



Institute of Health and Community Medicine

**Molecular Epidemiology of Tungro Viruses in East Malaysia and
Development of Serological Detection Tools for Diagnosis of Rice Tungro
Disease**

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**Doctor of Philosophy
2019**

UNIVERSITI MALAYSIA SARAWAK

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Molecular Epidemiology of Tungro Viruses in East Malaysia and
Development of Serological Detection Tools for Diagnosis of Rice Tungro
Disease

Yee Siew Fung

A thesis submitted

In fulfillment of the requirements for the degree of Doctor of Philosophy (Biotechnology)

Institute of Health and Community Medicine
UNIVERSITI MALAYSIA SARAWAK
2019

DECLARATION

The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ACKNOWLEDGEMENT

First and foremost, I would like to express my appreciation, gratitude and thanks to my supervisor, Dr. Magdeline Sum Henry and co-supervisor, Professor Dr. David Perera for their guidance, motivation, assistance and support throughout this research. To all the staff and students of the Institute of Health and Community Medicine, especially Fauziah, Nor, Nadia, Li Kuin, Ceci, Hamidah, Siti Aishah, Zila, May Ying, Milda and Dayana, thank you very much for your generosity and help in all forms in completing this research. A special thanks to Dr. Yuwana Podin for her constructive suggestions and encouragement during my dissertation writing period.

A thousand thanks to the Department of Agriculture (DOA), Sarawak and DOA Sabah for their support and the assistance provided for the success of this research. My gratitude especially to Dr. Lily Eng and Miss Evenni Poili, who believed in this research from the start and for their superb assistance.

Finally, I wish to thank my family and friends for their moral support and understanding, especially whenever I needed to retreat into my shelter to complete my research.

ABSTRACT

Malaysia aspires to be a rice self-sufficient nation as rice is the main food staple for her citizens. Thus, a rice disease outbreak can cause major losses for the rice granaries in the country. One of the viral rice diseases of concern in Malaysia is the rice tungro disease (RTD). RTD is caused by two viruses, namely the *Rice tungro bacilliform virus* (RTBV) and *Rice tungro spherical virus* (RTSV). The disease was detected in West Malaysia and Sabah in East Malaysia since the early twentieth century, and only recently in Sarawak in East Malaysia. RTD is very well-studied in West Malaysia, where the nucleotide sequences of both tungro viruses isolated from West Malaysia were published. In contrast, relatively little is known of RTD and the tungro viruses circulating in East Malaysia. Therefore, the aims of this study were to characterise the nucleotide sequences of tungro viruses isolated from East Malaysia and develop a RTD diagnostic method using in-house generated reagents such as sera and antigens in the form of recombinant proteins. The sequences of coat protein 1 (CP1), CP2 and CP3 of RTSV, and the open reading frame 1 (ORF1), ORF2, ORF3 (CP gene) and ORF4 of RTBV isolated from East Malaysia were characterized and compared with sequences of other published isolates by phylogenetic analysis. The identities and nucleotide sequences of fourteen RTBV and five RTSV isolates from East Malaysia determined from this study could be the first record of tungro viruses from East Malaysia. Then, recombinant proteins were generated by cloning and expressing CP genes of RTSV and RTBV isolated from Sabah using the SUMO fusion expression system. The tungro isolates selected for cloning and protein expression had high similarities in the nucleotide and deduced amino acid sequences with all tungro isolates from East Malaysia. This was to ensure that the antibodies generated from the

selected tungro isolates are able to detect both tungro viruses from East Malaysia. Lastly, in-house polyclonal antibodies against tungro viruses were generated using the purified tungro viruses and the produced recombinant proteins. Two recombinant proteins that have potential to replace purified tungro virions as antigens in antibody production, and a reactive anti-serum for use in serological detection of RTD were finally obtained from the cloning and expression of recombinant CP of tungro viruses and production of antibodies against tungro viruses, respectively.

