UNIVERSITI PUTRA MALAYSIA

HETEROSIS AND COMBINING ABILITY FOR YIELD AND ANTHRACNOSE DISEASE RESISTANCE IN CHILLI HYBRIDS (Capsicum spp)

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By

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June 2022

Chairman : Shairul Izan Ramlee, PhD
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Chilli (*Capsicum* species*)* is an important spice and vegetable crop in Malaysia. In 2019, the self-sufficiency ratio for chilli was recorded at 30.8% indicating the importance of increasing chilli production in the country. Chilli production in Malaysia is hampered by many diseases. One of the major diseases in chilli is anthracnose. Anthracnose disease caused by *Colletotrichum* species is a devastating fungal disease that resulted in huge yield losses in chilli cultivation. Thus, this study was conducted to develop high yielding anthracnose disease-resistant chilli hybrids. The study is composed of four parts. In the first part of the study, 32 inbred lines were evaluated for yield and yield component traits under randomized complete block design (RCBD) with three replicates to assess the genetic variability among inbred lines and identify high yielding parents for F_1 hybrid production. Highly significant differences were observed among inbred lines for yield and yield-related traits indicating the high genetic variability. Inbred line, MICH PL CC 2018/33 was recorded the highest yield per plant followed by MICH PL 21. The trait, number of fruits per plant recorded the highest phenotypic coefficient of variance (PCV) and genotypic coefficient of variance (GCV) (78.01% and 53.97, respectively) followed by the yield per plant (53.09% and 34.29% respectively) confirming high genetic variability among inbred lines for these traits. In the second part, 32 inbred lines were included to evaluate the anthracnose disease severity. Four inbred lines, MICH PL CA 2018/3, MICH PL CA 2018/20, MICH PL CA 2018/21, MICH PL 35 and MICH PL CC 2018/17 were identified as resistant lines for anthracnose disease caused by *C. truncatum.* In the third part of the study, combining ability among inbred lines and the heterosis exhibited by the chilli hybrids for yield and anthracnose disease resistance were studied. The hybrids were obtained by crossing seven inbred lines that were selected based on the first and second parts of the study. A total of 23 genotypes consisting of 14 successful single cross hybrids, seven parents and two check varieties were evaluated in two environments for their yield and yield component traits. Simultaneously, anthracnose disease severity assessment was done for the chilli hybrids, parents and check varieties as the fourth part of the study. Under the combined data analysis over

two environments, significant general combining ability (GCA) was exhibited in female parents for the traits, number of fruits per plant and fruit length. The traits, number of fruits per plant, number of days to fruit maturity, stem diameter, anthracnose disease severity as green mature fruits and red ripened fruits in male parents recorded significant GCA. This indicated the high influence of additive genetic effect for these traits. Specific combining ability (SCA) variances were significant for traits, yield, number of fruits per plant, fruit length, fruit diameter, pericarp thickness, average fruit weight, number of days to first flowering, number of days to maturity, plant height, canopy width and number of primary branches indicating preponderance of non-additive genetic effect in the inheritance of these studied traits. Out of 14 produced hybrids,10 hybrids exhibited resistance to anthracnose disease at green mature fruit stage and red ripe fruit stage with 5 of them, H1, H3, H4, H6 and H8 showed comparable yield data with imported commercial hybrid variety SJ2-46. Hybrids, H8, H3, H4 and H6 exhibited 22.5%, 12.07%, 6.5%, 5.2% higher yield respectively when compared to local commercial open pollinated variety Kulai 907. Further, these five hybrids with high mean performance recorded high mid parent heterosis (MPH) and better parent heterosis (BPH) for yield and anthracnose disease severity indicating the availability of hybrid vigour for commercial production of high yielding hybrids with anthracnose resistance. Among the parental inbred lines, male parents, MICH PL CC 2018/3 and MICH PL CA 2018/21 were identified as good general combiners for the yield. Good general combiners for anthracnose disease resistance were MICH PLCC 2018/3, MICH PL CA 2018/20, MICH PL CA 2018/21 and MICH PL 35. Hybrids, H4, H10, H2 and H7 recorded significant positive SCA for yield. None of the hybrids was a good specific combiner for anthracnose disease severity. Anthracnose disease resistance in parents and hybrids were further confirmed using the molecular markers linked to anthracnose disease resistance gene. This was carried out using previously identified SSR and STS markers. The screening of the hybrids revealed that all hybrids which were identified as resistant by the disease severity test carry the resistant gene for anthracnose disease. In this study, it was proved that the yield and most of the yield-related traits are highly controlled by the non-additive genetic effect that could be exploited during heterosis breeding. Highly significant heterosis values for yield and most of the yield-related traits indicated the availability of hybrid vigour in chilli for hybrid production in commercial level. Comparatively high yield and anthracnose disease resistance against *C. truncatum*, the chilli hybrids H1, H3, H4, H6 and H8 could be promising candidates to include in multi-locational trials before releasing for commercial cultivation in Malaysia.

