 18% error, respectively. Zhang et al. (2005) used decision tree model for predicting pasture productivity and found it relatively more effective than many other traditional classification methods (e.g. regression analysis). In precision agriculture, non-destructive (RS) techniques are commonly used for obtaining larger data sets of plant growth pattern. These data sets are classified using different algorithms. Classification of plants based on the decision tree model has several advantages since it has no strict assumption for distributing dependent variables, it does not require transformation of the variables during non-linear analysis and can easily incorporate variation in inputs, soil conditions, and plant growth.

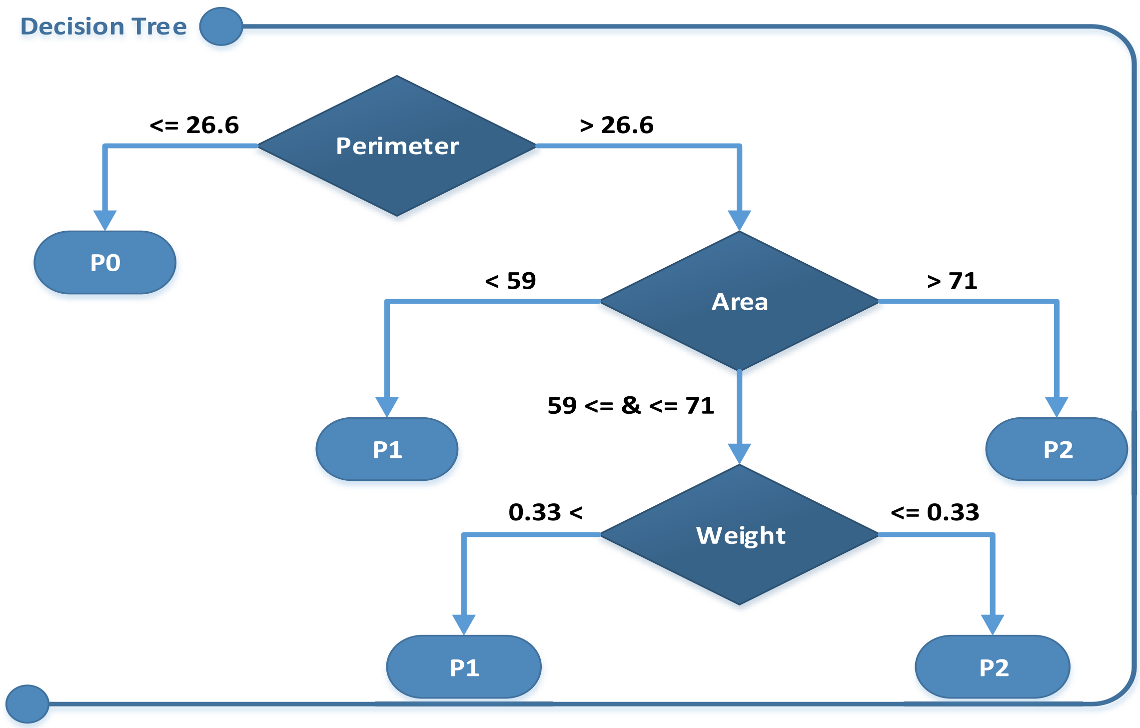


Figure 5.11: Clustering of cotton plants on the basis of leaf area, perimeter and dry weight using a decision tree model.

5.3.5 Estimating leaf P content using the method proposed in this study

R, G and B values and leaf dimensions are calculated by the modified RGB technique were used to estimate leaf P content using different algorithms for each crop; lettuce, tomato and cotton. To validate the efficiency of the modified RGB technique, the estimated P values (computed using the modified RGB technique) were compared against the original leaf P content (determined by destructive laboratory technique). Data showed significantly high correlation between the estimated and original P values for lettuce ($R^2 = 0.6278$), tomato ($R^2 = 0.7778$), and cotton ($R^2 = 0.8067$) (Figure 5.12A, B and C).

The readings were further validated using a single formula for all three species. Reasonably good R^2 values were obtained, which were 0.77 and 0.76 for lettuce and cotton, respectively, while the R^2 value for the tomato was only 0.53 (Table 5-2). These data indicate that customising the formula for individual species can produce more effective estimation of leaf P content. Data obtained with the proposed algorithm together with the LDA and decision tree models suggest that crop P content can be estimated using non-destructive methods. In addition, the method is simple and straightforward, which not only estimates the P content of different crops but also classifies plants on the basis of P application rates and assists in scheduling P application rates and time for crops.

