



**UNIVERSITI PUTRA MALAYSIA**

**BIOCHEMICAL AND MOLECULAR STUDIES OF ACTIVE AND  
PASSIVE DEFENSE SYSTEMS IN *MUSA ACUMINATA* L. CV. 'JARI  
BUAYA'**

**WAY CHIANG POH**

**FBSB 2006 26**



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**By**

**WAY CHIANG POH**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
Fulfilment of the Requirement for the Degree of Master of Science**

**September 2006**



**DEDICATED TO:**

*BELOVED A-MA, BROTHER AND SISTERS, ONG KOK ENG,*

*ALSO NOT MISSING THE LECTURERS AND FRIENDS.*

*WHO ALWAYS HAVE CONFIDENCE IN ME. THEIR FAITH AND  
ENDLESS SUPPORTS AS MY STRENGTH PILLARS THAT DIRECTING ME  
GONE THROUGH ALL THE OBSTACLES IN THE LIFE.*



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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**September 2006**

**Chairman: Mohd. Puad Abdullah, PhD**

**Faculty: Biotechnology and Biomolecular Sciences**

Fusarium wilt incidence constitutes one of the major constraints in the development of sustainable banana industry in the country. To date, a cost-effective measure of control for this disease is still not available and farmers are still depending heavily on the use of chemicals to minimize the problem. The use of resistant cultivar to enhance resistant to critical diseases and the reduction of chemicals usage holds the key to surge a better profit margin in the industry. The approach employed in this study was to enhance disease resistant of some of the existing banana cultivars in Malaysia by using biotechnology technique. The objectives of this study were isolation of genes related to plant defense system by using PCR, and analyses of resistant gene candidates (RGCs) in specific host-pathogen interaction, by using reverse transcription-polymerase chain reaction (RT-PCR). Five out of six of the putative diseases RGCs were differentially expressed in the 'Jari Buaya' (JB)- *Fusarium oxysporum* cubense race 4 (Focr4) interactions. RGC1 was induced and involved in this interaction. In addition, the



expression of RGC1 was not affected water stress. The transcripts level of RGC2 and RGC3 decreased as the infection progressed from 1 hour to 3 hours. The mentioned three different RGCs belong to the class of nucleotide binding site-leucine rich repeat (NBS-LRR) disease related proteins. Southern hybridization analyses depicted that these genes belonged to a small gene family. The other three RGCs, RGC4, RGC5 and RGC6 were classified in the kinase family. RGC4 and RGC5 were constitutively expressed in JB-Focr4 interaction and no expression was observed for RGC6. This is the first report of globally renowned Focr4 resistant of local banana crop 'Jari Buaya' at molecular level in plant active defense system.

The accumulation of phenolic compounds and often lignin in plant tissues especially the cell wall and vascular system, is an established plant response to fungal attack. This response has been hypothesized to play an important role in determining passive resistant. Focr4 resistant cultivar, 'Jari Buaya' possessed higher amount of phenolic compounds for both the intracellular and cell wall. However, the susceptible cultivar, 'Rastali' secreted more phenolics than the resistant cultivar into the environment. The results were in tandem with the phenylalanine ammonia lyase (PAL) enzyme activity. Lignin staining of banana roots unveiled that the resistant cultivar possessed higher PAL activity and more lignin deposition in the roots than the susceptible cultivar.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KAJIAN BIODIVERSITI DAN MOLEKUL SISTEM PERTAHANAN AKTIF DAN PASIF BAGI *MUSA ACUMINATA* L. CV. 'JARI BUAYA'**

Oleh

**WAY CHIANG POH**

**September 2006**

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Kejadian kelayuan *Fusarium* merupakan salah satu daripada pengekang utama dalam pembangunan industri pisang negara. Pada masa ini, kaedah pengawalan berkesan masih belum ada dan petani masih banyak bergantung kepada penggunaan bahan kimia untuk mengurangkan masalah ini. Penggunaan kultivar pisang yang tahan kepada penyakit-penyakit yang kritikal dan pengurangan penggunaan bahan-bahan kimia menjadi kunci kepada peningkatan keuntungan dalam industri ini. Pendekatan kita adalah dengan penggunaan teknik-teknik bioteknologi untuk meningkatkan ketahanan penyakit kultivar pisang yang sedia ada. Projek ini bertujuan untuk memencilkan gen-gen yang berkaitan dengan sistem pertahanan tumbuhan dengan menggunakan kaedah PCR bagi tujuan pengawalan penyakit dengan mengenalpasti calon gen ketahanan (RGC) yang diekspres dalam kajian pengekspresan gen secara 'RT-PCR' melibatkan interaksi perumah-patogen yang spesifik. Lima daripada enam calon gen ketahanan telah menampilkan corak ekspresi yang berlainan dalam interaksi Jari Buaya-*Fusarium oxysporum* (Focr4).



RGC1 diaruh dan kemungkinan besar terlibat dalam interaksi ini. Tambahan pula, pengekspresan RGC1 tidak dipengaruhi oleh tekanan air. Akan tetapi, bilangan transkrip untuk RGC2 dan RGC3 berkurangan setelah jangkitan berlanjutan dari satu jam ke tiga jam. Ketiga-tiga gen tersebut digolongkan dalam kumpulan protein berkaitan penyakit tapak pengikatan nukleotida-kaya leusina berulang (NBS-LRR). Analisis 'Southern' menunjukkan protein kumpulan ini adalah dalam famili protein kecil. Tiga gen lagi dikategorikan dalam kumpulan 'kinase'. RGC4 dan RGC5 diekspreskan sepanjang masa dalam interaksi ini dan tidak ada ekspresi diperlihatkan untuk RGC6. Ini merupakan laporan pertama yang menampilkan pengajian di peringkat biologi molekul yang melibatkan sistem pertahanan aktif pisang Jari Buaya yang diketahui umum sebagai rintang kepada Focr4.

