STATUS OF CITRUS CANKER IN ETHIOPIA AND MALAYSIA, AND CHARACTERIZATION OF THE CAUSAL AGENT

By

ESHETU DERSO KIDANU

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

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DEDICATION

To the memory of my mother the late Shiwaye Derso who sacrificed everything to bring me up with love and affection.

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

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Faculty: Agriculture

Citrus canker disease surveys were conducted between August to November 2003 in Ethiopia and between January to April 2004 in West Malaysia. The pathogens were isolated and identified based on morphological characteristics and pathogenicity tests on seedlings of citrus cultivars using isolates collected from West Malaysia. Biochemical characterizations of the isolates were also carried out using standard determinative tests. In Ethiopia, citrus canker was observed only on sour orange (*Citrus aurantium*) and Mexican lime (*C. aurantifolia)*. Disease incidence in the field on Mexican lime leaves in Ethiopia was 71.4% and severity was 26.8%; incidence on fruits was 30% and severity 21.25%. This is the first confirmed report of the disease in Ethiopia. In Malaysia, the disease was observed on Mexican lime (*C. aurantifolia)*, pomelo (*C. grandis*) and kaffier lime (*C. hysterix*). Overall, the disease incidence in Malaysia was of 36.5% and severity of 12.5% on leaves, while incidence on fruits was 18.7% and severity 7.5%. Growth of *X. axonopodis* pv. *citri* in yeast dextrose chalk agar (YDCA) was not as fast as in peptone

sucrose agar (PSA) or nutrient glucose agar (NGA). However, the former medium was very selective to X*anthomonas* species. There were highly significant differences in lesion size for cultivars ($P \leq 0.01$) but not for isolates. Significant positive regression ($P \leq 0.05$) was observed between lesion size and time after inoculation. Repeated measure analysis using general linear model (GLM) for correlation between times after inoculation was highly significant ($P \leq 0.01$). No variation in pathogenicity was observed among the isolates. Population sizes increased by over 2 Log cfu/lesion on Mexican lime and nearly by 1.5 Log units on sour orange and pomelo and remained around the initial inoculum level on calamondin. Significant positive correlations were observed between *X. axonopodis* pv. *citri* population and lesion size on sour orange ($r = 0.57$, $P = 0.024$), pomelo ($r = 0.73$, $P = 0.018$) and Mexican lime ($r = 0.76$, $P = 0.001$). The correlation was relatively the highest for Mexican lime ($r = 0.76$) and lowest for calamondin ($r = 0.25$). The interactions between isolates and cultivars were highly significant ($P \leq 0.01$). Tukey tests showed no significant differences in reaction to *X. axonopodis* pv. *citri* isolates between sour orange and pomelo, sour orange and sweet orange, Mexican lime and grapefruit, and also between calamondin and sweet orange. The six citrus test cultivars were all susceptible to the 15 *X. axonopodis* pv. *citri* isolates and citrus canker lesions were induced on the detached leaves. There was highly significant ($P \leq 0.01$) interaction between cultivars and all strains. Disease severity on detached leaves was significantly correlated ($P \leq 0.01$) with disease severity ratings in attached leaf test studies and was relatively the highest ($r = 0.97$) for Mexican lime. In the biochemical characterization study, all 15 isolates of *X. axonopodis* pv. *citri* showed similar results using standard determinative tests. Field host ranges for citrus canker in Ethiopia were Mexican lime and

sour orange, while in West Malaysia it appears to be wider than in Ethiopia. In conclusion, on the basis of their host range in seedling tests, morphological characteristics, pathogenicity tests, population growth in *planta* and biochemical characteristics, the 15 representative West Malaysian isolates of *X. axonopodis* pv. *citri* were characterized to be associated to the Asiatic type ("A" type) citrus canker. Sour orange, pomelo, Mexican lime, calamondin grapefruit and sweet orange were all susceptible to the 15 *X. axonopodis* pv. *citri* isolates.

