UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF CHLOROPHYLL AND NITROGEN CONTENT IN RICE LEAVES AT VARIOUS GROWTH STAGES

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By

MOHAMMADMEHDI SABERIOON

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the requirement for the Degree of Doctor of Philosophy

April 2014

DEDICATION

To my parents who have helped me to build the bridge

&

To my beloved wife, Asa, who has helped me to cross the bridge.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

DETERMINATION OF CHLOROPHYLL AND NITROGEN CONTENT IN RICE LEAVES AT VARIOUS GROWTH STAGES

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April 2014

Chairman: Professor Mohd Amin Mohd Soom, PhD, PEng

Faculty: Institute of Advanced Technology

Nitrogen (N) is an important input variable for rice farming. Concerns over the use of nitrogen have increased due to the increase in fertilizer costs and environmental pollution from excess nitrogen applications in paddy fields. Several methods are available to assess nitrogen in crops. However, they are either expensive, time consuming, inaccurate or needs specialists to operate the tools. Recently researchers suggest remote sensing of chlorophyll content in crop canopies as a low-cost alternative to determine plant N status. Among different remote sensing platforms, low altitude remote sensing (LARS), which is a relatively new concept of acquiring earth imagery at a low altitude using unmanned aerial vehicle (UAV), is an attractive method and being promoted by researchers to determine status of chlorophyll content and nitrogen in rice field. The high spatial and temporal resolution, fast way to collect data and relatively low data acquisition cost would suggest this platform as ideal tool for monitoring crops through out the plant's growth. Furthermore, researchers have found that visible wavelengths would be useful for determining nutrient status and biomass in crops. Thus, the main objective of this study was to develop and test new methods for determining the status of chlorophyll content and N in rice leaf by analyzing and considering information derived from the images captured using conventional digital cameras (Panasonic Lumix DMC-TZ10 and Basler Scout scA640-70fc) and a new generation of multispectral camera (Tetracam ADC). Digital photographs of the upper most collared leaf of rice (*Oriza sativa L.)* in both leaf and canopy scale, grown over a range of N treatments, were processed into all published indices and also I_{PCA}, which is a new index for rice developed in this study based on utilizing all three visible bands and principal component analysis. Also conventional digital camera mounted to an UAV was used to acquire image over the rice canopy for verification purpose. The results indicate that conventional digital camera (r = -0.81) and Tetracam ADC (r = 0.89) could be used as sensors to determine the status of chlorophyll content in rice plants through different growth stages. Also I_{PCA} shows significant negative correlation not only in leaf scale (r = -

0.81) but also in canopy scale when UAV was utilized to capture images (r = -0.78). This indicates that conventional low-cost digital cameras and Tetracam ADC cameras, both can be used for determining chlorophyll content and consequently monitor Chlorophyll and N content of the growing rice plants, and also offers a potentially inexpensive, fast, accurate and suitable tool for rice growers. Additionally, results exhibited that a low cost LARS system would be well suited for high spatial and temporal resolution images and data analysis for proper assessment of key nutrients in rice farming in a fast, inexpensive and non-destructive way. Hence, the results of this study could be a significant contribution to develop the site-specific management decision for in-season, variable rate fertilizer application towards sustainable agriculture.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENENTUAN KANDUNGAN KLOROFIL DAN NITROGEN DI DALAM DAUN PADI PADA PERINGKAT TUMBESARAN YANG BERBEZA

