



UNIVERSITI PUTRA MALAYSIA

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

ITEU MARGARET HIDAYAT

FSAS 2002 11

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

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.

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

by

ITEU MARGARET HIDAYAT

March 2002

Chairman : Professor Marziah Mahmood, Ph.D.

Faculty : Science and Environmental studies

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. annum* 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

ITEU MARGARET HIDAYAT

Mac 2002

Pengerusi : Profesor Marziah Mahmood, Ph.D.

Fakulti : Sains dan Pengajian Alam Sekitar

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 faktorannya 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 menunjukkan 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. annum* antara 0.042 hingga 0.443.

ACKNOWLEDGEMENTS

I am particularly indebted to the chairman of my committee: Professor Marziah Mahmood, Ph. D.; members of my committee: Professor Sariah Meon Ph.D. and Professor Datin Khatijah Mohd. Yusoff Ph.D., for their support, assistance, encouragement, and kindly providing research facilities for the study, and critically reviewed my thesis. My high appreciation is due to Professor Othman Omar, an independent examiner, for his careful examination and valuable suggestion for my thesis.

I wish to thank Malaysian Technical Cooperation Program (MTCP), Agency for Agriculture Research and Development (AARD), Ministry of Agriculture and Forestry Indonesia, through ARMP II Project, and Ms. Iteu M. Hidayat for providing me sponsorship throughout the study.

I wish to thank Breeders Dr. Chew B. Hock and Dr. Melor Rejab of MARDI, Malaysia, Dr. Anggoro Hadi Permadi and Ir. Yenni Kusandriani of RIV, Indonesia, for providing chilli pepper seeds for the experiments.

From Agency for Agriculture Research and Development (AARD) Indonesia, I am indebted and very grateful to the Directors of Lembang Research Institute for Vegetables for providing research facilities for the study, the Directors of Central Research Institute for Horticulture, ARMP II Project Leaders, the Committee of Human Resources Development AARD, for their tremendous support, understanding, and encouragement.



For sincere help, support and encouragement of Kak Armi Shamsuar, Dr. Azlan Jualang Gansau Abdullah, Dr. Aziz Ahmad, Dr. Ida Hanarida Somantri, Dr. Rusli Ibrahim, Ibu Ir. Soertini Soedjono MS, Cik Hafizah Mohd., and for letting their hands and time in one way or another of all wonderful people in Laboratory 235 Department Biochemistry and Microbiology, Lab. Plant Pathology, Ladang 2, Glasshouse of UPM, En. Ariff Zaidi Jusoh, Dr. Yunus Shukor, Mas Agung, Dr. Darlis, Dr. Maria Viva Rini, Ms. Wei Ling, Persatuan Pelajar Indonesia (PPI) UPM, and in particular, staffs of Research Institute for Vegetables, Lembang: Ir. Ineu, Isum and Pak Entu are highly appreciated. I am thankful for all their assistance without which I could not have completed my study.

Last but not least, I wish to thank all my family for their patient, understanding and faith on me. Alhamdu lillaahi !!!

TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	vii
APPROVAL SHEETS	ix
DECLARATION FORM	xi
LIST OF TABLES	xvi
LIST OF FIGURES	xvii
LIST OF PLATES	xx
LIST OF ABBREVIATIONS/NOTATIONS/GLOSSARY OF TERMS	xxi
CHAPTER	
I INTRODUCTION	1.1
II LITERATURE REVIEW	2.1
2.1. <i>Capsicum</i> spp.	2.1
2.1.1. Genetic Background and Economic Values	2.1
2.1.2. Germplasm and Breeding on <i>Capsicum</i> spp.	2.3
2.1.3. Breeding for Resistance to <i>Colletotrichum</i> spp. on Chilli Pepper	2.6
2.1.4. Genetic Markers	2.10
2.2. <i>Colletotrichum</i> spp.	2.17
2.2.1. Classification, General Characteristics, Host Range and Epidemiology	2.17
2.2.2. Infection Process	2.20
2.2.3. Anthracnose on Chilli Pepper	2.24
2.3. Plant-Pathogen Interaction	2.25
2.3.1. Resistance	2.31
2.3.2. Biochemical Changes in Defense Responses	2.33
2.3.2.1. Phenolics	2.33
2.3.2.2. Chlorophylls	2.35
2.3.2.3. Carotenoids	2.37
2.3.2.4. Protein	2.38
2.3.2.4.1. Peroxidase	2.39
2.3.2.4.2. Polyphenol oxidase	2.40
2.4.3.4.2. Chitinase	2.42
2.3.2.4.4. β -1,3-glucanase	2.46
2.4. Physiology of Fruit Ripening in Chilli Pepper	2.48



