



**UNIVERSITI PUTRA MALAYSIA**

**IDENTIFICATION AND CHARACTERIZATION OF FUNGAL SPECIES  
CAUSING SHEATH BLIGHT DISEASES ON PADDY ( *Oryza sativa* L. )**

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**BY**

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## CERTIFICATION

This project report entitled “**Identification and characterization of fungal species causing sheath blight disease on paddy ( *Oryza sativa* L.)** “ prepared by Mawardi bin Mahusin submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Science Horticulture.

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## LIST OF ABBREVIATIONS

|         |   |   |
|---------|---|---|
| BLAST   | = | Basic Local Alignment Search Tool                                 |
| DNA     | = | Deoxyribonucleic acid   |
| ITS     | = | Internal Transcribed Spacer                                       |
| PCR     | = | Polymerase Chain Reaction   |
| RNA     | = | Ribonucleic acid  |
| rNA     | = | Ribosomal ribonucleic acid  |
| NCBI    | = | National Center for Biotechnology Information                     |
| PDA     | = | Potato Dextrose Agar  |
| UPM     | = | Universiti Putra Malaysia   |
| MAFFT   | = | Multiple Alignment Program for Amino Acid or Nucleotide sequences |
| MEGA    | = | Molecular Evolutionary Genetics Analysis                          |
| BIOEDIT | = | Biological Sequence Alignment Editor                              |

## LIST OF UNITS

|    |   |                |
|----|---|----------------|
| %  | = | Percentage     |
| °C | = | Degree Celcius |
| Cm | = | Centimeter     |
| g  | = | Gram           |
| h  | = | Hour           |
| mM | = | Milimol        |
| ml | = | Mililitre      |
| mm | = | Milimeter      |
| μl | = | Microlitre     |

## ABSTRACT

Sheath blight disease is one of the major problem faced by paddy growers particularly in the tropics. The sheath blight disease became a major problem when the high-yielding rice cultivars were introduced in tropical Asia. Also, the pathogen causing this disease is favors warm temperature and high humidity conditions. This pathogen infects the leaf sheaths at the lower leaves near the water line. Later, these lesions expand, appear water-soaked lesions, and eventually the lesions turn brown-to-tan and dry out of sheaths. This study were focusing on isolation of fungal pure culture, then identify fungal pathogens to species level based on morphological characteristics and polymerase chain reaction (PCR) protocol using ITS4 and ITS5 primers and also to construct internal transcribed spacer (ITS) phylogeny of the isolated fungal species. To accomplish these objectives, infected paddy plants were collected and isolated on potato dextrose agar (PDA) and observing in vitro morphological and physiological characteristics. The morphological observation resulting in the detection of *Rhizoctonia solani* characteristics. To ensure the morphological result and determine the pathogen to species level, molecular observation were done by extracting the DNA genomic from fresh fungal mycelium using the CTAB protocol. The internal transcribed spacer (ITS) region of the ribosomal DNA was amplified using primers ITS4/ITS5. The PCR products of the ITS were sequenced and analyzed using BLAST nucleotide query in GenBank. The result obtained from molecular observation approving the fungal species claimed from morphological observation that causing sheath blight diseases on paddy is *Rhizoctonia solani*. This study is a significant step forward toward management recommendations in controlling sheath blight disease in rice-growing regions in Malaysia and adds information on the genetic diversity of this pathogenic fungi causing sheath blight disease.

