



**DEVELOPMENT OF YELLOW LEAF CURL DISEASE RESISTANT AND
HIGH YIELDING TOMATO (*Solanum lycopersicum* L.) HYBRID THROUGH
MARKER-ASSISTED SELECTION AND DIALLEL CROSSES**

By

HITIHAMY MUDIYANSELAGE VISHAKA THARANGANI WELEGAMA

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

August 2022

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DEDICATION

I dedicate this thesis to all my beloved family members for their continuous support, sacrifices and patience.



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

DEVELOPMENT OF YELLOW LEAF CURL DISEASE RESISTANT AND HIGH YIELDING TOMATO (*Solanum lycopersicum* L.) HYBRID THROUGH MARKER-ASSISTED SELECTION AND DIALLEL CROSSES

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August 2022

Supervisor : Professor Mohd Rafii Yusop, PhD
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Tomato Yellow Leaf Curl Disease (TYLCD) is a highly destructive disease in the most tomato cultivated areas globally, while becoming uncontrollable in tropical and subtropical regions. TYLCD is triggered by several Begomovirus species and it is transmitted by whitefly (*Bemisia tabaci*). TYLCD is controlled mainly by vector targeted pesticides, which leads to evolve pesticide resistant vector biotypes. Thus, resistant tomato varieties are the most sustainable way to control TYLCD. This study aimed to develop tomato F₁ hybrids with TYLCD resistance/ tolerance and higher yield. TYLCD causative Begomovirus in the study was confirmed as Pepper yellow leaf curl virus which has close homology with tomato yellow leaf curl virus. Forty tomato accessions were evaluated and characterized based on 15 traits of yield and yield components, morphological, physiochemical and TYLCD symptom severity in a randomized complete block design (RCBD) with 3 replications at Field 15, Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia and the best 9 accessions were selected as parental lines to produce F₁ crosses. The selected 9 accessions out of initial 40 were used to produce F₁ hybrids and, succeeded 18 F₁ hybrids and their 9 parental lines were screened to determine the presence of TYLCD resistant/tolerance namely, *Ty 1*, *Ty 2*, *Ty 3*, *Ty 4*, *ty 5* and *Ty 6* genes using gene specific primers. The results revealed the F₁ hybrids, T4 × T6, T6 × T4 and T7 × T15 possess homozygous resistance (RR) for *Ty 1*, *Ty 2* and *Ty 3* alleles with most promising tolerance to TYLCD, while T4 × T8, T4 × T9, T7 × T5, T8 × T4, T8 × T6, T8 × T9, T9 × T4 and T9 × T6 hybrids possess homozygous resistance only for *Ty 3* and *Ty 2* genes, which is expected to have substantial potential for TYLCD tolerance. The 18 F₁ hybrids with their 9 parental lines were evaluated in a glass house over 2 planting cycles in randomized complete block design (RCBD) with 3 replications. Highly significant differences were observed among all genotypes at $p \leq 0.01$ for all the traits. Out of the 18 F₁ hybrids and their 9 parental lines, only 10 F₁ hybrids and their 5 parental lines were applicable in to half diallel analysis. Estimated general combining ability (GCA) revealed parental lines T4 and T2 had the highest values in yield per plant trait, 276.34

g/plant and 112.11 g/plant respectively, whereas T8 is the best in terms of disease incidence (GCA: -0.17) and index for disease severity (GCA: -0.11) toward TYLCD resistance. Specific combining ability (SCA) analysis revealed two hybrids, namely T2×T4 (SCA: 838.90 g/plant) and T4×T8 (SCA: 424.83 g/plant) as the best performers in terms of yield traits, while hybrid T6×T9 was identified as the best in terms of disease incidence (SCA: -0.08) and index for disease severity (SCA: -0.008) on TYLCD resistance. Hybrid T4 ×T8 has superior yield (2.06 kg/plant) and TYLCD resistance is highly recommended for further large-scale evaluation before release as a new developed variety for commercial cultivation in Malaysia.