III	RESPONSES OF FRUITS OF FOUR LINES CHILLI PEPPER (<i>Capsicum</i> spp.) TO <i>C. capsici</i> and <i>C. gloeosporioides</i>	
3.1.	Introduction	3.1
3.2.	Materials and Methods	3.2
	3.2.1. Establishment of Plant Materials	3.2
	3.2.2. Inoculum Preparation	3.3
	3.2.3. Assessment for Resistance	3.4
3.3.	Results	3.5
	3.3.1. Width of Lesion	3.6
	3.3.2. Length of Lesion	3.11
	3.2.3. Percentage of Lesion	3.12
	3.2.4. Percentage of Sporulation	3.14
	3.2.5. Rate of Lesion Development	3.14
3.4.	Discussion	3.16
3.5.	Conclusion	3.20
IV	BIOCHEMICAL CHANGES ON CHILLI PEPPER FRUITS INOCULATED WITH <i>C. capsici</i> and <i>C. gloeosporioides</i>	
4.1.	Introduction	4.1
4.2.	Materials and Methods	4.3
	4.2.1. Establishment of Plant Materials	4.3
	4.2.2. Extraction and Determination of Chlorophylls and Carotenoids	4.7
	4.2.3. Extraction and Determination of Total Soluble Phenolics	4.7
	4.2.4. Extraction and determination of total soluble proteins (Basic and Acid Soluble)	4.8
	4.2.5. Peroxidase Activity Assay	4.9
	4.2.6. Polyphenol oxidase Activity Assay	4.10
	4.2.7. Chitinase Activity Assay	4.10
	4.2.8. β -1,3-glucanase Activity Assay	4.11
	4.2.9. PAGE Analysis of Total Soluble Protein, Peroxidase, and Polyphenol oxidase	4.12
4.3.	Results	4.14
	4.3.1. Samples Collection	4.14
	4.3.2. Lesion Development on Inoculated Fruits	4.14
	4.3.3. Biochemical Changes	4.17
	4.3.3.1. Chlorophylls and Carotenoids	4.18
	4.3.3.2. Total Soluble Phenolics	4.34
	4.3.3.3. Total Soluble Proteins (Basic and Acid Soluble)	4.40
	4.3.3.4. Peroxidase Activity	4.53
	4.3.3.5. Polyphenol oxidase Activity	4.53
	4.3.3.6. Chitinase Activity	4.53
	4.3.3.7. β – 1,3 – glucanase Activity	4.55
	4.3.3.8. PAGE Analysis of Total Soluble Protein, Peroxidase, and Polyphenol oxidase	4.60

4.4.	Discussion	4.64
4.4.1.	Chlorophylls and Carotenoids	4.66
4.4.2.	Total Soluble Phenolics	4.69
4.4.3.	Total Soluble Proteins (Basic and Acid Soluble)	4.72
4.4.4.	Peroxidase Activity	4.76
4.4.5.	Polyphenol oxidase Activity	4.78
4.4.6.	Chitinase	4.79
4.4.7.	β -1,3-glucanase	4.79
4.5.	Conclusion	4.79

V PEROXIDASE ACTIVITY OF 20 LINES/VARIETIES CHILLI PEPPER

5.1.	Introduction	5.1
5.2.	Materials and Methods	5.3
5.2.1.	Plant Materials	5.3
5.2.2.	Establishment of Plant Materials and Resistance Assessment	5.4
5.2.3.	Establishment of Plant Materials, Extraction, Assays for Total Basic Soluble Protein and Peroxidase	5.4
5.2.4.	Total Basic Soluble Protein and Peroxidase Activity of Leaves at Different Leaf Position	5.5
5.2.5.	Total Basic Soluble Protein and Peroxidase Activity of Leaves at Different Physiological Stages of Plant Development	5.5
5.2.6.	Total Basic Soluble Protein and Peroxidase Activity of Healthy and Infected Fruits of Line 327	5.5
5.2.7.	Polyacrylamide Gel Electrophoresis (PAGE) of Total Basic Soluble Protein and Peroxidase	5.6
5.2.8.	Relationships Between Peroxidase Activity on Leaves with Level of Resistance to <i>Colletotrichum</i> spp. on Fruits of 20 Lines/Varieties of Chilli Pepper	5.6
5.3.	Results	5.7
5.3.1.	Resistance to <i>Colletotrichum</i> spp. on 20 Lines/Varieties of Chili Pepper	5.7
5.3.2.	Total Basic Soluble Proteins and Peroxidase Activity of Leaves at Different Leaf Position	5.9
5.3.3.	Total Basic Soluble Protein and Peroxidase Activity of Leaves at Different Physiological Stage of Plant Development	5.9
5.3.4.	Total Basic Soluble Protein and Peroxidase Activity of Healthy and Infected Fruits Line 327	5.9
5.3.5.	Polyacrylamide Gel Electrophoresis (PAGE) of Total Basic Soluble Protein and Peroxidase	5.11
5.3.6.	Relationships Between Peroxidase Activity on Leaves with Level of Resistance to <i>Colletotrichum</i> spp. on Fruit of 20 Lines/Varieties of Chili Pepper	5.14
5.4.	Discussion	5.18
5.4.1.	Resistance to <i>Colletotrichum</i> spp. on Fruits of 20 Lines/Varieties of Chili Pepper	5.18