Oleh

MOHAMMADMEHDI SABERIOON

April 2014

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Nitrogen (N) merupakan suatu input yang penting dalam penanaman padi. Kebimbangan tentang penggunaan nitrogen telah meningkat disebabkan oleh peningkatan kos pembajaan dan pencemaran alam sekitar daripada penggunaan nitrogen berlebihan di sawah padi. Beberapa kaedah boleh menilai kandungan nitrogen pada tanaman. Walau bagaimanapun, kaedah tersebut mungkin terlalu mahal, perlu menggunakan masa yang lama, kurang tepat atau memerlukan kepakaran dalam mengendalikan alat atau kaedah tersebut. Terkini, para penyelidik telah mencadangkan kawalan penderiaan kandungan klorofil di dalam kanopi tumbuhan sebagai alternatif kos rendah untuk menentukan status N tanaman. Antara platform kawalan penderiaan yang agak berbeza adalah kawalan penderiaan beraltitud rendah (LARS) yang mana ianya merupakan konsep yang agak baharu dalam pencerapan imej bumi pada altitud rendah dengan menggunakan kenderaan udara tanpa pemandu (UAV), yang telah diberi perhatian dan dipromosikan oleh para penyelidik untuk menentukan status kandungan klorofil dan nitrogen di dalam sawah padi. Resolusi ruang dan masa yang tinggi, cara yang singkat untuk mencerap data dan kos pencerapan data yang agak rendah akan menjadikan platform ini sebagai alat yang cukup ideal untuk memantau tanaman sepanjang tumbesaran pokok. Selanjutnya, para penyelidik juga telah menemui bahawa panjang gelombang berkelihatan sangat berguna untuk menentukan status nutrien dan jisim bio di dalam tumbuhan. Maka, objektif utama kajian ini adalah untuk membangun dan menguji kaedah baharu bagi menentukan status kandungan klorofil dan nitrogen di dalam daun padi dengan menganalisis dan mempertimbangkan maklumat yang diperolehi dari imej yang diperolehi menggunakan kamera digital (Panasonic Lumix DMC-TZ10 dan Basler Scout scA640-70fc) dan sebuah kamera multispectral generasi baru (Tetracam ADC). Gambar digital di bahagian atas bagi kebanyakan daun padi (*Oriza sativa L.)* yang disimpan dalam kedua-dua skala daun dan kanopi, yang membesar dalam pelbagai rawatan nitrogen, telah diproses ke dalam semua indeks yang diterbitkan dan juga I_{PCA}, yang merupakan satu indeks baharu untuk padi yang dibangunkan di dalam kajian ini berdasarkan kepada pengunaan ketiga-tiga band

berkelihatan dan analisis komponen utama. Kamera digital yang dipasangkan pada kenderaan udara tanpa pemandu juga telah digunakan untuk mencerap imej di seluruh daun padi untuk tujuan pengesahan. Keputusan yang diterima menunjukkan bahawa kamera digital konvensional (r = -0.81) dan Tetracam ADC (r = 0.89) boleh digunakan sebagai penderia untuk mengenal pasti status kandungan klorofil di dalam pokok padi pada peringkat pembesaran yang berbeza. Tambahan lagi, I_{PCA} menunjukkan pertalian yang negatif bukan sahaja pada skala daun (r = -0.81) tetapi juga pada skala kanopi (r = -0.78) semasa UAV digunakan untuk mencerap imej. Ini menunjukkan bahawa kamera digital konvensional kos rendah dan kamera Tetracam ADC, kedua-duanya mampu untuk menentukan kandungan klorofil dan seterusnya memantau kandungan nitrogen bagi pokok padi yang ditanam dan juga menawarkan suatu alat berpotensi yang murah, pantas, tepat dan sesuai digunakan oleh para petani. Tambahan lagi, keputusan menunjukkan bahawa sistem LARS berkos rendah boleh disesuaikan untuk imej dengan resolusi ruang dan masa yang tinggi dan analisis untuk penilaian yang berkesan bagi data nutrien-nutrien penting dalam penanaman padi secara pantas, tidak mahal dan tidak memudaratkan. Maka, keputusan kajian ini merupakan satu sumbangan yang penting dalam membuat keputusan pengurusan ladang khusus untuk penggunaan baja dengan kadar boleh ubah pada musim pembajaan ke arah melestarikan pertanian.

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I certify that a Thesis Examination Committee has met on 24th April 2014 to conduct the final examination of Mohammadmehdi Saberioon on his thesis entitled "Determination of Chlorophyll Content and Nitrogen in Rice Leaves at Various Growth Stages" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

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DECLARATION

DECLARATION BY GRADUATE STUDENT

I hereby confirm that:

- This thesis is my original work
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DECLARATION OF MEMBER OF SUPERVISORY COMMITTEE

This is to confirm that:

- The research conducted and the writing of this thesis was under our supervision;
- Supervision responsibilities as stated in Rule 41 in Rules 2003 (Revision 2012-2013) were adhered to.

