DEVELOPMENT OF ANTHRACNOSE RESISTANT CHILI VARIETIES THROUGH MARKER-ASSISTED PEDIGREE SELECTION

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By

RAIHANA BINTI RIDZUAN

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

December 2018

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DEDICATION

I dedicate this thesis to my beloved husband, Mr. Halim bin Ahmad and my mother Mrs. Hairunnishah binti Mat Jaffari for your patience, 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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December 2018

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

Capsicum annuum (chili) is one of the most valuable vegetable crops worldwide. However, the quality and yield production of this crop is facing a significant challenge due to the most destructive fungal disease namely anthracnose. As results of anthracnose disease infection in chili production in Malaysia, this study was conducted to develop high yield and anthracnose resistant *C. annuum* genotypes through marker-assisted pedigree selection. The specific objectives of this study were to evaluate morpho-physiological and yield performances of 14 chili genotypes over two planting seasons, to determine virulent *Colletotrichum* species via pathogenicity test as well as to identify their species using morphological and molecular characteristics, to select parental lines based on anthracnose disease severity, biochemical content and antioxidant activity for crossing program, to identify SSR markers linked to anthracnose resistance in the parental, F_1 and F_2 populations, and to select high-yielding and anthracnose resistant genotypes in F_2 population. This study consists of four experiments, where in the first experiment on field evaluation of 14 genotypes, genotype AVPP9813 indicated the highest number of fruits and total yield per plant (130.13 fruits and 541.39 g) followed by Kulai 907 and Chili Bangi 5, (99.63 fruits, 502.64 g) and (110.00 fruits, 418.46 g) respectively. The highest genotypic (GCV) and phenotypic coefficient of variation (PCV) were shown by the fruit number per plant (49.71% and 66.04%, respectively). High heritability was observed in yield characters; fruit weight, length and girth and indicated high genetic advance. A total of eight groups were obtained from the cluster analysis. In the second experiment, five virulent isolates were successfully selected according to their degree of virulence with more than 15% anthracnose severity lesion (anthracnose severity score 7). The phylogenetic analyses from DNA sequence data based on the ITS regions clearly grouped three isolates as *C. truncatum*, one isolate as *C. fructicola*, and another one isolate as *C. sojae* with bootstrap support 100%, 98% and 100% respectively. In the third experiment, the responses of ripe fruits of 14 selected chili genotypes on these virulent *Colletotrichum* species revealed the resistance of genotypes SDP203-6-3, AVPP0009, AVPP0514, AVPP0705, AVPP0805 and AVPP9813 against all isolates with less than 2% anthracnose lesion (anthracnose severity score 1). In case of biochemical experiment, the capsaicinoids content and total

phenolic content were high in Chili Bangi 3 at ripe dry fruit while for antioxidant activity using beta carotene bleaching assay, SDP203 was the highest in ripe dry fruit. Two susceptible genotypes namely, Kulai 907 and Chili Bangi 3, and two resistant genotypes; AVPP0805 and AVPP9813 were selected for marker-assisted pedigree breeding program. In the fourth experiment, a total of 165 F_1 crosses and reciprocal plants were screened with one SSR marker (Hpms 2-24) linked to anthracnose resistant genes. Out of the 165 plants, 72 plants were confirmed carrying resistance genes. These plants were evaluated for morphological and yield traits, and were challenged against the most virulent *C. truncatum* isolate (Genebank accession number: MG016494). In term of yield and yield performances, F_1 hybrids especially Kulai 907 × AVPP0805 showed better results compared to those F_1 reciprocal crosses which were good in growth performances. Further, 24 high yield and resistant F_1 plants were selected and selfed to produce F_2 generation. The chi-square analysis based on 283 individual plants from F_2 population showed that it is no significance differences between observed and expected values with 1:2:1 ratio. This indicated that the anthracnose resistant gene is control by a single gene inheritance. Ten improved anthracnose resistant chili lines namely, AP-13, AP-18, AP-25, AP-37, AP-41, BP-23, BP-44, CP-36, DP-37, DP-51 and DP-57 were selected from F_2 generation showing high yield and anthracnose resistant plants for further breeding program.