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

**PEMBANGUNAN HIBRID TOMATO (*Solanum lycopersicum* L.) RINTANG
PENYAKIT KERINTING DAUN KUNING DAN BERHASIL TINGGI
MELALUI PEMILIHAN BANTUAN PENANDA DAN KACUKAN DIALEL**

Oleh

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Penyakit keriting daun kuning tomato (TYLCD) adalah penyakit yang sangat memusnahkan di kawasan penanaman utama tomato di seluruh dunia, serta menjadi tidak terkawal di kawasan tropika dan subtropika. TYLCD dicetuskan oleh beberapa spesies Begomovirus dan ia disebarkan oleh lalat putih (*Bemisia tabaci*). Pengawalan TYLCD dijalankan terutamanya dengan penggunaan racun perosak dengan menyasarkan vektor, tetapi telah mengakibatkan evolusi biotip vektor menjadi rintang kepada racun perosak. Oleh itu, varieti tomato yang rintang adalah cara yang paling mampan untuk mengawal TYLCD. Objektif kajian ini adalah untuk membangunkan hibrid tomato F₁ berhasil tinggi dan rintang/toleran TYLCD. Begomovirus penyebab TYLCD dalam kajian itu disahkan sebagai virus keriting daun kuning chili yang mempunyai homologi rapat dengan virus keriting daun kuning tomato. Empat puluh aksesori tomato telah dinilai dan dibuat pencirian berdasarkan 15 ciri hasil dan komponen hasil, morfologi, fisiokimia dan keterukan simptom TYLCD menggunakan rekabentuk blok penuh rawak (RCBD) dengan 3 replikasi di Ladang 15, Institut Pertanian Tropika dan Sekuriti Makanan, Universiti Putra Malaysia, dan 9 aksesori terbaik telah dipilih sebagai titisan induk dalam kacukan dialel untuk menghasilkan kacukan F₁. Daripada 40 aksesori, sembilan aksesori terpilih telah digunakan untuk menghasilkan kacukan F₁ dan, 18 hibrid F₁ telah berjaya dihasilkan berserta 9 titisan induknya disaring untuk menentukan kehadiran gen kerintangan/toleransi TYLCD iaitu gen *Ty 1*, *Ty 2*, *Ty 3*, *Ty 4*, *ty 5* dan *Ty 6* menggunakan penanda gen spesifik. Keputusan menunjukkan, hibrid F₁, T4 × T6, T6 × T4 dan T7 × T15 mempunyai kerintangan homozigot (RR) untuk alel *Ty 1*, *Ty 2* dan *Ty 3* dengan tahap toleran yang terbaik terhadap TYLCD, manakala hibrid T4 × T8, T4 × T9, T7 × T5, T8 × T4, T8 × T6, T8 × T9, T9 × T4 dan T9 × T6 mempunyai kerintangan homozigot hanya untuk gen *Ty 3* dan *Ty 2* yang dijangka mempunyai potensi yang besar untuk toleran terhadap TYLCD. Lapan belas hibrid F₁ dengan 9 titisan induknya telah dinilai dalam rumah kaca yang melibatkan 2 pusingan penanaman dalam rekabentuk blok lengkap rawak (RCBD) dengan 3 replikasi. Terdapat perbezaan yang sangat signifikan diperhatikan di antara semua genotip pada $p \leq 0.01$ untuk semua ciri. Daripada

18 kacukan F_1 dan 9 titisan induknya, hanya 10 kacukan F_1 dan 5 titisan induknya digunakan dalam analisis separa dialel. Anggaran kemampuan bergabung am (GCA) menunjukkan titisan induk T4 dan T2 mempunyai nilai tertinggi untuk ciri hasil sepokok, masing-masing 276.34 g/pokok dan 112.11 g/pokok, manakala T8 adalah yang terbaik dari segi kejadian penyakit (GCA: -0.17) dan indeks untuk keterukan penyakit (GCA: -0.11) terhadap kerintangan TYLCD. Analisis kemampuan bergabung khusus (SCA) mendapati dua hibrid iaitu T2×T4 (SCA: 838.90 g/pokok) dan T4×T8 (SCA: 424.83 g/pokok) sebagai prestasi terbaik dari segi ciri hasil, manakala hibrid T6×T9 dikenal pasti sebagai yang terbaik dari segi insiden penyakit (SCA: -0.08) dan indeks keterukan penyakit (SCA: -0.008) terhadap kerintangan TYLCD. Hibrid T4×T8 mempunyai hasil unggul (2.06 kg/pokok) dan kerintangan TYLCD, sangat disyorkan untuk dijalankan penilaian berskala besar sebelum diistiharkan sebagai satu varieti maju baharu untuk penanaman komersial di Malaysia.

