



UNIVERSITI PUTRA MALAYSIA

**DIFFERENTIAL REACTION OF HOSTS, GENE
CHARACTERIZATION AND MANAGEMENT OF
HUANGLONGBING USING CHEMICAL
TREATMENTS**

HAJI VAND SHOKROLLAH

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By

HAJI VAND SHOKROLLAH

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

September 2010



DEDICATION

Specially dedicated to:

My lovely wife (Mahin) for her endless and boundless love, understanding and encouragement throughout my study, my dear son (Pooya) how he gave me peace and motivation every time by saying “baba don’t turn on your laptop please” or “please don’t go to UPM today” and I said: I love you that’s why I do that, and my new born baby (Houra) and my dearest mother. To my strong, brave and loving father who already passed away, for all his inspiring words and to all his sacrifices for me and my family making me who I am today.



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: Associate Professor Thohirah Lee Abdullah, PhD

Faculty: Agriculture

Citrus is an important crop worldwide and the total production of citrus was about 105.4 million tons in 2006. Unfortunately many diseases have been threatening citrus. The disorder of citrus described as Huanglongbing (HLB) which is known as citrus greening disease is caused by the fastidious phloem limited bacterium in the genus *Candidatus Liberibacter* and caused severe damage on citrus in more than forty countries including Malaysia. Although HLB has been detected in citrus in Malaysia, there have been few reported studies in the country. Study on screening the local and non-local *Citrus* species in Malaysia would be useful to select the best rootstocks and interstocks against the HLB causing greening disease in *Citrus* species. There is also very little information on seed transmission of HLB disease, pathogen movement and the good agriculture practices there can control and reduce



greening disease severity of citrus. Seeds were collected from infected trees and germinated in a screenhouse condition. New leaves were collected two months after germination for detection of the HLB using conventional PCR. HLB was not detected in the seed and HLB is not a seed borne disease. The healthy *Citrus reticulata* (Limau madu) seedlings were used to detect the HLB pathogen movement. They were inoculated using grafting method and the HLB movement evaluated using PCR test after two months. HLB can reach to the roots system twenty six weeks after inoculation. This means that resistant or tolerant rootstocks maybe effective to control or to reduce the severity of HLB because rootstocks provide the root system for the scion. Screening of eighteen *Citrus* species for susceptibility to HLB was carried out through grafting. *C. reticulata* that showed typical symptoms of HLB was identified and confirmed by PCR test and used as a source of inoculums. Infected *Citrus* species showed low total biomass, plant height and stem diameter when compared to non-inoculated *Citrus* species. The *C. reticulata*, *C. sinensis*, and *C. microcarpa* showed lowest total biomass, plant height and stem diameter. HLB was detected on 15 species by PCR test, and the species were categorized into three groups; severe group from 58 to 80% severity, includes *C. reticulata*, *C. sinensis*, *C. reshni* (cleopatra), moderate group from 41 to 50% severity, includes *Furtunella* spp. (Kasturi Chini), *C. macrophylla*, *C. microcarpa* (Kasturi), mild group from 17 to 25% severity, includes *C. medica*, *C. aurantifolia* (Limau nipis), *Citrus* sp. (Limau naga), *C. jambhiri* and three *Citrus* sp. (Sr.No. 12, 15 and 16). HLB was detected in *C. aurantium* and *C. aurantifolia* by conventional PCR test (16% PCR test and without symptoms). Effects of rootstocks and interstocks against HLB disease were studied. The tolerant and resistant species were chosen for rootstocks and interstocks and evaluated using conventional and nested-

