UNIVERSITI PUTRA MALAYSIA

INTRA AND INTERSPECIFIC COMPATIBILITY, MORPHOPHYSIOLOGICAL AND THEIR GENETIC ANALYSIS IN EGGPLANT (Solanum melongena L.) GRAFTING FOR BACTERIAL WILT DISEASE RESISTANCE

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By

MUSA IBRAHIM

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

February 2021

DEDICATION

I dedicate this thesis to my late father Alh Musa Abba Shelleng, late mother Mrs Hannatu TB, beloved wife, Ikilima Abubakar and my children Abdullah, Musa and Hannatu for love and encouragement.

Thank you very much for everything

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

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Supervisor : Professor Mohd Rafii Yusop, PhD
Institute : Tropical Agriculture and Food Security

Solanum melongena (eggplant) is one of the most famous and widely cultivated vegetable crops in the world. However, the yield production of this crop is facing a great challenge worldwide including Malaysia, due to most destructive bacterial wilt disease, caused by *Ralstonia solanacearum*. This study was therefore, conducted to determine the genetic diversity of 42 eggplant genotypes and screening for bacterial wilt resistance eggplant genotypes (from Bangladesh, China, Malaysia. Nigeria and Thailand) and explored the effects of grafting as a potential eggplant breeding to improve fruit yield and quality traits. Among the genotypes used, 4 varieties are F1 (hybrids), 35 are commercial/cultivated and 3 are wild. The analysis of variance (ANOVA) showed that all the traits under study were highly significant variation among the 42 eggplant genotypes evaluated under open field condition. The MBC32 genotype was observed to have a high yield per hectare and yield per plant, with 39.78 t/ha and 2.98 kg/plant, respectively, followed by MBC72 with 37.51 t/ha and 2.81 kg/plant. The phenotypic coefficient of variation (PCV) values was greater than the genotypic coefficient of variation (GCV) values for all traits under study, thus, showing that the differences observed were not exclusively due to genetics but likewise influenced by environmental factors in the expression of the traits. Fruit weight character showed the highest GCV and PCV values (71.2% and 75.8% respectively). High estimated broad-sense heritability and genetic advance values were determined in yield per hectare, yield per plant, number of fruits per plant, fruit weight, fruit length and fruit diameter, indicating that higher of genetic inheritance and genotype selection based on these traits are effective. The 42 genotypes were grouped into four major clusters based on the morphological traits measured using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram. The diversity pattern and other performance of the genotypes MBC32, MBC72, MBC45, MBC41, MBC09 and MBC66 from the group I of cluster analysis may be taken into consideration as better parent for an efficient and effective hybridization programme of eggplant. The pathogenicity test based on disease severity index values at fourth weeks after inoculation indicated that genotypes MBC37,