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

HETEROSIS DAN KEUPAYAAN GABUNGAN UNTUK HASIL DAN KERENTANAN TERHADAP PENYAKIT ANTRAKNOSA UNTUK HIBRID CILI (*Capsicum* spp)

Oleh

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Cili (spesies *Capsicum*) merupakan tanaman rempah dan sayuran yang penting di Malaysia. Pada 2019, kadar sara diri bagi cili direkodkan sebanyak 30.8% dan ini menunjukkan betapa penting untuk pengeluaran cili di negara ini ditingkatkan. Secara umumnya, pengeluaran cili di Malaysia dihalang oleh pelbagai penyakit. Salah satu penyakit utama cili adalah penyakit antraknosa. Penyakit antraknosa adalah penyakit kulat daripada spesies *Colletotrichum* yang boleh mengakibatkan kehilangan hasil yang besar dalam penanaman cili. Oleh itu, kajian ini dijalankan untuk membangunkan cili hibrid yang rentan terhadap penyakit antraknosa serta berhasil tinggi. Di dalam bahagian pertama kajian, 32 titisan inbred telah dinilai untuk hasil dan sifat-sifat komponen hasil. Eksperimen dijalankan di bawah reka bentuk blok lengkap rawak (RCBD) dengan tiga replika untuk menilai kepelbagaian genetik di kalangan titisan inbred dan mengenal pasti induk yang menghasilkan hasil tinggi untuk pengeluaran hibrid F_1. Perbezaan yang sangat ketara telah diperhatikan di kalangan titisan inbred untuk hasil dan sifat berkaitan komponen hasil. Ini menunjukkan kepelbagaiann genetik yang tinggi di kalangan titisan inbred. Titisan inbred, MICH PL CC 2018/33 mencatatkan hasil tertinggi bagi setiap tumbuhan diikuti oleh MICH PL 21. Sifat seperti bilangan buah bagi setiap tumbuhan mencatatkan pekali fenotip varians (PCV) dan pekali varians genotip (GCV) (masing-masing 78.01% dan 53.97) diikuti oleh hasil setiap tumbuhan (masing-masing 53.09% dan 34.29%) mengesahkan kepelbagaian genetik yang tinggi di kalangan titisan inbred untuk sifat ini. Dalam bahagian kedua, 32 titisan inbred dimasukkan untuk menilai kerentanan penyakit antraknosa. Empat titisan inbred, MICH PL CA 2018/3, MICH PL CA 2018/20, MICH PL CA 2018/21, MICH PL 35 dan MICH PL CC 2018/17 telah dikenal pasti sebagai titisan yang tahan untuk penyakit antraknosa yang disebabkan oleh *C. truncatum*. Dalam bahagian ketiga kajian, keupayaan gabungan antara titisan inbred dan heterosis yangoleh hibrid kacukan untuk hasil dan rintangan penyakit antraknosa telah dikaji. Hibrid kacukan diperoleh dengan menyilang tujuh titisan inbred yang dipilih berdasarkan kajian di bahagian pertama dan kedua. Sebanyak 23 genotip yang terdiri daripada 14 hibrid kacukan silang tunggal yang berjaya, tujuh induk dan dua komersil

