



UNIVERSITI PUTRA MALAYSIA

***CHARACTERIZATION AND PATHOGENICITY OF *Rhizoctonia* SPP
ISOLATED FROM VARIOUS CROP SPECIES IN DIFFERENT
AGROECOSYSTEMS IN MALAYSIA***

OSAMAH ZAID ALI RASHED

FP 2017 60



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By

OSAMAH ZAID ALI RASHED

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfillment of the Requirements for the Degree of Master of Science**

September 2017

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DEDICATION

The author dedicated this work to almighty Allah. May Allah accept my effort and bless the time I spend it to contribute something could be useful.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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September 2017

Chairman : Prof. Datin Siti Nor Akmar Abdullah, PhD
Faculty : Agriculture

Rhizoctonia species is well known as a necrotrophic soilborne fungus prevalent in different agro-ecosystem worldwide. It has been reported as a destructive fungal pathogen that caused various types of diseases on a wide variety of crops. This study investigated *Rhizoctonia* isolates obtained from different crops and locations based on morphological traits, pathogenicity, molecular identification and genetic diversity characterization. Morphological traits revealed that majority of the isolates were multinucleated (MNR) except for two isolates were binucleated (BNR). Radial growth rate showed that all the isolates could cover the plate within 2-3 days and few isolates covered the plate within four days. Mycelium width ranged between 3.60 -7.33 μ m, while most of the culture texture appeared oppressed. Nevertheless, some were raised and fluffy. The culture colors were varied from white to light yellowish white or light brown to brown. Sclerotia color was light brown to dark brown and sclerotia distribution pattern was centered and rim concentrated to scatter while the intensity of sclerotia was high to low and some isolates did not produce. Based on identification of the ITS rDNA and *tef-1 α* genes different taxonomic groups were determined. Twenty-seven isolates were identified as *R. solani* AG-1 IA, four isolates as *R. solani* AG-1 ID, two isolates as *R. solani* AG-4 HG-I and one isolates as *R. solani* AG-2-2 IV. Two isolates were identified as binucleate *Rhizoctonia* AG-Fa and AG-A. Phylogenetic analysis using different algorithms separated *Rhizoctonia* spp. to the distinct clades. Binucleate *Rhizoctonia* AG-Fa clustered with *R. solani* isolates indicating close relationship with some taxonomic groups of *R. solani*. Variation was detected for ITS rDNA and *tef-1 α* gene sequences at 0.25 variation and 30% homopolymer level. This led to the identification of 50 SNPs and Indels for ITS while 28 SNPs and Indels were found for *tef-1 α* which indicated that ITS rDNA variation is greater than *tef-1 α* . Species-specific primer of ITS rDNA region has confirmed the identity of each anastomosis groups. The virulence among isolates of AG-1 IA was varied where strains of rice were more virulent than the strains of corn. Similarly, the virulent among

various anastomosis groups in this study showed that AG-1 IA, AG-4 GH-I and AG-2-2 IV were more virulent than AG-1 ID, AG-Fa and AG-A. Genetic variability was detected using RAPD, iPBS and ISSR markers. All molecular markers were able to show reasonable polymorphisms. There was no relationship between morphological traits, pathogenicity, geographical origin and genetic diversity. However, the clustering tree and PCA plot supported the separation based on taxonomic groups indicating that there are other factors which could play a significant role on genetic variation. The knowledge gathered in this study would be useful for developing crops that are resistant to *Rhizoctonia* diseases. This will assist in planning for the right crop rotation and proper disease management programs.



