UNIVERSITI PUTRA MALAYSIA

GENETIC ENGINEERING OF *MUSA SAPIENTUM* L. CV. NANGKA (AAB) FOR TOLERANCE TO FUSARIUM WILT

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By

FATEMEH MAHDAVI DARVARI

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

April 2011

DEDICATION

To my dearest parents, Abed and Shokooh

....who have raised me to be the person I am today

To my kindest husband, Ayyoub

…in all love, humility, and gratitude

To my lovely brother, Mostafa

…for his everlasting love and support

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

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Chairman: **Professor Maziah Mahmood, PhD**

Faculty: **Institute of Tropical Agriculture**

Bananas and plantains are the fourth most important crop in the world as well as Malaysia. In recent years, their production has been seriously threatened by Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *cubense* fungus, and it is one of the most important destructive diseases of banana (*Musa* spp.). Genetic engineering offers the greatest opportunity to the breeders to increase tolerance to Fusarium wilt. Hence, this study aims to produce a new banana cultivar tolerant to *F. oxysporum* f.sp. *cubense* race 4 through genetic engineering. Male inflorescence of Pisang Berangan (AAA), Pisang Nangka (AAB), Pisang Rastali (AAB) and Pisang Abu (ABB) as a potential explant for rapid micropropagation were used for *in vitro* propagation study. Considering the importance of cooking banana in the diet of the local people, Pisang Nangka (AAB), a cooking banana, was chosen for the genetic engineering study. Single cauliflower-like bodies cluster induced

from male flowers, in MS medium containing 8 mg/L of BAP, was used as target tissue for gene transfer. Hygromycin was applied as a selection agent since plasmids used in this study contain the hygromycin resistance gene. Physical and biological parameters which affect DNA delivery into the Pisang Nangka (AAB) single cauliflower-like bodies' have been optimised using *gfp* as reporter gene. The optimised bombardment parameters for the 'CLBs' clusters was 1100 psi, 9 cm target distance, 28 mmHg, 1 µm gold particle size, two times bombardment, 60 µg/µL amount of the gold particles per bombardment, 1.5 µg/µL DNA per bombardment and three days pre-culture prior to the bombardment. Rice thaumatin-like protein gene was cloned in pCambia 1304 binary vector by *Nco*I and *Pml*I restriction enzymes. Transformation of cloned rice thaumatin-like protein (*tlp*) gene was performed using particle bombardment. Integration of transgene was assessed by PCR amplification of *tlp* gene using its specific primer. Genomic southern blot hybridization confirmed the incorporation of the *tlp* gene in the host genome. RT-PCR revealed the expression of transgene in leaf tissue as well as root tissue in transformants.The 28 day old spores of *F. oxysporum* f.sp. *cubense* (Race 4) with 66% germinating capacity were used to estimate the transgenic Pisang Nangka (AAB) plantlets tolerance against Fusarium wilt. The percentage of disease incidence occurred in control plantlets was 89.1% after four weeks of infection while it was 29.4 % in transgenic plants. The results demonstrated that expression of rice thaumatin-like protein in transgenic banana plants enhanced resistanceto Fusarium wilt significantly.

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

KEJURUTERAAN GENETIK *MUSA SAPIENTUM* L. CV.NANGKA (AAB) UNTUK TOLERANSI TERHADAP LAYU FUSARIUM

Oleh

FATEMEH MAHDAVI DARVARI

April 2011

Pengerusi: Profesor Maziah Mahmood, PhD

Fakulti: Institut Pertanian Tropika

Pisang adalah tanaman keempat terpenting di dunia dan Malaysia. Pada tahun kebelakangan ini, hasil tanaman pisang sering kali diserang penyakit layu Fusarium. Penyakit ini disebabkan oleh kulat *Fusarium oxysporum* f.sp. *cubense* dan ia merupakan salah satu penyakit tanaman pisang yang paling merosakkan. Kejuruteraan genetik menawarkan peluang besar kepada penanam untuk meningkatkan kerintangan terhadap penyakit layu Fusarium. Oleh itu, tujuan kajian ini adalah untuk menghasilkan kultivar baru pisang yang rintang terhadap *F. oxysporum* f.sp. *cubense* race 4 melalui kaedah kejuruteraan genetik. Bunga jantan Pisang Berangan (AAA), Pisang Nangka (AAB), Pisang Rastali (AAB) dan Pisang Abu (ABB) sebagai berpotensi untuk eksplan mikropropagasi pantas digunakan untuk kajian propagasi in vitro. Oleh kerana kepentingan pisang masakan dalam diet

penduduk setempat, Pisang Nangka (AAB) iaitu pisang masakan, telah dipilih untuk kajian kejuruteraan genetik. Kelompok 'cauliflower-like body' tunggal yang dijanakan daripada bunga jantan telah digunakan sebagai tisu target untuk pemindahan gen. Hygromycin digunakan sebagai agen pemilih kerana plasmid yang digunakan dalam kajian ini mengandungi gen rintang. Parameter fisikal dan biologi yang mempengaruhi pemindahan DNA kepada kelompok 'cauliflower-like bodies' tunggal Pisang Nangka (AAB) telah dioptimumkan menggunakan gen reporter *gfp*. Parameter pembedilan yang optimum untuk kelompok 'CLBs' adalah 1100 psi, 9 cm jarak sasaran, 28 mmHg, 1 µm saiz partikel emas, dua kali pembedilan, 60 µg/µL jumlah partikel emas setiap pembedilan, 1.5 µg/µL DNA setiap pembedilan and tiga hari pra-kultur sebelum pembedilan. Gen protein 'thaumatin-like padi' telah diklonkan dalam vektor binari pCambia 1304 binary oleh enzim restriksi *Nco*I dan *Pml*I. Transformasi klon gen protein 'thaumatin-like padi' (*tlp*) telah dilakukan menggunakan bedilan partikel bombardment. Integrasi transgene telah dinilai oleh amplikasi PCR gen *tlp* menggunakan primer spesifiknya. Penghibridan 'genomic southern blot' hybridization mengesahkan kemasukan gen tlp dalam genom perumah. RT-PCR menunjukkan ekspresi transgene dalam tisu daun dan tisu akar dalam transforman. Spora *F. oxysporum* f.sp. *cubense* (Race 4) berumur 28 hari yang 66% mampu bercambah, telah digunakan untuk menganggar kerintangan planlet transgenik Pisang Nangka (AAB) terhadap penyakit layu Fusarium. Peratusan insiden penyakit yang berlaku dalam planlet kawalan adalah

89.1% selepas 4 minggu jangkitan sementara 29.4% di dalam pokok transgenik . Keputusan menunjukkan ekspresi protein 'thaumatin-like padi' dalam tumbuhan pisang transgenik meningkatkan kerintangan terhadap layu Fusarium secara signifikan.

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I certify that a Thesis Examination Committee has met on 5 April 2011 to conduct the final examination of Fatemeh Mahdavi Darvari on her thesis entitled "Genetic Engineering of Banana (*Musa* sp.) for Tolerance to Fusarium Wilt" in accordance with the universities and university colleges Act 1971 and the constitution of the Universiti Putra Malaysia [P.U. (A) 106] March 15, 1998. The Committee recommends that the candidate be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not submitted for any other degree at Universiti Putra Malaysia or other institutions.

FATEMEH MAHDAVI DARVARI

Date: 05 April 2011

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