PCR. HLB symptom was not observed when *C. grandis* used as a rootstock and *C. hystrix* used as an interstock and vice versa with *C. reticulata* as the scion. In this combination, dry matter, plant height and stem diameter also were significantly increased compared to combination with *C.aurantium* and *C. aurantifolia*. High percentage of disease severity was measured in combination between *C. aurantium* rootstock and *C. aurantifolia* interstock. *Ca. Liberibacter asiaticus* was detected in all combinations using nested PCR. Effects of chemical treatments on HLB infected *C. reticulata* orchard were conducted in Kuala Terengganu. The results showed a significant difference among the treatments. Fruit quantity and quality were significantly increased on treatments T4 (antibiotic + GA₃), T7 (antibiotic + GA₃ + foliar fertilizer) and T5 (antibiotic + foliar fertilizer) compared to fruit quantity and quality of T8 (control). Total thickness of cell wall also were significantly increased on T4, T7, T5 and T6 (GA₃ + foliar fertilizer) compared to T8 (control), T3 (foliar fertilizer), T2 (GA₃) and T1 (antibiotic). HLB bacteria were successfully detected using nested-PCR on all treatments. Spherical and rod shape of *Ca. Liberibacter asiaticus* bacteria were detected in sieve tube cells of phloem. Cross section of sieve tube showed abundant bacteria on the cells and it damaged cell wall and middle lamella. A new strain of HLB was successfully obtained and registered (GU133055) in NCBI gene bank as new strain of HLB from *C. reticulata* in Terengganu, Malaysia.

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

**PERBEZAAN TINDAK BALAS PERUMAH, PERWATAKAN GEN DAN
PENGURUSAN HUANGLONGBING DENGAN MENGGUNAKAN
RAWATAN KIMIA**

Oleh

HAJI VAND SHOKROLLAH

September 2010

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Limau merupakan tanaman yang penting di dunia dan jumlah pengeluaran limau adalah kira-kira 105.4 ribu tan pada tahun 2006. Malangnya pelbagai penyakit telah mengancam pengeluaran limau. Gangguan penyakit pada pokok limau yang dikenali sebagai Huanglongbing (HLB) juga diketahui sebagai “citrus greening disease” yang dijangkiti oleh bacteria penggali floem terhad yang tergolong dalam genus *Candidatus Liberibacter* dan menyebabkan kemusnahan yang teruk pada limau yang ada di lebih daripada 40 negara termasuk Malaysia. Walaupun HLB telah dikesan pada limau di Malaysia, kajian yang dikemukakan di negara ini masih kurang. Kajian dalam penyaringan spesis limau tempatan dan bukan tempatan di Malaysia bakal berguna dalam pemilihan pokok penanti dan pokok penanti tengah yang terbaik untuk mencegah patogen HLB yang menyebabkan “greening disease” pada limau. Maklumat mengenai penjangkitan penyakit HLB melalui biji benih, pergerakan patogen dan amalan pertanian yang baik untuk mengawal dan mengurangkan keterukan “greening disease” pada limau juga sangat kekurangan.

Biji benih telah dikumpul daripada pokok yang dijangkit dan dicambahkan dalam rumah saringan. Daun baru telah disampel dua bulan selepas percambahan untuk mengesan HLB dengan menggunakan PCR konvensional. HLB tidak dapat dikesan pada biji benih, maka HLB bukan penyakit yang berasal daripada biji benih. Anak pokok *Citrus reticulata* yang sihat telah digunakan untuk pergerakan patogen HLB. Anak pokok telah diinokulasikan melalui kaedah cantuman dan pergerakan HLB diperiksa selepas dua bulan dengan menggunakan ujian PCR. HLB dikesan di sistem akar 26 minggu selepas inokulasi. Ini bermaksud pokok penanti yang resistan atau toleran mungkin berkesan untuk mengawal atau mengurangkan keparahan HLB kerana pokok penanti membekalkan sistem akar untuk Sion. Penyaringan lapan belas spesis limau yang rentan terhadap penyakit HLB telah dijalankan melalui cantuman. *C. reticulata* (Limau madu) yang menunjukkan simptom khas HLB telah dikesan dan dipastikan melalui ujian PCR dan digunakan sebagai sumber inokulasi. Spesis limau yang dijangkit menunjukkan jumlah biomas, ketinggian dan diameter batang yang rendah berbanding dengan spesis limau yang sihat. *C. reticulata*, *C. sinensis*, dan *C. microcarpa* menunjukkan jumlah biomas, ketinggian dan diameter batang yang paling rendah. HLB telah dikesan pada 15 spesis limau dengan menggunakan ujian PCR dan dikategorikan kepada tiga kumpulan; kumpulan parah dengan indeks keparahan dari 58 hingga 80%, yang termasuk *C. reticulata*, *C. sinensis*, *C. reshni* (cleopatra); kumpulan sederhana dengan indeks keparahan dari 41 hingga 50%, yang termasuk *Furunculata spp.* (Kasturi Chini), *C. macrophylla*, *C. microcarpa* (Kasturi); kumpulan ringan dengan indeks keparahan dari 17 hingga 25%, yang termasuk *C. medica*, *C. aurantifolia* (Limau nipis), *Citrus sp* (Limau naga), *C. jambhiri* dan tiga *Citrus sp* (Sr.No. 12, 15 dan 16). HLB telah dikesan pada *C. aurantium* dan *C. aurantifolia* dengan menggunakan ujian PCR (16% dengan menggunakan ujian PCR