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LIST OF ABBREVIATIONS

ADC	:	Agriculture Digital Camera
AE	:	Agronomical Efficiency
AGB	:	Above Ground Biomass
ANOVA	:	Analysis of Variance
APE	:	Agro-physiological Efficiency,
ARE	:	Apparent Recovery Efficiency,
B	:	Blue
b	:	Normalized Blue
CCD	:	Charge-Coupled Device
CI	:	Chlorophyll Index
CI-G	:	Chlorophyll Index-Green
CM	:	Chlorophyll Meter
$CO(NH_2)_2$	:	Urea
CV	:	Coefficient of Variation
DAP	:	Days After Planting
DGCI	:	Dark Green Colour Index
ExG	:	Excessive Green Index
ExR	:	Excessive Red Index
G	:	Green
g	:	Normalized Green
GLI	:	Green Leaf Index
gNDVI	:	Green Normalized Difference Vegetation
GNDVI	:	Green Normalized Vegetation Index
GNSS		Global Navigation Satellite System
GPS		Geographic Positioning System
GRVI	:	Green-Red Vegetation Index
HSV	:	Hue, Saturation and Value
IADA		Integrated Agriculture Development Area
I_{KAW}	:	Kawashima Index
I_{PCA}	:	Principal Component Analysis Index
IR	:	Infrared
IRRI	:	International Rice Research Institute
LAI	:	Leaf Aerial Index
LARS	:	Low Altitude Remote Sensing
LCC	:	Leaf Colour Chart
LED	:	Light Emitting Diodes
LNC	:	Leaf Nitrogen Concentration
NDVI	:	Normalized Difference Vegetation Index
NGRDI	:	Normalized Green Red Difference Index
NH_4^+	:	Ammonium
NIR	:	Near Infrared
NO_3^-	:	Nitrate
NUE	:	Nitrogen Use Efficiency
PC	:	Principal Component
PCA	:	Principal Component Analysis
PE	:	Physiological Efficiency
PF	:	Precision Farming

R	:	Red
r	:	Normalized Red
RGB	:	Red, Green and Blue
RMSE	:	Root Mean Square Error
RS	:	Remote Sensing
SAT	:	Saturation
SAVI	:	Soil Adjusted Vegetation Index
SMLR	:	Stepwise Multiple Linear Regression
SPAD	:	Soil Plant Analysis Development
TGI	:	Triangular Greenness Index
UAV	:	Unmanned Aerial Vehicle
UCD-LCC	:	University of California-Leaf Colour Chart
UE	:	Utilization Efficiency
UV	:	Ultraviolet
VARI	:	Visible Atmospherically Resistance Index
VI	:	Vegetation Index
Vis	:	Visible Bands
VRT		Variable Rate Technology
ZAU-LCC	:	Zheijiang Agriculture University- Leaf Colour Chart

CHAPTER 1

INTRODUCTION

1.1. Background

Rice (*Oryza sativa L.*) is grown over an area of 156 million hectares worldwide, of which 85% of the area is in Asia (IRRI, 2006). It is estimated that 2.3 million farmers and their households, worldwide, depend on rice as their main source of income (Mohanty, 2010). The sustainability of a rice-based system is threatened because of inefficient use of inputs, especially water and fertilizers. Rice production consumes about 34 - 43% of the world's irrigation water (Bouman et al., 2007) and accounts for 14.6% of the world 's fertilizer use (Heffer, 2009). Heffer (2009) also shows that rice production accounts for about 15.8 % of total Nitrogen (N) usage in world, which is approximately 53.0 million tones as fertilizer. In other words, N is the most widely used fertilizer nutrient in rice cultivation and its consumption has increased significantly in the recent decades (FAOSTAT, 2009). Despite this upward trend in N fertilizer usage, the quantity of rice grain produced per unit of applied fertilizer N (partial factor productivity) is very low (Dobermann et al., 2002). One of the main reasons for low N use efficiency is an inefficient splitting of N doses coupled with N applications in excess of crop requirements (Varinderpal-Singh et al., 2010). Furthermore, N fertilizers are rapidly lost in flooded soil-water systems by volatilization and denitrification. When N fertilization is managed inefficiently, a large portion of the applied N can leak from soil-plant system to reach the atmosphere and water bodies, which leads to the creation of pollution problems.