## ABSTRAK

Penyakit hawar seludang adalah salah satu masalah utama yang dihadapi oleh pengusaha padi terutamanya di kawasan tropika. Penyakit hawar seludang ini menjadi masalah utama apabila kultivar padi yang berhasil-tinggi telah diperkenalkan di kawasan tropika Asia. Selain itu juga, patogen yang menyebabkan penyakit ini adalah sesuai pada suhu panas dan keadaan kelembapan yang tinggi. Patogen ini menjangkiti seludang daun pada daun yang lebih rendah berhampiran permukaan air. Kemudian, luka-luka ini berkembang, kelihatan luka-luka seperti terendam air, dan akhirnya luka-luka bertukar coklat dan kering. Kajian ini telah memberi fokus kepada pengasingan kultur tulen kulat, kemudian mengenalpasti patogen kulat sehingga ke peringkat spesies berdasarkan ciri-ciri morfologi dan tindak balas rantai polimerase (PCR) protokol menggunakan ITS4 dan ITS5 sebagai primer dan juga untuk membina filogeni Ruang Tertranskripsi Dalam (ITS) bagi spesies kulat. Untuk mencapai objektif ini, pokok padi yang dijangkiti telah diambil dan dipindahkan pada Potato Dextrose Agar (PDA) dan ciri-ciri morfologi dan fisiologi dalam vitro diperhatikan. Pemerhatian morfologi memberi keputusan penemuan ciri-ciri *Rhizoctonia solani*. Bagi memastikan keputusan morfologi dan menentukan patogen ke peringkat spesies, kajian molekul dilakukan dengan mengeluarkan genomik DNA daripada miselium kulat segar menggunakan protokol CTAB. Ruang Tertranskripsi Dalam (ITS) bagi DNA ribosom telah diperkuatkan menggunakan primers ITS4 / ITS5. Produk PCR daripada ITS telah disusun dan dianalisis menggunakan BLAST nukleotida dalam GenBank. Keputusan yang diperolehi daripada pemerhatian molekul mengesahkan spesies kulat yang dinyatakan daripada pemerhatian morfologi yang menyebabkan penyakit hawar seludang pada padi ialah *Rhizoctonia solani*. Kajian ini merupakan satu langkah ke hadapan yang penting ke arah cadangan pengurusan dalam mengawal penyakit hawar seludang di kawasan-kawasan padi di Malaysia dan menambah maklumat mengenai kepelbagaian genetik kulat patogen menyebabkan penyakit hawar seludang ini.



## CHAPTER 1

### INTRODUCTION

Paddy or the botanical name is *Oryza sativa* L. is an important crop and one of the major food supply to be consumed by the world's population especially in Asian region. Paddy was categorized in grass family that can produce seed called rice as the cereal grain and provide carbohydrate that consumed by the people as the staple food and source of energy. (Smith, Bruce D, 1998) The production of this foodcrop is the third largest in the world after sugarcane and maize based on FAOSTAT 2012 and this statistic shown that paddy is very important for the demand of the world population. Many varieties of paddy were produced for certain objective especially in increasing the yield quality and quantity, but majority of them come from the wild paddy species, which is *Oryza sativa*, the Asian species and cultivated worldwide while the other one is *Oryza glaberrima* which is limited in West Africa. (P.K. Subudhi *et al.*, 2006).. However, this valuable crop faced many problems that can cause reduction in quality and quantity of yield. This foodcrop are exposed to the abiotic and biotic factors including pest and diseases and may resulting bad effects.

There are lot of pest and diseases stated that can cause loss of yield and quality of paddy production such as planthopper, rice bugs and weevils, apple snails, rice ragged stunt by virus, rice blast by fungus and one of the major problem is sheath blight disease. This is shown that paddy crop are susceptible and exposed for pathogens that causing bad effects to the plants. (Sitch, 1990).

Sheath blight disease or locally known as “ Penyakit Hawar Seludang” is caused by a fungus.. This disease has been stated can infest paddy plant in Malaysia and other countries around the world. This disease can be described by observing the symptom appear on the sheath and leaf of the paddy plant. The lesion with water-soaked will form and become

browning or straw-colored on the sheath and leaf collar of paddy plant as the fungus infest the plant. This formation will continuous to paddy leaf and may causing reduction in yield qualities and quantities and also may cause the death of the paddy plants. (Agrios.,2005)

There are many researches published before stated that sheath blight disease on paddy plant are caused by *Rhizoctonia solani*. Hence, the main objective of this study is to identify the fungal causing agent of sheath blight disease of paddy. This main objective can be achieved by this sub-objectives for this study :

- (i) To isolate pure culture of fungal isolates causing sheath blight on paddy
- (ii) To identify fungal pathogens to species level based on morphological characteristics and polymerase chain reaction (PCR) protocol using ITS4 and ITS5 primers.
- (iii) To construct internal transcribed spacer (ITS) phylogeny of the isolated fungal species

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