UNIVERSITI PUTRA MALAYSIA

PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF Ralstonia solanacearum CAUSING BACTERIAL WILT OF TOMATO (Solanum lycopersicum variety cerasiforme) IN SELANGOR

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FP 2017 42

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SERDANG, SELANGOR DARUL EHSAN

2016/2017

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By

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A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science

Department of Plant Protection

Faculty of Agriculture

Universiti Putra Malaysia

2016/2017

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ACKNOWLEDGEMENT

First and foremost, I am grateful to the Almighty for the good health and well-being that were necessary to complete this thesis. I wish to express my sincere thanks to Prof. Dr. Abdul Shukor Juraimi, Dean of the Faculty, for the continuous encouragement. I am also grateful to my beloved supervisor, Dr. Dzarifah Mohamed Zulperi, from the Department of Plant Protection. I am extremely thankful and indebted to her for sharing her expertise, sincere and valuable guidance as well as encouragement. She spent a lot of her valuable time to read and review this thesis. I would also like to express my gratitude to all of the Department faculty members for their help and support. I am honoured to all laboratory staffs especially Mrs. Junaina Jaafar and Mr. Johari Mohd Sarikat for their technical assistance. Thank you to my parent, Mr. Zakaria Said and Mrs. Sa'diah Ali for the unceasing encouragement, support and attention.

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LIST OF ABBREVIATIONS

%	percent
°C	degree celcius
BLAST	Basic Local Alignment Search Tool
bp	base pair
cm	centimetre
DNA	deoxyribonucleic acid
DOA	Department of Agriculture
EDTA	ethylene-diamine-tetraacetic acid
EOL	Encyclopedia of Life
F	forward
R	reverse
FAMA	Federal Agricultural Marketing Authority
FAO	Food and Agriculture Organization
g	gram
h	hour
ha	hectare
H_2O_2	hydrogen peroxide
in	inches
kb	kilobase pair
KOH	Potassium hydroxide
Mb	megabase pair
min	minutes
ml	millilitre

MOA	Ministry of Agriculture
Mt	Megatonne
mm	milimeter
mM	milimolar
NA	nutrient agar
PCR	polymerase chain reaction
PP	posterior probability
rpm	rotation per minute
rDNA	ribosomal deoxyribonucleic acid
sec	seconds
spp.	species
SPA	sucrose peptone agar
TAE	tris-acetic EDTA
Taq	Thermus aquaticus
TM	melting temperature
TZC	tetrazolium chloride
V	voltan/volt
x g	gravity force
µg	microgram
µl	microliter
µM	micromolar
µm	micrometer
1bs.	pound

ABSTRACT

PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF *Ralstonia solanacearum* CAUSING BACTERIAL WILT OF TOMATO (*Solanum lycopersicum* variety *cerasiforme*) IN SELANGOR

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Cherry tomato (*Solanum lycopersicum* variety *cerasiforme)* is a vegetable from Solanaceae family. It is grown and eaten by humans nationwide. However, the results from survey at Fertigation Field show that almost all the cherry tomato plants died caused the outbreak of wilt disease on April, 2017. The disease is caused by *Ralstonia solanacearum (R. solanacearum)* and occurred in tropical, subtropical and temperate regions of the world. Many commercial tomato cultivars are highly susceptible to bacteria wilt and infections that resulted in wilting of the youngest leaves at the end of branches, yellowing of foliage, stunting and within two to three days, it will death. This study was conducted to isolate and identify *R. solanacearum* causing tomato bacterial wilt by using phenotypic and molecular characteristics. To achieve the objective, two samples of infected cherry tomato expressing bacterial wilt symptoms were collected from Fertigation Field located in UPM Serdang, Selangor. For phenotypic characterization, the morphology of the bacterial strains isolated from the infected cherry tomato fruits were identified as creamy white, mucoid, virulent, fluidal and irregular. On Kelman's tetrazolium medium, the colony was large, diffusible brown pigment, elevated and creamy white colonies with pink centre. Gram staining revealed that all strains were Gram-negative with rod shaped,

positive for KOH test, catalase test and Kovacs oxidase test. Pathogenicity test revealed that all fruits inoculated with *R. solanacearum* strains produced bacterial wilt symptoms as observed on naturally infected samples, whereas the control fruits remained asymptomatic. The 16S rDNA polymerase chain reaction (PCR) amplification by using 8F and 1492R primers was performed for molecular identification of four *R. solanacearum* strains produced amplicon of ~ 1400 basepair (bp). Sequencing analysis showed that all strains were 100% identical to *R. solanacearum* reference strains in Genbank database (Accession no. AF207891 and U28221). The phylogenetic analysis of 16S rDNA gene sequences clustered all strains into *R. solanacearum* reference sequences strains.