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

PENCIRIAN DAN PATOGENISITI *Rhizoctonia* SPP YANG DIPENCILKAN DARIPADA PELBAGAI SPESISES TANAMAN DARI AGROEKOSISTEM YANG BERBEZA DI MALAYSIA

Oleh

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September 2017

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Spesies *Rhizoctonia* dikenali sebagai kulat nekrofilik bawaan tanah yang lazimnya berada dalam berbeza-beza agro-ekosistem di seluruh dunia. Ia telah dilaporkan sebagai kulat patogen perosak yang menyebabkan pelbagai jenis penyakit kepada pelbagai jenis tanaman. Kajian ini menyiasat pencilan *Rhizoctonia* yang diperolehi daripada pelbagai spesies tanaman dan lokasi berbeza berdasarkan ciri-ciri morfologi, patogenisiti, identifikasi molekul dan pencirian kepelbagaian genetik. Ciri-ciri morfologi telah menunjukkan bahawa majoriti daripada pencilan tersebut adalah multi-ternukleus (MNR) kecuali dua daripada pencilan adalah bi-ternukleus (BNR). Kadar pertumbuhan radial menunjukkan bahawa semua pencilan boleh meliputi plat dalam masa 2-3 hari dan terdapat beberapa strain yang mampu meliputi plat dalam tempoh 4 hari. Kelebaran miselium adalah antara 3.60-7.33 μm , manakala kebanyakan tekstur kultur yang muncul telah ditindas. Walau bagaimanapun, ada beberapa kultur yang mampu membesar dan mengembang. Warna kultur adalah pelbagai dari warna putih kepada kuning cair keputihan atau dari warna coklat muda kepada coklat. Warna sklerotia adalah berwarna coklat muda kepada coklat gelap dan corak edaran adalah berpusat dan rim yang tertumpu telah tersebar, manakala keamatan sklerotia adalah dari tinggi kepada rendah dan terdapat beberapa pencilan tidak menghasilkan sklerotia. Berdasarkan identifikasi ITS rDNA dan *tef-1 α* gen bahawa kumpulan taksonomi yang berbeza telah ditentukan. Dua puluh tujuh pencilan diklasifikasikan sebagai *R. solani* AG-1 IA, empat pencilan sebagai *R. solani* AG-1 ID, dua pencilan sebagai *R. solani* AG-4 HG-I dan satu sebagai *R. solani* AG2-2 IV. Dua pencilan telah dikenalpasti sebagai binukleat *Rhizoctonia* AG-Fa dan AG-A. Analisis filogenetik menggunakan algoritma berbeza telah memisahkan *Rhizoctonia* spp. kepada klad yang berbeza. Binukleat *Rhizoctonia* AG-Fa berkelompok dengan strain *R. solani* menunjukkan hubungan yang rapat dengan beberapa kumpulan taksonomi *R. solani*. Perubahan variasi telah dikesan pada ITS rDNA dan jujukan gen *tef-1 α* pada variasi 0.25 dan tahap homopolymer 30%. Ini membawa kepada pengenalpastian 50 SNP dan Indels untuk ITS, manakala 28 SNP dan Indels telah didapati untuk *tef-1 α* yang menunjukkan

bahawa variasi rDNA ITS adalah lebih besar daripada *tef-1 α*. Kawasan primer spesies khusus ITS rDNA telah mengesahkan identiti setiap kumpulan anastomosis. Keagresifan antara pencilan AG-1 IA berbeza, iaitu di mana strain beras lebih virulen daripada strain jagung. Begitu juga, keagresifan di kalangan kepelbagaian kumpulan anastomosis dalam kajian ini menunjukkan bahawa AG-1 IA, AG-4 GH-I dan AG-2-2 IV lebih virulen daripada AG-1 ID, AG-Fa dan AG-A. Kepelbagaian genetik dikesan menggunakan RAPD, iPBS dan penanda ISSR. Semua penanda molekul dapat menunjukkan polimorfisme yang sewajarnya. Tidak ada sebarang hubungan antara ciri-ciri morfologi, patogenisiti, asal-usul geografi dan kepelbagaian genetik. Walau bagaimanapun, pengelompokan pokok dan plot PCA menyokong pemisahan berdasarkan kumpulan taksonomi yang menunjukkan bahawa terdapat faktor-faktor lain yang boleh memainkan peranan besar ke atas variasi genetik. Pengetahuan yang dapat diperolehi dalam kajian ini akan berguna untuk membangunkan tanaman yang tahan terhadap penyakit *Rhizoctonia*. Ini akan membantu dalam merancang kitaran tanaman yang betul dan dalam membina program pengurusan penyakit tanaman yang sesuai.