5.4.2.	Peroxidase on Leaves Based on Leaf Position and Physiological Development	5.20
5.4.3.	Peroxidase on Fruits	5.21
5.4.4.	Peroxidase Activity on Leaves with Level of Resistance to <i>Colletotrichum</i> spp. on Fruits	5.22
5.5.	Conclusion	5.23
VI	RAPD PROFILES ON 20 LINES/VARIETIES OF CHILLI PEPPER (<i>Capsicum</i> spp.)	
6.1.	Introduction	6.1
6.2.	Materials and Methods	6.3
6.2.1.	Plant Material Establishment	6.3
6.2.2.	DNA Extraction	6.4
6.2.3.	Primers	6.5
6.2.4.	PCR Conditions	6.5
6.2.5.	Analysis of PCR Products by Electrophoresis	6.6
6.2.6.	Data Analysis	6.6
6.3.	Results	6.7
6.4.	Discussion	6.11
6.5.	Conclusion	6.14
VII	GENERAL DISCUSSION AND CONCLUSION	
7.1.	General Discussion	7.1
7.2.	General Conclusion	7.13
	BIBLIOGRAPHY	B1
	APPENDICES	A1
	BIODATA	V1

LIST OF TABLES

Table	Page
3.1. Morphological Characteristics of Fruits of Four Lines Chilli Pepper	3.6
3.2. Lesion Width and Length at Three, Five and Seven Days Post Inoculation	3.10
3.3. Percentage of Lesion and Sporulation at Three, Five, and Seven Days Post Inoculation	3.13
4.1. Lesion Development on Chilli Pepper Fruits Line 327 and var. Cili Bangi2	4.16
4.2. Biochemical Changes of Chilli Pepper Fruits Line 327 and Var. Cili Bangi2 (control)	4.20
4.3. Non-Significant and Significant Relationships Between Biochemical Changes in Fruit Tissues and Their Resistance Level	4.23
4.4. Peroxidase and Polyphenoloxidase Profiles of Chilli Pepper Fruits Line 327 and Var. Cili Bangi2	4.63
5.1. Seed Sources, Level of Resistance to <i>Colletotrichum</i> spp., and Leaf Peroxidase of 20 Lines/Varieties Chilli Pepper (<i>Capsicum</i> spp.)	5.8
6.1. The Primers, Sequences, and PCR Products	6.7

LIST OF FIGURE

Figure	Page
2.1. Stages of Infection Process in <i>Colletotrichum</i> spp.	2.21
2.2. Schematic Representation During Interactions Between Plants-Pathogenic Fungi	2.27
2.3. Genes Involved in Plant-Pathogen Interaction	2.29
3.1. Rate of Lesion Development	3.15
4.1. Changes in Chlorophylls, Carotenoids and Total Soluble Phenolics of Fresh Healthy Chilli Pepper Fruits Line 327 and Var. Cili Bangi2	4.19
4.2. Chlorophylls Changes During Six Days Post Inoculation on Chilli Pepper Fruits Line 327	4.21
4.3. Relationships Between Chlorophylls Changes and Lesion Development During Six Days Post Inoculation on Line 327	4.24
4.4. Chlorophylls Changes During Six Days Post Inoculation on Chilli Pepper Fruits Var. Cili Bangi2	4.26
4.5. Relationships Between Chlorophylls Changes and Lesion Development During Six Days Post Inoculation on Var. Cili Bangi2	4.27
4.6. Carotenoids Changes During Six Days Post Inoculation on Chilli Pepper Fruits Line 327	4.29
4.7. Relationships Between Carotenoids Changes and Lesion Development During Six Days Post Inoculation on Line 327	4.30
4.8. Carotenoids Changes During Six Days Post Inoculation on Chilli Pepper Fruits Var. Cili Bangi2	4.32
4.9. Relationships Between Carotenoids Changes and Lesion Development During Six Days Post Inoculation on Var. Cili Bangi2	4.33
4.10. Total Soluble Phenolics Changes During Six Days Post Inoculation on Chilli Pepper Fruits Line 327	4.35