varieti telah dinilai dalam dua lokasi untuk hasil dan sifat-sifat dari komponen hasil. Pada masa yang sama, penilaian pada kerentanan penyakit antraknosa dilakukan untuk hibrid kacukan cili, induk dan komersil varieti dalam bahagian keempat kajian. Di bawah analisis data gabungan ke atas dua lokasi, keupayaan gabungan am (GCA) yang ketara telah dicapai oleh induk betina untuk sifat; bilangan buah setiap tumbuhan dan panjang buah. Sifat seperti bilangan buah setiap tumbuhan, bilangan hari hingga kematangan buah, diameter batang, kerentanan terhadap penyakit antraknosa pada buah matang hijau dan buah masak merah pada induk jantan merekodkan GCA yang ketara. Ini menunjukkan pengaruh kesan genetik aditif yang tinggi untuk sifat-sifat tersebut. Varians keupayaan penggabungan spesifik (SCA) adalah signifikan untuk ciri seperti hasil, bilangan buah setiap tumbuhan, panjang buah, diameter buah, ketebalan pericarp, purata berat buah, bilangan hari untuk berbunga pertama, bilangan hari untuk matang, ketinggian tumbuhan, kanopi lebar dan bilangan cawangan utama yang menunjukkan kelebihan kesan genetik bukan tambahan dalam pewarisan sifat yang dikaji ini. Daripada 14 hibrid kacukan yang dihasilkan, 10 hibrid kacukan menunjukkan ketahanan terhadap penyakit antraknosa pada peringkat buah matang hijau dan peringkat buah masak merah. Lima hibrid iaitu H1, H3, H4, H6 dan H8 menunjukkan data hasil yang setanding dengan varieti hibrid komersial yang diimport SJ2-46. Hibrid, H8, H3, H4 dan H6 menunjukkan hasil sebanyak 22.5%, 12.07%, 6.5%, 5.2% lebih tinggi jika dibandingkan dengan varieti pendebungaan terbuka komersial tempatan,Kulai 907. Seterusnya, kelima-lima hibrid kacukan dengan prestasi tinggi ini merekodkan heterosis induk pertengahan (MPH) tinggi dan heterosis induk (BPH) yang lebih baik untuk hasil dan kerentanan n terhadap penyakit antraknosa. Ini menunjukan menunjukkan potensi untuk pengeluaran komersial hibrid hasil tinggi serta rentan antraknosa. Antara titisan inbred induk, induk jantan, MICH PL CC 2018/3 dan MICH PL CA 2018/21 telah dikenalpasti sebagai penggabung am yang baik untuk hasil. Manakala, Penggabung am yang baik untuk kerentanan terhadap penyakit antraknosa ialah MICH PLCC 2018/3, MICH PL CA 2018/20, MICH PL CA 2018/21 dan MICH PL 35. Hibrid, H4, H10, H2 dan H7 merekodkan SCA positif yang ketara untuk hasil. Tiada satu pun daripada hibrid kacukan adalah penggabung khusus yang baik untuk kerintangan kepada penyakit antraknosa. Kerintangan penyakit antraknosa pada induk dan hibrid kacukan telah disahkan lagi menggunakan penanda molekul yang dikaitkan dengan gen yang rentan kepada penyakit antraknosa. Ini telah dijalankan menggunakan penanda molekul SSR dan STS yang dikenal pasti sebelum ini. Saringan kepada hibrid kacukan mendedahkan bahawa semua hibrid kacukan yang dikenal pasti sebagai rentan oleh ujian kerentanan penyakit mempunyai gen yang rentan untuk penyakit antraknosa. Dalam kajian ini juga, telah dibuktikan bahawa hasil dan kebanyakan ciri berkaitan hasil dikawal oleh genetik bukan aditif yang boleh dieksploitasi semasa pembiakan heterosis. Nilai heterosis yang sangat ketara untuk hasil dan kebanyakan ciri berkaitan hasil menunjukkan potensi hibrid dalam cili untuk pengeluaran hibrid di peringkat komersial di Malaysia. Adalah dicadangkan bahawa kacukan hibrid cili H1, H3, H4, H6 dan H8 berpotensi untuk memberikan hasil yang agak tinggi dan rentan pada penyakit antraknosa yang disebabkan oleh *C. truncatum* diuji selanjutnya di dalam pengujian pelbagai lokasi yang berskala besar sebelum dikeluarkan untuk penanaman komersial di Malaysia.