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

PEMBANGUNAN VARIETI CILI TAHAN PENYAKIT ANTHRAKNOS MELALUI PEMILIHAN PEDIGRI BANTUAN PENANDA

Oleh

RAIHANA RIDZUAN

Disember 2018

Penyelia: **Profesor Mohd Rafii Yusop, PhD**
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Capsicum annuum (cili) merupakan salah satu tanaman sayuran yang bernilai tinggi di seluruh dunia. Walau bagaimanapun, kualiti dan hasil pengeluaran tanaman ini menghadapi cabaran yang besar disebabkan oleh penyakit kulat iaitu antraknos. Akibat penularan jangkitan penyakit antraknos terhadap pengeluaran cili di Malaysia, kajian ini telah dijalankan untuk membangunkan genotip cili yang mempunyai hasil tinggi dan tahan terhadap penyakit antraknos melalui pemilihan pedigri bantuan penanda. Objektif bagi kajian ini adalah untuk menilai prestasi morfo-fisiologi dan hasil buah bagi 14 genotip cili dari dua musim penanaman, untuk menentukan spesies *Colletotrichum* yang virulen melalui ujian patogenisiti serta mengenal pasti isolat berdasarkan ciri-ciri morfologi dan molekular, untuk memilih waris induk yang terbaik berdasarkan peratusan keparahan penyakit antraknos, kandungan biokimia dan aktiviti antioksidan untuk program pembiakbakaan, untuk mengenal pasti penanda SSR yang dikaitkan dengan kerintangan terhadap antraknos dalam populasi induk, F_1 dan F_2, dan untuk memilih genotip yang mempunyai hasil tinggi dan tahan terhadap antraknos dalam populasi F_2. Kajian ini terdiri daripada empat eksperimen, di mana pada eksperimen pertama iaitu penilaian di lapangan ke atas 14 genotip cili, genotip AVPP9813 menghasilkan bilangan dan hasil buah sepokok yang tertinggi (130.13 buah dan 541.39 g) diikuti oleh Kulai 907 dan Chili Bangi 5, masing-masing dengan (99.63 buah dan 502.64 g) dan (110.00 buah dan 418.46 g). Genotip yang paling tinggi pekali variasi genotip (GCV) dan pekali variasi fenotip (PCV) ditunjukkan oleh bilangan buah setiap pokok (masing-masing 49.71% dan 66.04%). Nilai keterwarisan yang tinggi didapati untuk ciri-ciri hasil iaitu berat buah, panjang buah dan ukur lilit buah serta menunjukkan kemajuan genetik yang tinggi. Sejumlah lapan kumpulan telah dikelaskan berdasarkan analisis kluster. Dari eksperimen kedua, lima isolate kulat virulen berjaya dipilih mengikut tahap virulen iaitu lebih daripada 15% peratusan luka antraknos (skor luka antraknos 7). Analisis filogenetik daripada data urutan DNA berdasarkan kawasan ITS telah mengenalpasti tiga isolat sebagai *C. truncatum,* satu isolat sebagai *C. fructicola*, dan satu isolate lagi sebagai *C. sojae* dengan sokongan bootstrap 100%, 98% dan 100%. Dari eksperimen ketiga, tindak balas buah cili buah cili matang daripada 14 genotip cili terpilih terhadap tiga spesies *Colletotrichum* yang virulen tersebut menunjukkan kerintangan genotip SDP203-6-3,

