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DETECTION OF TUNGRO DISEASE IN RICE LEAF IN RELATION TO NITROGEN LEVEL USING LASER LIGHT BACKSCATTERING IMAGING

NUR AZIZAH BINTI BACHIK

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By

NUR AZIZAH BINTI BACHIK

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

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Chairman: Norhashila Hashim, PhD
Faculty: Engineering

Rice tungro disease (RTD) is one of the dangerous rice diseases that occurred due to two viruses i.e. rice tungro disease spherical virus (RTSV) and rice tungro disease baciliform virus (RTBV) which is transmitted by green leafhoppers (GLH). Hence, fast detection of this disease is required in order to prevent higher yield loss. The current conventional techniques i.e. serological and nucleic acid techniques are skill dependent and time consuming. Therefore, this study is proposed to investigate the capability of laser light backscattering imaging (LLBI) for detecting RTD in rice leaf. MR-219 rice cultivar was planted in a randomized complete block design (RCDB) with two factorial treatments i.e. inoculation (INO) and nitrogen (N) treatments with three replications. INO treatments including healthy (H), inoculation 1 (INO 1) (3 GLH/seedling) and inoculation 2 (INO 2)(5 GLH/seedling) while N treatments including N1 (0kg/ha), N2 (85 kg/ha), N3 (170 kg/ha) and N4 (250 kg/ha). The seedlings were inoculated with infected GLH during 20 days after planting (DAP). The data collection were carried out on youngest fully expanded leaf at where the readings were taken at three different location i.e. leaf tip, middle leaf and leaf base (4 cm interval).

The images then were analyse using image processing toolbox, MatlabR2013a (The Mathworks, Inc. Nattrick, MA, 2013a) to extract LLBI parameters from the images. The polymerase chain reaction (PCR) then was done as a confirmation of the RTD establishment. Results showed that the growth performance i.e. rice plant height and tiller number per hill were influenced by INO treatments but was not influenced by N treatments. Results also indicated significant difference between chlorophyll content and colour parameters i.e. lightness (L*) and chroma (C*) for INO treatments while only colour parameters i.e. red/green (a*) and hue (h^o) were significantly influenced by N treatments. The PCR result showed that the expected deoxyribonucleic acid (DNA) size (500 base pairs (bp)) of RTBV was amplified for inoculated rice plants. The LLBI parameters showed that all parameters except maximum intensity, minimum intensity and ratio were influenced by INO treatments while only minimum intensity was influenced by N treatments. There were significant difference between reading points on rice leaf for soil plant analysis development (SPAD) reading, colorimeter parameters and LLBI parameters for INO treatments but no significant for N treatments. The principal component analysis (PCA) and linear discriminant analysis (LDA) showed that LLBI is more appropriate for accessing RTD infection and sensitive in differentiate between reading points location on rice leaf. LDA classification rates for H, INO 1 and INO 2 of LLBI were 83.82%, 75.85% and 78.81%, the colorimeter were 85.60%, 74.46%, and 69.90% while SPAD were 80.95%, 51.19%, and 42.86% respectively.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGESANAN PENYAKIT PMV PADA DAUN PADI MENGIKUT ARAS NITROGEN MENGGUNAKAN PENGIMEJAN PEMBALIKAN CAHAYA LASER

Oleh

NUR AZIZAH BINTI BACHIK

Jun 2017

Pengerusi: Norhashila Hashim, PhD
Fakulti: Kejuruteraan

Penyakit merah virus (PMV) adalah salah satu penyakit padi merbahaya yang terjadi disebabkan oleh dua virus iaitu virus sfera PMV (RTSV) dan virus baciliform PMV (RTBV) yang dijangkitkan oleh bena hijau (GLH). Oleh itu, pengesanan yang cepat untuk penyakit ini diperlukan untuk mengelakkan kehilangan hasil yang lebih tinggi. Teknik-teknik konvensional semasa yang digunakan untuk mengesan PMV iaitu teknik serologi dan asid nukleik bergantung kepada kemahiran dan memakan masa. Oleh itu, kajian ini dicadangkan untuk menyiasat keupayaan pengimejan pembalikan cahaya laser (LLBI) untuk mengesan PMV pada daun padi. Kultivar padi MR-219 ditanam dalam blok yang direka bentuk secara rawak dan lengkap (RCDB) dengan dua rawatan faktorial iaitu rawatan inokulasi (INO) dan nitrogen (N) dengan tiga replikasi. Rawatan INO termasuk sihat (H), inokulasi 1 (INO 1) (3 GLH / anak benih) dan inokulasi 2 (INO 2) (5 GLH/ anak benih) sementara rawatan N termasuk N1 (0kg / ha), N2 (85 kg / ha), N3 (170 kg / ha) dan N4 (250 kg / ha). Anak benih diinokulasi dengan bena hijau yang telah dijangkiti PMV semasa 20 hari selepas menanam (DAP). Pengumpulan data telah dijalankan pada daun yang paling muda berkembang di mana bacaan telah diambil di tiga lokasi yang berbeza iaitu hujung daun, daun tengah dan pangkal daun (4 cm selang).