Additionally, rice cultivation in Malaysia follows a double cropping system. Under the double cropping system, availability of essential nutrients such as N for soil and plant is more necessary than under those obtained under the traditional system. This is because, during the off-season, the regeneration of N in soil takes place, making it suitable again for the next main-season rice crop to maintain a good level of productivity, so that farming operations can be carried out in a planned way. It means that, for a sustainable off-season and for sufficient plant nutrient availability during the growth stages, it is necessary to reduce distribution losses to an acceptable level in order to guarantee a reliable amount of important soil and plant nutrients throughout the field.

Sound management practices need to be established in order to improve N uptake and usage efficiently, which leads to higher grain yield, minimal N fertilizer losses and reduce cost of rice production. To achieve these goals, the monitoring of crop N status at various growth stages is important for improving the balance between crop's N demand and N supply from soil and fertilizer. Instruments to estimate leaf chlorophyll and N content such as chlorophyll meter (CM) (Gholizadeh et al., 2011), Dualex (Cartelat et al., 2005), Greenseeker (Johanson et al., 2002), Crop Circle ACD-210 and Yara N-sensor/Fieldscan can be used to manage the amount of N fertilizer applied to crops based on site-specific requirements. However, these instruments are time consuming, expensive and in some cases labor intensive. Remote sensing of chlorophyll content in crop canopies may help to provide a low-cost alternative for determining plant N status (Gitelson et al., 2005; Hatfield et al., 2008; Li et al., 2010a; Vigneau et al., 2011). Among the different remote sensing platforms, Low Altitude Remote Sensing (LARS), which is a relatively new concept of acquiring earth imagery at low altitude (Swain et al., 2007), is an attractive method for researchers and is being promoted for its capability for determining the status of chlorophyll and N content at the canopy scale. The high spatial and temporal resolution, fast data collection and relatively low data acquisition cost would suggest that this platform is an ideal tool for monitoring crops through out the plant's growth.

Furthermore, researchers have found that visible (Vis) wavelengths would be useful for determining nutrient status and biomass in crops (Xue et al., 2004; Hunt et al., 2011), because of spectral differences between vegetation and soil at green and red wavelengths. Thus, they proposed that conventional digital cameras or couple charged devices (CCDs) can be used as promising sensors (Hunt et al., 2005, 2013; Wang et al., 2013).

1.2. Problem statement

The lack of proper rice farming technologies has led farmers to apply management practices, such as applying fertilizers uniformly across the field or just splitting of N fertilizer during various growth stages. These are not efficient and could result in either insufficient or excess nutrient supply. Farmers also apply the same rate as in the previous season; and consequently, over application or under application of N fertilizer can be expected. These abovementioned practices are against the proper and sustainable agriculture practice, which can be achieved if the plant, soil and nutrient variations within a farm are considered. Therefore, the ability to improve management, such as high amounts and high cost of inputs, can lead to a significant effect on the profitability of a crop production operation. In addition, farmers must be aware of the environmental impact in modern agriculture. Nitrates that leach through the soil often end up in ground water, which is the same source that provides drinking water for many people. High levels of N in lakes can be harmful to aquatic life in many forms. If N in lakes can be matched to crop needs, agricultural contributions to water pollution will be decreased as a result. Thus, using the ability of precision farming (PF) can provide the evaluation of crop nutrients, such as N, to the farmers in order to reduce environmental pollution.

Most of the available techniques and tools to measure chlorophyll and N content in plants are destructive, time consuming and expensive. Paddy farmers cultivate two rice crop seasons in a year in Malaysia but techniques such as laboratory plant analyzing or Yara N-sensor/Fieldscan cause a delay in providing status of N during in-season. This makes them difficult for farmers to apply the necessary plan for optimum fertilizer and will not fulfill the concept of PF for specific variable rate application. Moreover, they are destructive which make them imperfect for farmers. Other available tools such as SPAD meter, Greenseeker, Dualex and Crop circle ACD-210 are expensive and still need field references. Since the variability of plant nutrients need to be measured rapidly and the information should be conveyed to the farmers before each split of fertilizer applied, therefore there is a need to study the new techniques or build a new instrument to overcome the above-mentioned problems.