MBC55, MBC64, MBC66, MBC51, MBC51, MBC52 and MBC72 had the highest level of resistance to the virulent *Ralstonia solanacearum* strain, UPMSE 16. As such, the genotypes were confirmed as an appropriate source for the breeding of bacterial wilt disease resistant varieties and also the candidate for a potential source of resistant rootstock for eggplant grafting. Among the SSR used, the average polymorphic information content was 0.373 and it ranged from 0.365 to 0.375. Dendrogram analysis classified these 42 eggplant genotypes into seven main clusters based on SSR marker. It was revealed that the co-dominant markers such as SSR proved to be high effective tool in discriminating between resistant and susceptible genotypes, and classifying these genotypes based on genetic diversity. The grafted plants with MBC50, MBC51 and MBC52 rootstock have proven a remarkable resistance result against the UPMSE 16 strain. The results showed that interspecific grafted genotypes derived from scions grafted onto wild relative rootstocks were produced higher yield as compared to non-grafted and intra self-grafted genotypes. General, the use of rootstocks resulted in higher total and marketable fruit yield compared to the non-grafted and self-grafted scion plants, respectively. In particular, MBC50 and MBC52 rootstock conferred the highest vigour to the scion, resulting in increasing for total and marketable fruit yield, number of fruits, average fruit weight, and fruit length and diameter. The remarkable compatibility and vigour of the rootstock with scion led to the improvement in total and marketable fruit yield. The result of proximate analysis indicated that eggplant of MBC41 grafted onto MBC50, and MBC44 grafted onto MBC50 had a higher protein content in open field and glasshouse conditions respectively, whereas MBC28 grafted onto MBC50 had higher fat content under both the cropping conditions. There was notable effect of grafting MBC66/MBC50, MBC28/MBC50, MBC41/MBC50 and MBC41/MBC51 on the total soluble solid, fruit firmness, pH and moisture content of the fruits, respectively. Furthermore, the organic chemical compound 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was higher in fruits of MBC09/MBC50, while grafted MBC09/MBC51 had remarkably higher ascorbic acid content. High total flavonoid content was recorded in MBC05/MBC50, whereas the level of total phenolic content was found to be higher in MBC44/MBC51 and MBC44/MBC52. Hence, from this study, the use of 'MBC50, MBC51 and MBC52' wild rootstocks for grafting displayed resistant to bacterial wilt, good vigour and high yield attributes as well as extend fruit quality. The development of interspecific new eggplant rootstock genotypes from highly resistant wild eggplant genotype (MBC50, MBC51 and MBC52) with *Solanum melongena* (MBC37, MBC55, MBC64 and MBC66) as possible substitute to those commonly used *Solanum torvun* will exhibit high and uniform germination, vigour and strong rooting eggplant genotypes for future grafting programme

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

KESERASIAN CANTUMAN TERUNG (*Solanum melongena* L.) SECARA INTRA DAN INTERSPESIFIK, MORFO-FISIOLOGI DAN ANALISIS GENETIK TERHADAP KERINTANGAN PENYAKIT LAYU BAKTERIA

Oleh

MUSA IBRAHIM

Februari 2021

Pengerusi : Profesor Mohd Rafii Yusop, PhD
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Solanum melongena (terung) adalah salah satu tanaman sayuran yang paling terkenal dan ditanam secara meluas di dunia. Walau bagaimanapun, pengeluaran hasil tanaman ini menghadapi cabaran besar diseluruh dunia termasuk Malaysia, kerana kemusnahan yang teruk oleh penyakit layu bakteria, yang disebabkan oleh *Ralstonia solanacearum.* Oleh itu, kajian ini dijalankan untuk mengenal pasti kepelbagaian genetik empat puluh dua genotip pokok terung dan menyaring genotip terung (berasal daripada Bangladesh, China, Malaysia, Nigeria dan Thailand) yang rintang terhadap penyakit layu bakteria serta mengkaji kesan teknik cantuman pembiakbakaan terung yang berpotensi meningkatkan hasil dan kualiti buah. Antara genotip terung yang digunakan, empat varieti adalah genotip hybrid F_1, 35 genotip adalah genotip komersial/ditanam dan tiga genotip adalah liar. Analisis varians (ANOVA) menunjukkan bahawa semua ciri yang dikaji memberikan variasi yang sangat signifikan dikalangan 42 genotip terung yang dinilai di persekitaran ladang terbuka. Genotip MBC32 telah menghasilkan hasil tinggi per hektar dan hasil per pokok, masing-masing dengan 39.78 t/ha dan 2.98 kg/pokok, diikuti MBC72 dengan 37.51 t/ha dan 2.81 kg/pokok. Nilai pekali variasi fenotipik (PCV) adalah lebih besar daripada nilai pekali variasi genotip (GCV) untuk semua sifat yang dikaji, yang menunjukkan bahawa perbezaan variasi tersebut adalah tidak sepenuhnya disebabkan oleh genetik tetapi juga dipengaruhi oleh faktor persekitaran dalam pengekspresan ciri-ciri tersebut. Ciri berat buah menunjukkan nilai GCV dan PCV tertinggi (masing-masing 71.2% dan 75.8%). Nilai anggaran heritabitili luas dan kemajuan genetik yang tinggi telah ditentukan dalam ciri-ciri hasil per hektar, hasil per pokok, bilangan buah per pokok, berat buah, panjang buah dan diameter buah, yang menunjukkan bahawa ciri tersebut mempunyai keterwarisan genetik yang tinggi dan pemilihan genotip berdasarkan ciri-ciri tersebut adalah berkesan. Empat puluh dua genotip terung telah dikelompokkan kepada empat kluster utama berdasarkan ciri-ciri morfologi yang dinilai menggunakan dendogram Kaedah Tak Berpemberat Kumpulan Berpasangan dengan Min Aritmetik (UPGMA). Corak diversiti dan prestasi genotip MBC32, MBC72, MBC45, MBC41, MBC09 dan MBC66 daripada kumpulan 1 analisis kluster boleh dipertimbangkan sebagai pokok induk yang lebih efisien dalam program