ABSTRAK

Tomato ceri (*Solanum lycopersicum* varieti *cerasiforme*) adalah sayuran dari keluarga Solanaceae. Ia ditanam dan dimakan oleh manusia di seluruh negara. Walau bagaimanapun, hasil daripada kajian di Ladang Fertigasi menunjukkan bahawa hampir semua ceri tumbuh-tumbuhan tomato mati disebabkan wabak penyakit layu pada April, 2017. Penyakit ini disebabkan oleh *Ralstonia solanacearum (R. Solanacearum)* dan berlaku di kawasan tropika, subtropika dan kawasan sederhana di dunia. Banyak kultivar tomato komersial adalah sangat mudah terdedah kepada layu bakteria dan jangkitan, yang menyebabkan daun muda layu pada akhir cabang, daun kekuningan, terbantut dan dalam masa dua hingga tiga hari, ia akan mati. Kajian ini telah dijalankan untuk mengasingkan dan mengenal pasti *R. solanacearum* dengan menggunakan fenotip dan ciri-ciri molekul. Untuk mencapai matlamat tersebut, dua sampel tomato ceri yang dijangkiti menyatakan simptom layu telah diambil dari Ladang Fertigasi terletak di UPM Serdang, Selangor. Untuk pencirian fenotip, morfologi strain bakteria yang diasingkan daripada buah-buahan tomato ceri yang dijangkiti telah dikenal pasti sebagai berkrim putih, berlendir, virulen, cecair dan tidak sekata. Pada medium tetrazolium Kelman ini, koloni adalah koloni putih yang besar, pigmen coklat boleh resap, *elevated* dan putih berkrim dengan pusat merah jambu. 'Gram staining' mendedahkan bahawa semua strain adalah Gram-negatif dengan berbentuk rod, positif untuk ujian KOH, ujian catalase dan ujian 'Kovacs oxidase'. Ujian kepatogenan mendedahkan bahawa semua buah-buahan disuntik dengan strain *R. solanacearum* menghasilkan simptom layu bakteria seperti yang diperhatikan pada sampel dijangkiti secara semula jadi, manakala buah-buahan kawalan kekal asimptomatik. 16S rDNA amplikasi tindak balas rantai polymerase (PCR) dengan menggunakan primer 8F dan 1492 dijalankan untuk mengenal pasti

molekul empat jenis *R. solanacearum* menghasilkan amplicon ~ 1400 basepair (bp). Analisis urutan menunjukkan bahawa semua strain adalah 100% sama dengan rujukan strain *R. solanacearum* di dalam pangkalan data bank gen. (Accession no. AF207891 and U28221). Analisis filogenetik urutan gen 16S rDNA berkelompok semua strain ke *R. solanacearum* urutan rujukan strain.

CHAPTER 1.0

INTRODUCTION

1.1 Background of the study

Cherry tomato is a small round fruited tomato, regarded as an intermediate genetic mix between wild currant-type and domesticated garden tomato (Nagpur, 2015). It become a perennial in its native habitat, and grown annually in temperate climates (Warrier,2013). This plant is typically grown in Cameron Highlands, Malaysia and is already planted commercially in the lowlands (Adam *et.al*, 2015*).* There is an area of 23.567 hectares of tomato plants with a production of 43.987 metric tons in 2009 in Malaysia (Anem, 2015). Tomato production in Malaysia seem cannot accommodate large population of Malaysia and there would appear to be only limited likelihood of any dramatic developments in Malaysia's fresh tomato industry in the future (Lucia, 2016).

1.2 Statement of the Problem

Bacteria wilt is one of the major diseases of cherry tomato and other the Solanaceae family (Champoiseau, 2008). The disease is caused by the bacterium *R. solanacearum* (Seleim, 2014), where it limits the production of many economically import crops, including tomato (Elphinstone, 2005). This bacterium is devastating during the warm wet period in the tropics and subtropics and causes economic losses too many host (Tiwari, 2012). Bacterial wilt of tomato can cause 60 to 100% loss in yield (Popoola, 2015).

1.3 Significance of the study

Cherry tomato is an important fruit in Malaysia for domestic markets. Since Malaysia is experiencing lack of studies of bacteria wilt disease on cherry tomato, it is crucial to identify the caused agent infecting cherry tomato. Results obtained from this study will become an important updated documentation on *R. solanacearum* infection on cherry tomato plant in Malaysia via phenotypic and molecular approaches.

1.4 Objectives of the study

This study was carried out to achieve the following objectives:

1. To isolate and identify *Ralstonia* spp. infecting cherry tomato by using phenotypic characterization
2. To determine the genetic relationship of *R. solanacearum* strains isolated via molecular and phylogenetic approaches

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