



UNIVERSITI PUTRA MALAYSIA

**PATHOTYPES OF PSEUDOPERONOSPORA CUBENSIS (BERK. ET
CURT.) ROSTOW, CAUSAL AGENT OF DOWNY MILDEW AND
INDUCED DEFENSE RESPONSES IN THE HOST**

MANSOUR SALATI

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ROSTOW, CAUSAL AGENT OF DOWNY MILDEW AND INDUCED
DEFENSE RESPONSES IN THE HOST**

By

MANSOUR SALATI

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

March 2011



DEDICATION

WITH LOVE AND APPRECIATION TO:

My Beloved Wife: Maryam Hosseini

My Son: Amir Hossein



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

**PATHOTYPES OF *PSEUDOPERONOSPORA CUBENSIS* (BERK. ET CURT.)
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Chairman : Wong Mui Yun, PhD

Faculty : Agriculture

There is abundant literature on aspects of molecular biology which tended to focus on taxonomic and phylogenetic studies in Oomycete. However, information on local intraspecific populations or pathotype levels of *Pseudoperonospora cubensis*, the causal agent of downy mildew of cucurbit, is lacking. Host specificity identification is an important aspect of downy mildew disease management, especially for plant breeding purposes and in studies on population biology. Surveys were conducted at five states of West Malaysia to study *P. cubensis* pathotypes during 2008 and 2009. The sampling sites included farms where cucurbits, such as *Cucumis sativus*, *Cucumis melo*, *Luffa cylindrica* and *Trichosanthes cucumerina* were grown. Germination test confirmed that only 13 of 29 isolates have the ability to sporulate, and were used for further studies.



The results of the present investigation using leaf disc assay showed that there was high variability in *P. cubensis* pathotypes in West Malaysia (12 pathotypes from 13 isolates in five states). The majority of the isolates obtained in this study were categorized in medium and high pathogenicity groupings, and this illustrates the potential of this pathogen in invading cucurbit fields in tropical regions. Morphological characterization indicated that there is no relationship between pathotype and the size and shape of sporangia. Molecular characterization of the pathotypes based on ITS region revealed that the percent of homology among the 13 isolates and similar sequences from GenBank was high (99%). Phylogenetic analysis of the 13 isolates based on neighbor-joining method on ITS regions revealed five groupings. The highest variation in nucleotide sequence was found in the ITS2 region followed by ITS1, and 5.8S region served as a conserve region with no variation in nucleotide sequence. The phylogram from 13 sequences of COX-II region based on neighbor-joining method categorized isolates into three groupings. The results indicated that both selected regions were inadequate to be used for differentiation of *P. cubensis* pathotypes. The newly designed species-specific primers on ITS and COX-II regions successfully amplified (528 and 253 bp) rDNA and mitochondria of *P. cubensis*, respectively. However, no PCR products were obtained for the new designed sets when the primers were tested against different isolates of fungi as negative control including *Fusarium solani*, *Phomopsis langicola*, *Lasiodiplodia theobromae*, *Fusarium oxysporum*, *Pythium sp.*, *Phytophthora sp.*, *Pyricularia oryzae*, *Aspergillus flavus* and *Aspergillus niger*.

A higher activity on chitinase and glucanase enzymes was detected on watermelon than cucumber plants at 12 hours after treatment, only with chitosan as an inducer of plant



resistance. The results of nitric oxide (NO) and chitinase and glucanase enzymes detection in competitive study showed that increased amount of NO was detected in plants treated with chitosan. Chitinase and glucanase enzymes were also produced in chitosan treated plants with a peak at 12 hours after inoculation with the pathogen. The peak of NO generation in chitosan treatment was 4 hours earlier than the peak of chitinase and glucanase detection. Expression of chitinase and glucanase genes was marked at 4 hours before maximum detection of chitinase and glucanase enzymes, respectively. The NOA expression was conducted based on amplification of NOA associated protein which was designed based on tobacco and potato. Disease assessment confirmed that production of NO in the NO donor treatment (chitosan) plays a critical role in mediating the defense responses in cucumber against downy mildew disease with 58.1% protection. Disruption in NO production would have negative effects on chitinase and glucanase enzymes levels and increase disease incidence up to 60.6% and 71.1% for LNAME and CPTIO treatment, respectively in comparison with control (96.6%). These results demonstrated the there is a relationship between chitinase and glucanase enzymes detection and NO emission in cucumber. The transcript of NOA led to production of NO which suppressed disease incidence. This provides additional evidence for over expression of NOA in cucumber for future study.



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

**PATOTIP *PSEUDOPERONOSPORA CUBENSIS* (BERK. ET CURT.) ROSTOW,
PENYEBAB PENYAKIT KULAPUK DOWNY DAN RESPON KETAHANAN
TERANGSANG PADA PERUMAH**

Oleh

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Terdapat banyak literatur tentang aspek- aspek biologi molekul yang cenderung untuk memberi fokus pada kajian taksonomi dan filogenetik dalam ‘Oomycete’. Namun, maklumat tentang populasi tempatan intraspesifik atau peringkat patotip *Pseudoperonospora cubensis*, penyebab penyakit kulapuk downy pada tanaman ‘cucurbit’, kurang. Pengenalan kekhususan perumah merupakan aspek penting dalam pengurusan penyakit kulapuk downy, terutamanya untuk tujuan pembiakbakaan tanaman dan kajian ke atas biologi populasi. Tinjauan telah dilakukan di lima negeri yang terletak di Malaysia Barat untuk mempelajari patotip *P. cubensis* pada tahun 2008 dan 2009. Penyampelan dilakukan di kawasan- kawasan penanaman ‘cucurbit’, seperti *Cucumis sativus*, *Cucumis melo*, *Luffa cylindrica* dan *Trichosanthes cucumerina*. Ujian percambahan membuktikan bahawa hanya 13 daripada 29 isolat mempunyai kemampuan untuk bercambah, dan ia digunakan dalam kajian lanjut.