Imej-imej yang telah diambil dianalisis menggunakan toolbox pemprosesan imej, MatlabR2013a (Mathworks, Inc. Nattrick, MA, 2013a) untuk mengekstrak parameter LLBI dari imej. Tindak balas rantaian polimerase (PCR) kemudian dilakukan sebagai pengesahan jangkitan PMV pada daun padi. Hasil kajian menunjukkan bahawa prestasi pertumbuhan iaitu ketinggian tanaman padi dan bilangan anak padi setiap bukit dipengaruhi oleh rawatan INO tetapi tidak dipengaruhi oleh rawatan N. Keputusan juga menunjukkan perbezaan yang signifikan antara kandungan klorofil dan parameter warna iaitu keterangan cahaya (L*) dan kroma (C*) untuk rawatan inokulasi manakala hanya parameter warna iaitu merah/hijau (a *) dan hue (h^o) dipengaruhi dengan ketara oleh rawatan N. Hasil PCR menunjukkan bahawa saiz asid deoksiribonukleik (DNA) (500 pasangan asas (bp)) daripada RTBV seperti yang dijangka telah digandakan bagi pokok padi yang diinokulasi. Parameter LLBI menunjukkan bahawa semua parameter kecuali intensiti maksimum, intensiti minimum dan nisbah dipengaruhi oleh rawatan inokulasi manakala hanya intensiti minimum dipengaruhi oleh rawatan N. Terdapat perbezaan yang signifikan antara lokasi titik bacaan pada daun padi bagi bacaan pembangunan analisis tanah tumbuhan (SPAD), parameter kolorimeter dan parameter LLBI untuk rawatan inokulasi tetapi tidak signifikan untuk rawatan N. Analisis komponen utama (PCA) dan analisis diskriminan linear (LDA) menunjukkan bahawa LLBI adalah lebih sesuai untuk mengakses jangkitan PMV dan sensitif dalam membezakan antara lokasi titik bacaan pada daun padi. Kadar klasifikasi LDA untuk H, INO 1 dan INO 2 LLBI masing-masing adalah 83.82%, 75.85% dan 78.81%, colorimeter adalah 85.60%, 74.46% dan 69.90% manakala SPAD adalah 80.95%, 51.19% dan 42.86%.

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I certify that a Thesis Examination Committee has met on 20 June 2017 to conduct the final examination of Nur Azizah binti Bachik on her thesis entitled "Detection of Tungro Disease in Rice Leaf in Relation to Nitrogen Level using Laser Light Backscattering Imaging" 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 Master of Science.

Members of the Thesis Examination Committee were as follows:

Muhammad Razif bin Mahadi @ Othman, PhD
Senior Lecturer
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

Siti Khairunniza binti Bejo, PhD
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Internal Examiner)

Md Shafiquzzaman Siddiquee, PhD
Senior Lecturer
University Malaysia Sabah
Malaysia
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 8 August 2017

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Norhashila Hashim, PhD
Senior Lecturer
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

Aimrun Wayayok, PhD
Senior Lecturer
Faculty of Engineering
Universiti Putra Malaysia
(Member)

Hasfalina Che Man, PhD
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)

____
ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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Name of Member of Supervisory Committee: ______________________________