penghibridan terung yang efektif. Ujian patogenik berdasarkan nilai indeks keparahan penyakit pada minggu keempat setelah inokulasi menunjukkan bahawa genotip MBC37, MBC55, MBC64, MBC66, MBC51, MBC51, MBC52 dan MBC72 mempunyai tahap kerintangan tertinggi terhadap strain virulen *R. solanacearum*, UPMSE 16. Oleh itu, genotip MBC64, MBC66, MBC51, MBC51, MBC52 dan MBC39 adalah di disahkan sebagai sumber yang sesuai untuk digunakan dalam pembiakbakaan varieti yang rintang penyakit layu bakteria dan juga sebagai sumber potensi pokok penanti untuk cantuman terung. Antara penanda SSR yang digunakan, purata kandungan informasi polimorfik adalah 0.373 yang berada di antara julat 0.365 dengan 0.375. Analisis dendogram mengklasifikasi 42 genotip terung kepada tujuh kuster utama berdasarkan penanda SSR. Hal ini juga menemukan penanda ko-dominan seperti penanda SSR dibuktikan sebagai alat efektif dalam memilih antara genotip rintang dan genotip yang mudah terkena penyakit serta membezakan berdasarkan diversiti genetik. Pokok cantuman dengan pokok penanti MBC50, MBC51 dan MBC52 telah dibuktikan memberi kerintangan yang baik terhadap strain UPMSE 16. Hasil kajian menunjukkan bahawa genotip cantuman interspesifik yang berasal dari sion yang dicantumkan pada pokok penanti relatifnya liar menghasilkan hasil yang lebih tinggi berbanding dengan genotip yang tidak dicantumkan dan cantuman swa secara intra. Secara umumnya, penggunaan pokok penanti telah mengeluarkan hasil buah dan hasil yang boleh dipasarkan lebih tinggi berbandingkan dengan pokok yang tidak dicantumkan dan cantuman swa secara intra. Khususnya, pokok penanti MBC50 dan MBC52 memberikan kecergasan tertinggi kepada sion, yang telah memberikan peningkatan penghasilan jumlah hasil buah, buah yang boleh dipasarkan, bilangan buah, purata berat buah dan, diameter dan panjang buah. Keserasian dan kecergasan dari pokok penanti dengan sion telah menyebabkan peningkatan kualiti buah dan jumlah hasil yang dapat dipasarkan. Keputusan analisa proksimat menunjukkan bahawa terung MBC41 yang dicantumkan ke MBC50, dan MBC44 yang dicantumkan ke MBC50 masing-masing memberikan kandungan protein yang lebih tinggi dari persekitaran ladang terbuka dan rumah kaca, manakala MBC28 yang dicantumkan ke MBC50 mempunyai kandungan lemak yang lebih tinggi dalam kedua-dua persekitaran penanaman tersebut. Terdapat banyak kesan cantuman pada MBC66/MBC50, MBC28/MBC50, MBC41/MBC50 dan MBC41/MBC51 terhadap jumlah pepejal larut, ketegangan buah, pH dan kandungan kelembapan buah terung. Selanjutnya, sebatian kimia organik 2,2-diphenyl-1-picrylhydrazyl (DPPH) adalah lebih tinggi pada buah cantuman MBC09/MBC50, manakala cantuman MBC09/MBC51 mempunyai kandungan asid askorbik yang tinggi. Jumlah kandungan flavonoid yang tinggi dicatatkan dalam cantuman MBC05/MBC50, sedangkan tahap kandungan fenolik yang lebih tinggi pada MBC44/ MBC51 dan MBC44/ MBC52. Oleh itu, daripada kajian ini, penggunaan MBC50, MBC51 dan MBC52 sebagai pokok penanti liar untuk cantuman menunjukkan kerintangan terhadap penyakit layu bakteria, kecergasan tinggi, hasil dan kualiti buah yang tinggi. Pembangunan interspesifik genotip pokok penanti terung baru daripada pokok penanti genotip terung liar (MBC50, MBC51 dan MBC52) dengan *Solanum melongena* (MBC37, MBC55, MBC64 dan MBC66) sebagai pengganti pokok terung *Solanum torvun* yang sering digunakan akan menghasilkan genotip terung dengan percambahan yang tinggi dan seragam, kecergasan dan pengakaran yang kuat untuk program percantuman akan datang.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the award of degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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ANOVA	Analysis of variance
cm	Centimetre
°C	Degree centigrade
DNA	Deoxyribonucleic acid
et al	*et al*ia
FAO	Food and agriculture organization
h	hours
Kg	Kilogram
L	Lines
LSD	Least significant difference
µ	Micro
µl	microlitre
MBC	Malaysia eggplant collection
mg	milligram
ml	millilitre
Mol	mole
SA	Sodium azide
SAS	Statistical Analysis System
SE	Standard Error
spp	Species
CS	Centre of collection