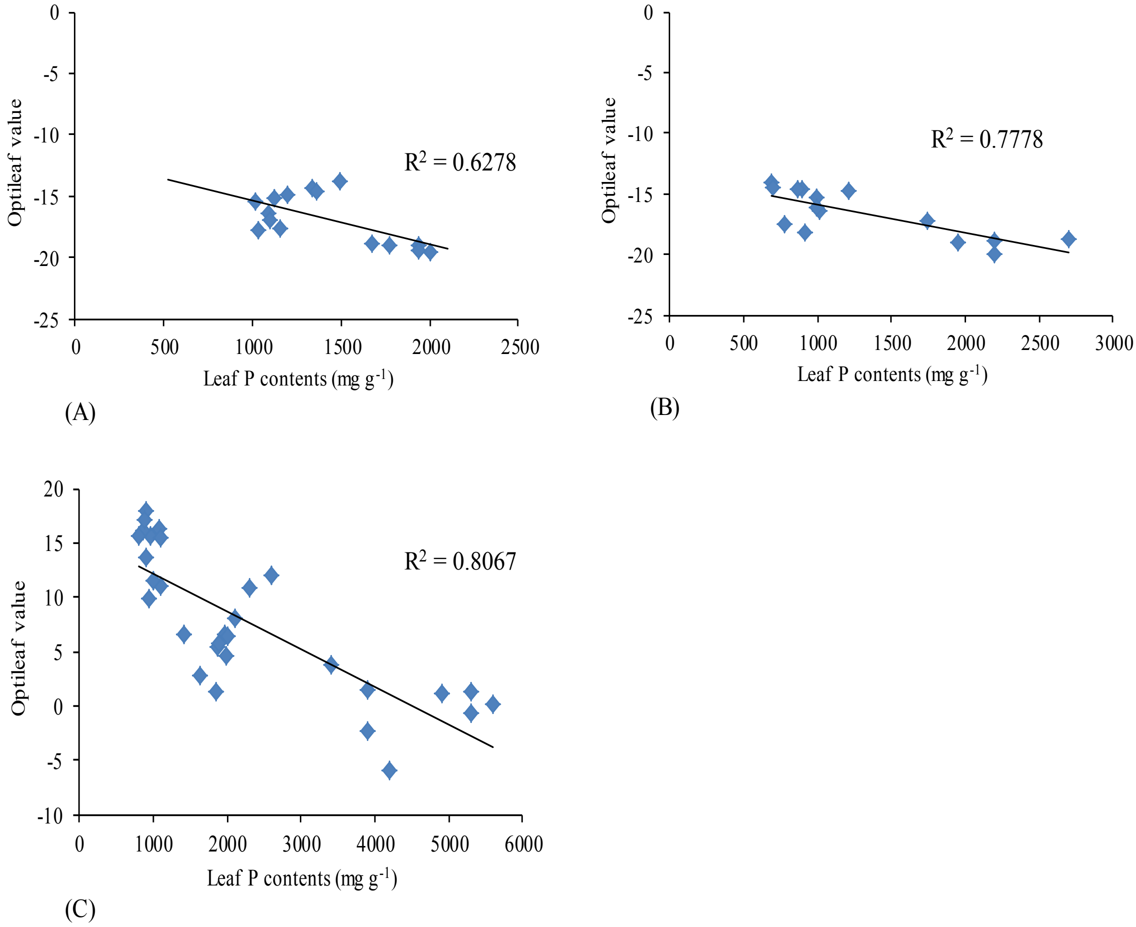


Figure 5.12: Correlation between leaf P content (laboratory based analysis) and values estimated by the modified RGB technique in (A) lettuce, (B) tomato and (C) cotton.

Table 5.2: Correlation coefficient (R^2) values between estimated (the modified RGB technique) and original (laboratory based method) P content for three crops, lettuce, tomato and cotton

Crop	R^2 value
Lettuce	0.77
Tomato	0.53
Cotton	0.76

5.4 Summary

The present study showed that crop P requirements may be estimated on the basis of simple morphological characteristics, such as leaf colour and dimensions. Due to ease of collecting data of leaf colour and dimension using a simple scanner, it is suggested that non-destructive methods can be used for the estimation of crop P requirements. Using different features and RGB data to train linear discriminant and decision tree models, we successfully classified cotton, lettuce and tomato plants on the basis of P and anthocyanin content. In addition, our proposed algorithms for estimating leaf P showed good relationships with the true leaf P content, indicating that this non-destructive method can efficiently estimate the P content of crops growing under variable P levels.