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LIST OF ABBREVIATIONS

a*	red/green coordinate
A_I	Illuminated area
$A_{260/280}$	Ratio of absorbance at 260 nm and 280 nm
ANOVA	Analysis of variance
b*	yellow/blue coordinate
bp	Base pair
BIS	Backscattering Imaging System
CV	Computer vision
CCD	Charged-couple device
C*	Chroma
D	Diameter
DNA	Deoxyribonucleic acid
DAP	Days after planting
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
GLH	Green leafhopper
H	Healthy
h^o	Hue
HIS	Hyperspectral imaging
HBI	Hyperspectral backscattering imaging
HSD	Tukey's honest significant difference
I_C	Reflectance intensity captured by CCD camera
I_L	Light intensity
INO 1	Inoculation 1
INO 2	Inoculation 2
IR	Infrared
I_R	Apparent, corrected light intensity on the fruit surface
K	Potassium
Kb	Kilobase
kg/ha	Kilogram per hectare
L*	Lightness
LCC	Leaf colour chart
LCD	Liquid crystal display
LDA	Linear discriminant analysis
LEDs	Light emitting diodes
LLBI	Laser light backscattering imaging
MARDI	Malaysian Agricultural Research and Development Institute
MBI	Multispectral backscattering imaging
MR219	Malaysian indica rice 219
N	Nitrogen
nm	Nanometer
NIR	Near infrared range
NIRS	Near infrared spectroscopy
P	Phosphorus

PCA	Principal component analysis
PCR	Polymerase Chain Reaction
PMV	Penyakit merah virus
r	Correlation coefficient
R^2	R-squared
RCBD	Randomized complete block design
RNA	Ribonucleic acid
RMSE	Root-mean-square error
ROI	Region of interest
RTBV	Rice tungro bacilliform virus
RTSV	Rice tungro spherical virus
RTD	Rice tungro disease
RS	Remote sensing
SPAD	Soil plant analysis development
TBE	Tris/Borate/EDTA
TIF	Tagged Image Format
TN1	Taichung native 1
UV	Ultraviolet
VA	Visual assessment
VIS	Visible
W	Week
WPI	Week post-inoculation

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Rice (*Oryza sativa*) is a cereal grain that is widely consumed as staple food. It is a predominant food for Asian countries as it is consumed by 2.9 billion people (Bunawan, Dusik, Bunawan, & Amin, 2014). Asia produces about 90% and above of the world rice production. The largest rice producer is China which followed by India, Indonesia, Bangladesh and Vietnam (Bunawan et al.,2014). In Malaysia, the rice production is around 2.5 million metric tonnes annually involving 300,000 farmers that cultivated on 674,928 ha land area (Rabu & Mohd Shah, 2013).

However, in major rice-growing countries, rice diseases remain a major threat to sustainable rice production. One of the serious diseases that widely spread in South and South-east Asian countries is rice tungro disease (RTD). Rice tungro disease (RTD) was found to be associated with two distinct viruses i.e. rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (Bunawan et al., 2014). This disease is transmitted via leafhopper as a vector (Mohd Daud, Jozani, & Arab, 2013).

The typical symptoms of the infected rice plant with RTD are stunting, yellow to orange leaves discolouration, reduced in number of tillers, panicles sterility, and the appearance of irregular-shaped dark brown specks on the leaves (Bunawan et al., 2014). A conventional method for RTD detection in the field is visual observation. However, the disease is difficult to identify as the symptoms are not similar from one infected varieties to another. It is also sometimes being misdiagnosed as a non-pathogenic disorder as the appeared symptoms are similar to the symptoms that are exhibited due to overwatering, nutritional deficiencies, or insect damage (Azzam, 2002 ; Bunawan et al., 2014).

It was reported that leaf colour can be used to indicate the plant nutrient and health status and the changes of the colour is closely related to the amount and proportion of nitrogen (N) content (Senoaji & Praptana, 2013). Furthermore, N content has been identified as one of the main causes that attract pest to host under a canopy (Lu et al., 2005). N is one of the vital minerals for growth of plant that is extremely important for encouraging root development, flowering and ripening. Deficiency of N results in stunted and slow growth, and chlorosis with small unit leaf area (Lu et al., 2005). As N is mobile, the older leaves exhibit chlorosis and necrosis with the appearance of light green to yellow discoloration symptoms earlier than the younger leaves.

There are several laboratory methods that being used to detect the RTD in rice plants i.e. serological method, nucleic acid technique and electron microscopy (Nath et al, 2000 ; Takahashi et al., 1993; Hibino, Roechan, & Sudarisman, 1978). All of these methods are destructive, time consumed in which it needs one to two days period to get the result and requires high cost and skills as well as very sensitive to contamination. As colour is one of the symptoms for the detection of N content as well as RTD, the presence of N in paddy leaf and RTD infection probably can be detected rapidly, accurately and non-destructively using optical imaging method.