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LIST OF ABBREVIATIONS

AFLPs	Amplified Fragment Length Polymorphism
ANOVA	Analysis of variance
AVRDC	Asian Vegetable Research and Development Center
AYVMV	Ageratum Yellow Vein Malaysia Virus
CAPS	Cleaved Amplified Polymorphic Sequence
CTAB	Cetyl Trimethyl Ammonium Bromide
DFLO	Days to 50% flowering
DFRU	Days to 50% fruiting
DFRUMT	Days to 50% fruit maturity
DI	Disease incidence
DOA	Department of Agriculture
EC	Electrical conductivity
FAO	Food Agriculture Organization
FPP	Fruits per plant
FRLEN	Fruit length
FRTFIR	Fruit firmness
FRWID	Fruit width
GCA	General combining ability
GS	Genomic selection
GWS	Genome-wide selection
IDS	Index for disease severity
InDels	Insertion Deletions
IPGRI	International Plant Genetic Resource Institute
LSD	Least significant differences
MABC	Marker-assisted backcrossing

MAPS	Marker-assisted pedigree selection
MARDI	Malaysian Agricultural Research and Development Institute
MARS	Marker-assisted recurrent selection
MAS	Marker-assisted selection
NTSYS	Numerical Taxonomy Multivariate Analysis System
PCR	Polymerase Chain Reaction
PepGMV	Pepper golden mosaic virus
PepLCV	Pepper leaf curl virus
PERITH	Pericarp thickness
PFW	Per fruit weight
PHT	Plant height at 50% flowering
PHYVV	Pepper huasteco yellow vein virus
PLCAV	Pepper leaf curl Aceh virus
PPY	Per plant yield
QTL	Quantitative trait loci
RCBD	Randomized Complete Block Design
RFLPs	Restricted Fragment Length Polymorphism
SAHN	Sequential Agglomerative Hierarchic and Non-overlapping
SCA	Specific combining ability
SCAR	Sequence Characterized Amplified Region
SNP	Single Nucleotide Polymorphism
TLCJV- Ageratum	Tomato leaf curl Java virus - Ageratum
TYLCD	Tomato Yellow Leaf Curl Disease
TYLCV	Tomato Yellow Leaf Curl Virus
UPGMA	Unweighted Pair Group Method with Arithmetic means

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Tomato which belongs to family Solanaceae has become the most important vegetable in the world due to its high nutrition and commercial value (Melomey *et al.*, 2019) with an annual global production of approximately 182 million metric tons in a harvested area around 4.8 million ha (FAO, 2018).

Malaysian tomato industry has shown steady development through the past decade and the cultivation area is approximately 1300 hectares (Rahim *et al.*, 2017) where the major cultivation regions are Jajahan Lojing, Kelantan; Cameron Highlands, Pahang and Kundasang, Sabah (Radam *et al.*, 2015).

Tomato crop is affected by number of biotic (pest and diseases) and abiotic stresses such as high and low moisture, heat, temperature, salinity and nutrient imbalances (Foolad, 2007). Among the biological stresses, viral diseases are considerably devastating. In particular, Tomato Yellow Leaf Curl Disease (TYLCD) caused by a group of Begomoviruses of family Geminiviridae is a major viral disease transmitted by silver leaf whitefly (*Bemisia tabaci* Gennadius) around the world (Dhaliwal *et al.*, 2020). TYLCD was first reported in Jordan Valley of Israel around nine decades ago (Cohen & Lapidot, 2007). At present TYLCD may cause symptom severity and yield losses depending on the varietal resistance, time of infection and many other biological, physiological causes and their interactions. Also, may cause up to 100 % yield reduction under uncontrolled conditions (Moriones and Navas-Castillo, 2000).

Cultivated tomato are extremely genetically poor due to occurrence of population bottle necks during domestication as a consequence of selection and also by genetic drift which is common for inbreeding species (Bai and Lindhout, 2007). Therefore, it is inevitably important to have large germplasm for an efficient screening of parental lines with variability in traits of interest. However, the selection of parental lines for higher fruit weight and larger fruit size is not much complicated as the trait is highly heritable and therefore could be easily selected phenotypically to utilize in breeding programmes (Foolad, 2007).