AVPP0009, AVPP0514, AVPP0705, AVPP0805 dan AVPP9813 terhadap semua isolat iaitu peratusan luka antraknos kurang daripada 2% (skor luka antraknos 1). Bagi eksperimen biokimia, kandungan capsaisinoid dan jumlah kandungan fenolik tinggi dapat dilihat pada buah matang yang kering genotip Chili Bangi 3 manakala untuk aktiviti antioksida, buah matang kering genotip SDP203 menunjukkan bacaan yang tertinggi. Dua genotip yang rentan antraknos iaitu Kulai 907 dan Chili Bangi 3, dan dua genotip rintang antraknos iaitu AVPP0805 dan AVPP9813 telah dipilih untuk program pembiakbakaan pedigri bantuan penanda. Dari eksperimen keempat, sebanyak 165 pokok daripada hibrid F_1 dan resiprokal telah diuji dengan satu penanda SSR (Hpms 2-24) yang dikaitkan dengan gen tahan antraknos. Daripada jumlah 165 pokok F_1 tersebut, 72 pokok telah disahkan membawa gen tahan antraknos. Kesemua pokok tersebut dinilai untuk ciri-ciri morfologi dan hasil buah, serta diuji ketahanan terhadap isolat *C. truncatum* (nombor aksesi Genebank: MG016494) paling virulen. Berdasarkan hasil dan komponen hasil buah, F_1 hibrid Kulai 907 × AVPP0805 menunjukkan hasil yang lebih baik berbanding dengan salingan F_1 yang menunjukkan ciri-ciri morfologi yang baik. Seterusnya, 24 pokok F_1 yang menunjukkan hasil buah yang tinggi dan ketahanan terhadap antraknos telah dipilih untuk menghasilkan generasi F_2. Analisis khi-kuasa dua berdasarkan 283 individu dari populasi F_2 menunjukkan bahawa tidak ada perbezaan yang signifikan antara nilai yang diperhatikan dan nilai yang dijangka dengan nisbah 1:2:1. Ini menunjukkan bahawa pewarisan gen tahan antraknos adalah secara gen tunggal. Sepuluh waris cili maju rintang antaknos iaitu AP-13, AP-18, AP-25, AP-37, AP-41, BP-23, BP-44, CP-36, DP-37, DP-51 dan DP-57 telah dipilih dari generasi F_2 yang mempunyai hasil buah yang tinggi dan tahan terhadap antraknos untuk program pembiakbakaan seterusnya.

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I certify that a Thesis Examination Committee has met on 20 December 2018 to conduct the final examination of Raihana Binti Ridzuan on her thesis entitled "Development of anthracnose resistant chili varieties through marker-assisted pedigree selection" in accordance with the Universities and University Colleges Art 1971 and the Constitution of the Universiti Putra Malaysia [P.U (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA	Analysis of Variance
AVRDC	World Vegetable Center
BHT	Butylated Hydroxytoluene
BPH	Better Parent Heterosis
cm	Centimeter
°C	Degree Celsius
DNA	Deoxyribonucleic Acid
et al.	*et alia*
F_1	First filial generation
F_2	Second filial generation
F-C	Folin-Ciocalteau
FAOSTAT	Food and Agriculture Organization (Statistics Division)
GAE	Gallic Acid Equivalence
GPS	Global Positioning System
g	Gram
g/L	Gram per liter
H	Hour
ITS	Internal Transcribed Spacer
L	Liter
LSD	Least Significant Differences
MABC	Marker-assisted Backcrossing
MAB	Marker-assisted Breeding
MAPS	Marker-assisted Pedigree Selection
MAS	Marker-assisted Selection
MPH	Mid Parent Heterosis
µM	Micro molar
mg	Milligram
mg/L	Milligram per liter
mm	Millimeter
min	Minute
M	Molar
PCR	Polymerase Chain Reaction
%	Percentage
AA%	Percentage of antioxidant activity
±	Plus minus
PDA	Potato Dextrose Agar
pH	Potentiometric hydrogen ion concentration
SAS	Statistical Analysis Software
SE	Standard Error
SSR	Simple Sequence Repeat
TPC	Total Phenolic Content
UFLC	Ultra-Fast Liquid Chromatography
UPM	Universiti Putra Malaysia

CHAPTER 1

INTRODUCTION

1.1 Introduction

Chili is one of the important economic vegetables worldwide. The world production in 2017 was approximately 36.1 million tonnes for fresh chili and 4.63 million tonnes for dry chili (FAOSTAT, 2017). The main chili producing countries are India, China, Indonesia, Mexico, Korea, Nigeria, Ghana, and Turkey. India stands as the largest producer with 35.5% of the world's dry chili production (FAOSTAT, 2016).