Keywords: Tungro, RTBV, RTSV, recombinant protein sequence, serology

Pembangunan Kaedah Diagnosis untuk Pengesanan dan Penyaringan Penyakit Tungro Padi di Malaysia Timur

ABSTRAK

Malaysia berinspirasi untuk menjadi sebuah negara yang dapat menampung keperluan beras nasional memandangkan beras merupakan makanan ruji penduduknya. Namun demikian, satu sebaran wabak penyakit padi boleh menyebabkan kerugian yang amat besar di jelapang-jelapang padi negara. Antara penyakit-penyakit virus pada padi yang dikhuatiri di Malaysia ialah penyakit tungro yang disebabkan oleh dua jenis virus, iaitu Rice tungro bacilliform virus (RTBV) and Rice tungro spherical virus (RTSV). Penyakit ini telah dikesan di Malaysia Barat dan di Sabah yang terletak di Malaysia Timur semenjak awal abad kedua-puluh. Penyakit tungro hanya dikesan baru-baru ini di Sarawak, Malaysia Timur. Kajian terhadap penyakit tungro di Malaysia Barat adalah lebih terperinci di mana jujukan nukleotida daripada kedua-dua virus tungro yang diisolat dari Malaysia Barat telah dilaporkan. Sebaliknya, maklumat mengenai penyakit tungro dan virus-virus tungro kurang diketahui di Malaysia Timur. Maka, penyelidikan ini dijalankan dengan objektif untuk mendapatkan jujukan nukleotida virus-virus tungro dari Malaysia Timur dan membangunkan satu kaedah mendiagnosa penyakit tungro dengan menggunakan reagen-reagen seperti serum dan antigen dalam bentuk protein rekombinan yang dihasilkan di dalam makmal. Jujukan protein kot 1 (coat protein 1) (CP1), CP2 dan CP3 pada RTSV, dan jujukan bingkai bacaan terbuka 1 (open reading frame 1) (ORF1), ORF2, ORF3 (gen CP) and ORF4 pada RTBV yang dipencil dari Malaysia Timur setelah dicirikan akan dibandingkan dengan jujukan-jujukan daripada isolat-isolat lain yang telah

diterbitkan melalui kaedah analisis filogenetik. Identiti dan jujukan nucleotida daripada empat belas isolat RTBV dan lima isolat RTSV dari Malaysia Timur yang dikenalpasti daripada penyelidikan ini mungkin merupakan pengekodan pertama untuk virus-virus tungro yang dipencil dari Malaysia Timur. Selepas itu, protein-protein rekombinan dihasilkan daripada pengklonan dan pengepresan gen-gen kod protein RTSV dan RTBV yang dipencilkan dari Sabah dengan menggunakan SUMO fusion expression system. Isolat-isolat tungro yang dipilih didapati mempunyai persamaan yang tinggi dalam jujukan nukleotida dan asid amino dengan isolat-isolat tungro yang lain dari Malaysia Timur. Pemilihan ini dilakukan untuk memastikan antibodi terhadap virus-virus tungro yang dijanakan dengan menggunakan isolat-isolat tungro yang terpilih dapat mengenali semua virus-virus tungro dari Malaysia Timur. Antibodi poliklonal l terhadap virus-virus tungro turut dijanakan di dalam makmal dengan menggunakan virus-virus tungro yang telah dituliskan, dan protein-protein rekombinan yang dihasilkan. Dua protein rekombinan yang berpotensi sebagai antigen untuk menggantikan virus-virus tungro sebagai antigen dalam penghasilan antibodi telah diperolehi untuk projek pengklonan dan ekspresi protein kod rekombinasi, dan satu anti-serum yang reaktif untuk digunakan dalam pengesanan penyakit tungro dengan kaedah serologi telah diperolehi daripada penjanakan antibodi terhadap virus-virus tungro.

Kata kunci: Tungro, RTBV, RTSV, jujukan protein rekombinan, serologi

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