dan tanpa simptom). Kesan pokok penanti dan penanti tengah terhadap penyakit HLB telah dikaji. Spesis yang toleran dan resistan telah dipilih sebagai pokok penanti dan pokok penanti tengah dan dinilai menggunakan konvensional dan nested PCR. Simtom HLB tidak dikesan apabila *C. grandis* digunakan sebagai pokok penanti dan *C. hystrix* digunakan sebagai pokok penanti tengah dan sebaliknya pada *C. reticulata* yang dijadikan sebagai sion. Dalam kombinasi ini, kandungan kering, tinggi pokok dan diameter batang juga meningkat dengan nyata sekali apabila dibandingkan dengan kombinasi *C. aurantium* dan *C. aurantifolia*. Peratus keterukan penyakit yang tinggi telah dikesan dalam kombinasi antara pokok penanti *C. aurantium* and penanti tengah *C. aurantifolia*. *Candidatus Liberibacter asiaticus* dikesan pada semua kombinasi dengan menggunakan nested PCR. Kesan rawatan kimia pada kebun *C. reticulata* yang dijangkiti HLB telah dijalankan di Kuala Terengganu. Keputusan yang dapat menunjukkan perbezaan yang nyata di antara rawatan. Kualiti dan kuantiti buah nyata meningkat pada rawatan T4 (antibiotik + GA₃), T7 (antibiotik + GA₃+ baja davn) dan T5 (antibiotik + baja davn) berbanding dengan kualiti dan kuantiti buah T8 (kawalan). Jumlah ketebalan dinding sel juga nyata meningkat pada T4, T7, T5 dan T6 (GA₃ + baja davn) berbanding dengan T8 (kawalan), T3 (baja davn), T2 (GA₃) dan T1 (antibiotik). Bakteria HLB telah berjaya dikesan dengan menggunakan Nested-PCR pada semua rawatan. *Candidatus Liberibacter asiaticus* bakteria yang berbentuk sfera dan rod telah dikesan di dalam sel tiub tapis floem. Keratan rentas tiub tapis menunjukkan banyak bakteria pada sel dan telah merosakkan dinding sel dan lamella tengah. Satu strain HLB yang baru telah berjaya didapati dan telah dihantar ke NCBI untuk pendaftaran (GU133055) sebagai strain baru HLB daripada *C. reticulata* di Terengganu.

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I certify that a Theses Examination Committee has met on 9/6/2010 to conduct the final examination of Haji Vand Shokrollah on his thesis entitled “Differential reaction of hosts, gene characterization and management of huanglongbing using chemical treatments” in accordance with the Universities and Universiti Colleges Act 1971 and the Constitution of the Universiti Pertanian Malaysia [P.U. (A) 106] 15 March 1990. The Committee recommends that the student be awarded the relevant degree of Doctor of Philosophy. Members of the thesis Examination 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 institution.

HAJI VAND SHOKROLLAH

Date: 6 September 2010

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