
**UNIVERSITI PUTRA MALAYSIA**

# *BIOLOGICAL CHARACTERIZATION AND GENETIC DIVERSITY OF Fusarium spp. ASSOCIATED WITH YELLOWING DISEASE IN BLACK PEPPER (Piper nigrum L.) IN MALAYSIA*

**SAHAR SHAHNAZI**

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**By**

**SAHAR SHAHNAZI**

**Thesis Submitted to the School of Graduate studies, Universiti Putra Malaysia in Fulfillment of the Requirement for the Degree of Doctor of Philosophy**

**June 2012**

**DEDICATION**

This thesis is dedicated to:

My beloved mother (Mahasti), father (Mohammad Hasan),

sister, brother, my husband and my brother in law

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

**BIOLOGICAL CHARACTERIZATION AND GENETIC DIVERSITY OF *Fusarium* spp. ASSOCIATED WITH YELLOWING DISEASE IN BLACK PEPPER (*Piper nigrum* L.) IN MALAYSIA**

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**June 2012**

**Chairman: Professor Sariah Meon, PhD**

**Faculty: Agriculture**

Yellowing disease or slow decline is one of the most important diseases of black pepper (*Piper nigrum* L.). To characterise the pathogen(s) responsible for yellowing disease of black pepper in Malaysia, 53 isolates of *Fusarium* were collected from the roots of diseased black pepper plants and from rhizosphere soils from major growing areas in Sarawak and Johor. 34 isolates of *F. solani* and 19 isolates of *F. proliferatum* were characterised and identified based on morphological characteristics and molecular techniques. DNA sequencing of the internal transcribed spacers (ITS1 and ITS2) and 5.8S ribosomal DNA (rDNA) regions was conducted to identify the *Fusarium* species. Nucleotide sequence

analysis of the ITS regions revealed that this molecular technique enabled identification of *Fusarium* at the species level as *F. solani* and *F. proliferatum.* A pathogenicity test on three-month-old black pepper seedlings (cv. Kuching) demonstrated that *F. solani* is the dominant pathogen of the disease, whereas *F. proliferatum* is non-pathogenic. ISSR analysis and FAME profiles were used to examine genetic and biochemical relationships of *Fusarium* isolates. ISSR marker was used in the genetic diversity analysis of 34 *F. solani* isolates and 19 *F. proliferatum* isolates. Results indicate that there is a high level of genetic variation among the isolates of *F. solani* and also among the isolates of *F. proliferatum*. Among three populations of *F. solani*, population II (Sarikei) had the highest genetic diversity levels, whereas population III (Kulai) had the lowest genetic diversity levels. The low similarity index value between the two clusters of *F. solani* isolates showed the high genetic variability among the isolates. Fatty acid analysis was carried out to characterize, differentiate and determine biochemical relationships between and among *F. solani* and *F. proliferatum* isolates. Fatty acid analysis showed palmitic acid, stearic acid, oleic acid and linoleic acid were the most abundant fatty acids in these species. The most predominant fatty acid was linoleic acid (37.44 %) in *F. solani* and oleic acid (39.81%) in *F. proliferatum.* Our study suggests that fatty acid profiles have the potential to be used as a diagnostic tool for *F. solani* and *F. proliferatum* and isolates of these species could be characterized and differentiated at species levels. Both principal component analysis and cluster analysis showed clear

biochemical relationships among isolates of these species. Some relationships between ISSR and FAME data were found. FAMEs data of *Fusarium* isolates supported ISSR findings. Both FAME and ISSR profiles can be used to study phenotypic and genetic diversity in *Fusarium* species and showed that there are some level of similarities between both techniques. The results of ISSR and FAME data showed that clusters were not related to geographic origin. The information obtained from FAME and ISSR data would help the breeder to develop resistant cultivars of black pepper, and also to assist in disease management.

