UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF RHIZOCTONIA ISOLATES AND EFFECTS OF SILICON AND PLANTING DISTANCE ON RICE SHEATH BLIGHT DISEASE MANAGEMENT

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CHARACTERIZATION OF *RHIZOCTONIA* ISOLATES AND EFFECTS OF SILICON AND PLANTING DISTANCE ON RICE SHEATH BLIGHT DISEASE MANAGEMENT

By

EI EI KHAING

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

March 2015

DEDICATION

Dedicated to:

(My Beloved Parents)

U Aung Nyunt and Daw Su Su Win

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

CHARACTERIZATION OF *RHIZOCTONIA* ISOLATES AND EFFECTS OF SILICON AND PLANTING DISTANCE ON RICE SHEATH BLIGHT DISEASE MANAGEMENT

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Chairperson: Associate Professor Zainal Abidin Mior Ahmad, PhD

Faculty: Agriculture

Sheath blight caused by the fungus *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatephorus cucumeris* (A.B.Frank) Donk has become an economically important disease of rice in tropical Asia, especially in intensive rice cropping systems and high nitrogen fertilizer applications. To sustain high yield, more nitrogen fertilizer input is needed and this will increase disease incidence. However, in modern agricultural practices it is highly important to manage diseases and pests using efficient methods with minimum harm to the environment. Although silicon has been reported to significantly reduce fungal diseases in crops and planting density is an important factor influencing yield of rice and disease development, this is not the practice adopted in Malaysia. This study focused on characterization of *Rhizoctonia* isolates in major rice growing areas and to evaluate the effect of silicon (Si), copper (Cu) and zinc (Zn) applications on MR219 and MR 253 cultivars and to investigate the effect of Si and spacing on sheath blight severity and yield improvement. A total of 16 isolates of *Rhizoctonia* were collected from various states in Peninsula Malaysia and studied for their morphological and molecular characteristics as well as test for pathogenicity. Colony growth rate were not significantly different among isolates but sclerotial features were varied among isolates. All 16 isolates were identified as *Rhizoctonia solani* through a Basic Local Alignment Search Tool (BLAST) search (with similarity ranging from 96 to 100%). Kelantan isolate (R12) was more virulent than all other isolates. Silicon treatment (24 g kg^{-1} soil) was found to significantly reduce disease severity by 17.16% for cultivar MR219 and 29.04% for MR253 compared to the respective control treatments. Si rate gave a yield of 56.2 g pot^{-1} for cultivar MR 219 and 27.56 g pot^{-1} for MR253 which were both significantly higher than the control of 30.09 g pot^{-1} for cultivar MR219 and 16.10 g pot^{-1} for MR253 respectively. Silicon treatment showed significantly higher lignin content of 6.62% in cultivar MR219, and 5.09% in MR253 compared to the two controls of the same cultivars of 3.60% and 3.33% respectively. These were also significantly higher compared to Cu and Zn treatments. From the results of the first experiment, Si treatment and MR219 cultivar giving the lowest sheath blight disease severity (30.49%) and improved yield were selected for study in a second glasshouse experiment. Analysis of data showed significant difference for disease incidence and disease severity were higher for 20 cm x 15 cm compared 25 cm x 25 cm spacing with or without application of Si. In the absence of disease, Si increased grain yield by 190.32 g pot^{-1} and 215.36 g pot^{-1} respectively for 25 x 25 cm and 20 x 15 cm spacing compared to the control of 145.61 g pot^{-1} and 153.24 g pot^{-1} respectively. However, in the presence of sheath blight disease, Si treatment gave a 48% and 90 % higher mean grain yield respectively for 25 x 25 cm and 20 x 15 cm spacing compared to the controls. *Chitinase* and *β-1, 3 gluc*anase were induced in Si treated plants with increasing time after inoculation. The highest level of *chitinase* and *β-1,3 -gluca*nase expression was recorded at two and four days after inoculation, respectively. This study showed the potential contribution for reducing sheath blight disease through manipulation of spacing (25 cm x 25 cm) and rice grain yield improvement via Si application.