There are several standard methods (whitefly mediated and non-whitefly mediated) have been developed to screen for TYLCD resistant/ tolerant tomato plants. Among which white fly mediated inoculation cage method is identified as a method with higher accuracy (Czosnek, 2007). Resistant level of each genotype could be estimated using the TYLCD symptom severity score within 4-8 week post inoculation period (Lapidot *et al.*, 2006).

Molecular marker assisted selection has become a popular tool in present day crop breeding for traits such as disease resistance as it is a low cost and fast selection tool with high accuracy (Barone, 2007). Early identification of tomato lines with Begomovirus resistant *Ty* genes allows breeders to accelerate cultivar development with TYLCD tolerance. Up to now, there are 6 genes (*Ty1*, *Ty2*, *Ty3*, *Ty4*, *ty5* and *Ty6*) with TYLCD resistance have been identified in tomato wild relatives and been introgressed to cultivated tomato (Yan *et al.*, 2021). Though, tomato wild relatives have been identified with TYLCD resistance; none of the cultivated tomato possess total resistance but only identified with field tolerance after introgression of the *Ty* genes. Therefore accumulation of more than one *Ty* gene in to a cultivar has led to higher protection from TYLCD causative Begomovirus attack.

TYLCD is induced by several Begomovirus species and it's important to confirm the disease causative species by specific molecular markers. Molecular confirmation of Begomovirus species is useful to evaluate the action of each *Ty* gene in host plant with respect to the confirmed Begomovirus. At the same time, molecular confirmation of Begomovirus helps to identify the evolutionary relationship with other similar Begomoviruses by phylogenetic analysis and to develop suitable control strategies.

Production of F₁ hybrids is a successful approach which results better tolerant cultivars for vegetables of family Solanaceae (Kaushik and Dhaliwal, 2018). Diallel analysis is extensively manipulating tool in tomato breeding to utilize the genetic variability and to identify better performing parents and hybrid combinations through measures of general combining ability (GCA), specific combining ability (SCA) and heritability studies (Kaushik and Dhaliwal, 2018; de Souza *et al.*, 2012).

1.2 Research Problem

Tomato growers in Malaysia practice an intense and continuous crop cultivation without keeping a fallow period (Radam *et al.*, 2015) hence led to an increase and survival of vector populations and thereby spread and prevalence of viral diseases. Apart from that, polyphagous nature of vector and virus, climate change and development of new vector and virus types have made difficult to break the disease cycle. At present, spreading of TYLCD is mostly controlled by vector targeted pesticides which leads to resistant build up in vectors, increasing of production cost and adverse effects to the environment (Firdaus *et al.*, 2012). However, when there is severe outbreak of vector; pesticides too become inefficient in vector control so that virus transmission continues (Lapidot and Friedmann, 2002).

In Malaysia there are few tomato varieties have been tested against the leaf curl virus where MF 8 showed more tolerant than MT 1 (Roff *et al.*, 2005). However, Malaysian tomato growers often rely on imported tomato seeds with high quality attributes besides its high cost (Radam *et al.*, 2015). Therefore, resistant/ tolerant tomato cultivar development is a timely need for economically sustainable tomato industry in Malaysia while it is the most effective way of controlling the damage caused by TYLCD.

1.3 Research objectives

The main objective of this study was to production of high yielding tomato hybrids with Tomato Yellow Leaf Curl Disease resistance/tolerance.

The specific objectives of the study were;

1. Genetic diversity analysis among forty tomato lines and screening best tomato lines for high yielding using yield and yield related parameters
2. Screening of best tomato lines with resistant/tolerant trait to TYLCD using Disease incidence (DI %) and Index for disease severity (IDS) parameters.
3. Molecular identification of tomato yellow leaf curl disease causative Begomovirus species
4. F₁ seed production using nine selected parental inbred lines and screening them for Begomovirus causative TYLCD resistant Ty genes using gene specific primers
5. Identification of best parental lines and F₁ hybrids using additive and dominant genetic variations (V_A and V_D), general combining ability (GCA), specific combining ability (SCA) and narrow sense heritability (h²) estimates

1.4 Expected outcome

Identification of tomato F₁ hybrid/s with TYLCD tolerance and higher yields to cultivate in Malaysia

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