6 CHAPTER SIX

6.1 Conclusions

Terrestrial plants respond to changing environmental stimuli by optimising primary production and modification in growth that is leaf colour and size. Thus, changes in these traits are commonly used as indicators of plant health under varying growth conditions. Estimation of crop growth and nutrient status is crucial for taking important decisions on crop management such as irrigation, fertilisation, harvesting. However, it has been a great challenge for crop managers to accurately estimate crop nutrient status, especially in large fields. Applying fertiliser at higher or lower rates can result in economic loss and environmental damage. In addition, changes in fertiliser application time can affect the overall crop management schedule. Laboratory-based methods for estimating crop growth and nutrient status are laborious, expensive and impractical for large fields. Despite the fact that destructive method using laboratory techniques (forexample Kjeldahl and Leco CHN analysis) are capable of accurately estimating the N content in plants, it cannot provide an instantaneous N estimation in the field as it requires special equipment and chemicals.

Various non-destructive methods are available for application; however, each of these methods has certain limitation and may not be applicable to different crops growing under variable environmental conditions. For example, results obtained from the most commonly used non-destructive leaf chlorophyll measuring technique in the field, SPAD-502 are found not to be optimal due to its small measuring area. In addition, the SPAD-502 reading may be influenced by stress-induced or varietal/species based difference in leaf chlorophyll content. Similarly, handheld crop sensor (Trimble) that displays NDVI (Normalized Difference Vegetation Index) readings for N estimation is less accurate when applied to some specific crops, such as cotton. Recently, image processing techniques as a method to detect leaf chlorophyll content and estimate crop health have attracted increased attention from researchers working in this field. In order to achieve higher correlation with the true chlorophyll readings, a controlled light environment is critical for the accuracy of most of these methods.

This study proposed to collect images using a simple handheld scanner to estimate leaf dimension and chlorophyll content. In addition, on the basis of these images, could be estimated the crop N and P requirements with an acceptable accuracy. Unlike most of the existing image processing based methods, our new technique does not require sophisticated system settings in terms of distance, angle and lighting condition. Moreover, this new method is portable and easy to use. With this method, total area, width, length, average width and perimeter of a variety of leaves were successfully estimated. The estimated leaf area showed significant positive correlation with the destructive leaf measuring technique (Li-Cor 3100). In addition, this method was more effective for measuring leaf perimeter and average width, which could not be estimated by the Li-Cor 3100. Validation of benchmark images and real leaf images showed that the modified RGB technique effectively estimated the various leaf metrics that were significantly similar to the true values.

Images collected by the handheld scanner were analysed using a Matlab code, and RGB (Red Green Blue) values of the leaves were recorded. Based on these RGB values, an algorithm was developed that could successfully estimate leaf chlorophyll and N content for different crops growing under variable environmental conditions. The algorithm was validated using three different crop species, tomato, lettuce and broccoli, and achieved a consistently better performance than the other image processing-based methods as well as the SPAD-502 chlorophyll meter under field and greenhouse conditions. An image processing based N estimation algorithm (IPNC) was also proposed for cotton growing under variable N conditions that achieved a relatively stronger correlation with the leaf N levels of cotton compared with the SPAD-502 and handheld crop sensor. Although IPNC and SPAD-502 produced quite a good estimation of the N treatment, N estimation at early stages of plant growth is a big challenge for most of the non-destructive methods. More experiments need to be conducted in order to further validate the IPNC method, and hence offer a new system to the cotton community that is inexpensive, yet reliable in estimating nitrogen levels.

This technique was further used for estimating P of crops. The leaf images collected through handheld scanner were successfully used to classify crops on the basis of P level, suggesting that data collected of plant morphological traits and leaf dimensions could be successfully used for estimating crop P requirements.

6.2 Suggestions for future research

The handheld scanner used for image collection consists of a simple machine that does not require special operating skills. The proposed technique provides more accurate estimation of crop growth and nutrient status than most of the existing non-destructive techniques. Chlorophyll and N content estimated by the modified RGB technique showed significantly strong correlations with the laboratory based methods that determined the true chlorophyll and N content. The consistency of the modified RGB technique for estimating chlorophyll and N content of a range of crops varying with leaf size, growth pattern and fertiliser requirement, making it an ideal choice for a broader range of crops. However, most of these experiments were conducted under greenhouse or on the small field scale and validity of this technique for larger field crops, especially for cotton, is still to be established. Manual data collection from larger fields, using this handheld scanner may not be feasible. Designing a scanner that can be mounted on a machine (tractor) may help in collecting leaf images from larger fields. In addition, new algorithms are also required for analysing the data from crop canopy. By optimising leaf scanner and devising an algorithm for field conditions, this technique can provide a more accurate and cost-effective solution for planning fertiliser application rates and timings.