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I certify that a Thesis Examination Committee has met on 28 September 2017 to conduct the final examination of Osamah Zaid Ali Rashed on his thesis entitled "Characterization and Pathogenicity of *Rhizoctonia* spp Isolated from Various Crop Species in Different Agroecosystems in Malaysia" 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.

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LIST OF ABBREVIATIONS

µl	Microliter
AFLP	Amplified Fragment Length Polymorphism analysis
AG	Anastomosis group
AM	Arbuscular mycorrhizal
AMOVA	Analysis of molecular variance
BLB	Bacterial Blight
BNR	Binucleate <i>Rhizoctonia</i>
BS	Brown spot
CIA	Chloroform: isoamyl-alcohol
cm	Centimeter
CRD	Completely randomized design
CTAB	Cetyltrimethylammonium bromide
EDTA	Ethylenediaminetetraacetic acid
FAME	Fatty acid methyl ester
FAO	Food and Agriculture Organization
H	Gene diversity
I	Shannon's Information Index
IGS	Intergenic spacer
iPBS	Primer binding site
IRRI	International Rice Research Institute
ISSR	Inter-simple sequence repeat
ITS rDNA	Internal transcribed spacer ribosomal DNA
Kg	Kilogram
LB	Leaf blast
mM	Millimolar
MOA	Ministry of agriculture
MNR	Multinucleate <i>Rhizoctonia</i>
Na	Observed number of alleles
NaCl	Sodium chloride
NaOCl	Sodium hypochlorite
NB	Neck blast

NCBI	National Center for Biotechnology Information
Ne	Effective number of alleles
NTD	Non-transcribed DNA
NP	Nitrogen phosphor
NPK	Nitrogen phosphor potassium
NTSYS	Numerical Taxonomy and Multivariate Analysis System
°C	Celsius
PAL	Phenylalanine ammonia lyase
PCA	Principle of coordinate analysis
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PDB	Potato dextrose broth
Pg	Picogram
PK	Phosphor potassium
RAPD	Random Amplification of Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism Analysis
rpm	Revolutions per minute
SAS	Statistical analysis software
SDW	Sterilized distal water
SHB	Sheath blight
SHR	Sheath root
SSR	Simple sequence repeat
<i>tef-1 α</i>	Translation elongation factor -1 alpha
uHe	Unbiased Expected Heterozygosity
UNR	Uninucleate <i>Rhizoctonia</i>
UPMGA	Unweighted pair group methods with arithmetic
URP	Universal rice primer
USD	United states dollar
WA	Water agar

CHAPTER 1

INTRODUCTION

1.1 General

In the last fifty years, the world has witnessed astonishing development in agriculture science and technology which leads to a remarkable contribution to the global food production. Reported statistical data showed a significant increase in crop production globally especially the essential crops such as wheat, rice and maize. Based on this progress, it was believed that each capita might be able to receive an adequate amount of nourishment (FAO, 2011). However, the present scenario is different. According to reported data on global crop production by FAO or by the Ministry of Agriculture (MOA) in Malaysia, world crop production increase is not enough to meet the sharp increase in population growth, especially in developing countries. The world population is expected to reach around 9 billion by 2050 (FAO, 2011). Evidently, imbalance between the food production globally and population, especially in recent decades, is considered as an alarm threatening the status of food security in the current century.

In general, the capacity of food production has encountered many challenges and perhaps impact negatively on total global food production worldwide. There are various obstacles which might bring food production backward unless if we are able to overcome these barriers. For example, there has been a significant drop in the ratio of the arable land area to global population size. Other challenges are affecting the agriculture output including water availability, soil health, and effects of climate change.