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

PENCIRIAN ASINGAN *RHIZOCTONIA* DAN KESAN SILIKON DAN JARAK TANAMAN DALAM PENYAKIT HAWAR SELUDANG PADI

Oleh

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Hawar seludang disebabkan oleh kulat *Rhizoctonia solani* Kuhn (teleomorf: *Thanatephorus cucumeris* (A.B. Frank) Donk telah menjadi penyakit padi yang penting dari segi ekonomi di Asia tropika, terutama yang mengamalkan sistem tanaman padi intensif dan pengunaan baja nitrogen tinggi. Untuk mengekalkan hasil tinggi, lebih banyat baja nitrogen diperlukan dan ini akan meningkatkan jangkitan penyakit. Walau bagaimanapun, dalam pertanian moden adalah penting untuk mengurus penyakit dan perosak menggunakan cara yang memberi bahaya yang terendah pada alam sekitar. Walaupun silikon telah dilaporkan boleh mengurangkan penyakit kulat pada tanaman secara ketara dan kepadatan tanaman adalah faktor penting mempengaruhi hasil padi dan perkembangan penyakit, ia bukanlah amalan penanaman di Malaysia. Kajian ini telah memberi tumpuan kepada pencirian asingan *Rhizoctonia* di kawasan tanaman padi utama dan menilai kesan penggunaan silikon (Si), kuprum (Cu) dan zink (Zn) pada kultivar MR219 dan MR253 dan untuk mengkaji kesan Si dan jarak tanaman pada keterukan hawar seludang dan peningkatan hasil. Sejumlah 16 asingan *Rhizoctonia* telah dikumpul dari beberapa negeri di Semenanjung Malaysia dan dikaji tentang ciri morfologi dan molekul bersama dengan ujian kepatogenan. Kadar pertumbuhan koloni tidak berbeza dengan bererti antara asingan tetapi bentuk sklerotium amat berbeza antara asingan. Kesemua 16 asingan di kenal pasti sebagai *Rhizoctonia solani* melalui pencarian BLAST (dengan persamaan berjulat dari 96 ke 100%). Ujian kepatogenan menunjukkan perbedaan patogenik yang bererti di antara 16 asingan. Dua kultivar padi MR219 dan MR253 di tanam di komplek rumah kaca Ladang 2, Universiti Putra Malaysia (UPM). Silika gel, kuprum sulfat dan zink sulfat di beri masing-masing pada kadar 24 g, 0.02 g, 0.03 g kg^{-1} tanah dan dibandingkan dangan rawatan kawalan tanpa dimasukkan bahan tambahan ini. Rawatan Si didapati menurunkan keterukan penyakit dengan bererti pada 17.16% untuk kultivar MR219 dan 29.04% untuk MR253 berbanding dengan rawatan kawalan yang berkenaan. Penggunaan silikon memberi hasil 56.2 g pot^{-1} untuk kultivar MR219 dan 27.56 g pot^{-1} untuk MR253 yang keduanya lebih tinggi bererti dari kawalan iaitu masing-masing 30.09 g pot^{-1} untuk kultivar MR219 dan 16.10 g pot^{-1} untuk MR253. Rawatan Si menunjukkan kandungan lignin yang lebh tinggi dan bererti iaitu 6.62% pada kultivar MR219, dan 5.09 % pada MR253 masing-masing berbanding dengan kawalan kultivar yang sama pada 3.60% dan 3.33%. Ini juga adalah tinggi dengan bererti berbanding dangan rawatan Cu dan Zn. Daripada keputusan percubaan pertama, rawatan Si dan kultivar MR219 yang memberi kesan penyakit hawar seludang yang terendah dan hasil terbaik telah dipilih

untuk kajian dalam percubaan rumah kaca yang kedua. Penelitian dibuat bagi jarak panjang dan antara barisan 20 cm x 15 cm dan 25 cm x 25 cm dan di nilai untuk pembaikan hasil padi. Analisis data menunjukkan perbezaan bererti untuk kejadian penyakit semasa pengeluaran daun anak dan keterukan penyakit adalah lebih tinggi untuk 20 cm x 15 cm berbanding dengan jarak 25 cm x 25 cm dangan atau tanpa penggunaan Si. Tanpa penyakit,silikon meningkatkan hasil padi pada 190.32 g pot^{-1} masing-masing untuk jarak dan 215.36 g pot^{-1} masing-masing untuk jarak 25 cm x 25 cm and 20 cm x 15 cm berbanding dengan kawalan masing-masing pada 145 g pot^{-1} dan 153.24 g pot^{-1}. Walau bagaimanapun, dengan kehadiran penyakit hawar seludang, rawatan Si memberi 48% dan 90% purata hasil bijirin yang lebih tinggi masing-masing untuk 25 cm x 25 cm dan 20 cm x 15 cm berbanding dengan kawalan. Kitinase dan β 1, 3-glukanase dirangsang dengan peningkatan masa selepas inokulasi. Paras ekspresi kitanase dan β-1, 3-glukanase paling tinggi di catat masing-masing pada 2 dan 4 hari selepas inokulasi. Kajian ini menunjukkan potensi sumbangan untuk mengurangkan peningkatan hawar seludang melalui pengubahan jarak (25 cm x 25 cm) dan pembaikan hasil bijirin padi dengan penggunaan Si.

