UNIVERSITI PUTRA MALAYSIA

LESION DEVELOPMENT, BIOCHEMICAL CHANGES AND GENETIC RELATIONSHIPS ASSOCIATED WITH RESISTANCE TO *COLLETOTRICHUM* SPP. ON CHILLI PEPPER (*CAPSICUM* SPP.)

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By

ITEU MARGARET HIDAYAT

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

March 2002

Dedicated to:

My mother and late father:
Ibu R. Y. Marie Hartati and Bapak R. J. D. Hidayat Prawiraatmadja

My late aunt and late uncle:
Ibu R. Mien Amalia Prawiraatmadja and Bapak Soetardjo Sindoemintardjo

My late grandmother:
Mak Entjeh binti Haji Basari

. . . . . who inspired, supported and gave me tremendous courage to be a well educated person

All teachers, friends and strangers
. . . . . who crossed their paths with mine, and thought me essential things for life

And

Ibu Pertiwi . . . . . Indonesia.

Abstract of the 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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Chairman : Professor Marziah Mahmood, Ph.D.

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Chilli pepper (*Capsicum* spp.) is one of the important vegetable crops, with have attractive features in the fruits: aesthetic quality of aroma, taste, color, texture, and also nutrients, minerals, vitamins and antioxidant properties. Resistance to *Colletotrichum* spp., causal agent of pre- and post harvest fruit rot diseases on commercial chilli pepper cultivars has not been reported, and screening for resistance could have been a difficult task. Based on lesion development (width, length, percentage of lesion, percentage of sporulation, and rate of lesion development), responses of detached green and red fruits of four lines of chilli pepper (P3, P5, 327 and 146) to spot and wound inoculation with *C. capsici* and *C. gloeosporioides* were evaluated in factorial experiments with complete randomized design.

Results indicated that there were different responses among the lines tested. The red fruits were more susceptible than the green fruits, and wound inoculation accelerated infection. *C. capsici* was less virulent than *C. gloeosporioides.* Lesion development can be used as components for assessment for resistance to *C. capsici* and *C. gloeosporioides*. Biochemical changes (chlorophylls, carotenoids, total soluble phenolics, total basic and acid soluble proteins and enzymes activities peroxidase, polyphenol oxidase, chitinase and β-1,3-glucanase) were studied on fresh (H) and incubated detached fruits: fresh fruits (HC), wounded fruits (HP)., and inoculated fruits with *C. capsici* and *C. gloeosporioides*. Tissue samples of line 327 at 30, 45, and 60 days after anthesis (DAA) and var. Cili Bangi2 at 60 DAA were collected from the incubated fruits at 2, 4 and 6 days of incubation or days post inoculation (DPI) of healthy (HI), surrounding lesion (TL) and lesion (L). Regression analysis between biochemical changes with lesion development indicated several significant relationships. However, total soluble phenolics of HI and TL, and HI of fruits line 327 at 30 DAA inoculated with *C. capsici* and *C. gloeosporioides* respectively indicated potential use as marker for the responses. Constitutive peroxidase activities on leaves of 20 lines/varieties chilli pepper did not show any significant relationship with level of resistance to anthracnose on their respective fruits. Furthermore, OPE primers were able to detect Cili api which belongs to *C. frutescens* with the highest genetic distance (0.500), and within lines/varieties of *C. annuum* with genetic distances varying from 0.042 to 0.443.

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

PERKEMBANGAN LESI, PERUBAHAN BIOKIMIA DAN HUBUNGAN GENETIK YANG BERKAITAN DENGAN KERESISTENAN TERHADAP *COLLETOTRICHUM* SPP. PADA CILI (*CAPSICUM* SPP.)

Oleh

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Cili (*Capsicum* spp.) adalah salah satu tanaman sayuran penting yang mempunyai ciri-ciri menarik pada buahnya: kualiti estetik aroma, rasa, warna, tekstur, dan juga nutrient, mineral, vitamin dan bahan-bahan anti oksidan. Keresistanan terhadap *Colletotrichum* spp., penyebab penyakit busuk buah sebelum dan selepas tuai pada cili komersial masih belum dilaporkan, dan saringan untuk keresistanan mungkin merupakan satu perkara yang sukar. Berasaskan perkembangan lesi (lebar, panjang, dan peratus lesi, peratus pensporaan dan kadar pembentukan lesi) gerak-balas di antara buah cili hijau dan merah yang terpisah untuk empat galur cili (P3, P5, 327 dan 146) terhadap inokulasi *C. capcisi* dan *C. gloeosporioides* secara titik dan luka, dalam eksperimen faktoran yang disusun berbentuk rawak sepenuhnya.