Laser light backscattering imaging (LLBI) system is an advancement of computer vision technology which uses laser light at different wavelength to detect physic-chemical properties in tissues. This technology combines vision system and spectral readings using laser diode light source which emits photons in the visible to near infrared range that produce backscattering images (Hashim et al., 2013). This technique has been proven for the detection of internal and external quality of fruit such as apples (Lu, 2004; Qing, Ji, & Zude, 2007; Qin & Lu, 2008) peach, pear, plums, tomatoes, zucchini, cucumber (Qin & Lu, 2008), citrus (Lorente, Zude, et al., 2013), kiwifruit (Baranyai & Zude, 2009), papaya (Udomkun et al., 2014) and banana (Hashim et al., 2013). As the changes of backscattering parameters could be used to detect the changes in pigment and fruit tissues that are related to quality, hence the potential of the system could be explored on its feasibility in the detection of crop diseases.

1.2 Problem Statement

RTD disease is a serious setback in the production of rice in Asia. This disease is transmitted by leafhopper that attack seriously on the N-enriched rice plants (Lu & Heong, 2009; Chau et al., 2003). It was reported that every fold increase in N application may increase plant hopper densities by 40-fold (Lu & Heong, 2009). Since RTD could causes severe damage such as “degenerated growth” and total loss of grain yields besides difficult to control, henceforth appropriate method for the detection and RTD management is required.

The current practice of detecting the diseases i.e. serological assay and nucleic acid are laborious, skill dependent, time consuming and costly. On the other hand, the current practices to control RTD which are by using insecticides and conventional resistance breeding offers inefficient solution. Application of heavy insecticide may result in not only masking the effects of natural biological control but also stimulating the development of insecticide resistance. Application of resistance breeding was not sustainable due to high disease pressure. Very often varieties with vector resistance were defeated by RTD as the vector population become adapted after their release (Bunawan et al., 2014). Thus, an approach of using LLBI for detection of RTD as well as N is proposed as a new monitoring device for fast and effective diagnostic technique.

1.3 Objective

The objective of this study is to detect rice tungro disease (RTD) infection in rice leaf as relation to nitrogen (N) treatments by using laser light backscattering imaging system (LLBI). The specific objectives of this study are stated as follows:

1. To determine RTD infection in rice leaf using reference methods i.e. SPAD chlorophyll meter and colorimeter
2. To determine RTD infection in rice leaf using LLBI
3. To investigate RTD infection as relation to N treatments
4. To compare the efficiency of RTD detection between SPAD chlorophyll meter, colorimeter and LLBI

1.4 Scope and Limitation of Study

This study is focused on the detection of RTD infection and influences of N treatments on RTD infection in rice leaf by using LLBI. The data collection is conducted about seven weeks starting from week 2 to week 9 of post-inoculation (WPI) i.e.36 until 78 days after planting (DAP). The sample used in this study is limited to Malaysian indica rice (MR219) variety.

1.5 Significance of the study

As the most devastating viral disease of rice in South and Southeast Asia, RTD become one of the significant fears to sustainable annual rice production in the world. Due to the increasing world population and subsequent increase in demand for food, identifying the causal agents, symptoms of disease and method of disease management of monitoring are important keys to understand how to reduce the economic damage caused by rice pathogens.

Since one of the symptoms of this disease is the changes of rice leaf colour which could be related to the N level of the paddy plant, application of LLBI for detection of the symptom is expected to be able to provide alternative solution. Hence, the application of LLBI is proposed in this study. The findings of this study could promote to the development of rapid and non-destructive tool, also a fundamental study for the detection of other similar diseases in agricultural produce and crops.

1.6 Thesis Layout

There are five chapters reported in this thesis. Chapter one highlights the background of food security and RTD in Asian countries and Malaysia, problem statements, scope of the study, the objectives of the study, hypothesis, and study interests. Chapter two follows by outlining the literature review of the techniques used for detection and monitoring RTD and N content. Chapter three explains the methodology of this study which includes planting rice plant, rearing of green leafhopper, and method of data collection. In the chapter four, the results obtained from the study are presented and discussed thoroughly. Lastly, summary and conclusions from this study as well as recommendations for further studies are derived in chapter five.

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