UTILIZATION OF MICROARRAY TECHNOLOGY FOR IDENTIFICATION OF DISEASE RESPONSE GENES IN BANANA (*MUSA* SPP.)

By

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UTILIZATION OF MICOARRAY TECHNOLOGY FOR IDENTIFICATION OF DISEASE RESPONSE GENES IN BANANA (*Musa S*pp.)

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May 2006

Chairman : Associate Professor Suhaimi Napis, PhD

Faculty : Biotechnology and Biomolecular Sciences

Banana is an important food crop in the world after cereals. In 2004, the Asia and Pacific region, Malaysia ranked 3rd for cultivated banana exporter, exporting mainly to Singapore, Hong Kong, Brunei and the Middle East. However, disease has become a major factor contributing to the declining banana industry in Malaysia. Fusarium wilt, primarily caused by *Fusarium oxysporum* f. sp. *cubense*, is a destructive disease, causing production loss of commercial banana cultivars not only Malaysia but worldwide. Up to now, no strategy has been found to effectively combat this disease. In this study, cDNA libraries for *Musa acuminata x balbisiana* cv Mutiara (banana Mutiara, AAB, *Fusarium* tolerant) and *Musa acuminata x balbisiana* cv Rastali (banana Rastali, AAB, *Fusarium* susceptible) were constructed. Five micrograms of mRNA from each banana variety was used in respective for cDNA library construction and 5000 clones from each library were randomly cored and amplified using PCR. Clones were then arrayed on glass slides and gene expression analysis was

carried out. Interesting clones were randomly selected for sequencing and homology search against available databases were made. The emphasis was give to clones that have putative function in pathogen response or are pathogenesis related following *Fusarium* fungal infection. The cDNA microarray analysis identified 55 *M. acuminata x balbisiana* cv Mutiara clones that were transcriptional responsive to the *Fusarium* fungus infection. Several functional types of genes, including those involved in defence response, cell structure, energy, transport, signal transduction and intracellular traffic were up-regulated after *Fusarium* fungus infection. Clones encoded proteins that are involved in primary metabolism, protein destination and storage were down-regulated after *Fusarium* infection. These expression profiles show defence signalling pathways of *M. acuminata x balbisiana* cv Mutiara against *Fusarium* involved considerable interaction between different signalling pathways. Activation of defence response to *Fusarium* fungal attack did not involve individual gene. Additionally, many clones encoding proteins with unknown functions were identified. Functional analysis of these genes could broaden the understanding of disease resistance mechanisms of *M. acuminata x balbisiana* cv Mutiara defence responses to Fusarium wilt of bananas and potentially introduce candidate prevent for this disease in application to molecular breeding.

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

PENGGUNAAN TEKNOLOGI MICROARRAY UNTUK PENGESAHAN GEN-GEN YANG BERTINDAK BALAS TERHADAP PENYAKIT DALAM PISANG (*Musa S*pp.)

Oleh

LIM KEAN JIN

Mei 2006

Pengerusi : Profesor Madya Suhaimi Napis, PhD

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Pisang merupakan salah satu tanaman kormesial yang penting di dunia. Malaysia merupakan pengeksport pisang yang ke-tiga terbesar di antara rantau Asia dan Pasifik. Hasil tanaman ini dieksport ke negara Singapura, Hong Kong, Brunei and Timur Tengah. Penyakit merupakan salah satu faktor utama yang menyebabkan pengurangan hasil tanaman pisang di Malaysia. “Fusarium wilt” adalah satu penyakit yang disebabkan oleh kulat *Fusarium oxysporum* f. sp. *cubense* yang mana boleh memusnahkan tanaman pisang dan menyebabkan kerugian hasil tanaman kormesial ini bukan setakat pengindustrian di Malaysia tetapi juga di seluruh dunia. Pada masa ini, masih tiada satu kaedah yang berkesan untuk mengatasi masalah tersebut. Dalam penyelidikan ini, perpustakaan DNA pelengkap untuk *Musa acuminata x balbisiana* cv Mutiara (pisang Mutiara, AAB, yang rintang kepada *Fusarium*) dan *Musa acuminata x balbisiana* cv Rastali (pisang Rastali, AAB, yang tidak rintang kepada *Fusarium*)

telah dihasilkan. Lima mikrogram mRNA daripada dua variati pisang tersebut diguna untuk menjana dua perpustakaan DNA pelengkap secara berasingan. 5000 klon telah dipilih secara rawak daripada perpustakaan tersebut dan mengamplikasinya dengan teknik PCR. Klon-klon dicetak di atas kepingan kaca dan analisis pengekspresan gen-gen dijalankan. Klon-klon yang diminati dipilih secara rawak untuk penjujukan DNA dan pencarian homologi dengan pengkalan data yang sedia ada. Tumpuan telah diberikan kepada klon-klon yang berpontensi dalam respon kepada patogen atau berkaitan dengan patogenesis selepas diserang oleh kulat *Fusarium*. Dalam kajian ini, didapati 55 klon *M. acuminata x balbisiana* cv Mutiara yang mempunyai tindak balas terhadap kulat *Fusarium* telah diidentifikasi melalui analisasi “microarray”. Diantaranya klon-klon yang dapat diidentifikasi termasuk juga yang mempunyai fungsi berkaitan dalam reaksi kerintangan, struktur sel, tenaga, pengangkutan, trasduksi isyarat serta trafik “intracellular” didapati dirangsang selepas serangan kulat *Fusarium*. Sebaliknya, klon-klon yang mengekodkan protein seperti metabolisma permulaan, destinasi protein serta penyimpanan protein telah digundah selepas penyerangan kulat *Fusarium*. Profil-profil ekspresi ini menunjukan bahawa mekanisma rintangan *M. acuminata x balbisiana* cv Mutiara yang terlibat mempunyai pelbagai hubung kait dengan mekanisma yang lain. Pengaktifan tindak balas ketahanan oleh *M. acuminata x balbisiana* cv Mutiara kepada penyerangan *Fusarium* tidak hanya melibatkan gen tertentu sahaja. Dalam kajian ini, banyak klon yang tidak diketahui fungsi ramalan juga dapat diidentifikasi. Analisis fungsi klon-klon ini membolehkan pemahaman lebih

baik dalam mekanisma sistem kerintangan *M. acuminata x balbisiana* cv Mutiara kepada penyakit “Fusarium wilt”. Dengan ini, tanaman pisang yang berpotensi rintang terhadap penyakit ini dapat diperkenalkan dalam program biakbaka.

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I certify that an Examination Committee has met on **11th May 2006** to conduct the final examination of **Lim Kean Jin** on his **Master of Science** thesis entitled "**Utilization of Microarray Technology for Identification of Disease Response Genes in Banana (*Musa* Spp.)**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis 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 are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for equations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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LIM KEAN JIN

Date: 12 June 2006

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