Hasil kajian menunjukkan perbezaan gerak-balas di antara galur cili yang diuji. Buah merah adalah lebih rentan berbanding buah hijau, dan inokulasi luka mempercepatkan jangkitan. *C. capcisi* adalah kurang virulen berbanding *C. gloeosporioides*. Perkembangan lesi boleh digunakan sebagai komponen untuk menilai keresistanan terhadap *C. capcisi* dan *C. gloeosporioides*. Perubahan biokimia (klorofil, karotenoid, fenolik larut penuh, protein-larut penuh alkali dan asid dan aktiviti enzim peroksidase, polifenol oksidase, kitinase dan β-1,3-glucanase) telah dikaji pada buah cili terpisah segar (H) dan yang diinkubasi: buah sihat (HC) dan buah luka (HP), dan buah yang diinokulasi dengan *C. capcisi* dan *C. gloeosporioides*. Sampel tisu-tisu dari buah cili terpisah galur 327 pada 30, 45 dan 60 hari selepas anthesis (DAA) dan var. Cili Bangi2 pada 60 DAA dikumpulkan dari buah yang diinkubasi pada 2, 4, dan 6 hari inkubasi atau hari selepas infeksi (DPI) pada tisu sihat (HI), tisu sekeliling lesi (TL) dan tisu lesi (L). Analisis regresi diantara perubahan biokimia dengan perkembangan lesi menunjukkan beberapa hubungkait yang bermakna. Walau bagaimanapun, fenolik larut penuh dalam HI dan TL, dan HI buah galur 327 pada 30 DAA yang diinokulasi dengan *C. capcisi* dan *C. gloeosporioides* masing masing menunjukan potensi kegunaan sebagai penanda dalam gerak balas. Aktiviti peroxidase konstitutif dalam daun dari 20 galur/varieti tidak menunjukkan hubungkait langsung dengan paras keresistanan terhadap antraknos pada buah masing masing. Lagi pun primer OPE telah berjaya mengesan Cili Api yang termasuk dalam *C. frutescens* dengan indeks jarak genetik yang tinggi (0.500), dan jarak genetik di antara galur/varieti *C. annuum* antara 0.042 hingga 0.443.

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ACC	1-aminocyclopropane-1-carboxylate
AVRDC	Asia Vegetable Research Center Development
avr gene	avirulence gene
bp	base pair
°C	Centigrade
cDNA	complementary DNA
cm	centimeter
DAA	Days after anthesis
DPI	Days post infection
DAT	Days after transplanting
dATP	deoxy adenine triphosphate
dCTP	deoxy cytosine triphosphate
dTTP	deoxy thymine triphosphate
dGTP	deoxy guanine triphosphate
dd H_2O	double distilled water
DMRT	Duncan's Multiple Range Test
DNA	deoxyribo nucleic acid
EC	enzyme commision
EDTA	ethylene diaminetetra acetic acid
FAO	Food and Agriculture Organization
FW	Fresh Weight
g	gram
GATA	guanine, adenine, thymine, adenine
GRSU	Genetic Resources and Seed Unit of AVRDC
h	hour
HR	Hypersensitive
IDPM	Integrated Pest Disease Management
INA	2,6-dichloroisonicotinic acid
ISR	Induced systemic resistance
kD	kiloDalton
kg	kilogram (10^3 gram)
l	litre
M	Molar (10^3 mM; 10^6 M)
m	metre (10^2 cm; 10^3 mm)
major gene	a gene which is inherited in a Mendelian manner and whose allelic forms give qualitatively distinct phenotypes (Jones et al., 1997).
MARDI	Malaysia Agricultural Research and Development Institute
minor genes	the genes that contribute to the complex phenotypes (usually polygenes) (Jones et al., 1997).
ml	mililitre
mm	milimetre
mM	milimolar
Mt	Metric ton
NPK	Nitrogen, Phosphorous, Potassium
NaCl	sodium chloride