CHAPTER 1

INTRODUCTION

1.1 Introduction

Eggplant (*Solanum melongena L*.) is one of the important vegetables belonging to the family Solanaceae, which comprises other significant crop species including tomato and potato. It is widely cultivated and freshly consumed in Malaysia and other part of the world (Sulaiman *et al*., 2020). It originated India and showed secondary diversity in South East Asia. Globally, the eggplant is ranked as third most important crop from Solanaceae family after potato and tomato with an annual production of 55,197,878.00 million tons (FAO, 2020), increased by 1.00 % against the previous year. This global eggplant production peaked in 2019, and the growth trend pattern is likely to be on a continuous increase. China and India are the leading in eggplant producing countries in the world followed by Egypt, Turkey, Iran, Indonesia, Japan, Italy, Iraq and Philippines (FAO, 2020).

Genetic variability plays an important role in a crop in selecting the best genotypes for making rapid improvement in yield and other desirable characters as well as to select the potential parents for hybridization programmes. The progress in breeding for economic traits that are mostly polygenically controlled and hence environmentally influenced is determined by the magnitude and nature of their genetic variability. Eggplant cultivars differ in fruit size, shape, and colour. It was considered among the healthiest fruit since it contains bioactive compounds, and a good source of vitamins and minerals (Raigón *et al.,* 2008; Plazas *et al.,* 2014b; Docimo *et al.,* 2016) and placed among the ten main fruits in terms of oxygen radical absorbance capacity (Cao *et al.,* 1996). Several studies have also shown that phenolic acids are chiefly in the flesh fruit of eggplant, Plazas *et al.,* (2013) and Stommel *et al.,* (2015) and anthocyanin in the skin of the fruit (Mennella *et al.,* 2012) which are also beneficial for humans (Plazas *et al.,* 2013; Braga *et al.,* 2016). Eggplant possesses medical properties and has been recommended as a great remedy to cure liver associated illness and diabetes (Sabolu *et al.,* 2014). Eggplant fruit contains ascorbic acid and phenolic compounds, both of which are powerful antioxidants (Vinson *et al.*, 1998). Eggplants have a significant nutritional value due to its composition, viz are minerals like potassium, calcium, sodium and iron (Mohamed *et al*., 2003; Raigon *et al*., 2008) as well as fibre content (Sanchez-Castillo *et al*., 1999). Grafting imprints resistance to pathogenic agents and soil pathogens, tolerance to abiotic stress factors, enhance uptake of water and nutrients and increases the graft vigour (Lee, 1994 and King *et al*., 2010). Grafting of eggplant cultivars on perennial and wild Solanaceous plants has been shown to increase fruit production and availability period of the fruits (Lee, 1994 and Gisbert *et al.,* 2011). The knowledge of graft incompatibility in solanaceous vegetables that resulted in a failure of graft union between rootstock and scion has been reported (Jeffree and Yeoman, 1982). Little is known about the mechanism of graft union formation (Fernandez-Garcia *et al*., 2003). The formation of the graft union is critical for proper growth and development of the grafted seedling.