One of the most critical challenges is crop losses due to pests and diseases, which is severely affecting the quantity and quality of potential crop production worldwide. Annually, 20-40% direct losses of crop production globally occur because of pests, plant diseases and weeds (Oerke, 2006). The total anticipated lost by plant diseases yearly is about 220 billion USD worldwide. When we add 6-12% crop losses after harvesting especially in tropical countries, the percentage of losses at the stage of post-harvest is higher (Agrios, 2005).

Historically, plant diseases have caused severe hunger in past decades. One of the most well-known phytopathogens was the late blight of potato in Ireland as the epidemic disease destroyed the whole potato plantation and caused starvation among people, the population was reduced from 8 to 6 million. In Ireland and some European countries, potato was a staple diet at that time. Another example of crop losses was due to the epidemic of disease, coffee rust in South-east Asia which attacked coffee plantation and reduced the yield from 228 to 101 kg /acre.

Rice considered as the most strategic crop in diverse part of Asia and it is also widely consumed in other parts of the world. The most critical diseases that can decrease the yield of rice caused by fungi e.g. sheath blight (SHB), brown spot (BS) and sheath root (SHR). Meanwhile, other diseases, e.g., bacterial blight (BLB), leaf blast (LB) and neck blast (NB) caused lesser yield losses. For example, sheath blight disease caused yield losses about 5-10% whereas bacterial blight about 0-1.7% (Savary et al., 2012).

The exact and possible yield losses were estimated between the periods 2001 to 2003 of some important crops. Generally, the highest potential losses were caused by weed (34%), while animal, pest and pathogens together caused lower losses than weed from 16% to 18%. Nonetheless, the possibility to control weeds is higher than of controlling pests and phytopathogens (Oerke, 2006). Despite of the intensive usage of pesticide in the last century, it did not reduce the crop losses considerably. Consequently, crop production for human consumption is at risk unless if we strive earnestly to improve the productivity of crops and minimize the crops losses accrue by pathogenic microbes and parasitic plants.

Understanding the concept of pest management and reducing the regular application of pesticide should be considered in order to minimize crop losses and the usage of pesticide which could be accepted at economic level (Oerke. 2006). Prior to any efforts to control or manage the diseases in the field, it is essential to identify the disease and causal agent accurately. Identification aspect is one of the most critical studies in phytopathology, as incorrect identification may lead to inaccurate disease control measures and that will increase crop losses and eventually, time and efforts will be wasted (Riley et al., 2002).

Rhizoctonia solani and *Rhizoctonia* spp. are well known as necrotrophic soilborne pathogenic fungus on many important crops such as rice, corn and popular vegetables. Different anastomosis groups of *Rhizoctonia* species were isolated and reported as the casual pathogen to various crop diseases such as sheath blight on rice and banded leaf blight on corn caused by *R. solani* AG-1 IA. So far, in Malaysia anastomosis groups of *Rhizoctonia* species have not been studied and screened sufficiently. *R. solani* AG-1 was reported as a causal pathogen of foliar blight on durian in Malaysia (Kwee, 1990). Thuan *et al.* (2008) reported *R.solani* AG-1 ID as the casual pathogen of leaf blight of durian in Vietnam. Therefore, this project objective is to screen and characterize the anastomosis groups of *Rhizoctonia* species in Malaysia isolated from different crop species and geographical regions.

1.2 Objectives of the study

This project aimed to identify and characterize *Rhizoctonia* spp isolated from a different type of crops and locations based on following objectives:

- To characterize *Rhizoctonia* spp. isolated from different hosts based on morphological traits.
- To identify and study genetic variability of *Rhizoctonia* spp. using molecular tools.
- To assess the pathogenicity of *Rhizoctonia* spp. isolated from various hosts.



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