MONITORING SPATIAL DISTRIBUTION OF NONPATHOGENIC *FUSARIUM OXYSPORUM* IN THE RHIZOSPHERE AND ROOTS OF BANANA USING β-D-GLUCURONIDASE AND GREEN FLUORESCENT PROTEIN

By

WONG PUI SEE

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

March 2005

DEDICATION

I dedicate this study to my parents, sisters and brother for their love and affection.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

MONITORING SPATIAL DISTRIBUTION OF NONPATHOGENIC *FUSARIUM OXYSPORUM* IN THE RHIZOSPHERE AND ROOTS OF BANANA USING β-D-GLUCURONIDASE AND GREEN FLUORESCENT PROTEIN

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Chairman: Professor Sariah Meon, PhD

Faculty: Agriculture

This study was conducted to monitor the spatial distribution of nonpathogenic isolate of *Fusarium oxysporum* (Fo4), using a detectable marker. Fo4 was transformed by GUS gene fusion system (*Escherichia coli* β-D-glucuronidase gene) and GFP (Green Fluorescent Proteins). The GUS was detected by 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Gluc). The GFP activity was detected under a fluorescence microscope. There was no different in the cultural and morphological characteristics between the transformed and non-transformed Fo4. The antagonistic activity against *Fusarium oxysporum* f. sp. *cubense* Race 4 (FocR4) was maintained at values within the range of 56.98–41.56% based on the Percentage Inhibition of Radial Growth (PIRG) *in vitro*. DNA polymorphism (RAPD) screening by the primers OPC-15 and OPC-11, confirmed that no mutation had occurred after the transformation and, band GUS-F vs. GUS-R and 5-GFP vs. 3-GFP showed that transformed Fo4 still carried the GUS and GFP gene after five consecutive conidiation/ generation cycles in culture.

The frequency of re-isolation on PCNB medium amended with 40 µg/ml X-Gluc and 200 µg/ml hygromycin B was highest (45.48 x 10^3 cfu/g roots) in the rhizosphere of banana plantlet at day 24, and remained to stabilize at between 37.24 x 10^3 cfu/g roots to 34.52 x 10^3 cfu/g roots until the end of sampling at day 36. The transformed Fo4 can be detected inside the root tissues even 4 days after inoculation although the colony forming units (cfu) (1 cfu/g roots) was substantially lower than that detected on the root surface (11.76 x10^3 cfu/g roots), suggesting that they were colonizing the root and living on the root exudates. The population of transformed Fo4 in the non-rhizosphere soil initially fell sharply with the time of sampling, but thereafter remained stable (4.38-18.00 x 10^4 cfu/g soil) until 36 days of sampling.

Effect of transformed and non-transformed Fo4 on plant growth and development of *Fusarium* wilt was conducted using 10 week-old tissue cultured nursery banana seedlings cv. *Berangan*. The failure to produce *Fusarium* wilt symptom confirmed this strain was nonpathogenic to banana seedlings. The seedlings inoculated with FocR4 were pathogenic to cv. *Berangan* with 100% disease severity. However, the seedlings which had been inoculated with either transformed or non-transformed Fo4 before challenged with FocR4 did not show significant differences ($P>0.05$) in the disease severity, confirmed the stability of the transformed GUS activity and GFP. This study suggests the possibility of using the GUS and GFP genes as visible detectable reporter genes for direct monitoring of the spatial distribution of a potential antagonist in the rhizosphere and roots of banana.

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

PEMANTAUAN TABURAN SPATIAL *FUSARIUM OXYSPORUM* BUKAN PATOGEN DALAM RIZOSFERA DAN AKAR PISANG MENGGUNAKAN β-D-GLUKURONIDAS DAN PROTEIN FLUORESCENS HIJAU

Oleh

WONG PUI SEE

Mac 2005

Pengerusi: Profesor Sariah Meon, PhD

Fakulti: Pertanian

Kajian ini dijalankan untuk memantau taburan *Fusarium oxysporum* (Fo4) bukan patogen menggunakan gen GUS (*Escherichia coli* β-D-glukuronidas gen) dan GFP (protein fluorescens hijau). GUS dikesan menggunakan 5-bromo-4-kloro-3-indolyl-β-D-glukuronida (X-Gluc). Aktiviti GFP dikesan di bawah mikroskop fluorescens. Tiada perbezaan dari segi ciri-ciri kultur dan morfologi di antara Fo4 transforman dan bukan transforman. Aktiviti antagonistic terhadap *Fusarium oxysporum* f. sp. *cubense* Race 4 (FocR4) dikekalkan pada nilai antara 56.98-41.56% berdasarkan Peratusan Perencatan Pertumbuhan Radial (PIRG) secara *in vitro.* Analisis polimorfisme DNA (RAPD) menggunakan primer OPC-15 dan OPC-11 membuktikan tiada mutasi berlaku selepas transformasi, dan jalur GUS-F lawan GUS-R and 5-GFP lawan 3-GFP menunjukkan Fo4 transforman masih membawa gen GUS and GFP sepanjang lima kitaran generasi dalam kultur.

Kekerapan pemencilan semula pada medium PCNB yang mengandungi 40 µg/ml X-Gluc dan 200 µg/ml hygromycin B adalah tertinggi (45.48 x 10^3 cfu/g

akar) di dalam rizosfera pada hari ke-24, dan seterusnya stabil antara 37.24 x 10^3 cfu/g akar ke 34.52 x 10^3 cfu/g akar sehingga akhir tempoh penyampelan pada hari ke-36. Fo4 transforman dikesan dalam tisu akar pada hari ke-4 (1 cfu/g akar) selepas penginokulatan sungguhpun unit pembentukan koloni (cfu) adalah rendah daripada yang dikesan pada rizosfera (11.76 x10^3 cfu/g akar), menunjukkan Fo4 transforman boleh hidup pada eksudat akar. Populasi Fo4 transforman di dalam tanah pada mulanya menurun dengan mendadak mengikuti tempoh penyampelan, tetapi kekal stabil selepas itu (4.38-18.00 x 10^4 cfu/g tanah) sehingga hari ke-36 penyampelan.

Kesan Fo4 transforman dan bukan transforman ke atas pertumbuhan dan pembentukan layu *Fusarium* diuji pada anak benih pisang cv. *Berangan* berumur 10 minggu. Tiada pembentukan gejala penyakit layu dicatatkan, membuktikan bahawa ia adalah tidak patogenik. Anak benih pisang cv. *Berangan* yang diinokulat dengan FocR4 mencatatkan 100% pembentukan keterukan penyakit layu *Fusarium*. Tetapi apabila anak pokok diinokulat dengan Fo4 transforman atau bukan transforman terlebih dahulu sebelum dilawan dengan FocR4, tiada perbezaan bererti ($P>0.05$) dicatatkan dalam penilaian keterukan penyakit. Ini membuktikan kestabilan transforman Fo4 dan aktiviti GUS dan GFP. Kajian ini mencadangkan kemungkinan menggunakan gen GUS dan GFP sebagai gen pengesan yang nyata untuk pemantauan taburan spatial antagonis yang berpotensi dalam rizosfera dan akar pisang.

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I certify that an Examination Committee has met on 30th March 2005 to conduct the final examination of Wong Pui See, on her Master of Science thesis entitled "Monitoring the Spatial Distribution of Nonpathogenic *Fusarium oxysporum* in the Rhizosphere and Roots of Banana Using β-D-glucuronidase and Green Fluorescent Protein" 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 requirements 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 quotations 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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WONG PUI SEE

Date:

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