4.11. Relationships Between Total Soluble Phenolics Changes and Lesion Development During Six Days Post Inoculation on Line 327	4.36
4.12. Total Soluble Phenolics Changes During Six Days Post Inoculation on Chilli Pepper Fruits Var. Cili Bangi2	4.38
4.13. Relationships Between Total Soluble Phenolics Changes and Lesion Development During Six Days Post Inoculation on Var. Cili Bangi2	4.39
4.14. Changes in Basic Soluble and Acid Soluble Proteins of Fresh Healthy Chilli Pepper Fruits Line 327 and Var. Cili Bangi2	4.41
4.15. Total Basic Soluble Proteins Changes During Six Days Post Inoculation on Chilli Pepper Fruits Line 327	4.42
4.16. Relationships Between Total Basic Soluble Proteins Changes and Lesion Development During Six Days Post Inoculation on Line 327	4.43
4.17. Total Basic Soluble Proteins Changes During Six Days Post Inoculation on Chilli Pepper Fruits Var. Cili Bangi2	4.45
4.18. Relationships Between Total Basic Soluble Proteins Changes and Lesion Development During Six Days Post Inoculation on Var. Cili Bangi2	4.46
4.19. Total Acid Soluble Proteins Changes During Six Days Post Inoculation on Chilli Pepper Fruits Line 327	4.48
4.20. Relationships Between Total Acid Soluble Proteins Changes and Lesion Development During Six Days Post Inoculation on Line 327	4.49
4.21. Total Acid Soluble Protein Changes During Six Days Post Inoculation on Chilli Pepper Fruits Var. Cili Bangi2	4.51
4.22. Relationships Between Total Acid Soluble Protein Changes and Lesion Development During Six Days Post Inoculation on Var. Cili Bangi2	4.52
4.23. Chitinase Activity Changes During Six Days Post Inoculation on Chilli Pepper Fruits Line 327	4.54
4.24. Chitinase Activity Changes During Six Days Post Inoculation on Chilli Pepper Fruits Var. Cili Bangi2	4.56
4.25. β -1,3-glucanase Activity Changes During Six Days Post Inoculation on Chilli Pepper Fruits Line 327	4.58

4.26.	β -1,3-glucanase Activity Changes During Six Days Post Inoculation on Chilli Pepper Fruits Var. Cili Bangi2	4.59
4.27.	Peroxidase and Polyphenoloxidase Profiles on Native PAGE	4.62
5.1.	Total Protein and Peroxidase Activity on Leaves of Chilli Pepper Line 327	5.10
5.2.	Peroxidase Profiles on Twenty Lines/Varieties of Chilli Pepper	5.14
5.3.	A Dendogram of Genetic Distances Among 20 Lines/Varieties of Chilli Pepper Based on Peroxidase profiles	5.15
5.4.	Relationship Between Level of Resistance to <i>C. capsisi</i> of Green and Red Fruits of 20 Lines/Varieties of Chilli Pepper with Their Respective Peroxidase Level on Leaves	5.16
5.5.	Relationship Between Level of Resistance to <i>C. gloeosporioides</i> of Green and Red Fruits of 20 Lines/Varieties of Chilli Pepper with Their Respective Peroxidase Level on Leaves	5.17
6.1.	A Dendogram of Genetic Distance Among 20 Lines/Varieties Chilli Pepper	6.10

LIST OF PLATE

Plate	Page
3.1. Morphological Characteristics of Fruits of line P5, 327, P3 and 146	3.7
3.2. Cultures and Conidia of <i>C. capsici</i> and <i>C gloeosporioides</i>	3.8
3.3. Lesion Development	3.9
4.1. Chilli Pepper Fruits Harvested at 30, 45 and 60 DAA (Days After Anthesis) of Line 327 and Var. Cili Bangi2	4.5
4.2. Chilli Pepper Fruits Line 327 and Var. Cili Bangi2 Prior to Homogenization	4.6
4.3. Chilli Pepper on Intact Plants of Line 327 and Var. Cili Bangi2	4.15
5.1. Native Protein and Peroxidase of Leaf Line 327	5.12
5.2. Native Protein and Peroxidase of 20 Lines/Var. Chilli Pepper	5.13
6.1. RAPD Profiles Generated by OPE 12, OPE 14, OPE 16, and OPE 20	6.9

LIST OF ABBREVIATIONS/NOTATIONS/GLOSSARY OF TERMS

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 ₂ O	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 = $1\mu\text{mol min}^{-1}$ = 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 *et al.*, 1990; Stommel and Griesbach, 1993; Rubatzky and Yamaguchi, 1997; Osuna-Garcia *et 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 *et 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 *et 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