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I certify that a Thesis Examination Committee has met on 30th March 2015 to conduct the final examination of EI EI KHAING on her thesis entitled "**Characterization of *Rhizoctonia* isolates and Effects of Silicon and Planting Distance on Rice Sheath Blight Disease Management**" in accordance with the Universities and University College 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 Doctor of Philosophy.

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LIST OF ABBREVIATIONS

Symbols

ADF	Acid detergent fiber
ADL	Acid detergent lignin
AM	Ante meridiem
ßGlu	B 1,3 glucanase
bp	Base pair
BLAST	Basic Local Alignment Search Tool
cDNA	Complementary DNA
Chn	Chitinase
CTAB	Cetyltrimethyl ammonium bromide
cv.	cultivar
d	Day
DAI	Days after inoculation
DAP	Days after planting
DNA	Deoxyribonucleic acid
DOA	Department of Agriculture
DS	Disease scoring
E value	Expect value
Ed	Endodermis
Ep	Epidermis
Gns1	Glucanase gene
g kg^{-1}	Gram per kilogram
gL^{-1}	Grams per liter
h	Hour
HRLH	Highest Relative Lesion Height
ITS	Internal Transcribed Spacer
kb	Kilo byte
LSD	Least Significant Difference
M	Molarity
ME	Mercaptoethanol
MARDI	Malaysian Agricultural Research and Development Institute
ml	Milliliter
m^2	Meter square
mM	Millimolar
mRNA	Messenger Ribonucleic acid
MT	Metric tons
Mx	Metaxylem
NCBI	National Center for Biotechnology Information
NJ	Neighbouring-joining
ngµl-1	Nangogram per microliter
Ph	Pholem
PI	Panicle initiation
PR	Pathogenesis-Related
Px	protoxylem
PVP	Polyvinylpyrrolidone
RCBD	Randomized Complete Block Design

HRLH	Highest relative lesion height
RNA	Ribonucleic acid
ROL	Radial oxygen loss
rpm	Rotation per minute
SDS	Sodium Dodecyl Sulfate
SDW	Sterile Distilled Water
SE	Standard Error
SEM	Scanning Electron Microscope
Smp	Spongy mesophyll
SRI	System of Rice Intensification
TAE	Tris-acetate-EDTA
TBE	Tris-borate-EDTA
TBA	Tertiary butyl alcohol
USDA	United States Department of Agriculture
UV	Ultra violet
w/v	Weight per volume

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa* L.) is staple food for more than half of the world's population (Zhao et al., 2011; FAO, 2014). Rice is cultivated mainly in irrigated, lowland ecosystems, and in upland or dry ecosystems (Maclean, et al., 2002). In Malaysia, most of the rice is planted as wet paddy while upland rice or dry paddy is very small and mostly in Sarawak and Sabah (Yahya, 2001). At present, rice self-sufficiency level is around 73 % with an average yield of 3.71 t ha^{-1} which is below the potential level (Akinbile et al., 2011). Therefore, research is urgently needed to improve and increase rice yields in order to supply enough food for the growing world population. Most of the granary areas in Malaysia are well established with irrigation systems and farmers practice double cropping with high-yielding varieties such as MR219 or MR220 (Ho et al., 2008). However, farmers are often intensifying land use without proper nutrient management practices which results in depletion of nutrients from soils linked to yield decline. Severe soil micronutrients deficiency has been reported to greatly affect rice production in the main granary areas in Malaysia (Zulkefli et al., 2004).

Furthermore, balanced nutrition does not only help to achieve better yield in crop production but also allows plants to protect themselves from new infections by disease (Narayanasamy, 2002; Agrios, 2005). It is predictable that judicious and proper use of fertilizers can noticeably increase rice yield and grain quality (Alam, 2009). Fertilizer is a very important input for intensive rice production and the profitability of rice production systems. Appropriate fertilizer inputs are therefore not only for getting high grain yield but also for attaining maximum profitability (Khuong, 2008). In a more specific study of nutrients, the micronutrients have been found to be equally important as macronutrients although they are required in minute quantities. Malaysian farmers frequently apply nitrogen (N), Phosphorus (P) and Potassium (K) fertilizers that are widely supported by subsidies from the government but the applications of beneficial and micronutrients such as Si, Cu and Zn are not a common practice. Nutrient imbalance with high nitrogen application in the planting of high-yielding rice varieties were known to cause severe disease infestation in the major rice planting area in Malaysia (Liew et al.,2012).