In Malaysia, two species of *Capsicum*; *C. annuum* (*cili besar*) and *C. frutescens* (*cili padi*) were widely grown but *C. annuum* is planted in large scale to meet the local demand. A remarkable decrease of chili production was observed in 2017 with 27,358 tonnes produced from 3,067 ha which was up to 37% reduction as compared to previous year (DOA, 2017). Most of chili production is affected by biotic factors, such as phytopathogenic fungi, bacteria, viruses, weeds and other pests such as root-knot nematodes, aphids and thrips; and abiotic factors, such as temperature, moisture and rainfall. One of the major biotic stresses in chili production is anthracnose disease which occurred during pre-harvest and post-harvest stages (Shin *et al*., 2000; Oanh *et al*., 2004; Sharma *et al*., 2005; Taylor *et al*., 2007).

Anthracnose is a fungal seed-borne disease caused by *Colletotrichum* spp. that has marketable yield loss of approximately 50% of the chili production in Malaysia (Sariah, 1994), 35% in Indonesia (Sastrosumarjo, 2003) and 80% in Thailand (Poonpolgul and Kumphai, 2007). Several reports were documented in United States and Brazil on major destructive of chili production due to this disease (Harp *et al*., 2008; Tozze and Massola, 2009).

Several strategies have been anticipated to control the anthracnose including rotation cropping system, biological and chemical fungicides. Biological fungicides such as the combination of plant extracts neem (*Azadirachta indica*), mahogany (*Swietenia mahagoni*) and garlic (*Allium sativum*) was reported to have shown a significant impact in controlling chili anthracnose (Rashid *et al*., 2015). Chemicals fungicides such as propiconazole (0.1%) (Gopinath *et al*., 2006; Yadav *et al*., 2014), mancozeb (0.2 %) (Linu and Jisha, 2006), fludioxonil (Gao *et al*., 2017) have been used to control anthracnose disease. Although chemical fungicide application is the most popular method of controlling anthracnose disease, however, they are considered uneconomical and not sustainable for small-holder farmers due to their high risks to the human health and environment (Montri, 2009; Setiawati *et al*., 2011).

One of the sustainable and significant strategies to reduce the crop losses is to cultivate resistant chili genotypes. Marker assisted selection (MAS) is one of strategies applied for durability of resistance in a cultivar. The employment of MAS in chili breeding programs appears to provide advantages for selection during the juvenile phase, for pyramiding disease resistance genes, and replacing expensive, time-consuming phenotyping selection (Eibach *et al*., 2007; Collard and Mackill, 2008; Rasheed *et al*., 2017; . Nevertheless, the successful of MAS in crop improvement is challenging since it depends on the association of target traits and genotyping in well-structural breeding programs (Yang *et al*., 2016).

1.2 Problem statements

Malaysia is still importing chilies from neighboring countries due to insufficient local production. To reach up almost 50 thousand tonnes per year demand, it is necessary to increase both cultivation area as well as crop productivity (Awang *et al*., 2015). One of the most constrain factor to increase chili production in Malaysia is due to anthracnose disease. However, there are lack of available high yield and anthracnose resistant chili genotypes for commercial cultivation in local environment. Cultivation of resistant chili genotypes can reduce the use of chemical fungicide application, decrease the cost of production and increase profit. One of the efficient method for varietal development is the used of marker-assisted selection (MAS). MAS speed up the selection process and reduce genetic drag in the segregating population. The application of molecular markers is a low-cost, high-throughput method to detect disease resistance genes, permitting for the introgression of genes into susceptible genotypes and the pyramiding of multiple genes into individual lines.

1.3 Research objectives

The main objective of this study was to develop high yield and anthracnose resistant chili genotypes through marker-assisted pedigree selection (MAPS).
The specific objectives were:

1. To evaluate morpho-physiological and yield performances of 14 chili genotypes over two planting seasons.
2. To determine virulent *Colletotrichum* species based on pathogenicity and phylogeny.
3. To select parental lines based on anthracnose disease severity, biochemical content and antioxidant activity for crossing program.
4. To identify SSR markers linked to anthracnose resistance in the parental, F_1 and F_2 populations.
5. To select high-yielding and anthracnose resistant lines in F_2 population.

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