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

**PENCIRIAN SECARA BIOLOGI DAN KEPELBAGAIAN GENETIK *Fusarium* spp. YANG DIKAITKAN DENGAN PENYAKIT KEKUNINGAN PADA LADA HITAM (*Piper nigrum* L.) DI MALAYSIA**

Oleh

**SAHAR SHAHNAZI**

**Jun 2012**

**Pengerusi: Profesor Sariah Binti Meon, PhD**

**Fakulti: Pertanian**

Penyakit kekuningan atau kemerosotan perlahan adalah salah satu daripada penyakit penting pada lada hitam (*Piper nigrum* L.). Untuk pencirian patogen penyebab penyakit kekuningan lada hitam di Malaysia, 53 isolat telah dikumpul daripada akar lada hitam terjangkit dan juga tanah rizosfera daripada kawasan penanaman utama di Sarawak dan Johor. 34 isolat *F. solani* dan 19 isolat *F. proliferatum* telah dicirikan dan dikenalpasti berdasarkan ciri-ciri biologi dan mengunakan kaedah molekular. Jujukan DNA daripada kawasan "Internal transcribed spacers" (ITS1 dan ITS2) dan "5.8S ribosomal DNA" (rDNA) telah dijalankan untuk mengenalpasti spesis *Fusarium.* Analisis jujukan nukleotida pada kawasan ITS mendedahkan bahawa kaedah molekular ini berupaya

mengenalpasti *Fusarium* sehingga ke peringkat spesis seperti *F. solani* dan *F. proliferatum*. Ujian patogenisiti pada pokok lada hitam (cv. Kuching) berusia tiga bulan menunjukkan bahawa *F. solani* adalah patogen dominan bagi penyakit ini manakala *F. proliferatum* adalah bukan patogen. Analisis ISSR dan pemprofilan FAME telah digunakan untuk menentukan kepelbagaian genetik dan perhubungan biokimia di kalangan isolat *Fusarium*. Penanda ISSR telah digunakan dalam analisis kepelbagaian genetik bagi 34 isolat *F. solani* dan 19 isolat *F. proliferatum.* Keputusan menyatakan bahawa terdapat variasi genetik yang tinggi di kalangan isolat *F. solani* dan *F. proliferatum.* Di antara ketiga-tiga populasi *F. solani*, populasi II (Sarieki) mempunyai paras kepelbagaian genetik yang tinggi manakala populasi III (Kulai) mempunyai kepelbagaian genetik yang rendah. Nilai indek kesamaan yang rendah di antara dua kluster isolat *F. solani* telah ditunjukkan tetapi mempunyai perbezaan yang tinggi di kalangan isolat. Analisis asid lemak telah dijalankan untuk tujuan pencirian, pembezaan dan menentukan perhubungan biokimia di antara dan di kalangan isolat *F. solani* dan *F. proliferatum*. Analisis asid lemak menunjukkan bahawa asid palmitik, asid sterik, asid olik dan asid linolik adalah asid lemak yang utama bagi bagi kedua-dua spesis. Asid lemak linolik (37.44%) adalah paling banyak pada *F. solani* dan asid lemak olik (39.81%) pada *F. proliferatum*. Kajian kami mencadangkan bahawa pemprofilan asid lemak mempunyai potensi untuk digunakan sebagai alatan diagnostik bagi kedua-dua *F. solani* dan *F. proliferatum* dan isolat bagi kedua-duanya boleh diciri dan dibezakan hingga ke paras

subspesis. Kedua-dua kaedah analisis iaitu analisis prinsip bahagian dan analisis kluster, jelas menunjukkan perhubungan biokimia di kalangan isolat bagi kedua-dua spesis. Sejumlah perhubungan di antara data ISSR dan FAME telah ditemui. Data FAME bagi isolat *Fusarium* menyokong penemuan ISSR. Kedua-dua kaedah iaitu FAME dan ISSR boleh digunakan untuk mengkaji ciri-ciri finotip dan kepelbagaian genetik bagi spesis *Fusarium* dan menunjukkan terdapat sejumlah paras kesamaan di antara kedua-duanya. Keputusan ISSR dan FAME menunjukkan kluster isolat tidak berkait dengan kawasan geografi asalnya. Maklumat yang diperolehi daripada FAME dan ISSR boleh membantu pembiak mendapat kultivar rintang bagi lada hitam dan juga membantu dalam pengurusan penyakit.

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I certify that a Thesis Examination Committee has met on 7 June 2012 to conduct the final examination of Sahar Shahnazi on her thesis entitled "Biological characterization and genetic diversity of *Fusarium* spp. associated with yellowing disease in black pepper (*Piper nigrum* L.) in Malaysia" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the doctor of philosophy.
Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment 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 concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

**SAHAR SHAHNAZI**

Date: 7 June 2012

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