Rice production is being hampered infection by different diseases. Among them, Sheath blight disease caused by *Rhizoctonia solani* Kuhn, is a major constraint to rice production, especially in intensified cropping systems (Jayaprakashvel and Mathivanan, 2012). Sheath blight is a major disease of rice in many countries including Bangladesh, USA and Malaysia, affecting more than 50% of global rice production areas (Groth et al., 1991; Latif, et al., 2011). Under favorable conditions, sheath blight caused up to a 50 % decrease in yield losses each year around the world (Zheng et al., 2013). IRRI observed that sheath blight has also caused a yield loss of 6% in tropical Asia (IRRI, 2009b). Groth and Lee (2003) reported that increase in sheath blight disease severity is attributed to cultural practices of modern rice production, intensive nitrogen fertilization, high plant populations' common in direct seeded rice culture and rotation with soybean.

Unfortunately, at present, there is no known complete resistance rice varieties to control sheath blight (Adhipathi et al., 2013) while there are no suitable economic disease management measure (Banerjee et al., 2012).So far, control of the disease has relied mainly on the use of chemical fungicides when affordable by farmers (Savary and Mew, 1996). However, this option is not considered sustainable because of the pesticide residue problems and the potential risk of emergence of pathogen populations that are resistant to fungicides. Several cultural practices have been applied to minimize the intensity of sheath blight. They include use of resistant cultivars, crop rotation, solarization, reduced close spacing of hills in transplanted rice, biological control with *Trichoderma* spp. and fluorescent *Pseudomonas*, and induced resistance with avirulent strains of *R.solani* or different chemicals and nutrients (Belmar et al., 1987; Roy, 1996; Kumar et al., 2009). Micronutrient deficiencies in crop had markedly increased due to intensive cropping, loss of fertile top soils and nutrients through leaching and surface runoff (Somani, 2008). Efficacy of micronutrients against sheath blight was reported by Bhattacharya and Roy (2001), Ganguli and Sinha (2003), Liew et al. (2012) and Salam et al.(2010).

Silicon (Si) is considered as an essential element for rice and known to provide beneficial effects under conditions of multiple abiotic and biotic stresses (Ma and Yamaji, 2006).Application of complete Si fertilizer not only increases rice yield but also decreases the incidence of rice fungal diseases (Rodrigues and Dantnoff , 2005;Wang Mei Qing, 2005). Absorption of Si increase cell wall thickness below the cuticle imparting the mechanical resistance to the penetration of fungi, decrease in transpiration (Yoshida et al.,1962), and the leaf angle improvement, producing more erect leaves, thus reducing self-shading, especially under high nitrogen rates (Yoshida et al., 1969) had been proven to give maximum growth and yield (Epstein, 1994).

Currently, system of rice intensification (SRI's) principles and practices are being applied in many rice growing areas using mechanical operations (Sharif, 2011). Sheath blight epidemic are favored by close spacing of hills in the case of transplanted rice (Kannaiyan and Prasad, 1983; Castilla et al., 1996), and by high sowing rates in the case of directs seeded rice (Mithrasena and Adikari, 1986). The manipulation of rice canopy structure offers promising possibilities for keeping sheath blight pathogens under control (Tivoli et al., 2013). Therefore, planting density is considered an important factor for determining rice yield and disease development.

Due to the changing socio-economic conditions around the world, grain yield reduction is not affordable by agricultural systems (Ahmad et al., 2013). Major nutrients (N, P, K) applications are already in practice at optimum level in Malaysia but yield gap is still present which justifies the need to venture into micronutrient applications in the rice production system. Studies on improving yield of rice affected by sheath blight disease in Malaysia have not been done. The recommendations of fertilizers and rice varieties were suggested by the staffs of the Department of Agriculture in a granary area in Selangor. This study, therefore, investigated whether this concept of cultural control can provide a sound and verifiable method for controlling sheath blight disease in rice production. It will also consider the change in planting density that will subsequently lead to improvement in yield. Environmentally friendly element in relation to soils, fertilizers and plant nutrition were considered.The main objectives of this study were:

i. To identify rice sheath blight fungus (*Rhizoctonia solani*) by using morphology and molecular method.
ii. To determine the effects silicon (Si), copper (Cu) and zinc (Zn) applications on sheath blight disease severity and rice yield.
iii. To evaluate the effect of Si and plant spacing on sheath blight disease severity and yield improvement.
iv. To evaluate the resistance of rice variety treated with silicon in defence of sheath blight disease.

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