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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 degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Declaration by the Members of Supervisory Committee

This is to certify that:

- The research conducted and the writing of the thesis was under our supervision
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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphic
ANOVA	Analysis of Variance
AVRDC	Asian Vegetable Research and Development Center
BPH	Better Parent Heterosis
°C	Degree Celsius
CAPS	Cleaved Amplified Polymorphic Sequence
CTAB	Cetyltrimethyl Ammonium Bromide
Cm	Centimeter
DOA	Department of Agriculture
EF	Elongation Factor
FAOSTAT	Food and Agriculture Organization (Statistics Division)
GCA	General Combining Ability
GS	Glutamine Synthetase
GAPDH	Glyceraldehydes-3-Phosphate Dehydrogenase
G	Gram
ISSR	Inter Simple Sequence Repeat
ITS	Internal Transcribed Space region
LSD	Least Significant Difference
MARDI	Malaysian Agricultural Research and Development Institute
MAS	Marker Assisted selection
mm	Millimeter
%	Percentage
PCR	Polymerase chain reaction

PDA	Potato Dextrose Agar
QTL	Quantitative Trait Loci
rDNA	Ribosomal Deoxyribonucleic acid
STS	Sequence-Tagged Sites
SAS	Statistical Analytical Software
SCA	Specific Combining ability
SCAR	Sequence Characterized Amplified Region
SSR	Simple Sequence Repeat
TBE	Tris-Borate-Ethylenediaminetetraacetic acid
TEF	Translation Elongation Factor
UPM	Universiti Putra Malaysia
USA	United States of America

CHAPTER 1

INTRODUCTION

1.1 Background

Chilli (*Capsicum* sp.) is one of the most cultivated spices and vegetable crops. Chilli plant belongs to the family *Solanaceae* and it originated from South and Central America (Siddappa et al., 2019; Thakur et al., 2019; Sindhusha & Rawat, 2020). The genus *Capsicum* consists of many species, however, only three species are widely used and valued for their pungency, aroma, colour and sensory attributes. The three species are notably *Capsicum annuum*, *Capsicum chinense*, *Capsicum frutescens* (Ananthan et al, 2018). Chilli has been cultivated on more than 1.3 million hectares and the annual global production for chilli is about 36 million tonnes. Asian region itself contributes to almost 23.9 million tonnes of hectares with 1.3 million cultivated lands (FAOSTAT, 2020). In Malaysia, *C. annuum* and *C. frutescens* are the most cultivated species with an annual production of 27,631 metric tonnes in 2019 (Department of Agriculture, 2019).

Hybrid chilli varieties are very popular among farmers worldwide. This is due to their high yielding ability with increased fruit weight and high number of fruits per plant, uniform fruit size, early maturity and very attractive uniform fruit colour (Muthumanickam & Anburani, 2017). Hybrid varieties are developed by the exploitation of hybrid vigour. Hybrid vigour is the increase in size, fertility and overall productivity over the parental inbred lines (Fujimoto et al., 2018). Hybrid vigour or heterosis depends on the existence of dominance effect directly and indirectly through interaction effect involving dominance effect at different loci and prediction of heterosis is very important to know the pattern of inheritance of the valuable agronomic traits of hybrids along with their parents (Singh, 2015). Yield and other important agronomic traits in chilli can be improved through heterosis breeding (Bhutia et al., 2015,). Heterosis has been widely used in crop improvement to increase yield, quality and adaptability of hybrids and it is applied for a large number of crop species including chilli (Dhaliwal et al., 2015; Rohini & Lakshmanan, 2016). In heterosis breeding, Homozygous genotypes called inbred lines are used (Shuro, 2017). Genetic diversity followed by combining ability is considered as selection criteria of inbred lines in hybrid breeding programs (Bhutia et al., 2015; Sreenivas et al., 2019a).

Combining ability is the capacity of a genotype to transmit superior performance to its crosses. This concept of combining ability was first time proposed in maize (*Zea mays*) by Richey and Meyar (Richey & Meyar, 1925). The most important and effective tool to identify the superior parental inbred lines for hybridization is the combining ability analysis which includes general combing ability (GCA) and specific combining ability (SCA) (Fasahat et al., 2016). It provides the information on selecting the parents for hybridization and the nature and magnitude of gene action responsible for the expression of important economic traits (Istipliler et al., 2015). Selection of suitable parent lines is the most critical task for the plant breeders who are dealing with hybrid development.

Therefore, it is necessary to find out the combining ability of the inbred lines for yield and other desired traits in chilli for effective transfer of genes into F_1 progeny (Thilak et al., 2019a).

Yield is a very complex trait and it is controlled by a large number of components traits. Therefore, it is very important to know the interrelationship between yield and its component traits to do proper selection of genotypes to increase the yield (Shweta, et al., 2018). Chilli fruit yield is correlated with other agronomic characters, plant height, fruit set percentage, number of fruits per plant, fruit weight, fruit length, days to green fruit maturity, number of secondary branches per plant and number of primary branches (Bijalwan and Mishra, 2016; Shweta, et al., 2018). Chilli fruit contains a high amount of vitamins C and E, carotenoids, flavonoids and capsaicinoids. It is used as a colouring agent in the food industry. Further, it has medicinal properties with huge potential in the pharmaceutical industry (Srivastava & Mangal, 2019).