nkat	nano katal enzyme activity. One nkat equal to amount of enzyme catalyzing one nM product equivalent in one second under assay conditions.
nm	nanometer
nM	nanomolar (10^{-2} molar)
P	probability
PAGE	Polyacrylamide Gel Electrophoresis
PCR	Polymerase chain reaction
pg	picogram(10^{-4} gram)
POX	Peroxidase
PPO	Polyphenol oxidase
PR protein	Pathogenesis-related protein
QTL	Quantitative Trait Loci
Quatitative resistance:	resistance is expressed as compatible and incompatible interactions, can be distinguished with sharply defined phenotypes, also called differential resistance. Monogenic dominant or recessive, mostly due to effect of a single gene (major gene).
Quantitative resistance:	resistance is expressed as a continuous range distribution between extremes which may even lie outside the mean range of the parents. Some times refers as general or horizontal resistance, mostly due to polygenic (minor genes)
R	Resistance is the ability of the host to suppress or retard the activity of the invading pathogen
R^2	Regression line
RAPD	Random Amplified Polymorphic DNA
RDA	Recommended Dietary Allowance
RE	Recommended Equivalent
R_f	Relative front
RIV	Research Institute for Vegetables
rpm	rotation per minute
SA	Salicylic acid
SAR	Systemic acquired resistance
spp.	Species
Susceptible	
T	Tolerance
TAE	Tris-HCl-glacial acetic acid -EDTA
TE	Tris-EDTA
TEMED	N,N,N,N-tetramethylethylenediamine
Tolerant	Ability of plants to produce a good crop even when they are infected with pathogen
U	unit of enzyme activity. One unit equal to change in 0.1 absorbance/minute/mg protein. 1U = 1µmol min^{-1} = 16.67 nkat
µg	microgram(10^{-3} gram, 10^{-6} kg)
µl	microlitre (10^{-3} ml, 10^{-6} l)
UPM	Universiti Putra Malaysia
UV	Ultra violet
v/v	Volume/volume
w x l x h	Width x length x height
y	Regression equation

CHAPTER I

INTRODUCTION

Chilli pepper (*Capsicum* spp.), also known as chile, chillies, aji, pimiento, paprika, capsicum, and chilli pepper, is one of the most important horticultural crops (Pickersgill, 1991; Sauer, 1993). The fruit characteristics such as shapes, colors, pungency, flavor, oleoresin, nutrient and minerals, α and γ tocopherol, and antioxidant contents diversify the use of chilli pepper, such as vegetable, spices, medicine, and an ornamental crop (Bagget and Kean, 1988; Bosland e*t al.,* 1990; Stommel and Griesbach, 1993; Rubatzky and Yamaguchi, 1997; Osuna-Garcia e*t al.,* 1998; Klein and Kurilich, 2000).

Although its vitamin A content is lower than that in carrots, its vitamin C is much higher compared to tomato and other vegetables (Appendix A1). Its ascorbic acid and carotenoids contributing 124 – 338% of the RDA for vitamin C and 0.33 - 336 RE/100 g of pro-vitamin A activity respectively (Howard e*t al.,* 2000). Thus, chilli pepper fruits could contribute as an antioxidant effect through food consumption, of which if taken routinely can lower the risk of cancer and cardiovascular diseases, and immune depression (Ramesh e*t al.,* 1999; Wargovich, 2000).

The pungency is due to capsaicinoids with the pungent principle capsaicin ($C_{18}H_{27}NO_3$), volatile aromatic compounds, and the flavor is mostly

due to capsanthin ($C_{40}H_{58}O_3$) (Rubatsky and Yamaguchi, 1997). Most of non-pungent types are used as vegetable and food coloring.

However, production of chilli pepper in hot and humid tropical regions has been hampered by pests and diseases (Poulos, 1992). Anthracnose is one of the most important diseases that caused 10 – 60% yield loss, which causes pre- and post-harvest losses on chilli pepper fruits (Mah, 1985; Sariah, 1994a). The causal agent of the disease is a fungus *Colletotrichum* spp. Cultural practices and fungicides have been applied in order to minimize the damage and to protect the crops (Cheah *et al.,* 1992; Vos, 1994). On the other hand, there has been growing concern that the excessive application of pesticides causes harmful effects to the environments and human health. Therefore, utilization of resistant varieties combined with other integrated pest management components provides one alternative to overcome the problem.

Breeding for disease resistance to anthracnose governed by polygenes is in progress (Chew *et al*, 1992; AVRDC, 1997; 1998; 1999). The goal is to introduce resistance genes into commercial varieties, however, presently no variety/cultivar with resistance to anthracnose is yet available (Hartman *et al*., 1992; AVRDC, 1998). This may be due to the resistant source has not been fully explored and exploited (Palloix, 1992); interspecies crossing barriers (Poulos, 1994; Pickersgill, 1997); knowledge