1.3. Objectives

The general aim of this study was to seek the appropriate tools to expedite the adoption of PF for double cropping rice cultivation. The research also aimed at developing a new method for monitoring N and chlorophyll content in paddy field in an easy, fast, inexpensive and non-destructive way. Prediction models were then developed for SPAD meter values and rice yield within the paddy fields by multispectral vegetation indices (VIs), colour VIs and colour-model indices derived from Tetracam ADC and commercial digital camera respectively. Following these aims, the specific objectives of the study were as follows:

1. To estimate the chlorophyll and nitrogen status at different rice growth stages, using a low-altitude RS images obtained by new generation of multispectral camera (Tetracam ADC).
2. To identify the relationship between colour VIs, colour model indices, SPAD and chlorophyll content by using charged-coupled devices and compare differences between them.
3. To develop a new index for determining rice chlorophyll content and nitrogen status from the leaf colour image captured with a conventional digital camera and compare with the use of SPAD-502 measurements and other available colour indices and colour VIs at leaf scale in the laboratory and using UAV at canopy scale.

1.4. Scope and limitations of the study

In PF management, appropriate methods for monitoring key nutrients in plant are valuable to avoid expanding soil and water pollution and produce higher grain yield with higher profit. Thus, the scope of this study was set to use the Tetracam ADC and conventional digital camera as two non-disruptive and cost effective tools to introduce new method to acquire data for determining status of N and chlorophyll content in paddy fields. These devices would help paddy farmers to estimate N and chlorophyll content faster, easier, cheaper and in non-destructive fashion.

In order to achieve the objectives and scopes of this study, an integrated methodology was developed. Tetracam ADC was used to determine the relationship between VIs, which are combining of visible, NIR bands, and chlorophyll content. Tetracam ADC is a digital camera utilizing charged couple device (CCD), with its infrared rejection filter replaced by a blue rejection filter. Two different coupled charged devices (CCDs) were also used to evaluate the relationship between chlorophyll content as in-direct parameter for determining the N status in rice by using all known colour spaces, colour indices in visible bands, and filtering the best colour index and the most excellent evaluation model. Also the difference between the two different cameras for various models was compared. A new method was developed and tested to determine the status of chlorophyll content by analyzing and utilizing all visible bands derived from images captured using conventional digital cameras in leaf and canopy scale. The study was limited to only one rice-growing season.

1.5. Significance of study

Monitoring of nutrient status could improve the management of environmental impacts, agriculture inputs, as well as time efficiency, leading to more profitability of rice farming. Moreover, it could be a significant contribution to develop site-specific management and variable rate technology, which will lead to sustainable agriculture. Since Tetracam ADC and conventional digital camera have been used in this study, they promote new possibility to use them as reliable sensors for determining status of nutrients in paddy fields. Such development could prove to be useful for developing variable rate technology and site-specific management decisions. Besides, since the imagery using digital cameras, which are mounted to Unmanned aerial vehicle (UAV), can be achieved anytime and anywhere, this methodology can be improved further to real time model approach. Hence, this could be valuable contribution to the determination of plant nutrition, rice field forecasting applications and PF of paddy field.

1.6. Organization of the thesis

The first chapter focuses on the general introduction regarding world rice production and the importance of nitrogen monitoring in rice paddy fields. The problem statement, objectives and scope are highlighted. The second chapter concentrates on literature review, description of rice growth and development and role of nitrogen and its importance in plant growth. Moreover, all available methods to evaluate plant’s nitrogen are discussed. Also previous studies on nitrogen assessment using remote sensing and image processing techniques are explained. Finally, at the end of this chapter, the role of vegetation indices for determining status of nutrients in crops is discussed.

Chapter three covers the capability of new generation of multispectral cameras (Tetracam ADC) for estimating nitrogen and rice grain yield. Assessment of relationship between all known colour spaces, colour indices in visible bands and chlorophyll and nitrogen content in rice plant for filtering the best colour indices and the most excellent evaluation model is explored in chapter four. Then, new method to determine the status of chlorophyll and N content for rice leaf, which was developed in this study, is explained in this chapter. Moreover, similarities of the images obtained by two different digital cameras are compared in chapter four.

Chapter five describes the capability of LARS system with unmanned aerial vehicle (UAV) to acquire images in visible bands for determining chlorophyll content status of rice plant in canopy scale. Furthermore, the application I_{PCA} over the canopy of paddy fields is examined in this chapter. The conclusion and recommendations for future works are given in chapter six. Lastly, references and appendices followed by bio-data of the author and list of publications from this study are presented at the end of this thesis.

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