Yield in chilli in the world is highly affected by biotic stresses such as bacterial, viral and fungal diseases under field conditions (Nanda, et al., 2016). Among the fungal diseases, anthracnose is a fungal disease in chilli caused by *Colletotrichum* species is one of the major diseases in chilli production throughout the world and breeding for durable anthracnose resistance is very important (Silav et al., 2019). This pathogen is known to cause yield losses of up to 50% worldwide, especially in major chilli cultivating areas in tropical and sub-tropical regions (Saxena et al., 2016). Three *Colletotrichum* species, *C. truncatum* (syn. *Colletotrichum capsici*), *C. scovillei* (previously identified as *C. acutatum*) and *C. siamense* (previously identified as *C. gloeosporioides*) have been reported as the major causal agents of chilli anthracnose disease in chilli (Lin et al., 2016; Mongkolporn, 2019). Among these *Colletotrichum* species, *C. truncatum* is the most prevalent species in major chilli cultivating areas in the tropical and sub-tropical region (Diao et al., 2017; Chinthagunta & Zacharia, 2018; De Silva et al., 2019; Welideniya et al., 2019). Even though physical, biological and chemical control methods can be applied to control this disease, development of resistant varieties is the most economical and sustainable method to control this disease (Mishra et al., 2019b; Zhao et al., 2020). Pairing heterosis breeding with Marker Assisted Selection (MAS) to develop anthracnose disease-resistant chilli varieties may help to increase the efficiency of the variety development process (Dhaliwal et al, 2015; Suwor et al., 2017).

1.2 Problem Statement

Anthracnose disease in chilli is one of the major problems in chilli cultivation in Malaysia. This disease results in huge post-harvest yield losses in the country (Ali et al., 2016 ; Noor & Zakaria, 2018). Among the *Colletotrichum* species that can cause anthracnose disease, *C. truncatum* is the common to be found in Malaysia (Noor & Zakaria, 2018). Resistance to *Colletotrichum* species in chilli pepper cultivars has not been reported in Malaysia (Ridzuan et al, 2018b). The average production of chilli in the country is only 9.7 t/ha. This number is very low in the comparison with other top chilli producing countries (Department of Agriculture, Malaysia, 2018). chilli has the highest import dependency ratio Among the vegetable crops, with a value of 73.6 % (Department

of Statistics, Malaysia, 2019). Looking at the current situation, there is a need to develop high yielding chilli varieties with anthracnose disease-resistant for commercial cultivation particularly in Malaysia. Heterosis breeding method can be applied to achieve both high yield and anthracnose disease resistance. In order to develop high yielding anthracnose disease-resistant chilli hybrid varieties, it is necessary to identify anthracnose resistant chilli breeding lines using genetically diverse germplasm. However, finding the resistant germplasm is a critical problem since only a very few germplasms have been identified with anthracnose disease resistance in the world. Thirty-two chilli inbred lines were used for this study and these inbred were developed paying major attention on high yield and anthracnose disease resistance under natural environmental conditions (Department of Agriculture, Sri Lanka, 2018). Therefore, there is a big potential to develop high yielding anthracnose disease-resistant chilli hybrids using these inbred lines. Further, identification of molecular markers linked to anthracnose disease resistance will help to increase the efficiency of heterosis breeding for high yield and anthracnose disease resistance.

1.3 Objectives

The main objective of this study is to develop F_1 chilli hybrid/hybrids with high yield and anthracnose resistance through heterosis breeding.

The specific objectives are:

1. To assess the genetic variability of 32 inbred lines for yield and yield component traits
2. To isolate and identify the *Colletotrichum* species, *C. truncatum* and anthracnose disease severity assessment of 32 inbred lines in chilli
3. To quantify the gene effect and heritability for yield, yield-related traits & anthracnose resistance in chilli hybrids derived from the selected inbred lines
4. To estimate the combining ability among inbred lines and heterosis exhibited by the chilli hybrids derived from chilli inbred lines selected for yield, yield-related traits and anthracnose disease resistance
5. To Confirm the anthracnose disease resistance in chilli hybrids using linked Simple Sequence Repeats (SSR) markers for anthracnose disease resistance

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