Hasil yang diperolehi daripada ujian cakera daun menunjukkan bahawa terdapat perbezaan yang tinggi dalam patotip *P. cubensis* di Malaysia Barat (12 patotip dari 13 isolat di lima negeri). Sebahagian besar isolat yang diperolehi dalam kajian ini dikategorikan dalam kumpulan kepatogenan sederhana dan tinggi, dan ini menggambarkan potensi patogen ini dalam pencerobohan kawasan penanaman 'cucurbit' di kawasan tropika. Ciri-ciri morfologi menunjukkan bahawa tidak ada hubungan antara patotip dengan saiz dan bentuk sporangium.

Ciri-ciri molekul patotip berdasarkan kawasan ITS mendedahkan bahawa peratus homologi antara 13 isolat dengan urutan nukleotida yang sama dari GenBank adalah tinggi (99%). Analisis filogenetik yang dijalankan ke atas 13 isolat melalui kaedah 'neighbor- joining' terhadap kawasan ITS menghasilkan lima kumpulan. Variasi tertinggi dalam urutan nukleotida dijumpai di kawasan ITS2 diikuti oleh ITS1, manakala 5.8S berfungsi sebagai kawasan terpelihara tanpa variasi dalam urutan nukleotida. 'Phylogram' yang dibentuk daripada 13 urutan nukleotida kawasan COX-II melalui kaedah 'neighbor- joining' dikategorikan menjadi tiga kumpulan. Keputusan kajian ini menunjukkan bahawa kedua-dua kawasan yang terpilih tidak cukup untuk membezakan patotip *P. cubensis*. Primer 'species- specific' yang direka bagi kawasan-kawasan ITS dan COX-II berjaya mengamplifikasi (528 dan 253 bp) rDNA dan mitokondria *P.cubensis*, masing-masing. Namun, produk PCR tidak diperolehi apabila primer 'species- specific' diuji terhadap pelbagai isolat kulat sebagai kawalan negatif iaitu *Fusarium solani*, *Phomopsis langicola*, *Lasiodiplodia theobromae*, *Fusarium oxysporum*, *Pythium* sp., *Phytophthora* sp., *Pyricularia oryzae*, *Aspergillus flavus* dan *Aspergillus niger*.



Satu aktiviti yang lebih tinggi terhadap enzim kitinase dan glukukanase telah dikesan pada tanaman tembikai berbanding dengan tanaman timun, 12 jam selepas rawatan dengan 'chitosan' sahaja sebagai agen pencetus ketahanan tanaman. Keputusan kajian persaingan bagi mengesan enzim-enzim nitrik oksida (NO), kitinase dan glukukanase menunjukkan bahawa peningkatan jumlah NO dikesan pada tanaman yang dirawat dengan 'chitosan'. Enzim-enzim kitinase dan glukukanase juga dihasilkan dalam tanaman yang dirawat dengan 'chitosan', di mana ia mencapai puncak pada 12 jam selepas penginokulatan patogen dijalankan. Puncak bagi generasi NO dengan rawatan 'chitosan' adalah 4 jam lebih awal daripada puncak pengesanan kitinase dan glukukanase. Pengekspresan gen kitinase dan glukukanase telah ditandakan 4 jam sebelum pengesanan maksimum enzim kitinase dan glukukanase. Pengekspresan NOA dilakukan berdasarkan amplifikasi protein yang berkaitan dengan NOA yang direka berdasarkan tembakau dan ubi kentang. Penilaian penyakit menegaskan bahawa pengeluaran NO dalam rawatan penderma NO ('chitosan') memainkan peranan penting dalam pengantaraan respon pertahanan dalam timun terhadap penyakit kulapuk downy dengan perlindungan sebanyak 58.1%. Gangguan dalam pengeluaran NO mungkin mempunyai kesan negatif terhadap tahap enzim kitinase dan glukukanase dan meningkatkan kejadian penyakit sehingga 60.6% dan 71.1% untuk rawatan LNAME dan CPTIO masing-masing berbanding dengan kawalan (96.6%). Keputusan ini menunjukkan wujudnya hubungan antara pengesanan enzim kitinase dan glukukanase dan pembebasan NO dalam timun. Transkrip NOA menyebabkan pengeluaran NO yang mengurangkan kejadian penyakit. Hasil kajian ini memberikan bukti tambahan kepada pengekspresan lebih NOA pada tanaman timun untuk kajian di masa depan.

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I certify that a Thesis Examination Committee has met on 29th March 2011 to conduct the final examination of Mansour Salati on his thesis entitled“ Pathotypes of *Pseudoperonospora cubensis* (Berk. et Curt.) Rostow, Causal Agent of Downy Mildew and Induced Defense Responses in the Host” in accordance with the Universities and University Colleges 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.

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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.

MANSOUR SALATI

Date: 29 March 2011



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