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Table 4-3: Actual and estimated length and width of leaf images calculated by various techniques

Leaf number	Actual length	Li-Cor 3100	Error	Modified RGB technique	Error	Actual width	Li-Cor 3100	Error	Modified RGB technique	Error
1	6.9	7.1	2.89	6.34	-8.07	3.7	3.6	-2.70	3.69	-0.02
2	2.7	3.1	14.81	2.50	-7.16	2.1	2.1	0	2.05	-2.04
3	4.7	5.9	25.53	4.20	-10.63	5.1	5.1	0	5.07	-0.58
4	5.5	6.4	16.36	5.20	-5.30	2.5	2.5	0	2.46	-1.46
5	5.1	6.1	19.60	4.89	-4.02	2.3	2.4	4.34	2.39	4.15
6	4.5	5.8	28.88	4.12	-8.35	2.0	1.9	-5.00	1.94	-2.65
7	3.7	7.1	91.89	3.43	-7.07	0.4	0.4	0	0.38	-4.77
8	7.2	8.7	20.83	6.85	-4.85	3.1	3.1	0	3.14	1.30
9	6.8	7.5	10.29	6.40	-5.85	2.4	2.4	0	2.40	0.16
10	5.8	6.1	5.17	5.44	-6.11	4.4	4.4	0	4.37	-0.53
11	5.2	5.4	3.84	4.86	-6.36	4.0	3.9	-2.5	3.95	-1.17
12	6.1	6.6	8.19	5.86	-3.93	1.3	1.4	7.69	1.33	2.87
13	8.3	8.9	7.22	7.96	-3.99	10.1	10.1	0	10.11	0.15
14	4.6	5.0	8.69	4.39	-4.45	1.9	2.1	10.52	1.91	0.68
15	6.9	7.1	2.89	6.55	-5.00	6.2	6.2	0	6.21	0.21
16	4.7	5.2	10.63	4.52	-3.78	2.0	2.0	0	1.97	-1.38
17	4.2	4.8	14.28	3.94	-6.04	4.0	4.1	2.5	4.07	1.79
18	2.6	3.0	15.38	2.44	-5.87	1.3	1.3	0	1.33	2.87

19	8.4	8.8	4.76	7.84	-6.64	10.4	10.2	-1.92	10.41	0.11
20	6.5	6.9	6.15	6.15	-5.28	6.5	6.0	-7.69	6.45	-0.63
21	7.1	7.9	11.26	6.56	-7.56	8.1	8.1	0	8.10	0.01
22	7.3	8.1	10.95	6.90	-5.45	5.2	5.1	-1.92	5.12	-1.35
23	8.5	9.1	7.05	8.07	-5.05	10.1	9.8	-2.97	10.04	-0.51
24	2.7	3.4	25.92	2.49	-7.47	1.5	1.5	0	1.47	-1.80
25	4.7	5.6	19.14	4.42	-5.94	5.3	5.3	0	5.29	-0.17
26	5.5	5.7	3.63	5.12	-6.84	2.2	2.1	-4.54	2.12	-3.42
27	4.9	5.1	4.08	4.48	-8.40	0.7	0.9	28.57	0.71	2.78
28	3.6	3.9	8.33	3.37	-6.14	0.4	0.5	25.00	0.49	24.85
29	4.0	4.3	7.50	3.75	-6.00	0.8	0.8	0	0.83	4.75
30	6.6	7.0	6.06	5.99	-9.15	1.9	1.9	0	1.81	-4.65
31	2.3	2.5	8.69	2.11	-7.95	1.3	1.3	0	1.24	-4.28
32	2.5	3.1	24.00	2.46	-1.42	1.8	1.9	5.55	1.83	2.05
33	10.5	11.0	4.76	10.10	-3.78	2.7	2.7	0	2.74	1.57
34	3.8	4.1	7.89	3.82	0.72	3.6	3.6	0	3.74	4.16
35	4.8	5.6	16.66	4.50	-6.14	2.4	2.4	0	2.42	1.22
36	12.8	13.9	8.59	12.50	-2.28	4.6	4.7	2.17	4.65	1.21
37	9.4	9.7	3.19	9.23	-1.71	4.8	4.9	2.08	4.79	-0.01
38	4.4	4.5	2.27	4.31	-1.84	0.4	0.5	25.00	0.44	12.15
39	9.0	9.4	4.44	8.84	-1.67	3.3	3.3	0	3.24	-1.75

40	10.5	11.6	10.47	10.21	-2.73	2.2	2.3	4.54	2.19	-0.34
Average error			12.83		5.39			2.21		0.88
SD			14.66		2.36			7.61		4.88

SD=standard deviation