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

STATUS KANKER LIMAU DI ETHIOPIA DAN MALAYSIA, DAN PENCIRIAN AGEN PENYEBAB

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Tinjauan penyakit kanker limau telah dijalankan di Ethiopia dan Malaysia. Pemencilan, pencirian morfologi dan ujian patogenisiti pada anak benih kultivar limau telah dijalankan. Pencirian biokimia strain turut dijalankan menggunakan ujian penentuan paiwai. Di Ethiopia, kanker limau hanya didapati pada limau masam (*Citrus aurantium*) dan limau nipis (*C. aurantifolia*). Kejadian penyakit pada daun di Ethiopia ialah 71% dan keterukan adalah 26.8%; kejadian pada buah ialah 30% dan keterukan 21.25%. Ini adalah laporan pertama yang sah mengenai penyakit di Ethiopia. Di Malaysia, penyakit ini didapati pada *limau nipis (C. aurantifolia*), limau bali (*C. grandis*) dan limau purut (*C. hysterix*). Secara keseluruhannya kejadian penyakit kanker limau di Malaysia adalah 36.5% pada daun dengan keterukan 15.1%, manakal a kejadian pada buah adalah 18.7%. Pertumbuhan di atas Yeast dextrose chalk agar (YDCA) tidak sepantas seperti di atas agar sukrosa peptone (PSA) atau agar glucosa nutrient (NGA). Terdapat perbezaan yang

sangat bererti di antara saiz lesi bagi kultivar ($P \leq 0.01$) tetapi bukan untuk strain. Regrasi positif yang bererti ($P \leq 0.05$) telah didapati di antara saiz lesi dan masa selepas inokulasi. Analisis pengukuran berulang menggunakan model linear umum (GLM) untuk korelasi di antara masa selapas inokulasi adalah sangat bererti ($P \leq 0.01$). Tiada perbezaan dilihat dari segi patogenisiti antara strain. Saiz populasi meningkat lebih 2 log cfu/lesi pada limau nipis dan hampir 1.5 log unit pada limau masam dan limau bali manakala kekal di paras inokulum permulaan bagi limau kasturi. Korelasi positif yang bererti telah dilihat di antara populasi *X. axonopodis* dengan saiz lesi pada limau masam ($r = 0.58$, $P = 0.024$), limau bali ($r = 0.73$, $P = 0.018$) dan juga limau nipis ($r = 0.76$, $P = 0.001$). Korelasi ini adalah paling tinggi untuk limau nipis ($r = 0.76$) dan terendah bagi limau kasturi. Interaksi di antara strain dan kultivar-kultivar limau adalah sangat bererti ($P \leq 0.01$). Ujian-t menunjukkan tiada berbezaan bererti dari segi tindak balas terhadap pencilan *X. axonopodis* pv. *citri* kultivar limau masam dan limau bali, limau masam dan sweet orange ; limau nipis dan grapefruit, serta limau kasturi dan sweet orange Navel. Keenam-enam kultivar limau yang diuji adalah peka kepada 15 pencilan *X. axonopodis* pv *citri* dan lesi kanker limau telah terangsang pada daun terpisah. Interaksi sangat bererti ($P \leq 0.01$) antara kultivar dan semua pencilan Terukan simptom pada ujian daun terpisah menunjukkan korelasi bererti ($P \leq 0.01$) dengan kadar keterukan simptom kajian ujian daun terlekat. Korelasi yang tertinggi ($r = 0.97$) adalah pada limau nipis. Dalam ujian pencirian secara biokimia kesemua 15 pencilan *X. axonopodis* pv. *citri* menunjukkan keputusan yang sama dangan menggunakan ujian penentuanpiawai. Julat perumah kanker limau di Ethiopia adalah limau nipis dan limau masam, manakala di Malaysia Barat ia kelihatan lebih luas berbanding di Ethiopia. Selanjutnya, berdasarkan julat

perumah ujian anak benih, ciri morfologi, ujian patogenisiti, pertumbuhan populasi dalam tumbuhan dan ciri biokimia, 15 wakil pencilan *X. axonopodis* pv. *citri* Malaysia Barat telah dicirikan sebagai berkaitan dengan kanker limau jenis Asiatic (Jenis "A"). Limau manis kesemuanya adalah peka kepada 15 pencilan *X. axonopodis* pv. c*itri*.

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I certify that an Examination Committee has met on 3rd May 2006 to conduct the final examination of Eshetu Derso Kidanu on his Doctor of Philosophy thesis entitled "Status of Citrus Canker in Ethiopia and Malaysia, and Characterization of the Causal Agent" 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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DECLARATION

I hereby declare that the thesis is based on my original work except for quotation 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 any other institutions.

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ESHETU DERSO KIDANU

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