Graft compatibility needs proper alignment of the vascular systems at the graft union of rootstock and scion to keep the scion alive until complete healing of the graft union occurs (Lee *et al.,* 2010). Soon after grafting, rapid healing of the graft union is necessary so that the scion may be supplied with water and nutrients from the rootstock because it is very susceptible to wilting which may result in death (Rivard and Louws, 2006b). Therefore, proper identification of size, age and type of plants to be grafted is the most important factor for successful compatibility (Besri, 2008). The criteria for crop improvement are the genetic variability which provides a wider scope for selection. Thus, the efficacy of selection is dependent upon the nature, extent and degree of genetic variability present in the material and the extent to which it is heritable. The selection of a particular yield contributing traits was based on the nature of genetic divergence, information on heritability and genetic advance. Therefore, it is necessary to know genetic variability, heritability and genetic advance (Mohanty (2002), Baswana *et al.* (2002) and Kumar *et al.* (2012). *Ralstonia solanacearum* is a common soil-borne bacterial disease in tropical, subtropical and some temperate regions (Fegan and Prior, 2005), and also a significant limiting factor for the productions of many crop plants worldwide (Agrios, 1997). This organism is the causal agent of bacterial wilt in eggplant, tomato, potato and tobacco, and some ornamentals and moko disease of banana (Stevenson *et al*., 2001). The species as a whole has a very wide host range, but diverse pathogenic races exist within the species occur with the preferences of the hosts.

In general, the pathogen infects hundreds of plant species including 44 families (Hayward, 1991), and economically valuable host plants in the Solanaceae family (Stevenson, *et al*., 2001). The identification of polymorphisms between cultivars and breeding lines is important for molecular breeding. However, a low degree of polymorphism has been recorded in solanaceous plants among cultivars and intraspecific lines (Nunome *et al*. 2003, Stagel *et al*. 2008). Studies of SSR marker use for the detection of diseases such as bacterial wilt in eggplants are very weak, literature research shows that SSR makers have used either molecular diversity or purity analysis of hybrids in eggplants. Therefore, SSR markers were used to detect eggplant genotypes immune to bacterial wilt.

1.2 Problem statement

Eggplant is a famous and widely cultivated vegetable crop among the Malaysian. Despite the high-profit potentials, major problems have reduced its production including *Ralstonia solanacearum, Fusarium oxysporum*, high temperature and lack of consistent high yielding varieties. With the restriction of using synthetic pesticides and scenarios of lack of effective growing genetic disease resistance varieties. Grafting of high yielding scion with resistant and vigourous rootstock have been chosen to manage the bacterial wilt and increase productivity.

1.3 Research objectives

I. The main objective of this study was to identify new potential rootstock and high yielding scion for eggplant production
II. To improve the protocol for intra and interspecific compatibility
III. To study their genetic analysis in eggplant grafting.

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