Pengumpulan sebatian yang lazimnya fenol dalam tisu tumbuhan terutamanya di dinding sel dan sistem vaskular merupakan reaksi tumbuhan terhadap serangan kulat yang telah diketahui, dan ia telah dihipotesis memainkan peranan penting dalam sistem pertahanan pasif tumbuhan kultivar rintang Focr4, Jari Buaya mempunyai kandungan sebatian fenol yang tinggi untuk kedua-dua jenis, intrasel dan yang terikat pada dinding sel. Bagaimanapun, Rastali ialah kultivar rentan Focr4 merembes lebih banyak sebatian fenol berbanding kultivar rintang Focr4. Keputusan tersebut selari dengan kajian enzim fenilalanina amonia liase (PAL) dan kajian histologi pewarnaan lignin yang menunjukkan kultivar rintang mempunyai aktiviti PAL yang tinggi dan memendapkan lebih banyak lignin pada tisu akar berbanding kultivar rentan.



## ACKNOWLEDGEMENTS

From the bottom of my heart, I would like to express my utmost gratitude to Dr, Mohd. Puad Abdullah, for giving me this golden opportunity to be involved in this banana research project. Throughout these 3 years in execution of this research, the accumulative experiences, sweets and sour, and knowledge were gradually built up the strong pillars that could possibly help me in excelling myself in my career advancement. I wish to thank to Professor Dr. Sariah Meon and Professor Dr. Maziah Mamood for their ceaseless and precious technical guidance, constructive advice and creative idea.

My sincere appreciation is also extended to Professor Khatijah and Dr. Suhaimi to give me green light to fully access to their laboratories' devices and instruments. Without them, that would be hard to carry out my experiments smoothly. Last but not least, special thanks to Dr. Parameswari and Dr. Ho Chai Ling. Not to forget about United Plantation for their unlimited resources supply especially banana Jari Buaya plantlets for research purposes.

Speechless thankful to all the lab mates in plant biotechnology laboratory, virology laboratory, cell and molecular biology laboratory, we have gone through so much good and bad, happiness and sadness accompanying with all sorts of courage and discouragement. Uncountable assistance from the UPM officers in Faculty of Biotechnology and Biomolecular Sciences, Plant Protection department in Faculty of Agricultural, Institute Biosciences were much appreciated.





I certify that an Examination Committee has met on 26<sup>th</sup> September 2006 to conduct the final examination of Way Chiang Poh on his Master of Science thesis entitled “Biochemical, and Molecular Studies of Active and Passive Defense Systems in *Musa acuminata* L. CV. ‘Jari Buaya’ in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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## **DECLARATION**

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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**WAY CHIANG POH**

Date: 7<sup>th</sup> NOVEMBER 2006



## TABLE OF CONTENTS

	<b>Page</b>
<b>DEDICATION</b>	ii
<b>ABSTRACT</b>	iii
<b>ABSTRAK</b>	v
<b>ACKNOWLEDGEMENTS</b>	vii
<b>APPROVAL</b>	viii
<b>DECLARATION</b>	x
<b>LIST OF TABLES</b>	xiv
<b>LIST OF FIGURES</b>	xv
<b>LIST OF ABBREVIATIONS</b>	xviii

### CHAPTER

<b>I</b>	<b>INTRODUCTION</b>	<b>1</b>
<b>II</b>	<b>LITERATURE REVIEW</b>	<b>4</b>
	Banana ‘Jari Buaya’ Cultivar Genotype	
	Resistant ( <i>R</i> ) Genes in Combating Disease Problems in Banana Industry	4
	Classes, Properties and Functions of Resistant <i>R</i> -Gene	5
	Conserved Domains of <i>R</i> -Gene	8
	Plant Defense System	12
	Genomic Perspective of Relationship between Phenylpropanoid Pathway and Plant Defense	14
	Plant-Pathogen Interaction (Gene-for-Gene Model)	16
	Signal Transduction	20
	Changes in Gene Activity	22
	Identification and Characterization of RGCs	23
	Perspectives on Molecular Genetic Analysis of <i>R</i> -gene or Candidates (RGCs)	25
<b>III</b>	<b>IDENTIFICATION AND GENE EXPRESSION STUDIES OF PUTATIVE RESISTANT GENE CANDIDATES (RGCS) FROM BANANA CULTIVAR, ‘JARI BUAYA’ RESISTANT TO <i>FUSARIUM OXYSPORUM CUBENSE</i> RACE 4 (Focr4)</b>	<b>26</b>
	Introduction	26
	Materials and Methods	29
	Plant Materials	29
	Fungus Isolate	29
	Genomic DNA Isolation from Banana ‘Jari Buaya’ Leaves	29
	Total RNA Isolation from ‘Jari Buaya’ Root	31



Qualitative and Quantitative Analysis for Genomic DNA and RNA Extract	31
Oligonucleotide Primer Design	32
Isolation of RGC Gene Fragments by PCR	34
Cloning of PCR Products	35
Analyses of RGCs Sequences	35
RT-PCR Gene Expression Study	36
Analysis of DNA by Southern Hybridization	37
Microbiological Methods	39
Enzyme Assay of Phenylalanine Ammonia Lyase (PAL)	43
Protein Content Determination: The Bradford Method	44
Results and Discussions	44
Isolation of 'Jari Buaya' Resistant Gene Candidates (RGCs)	44
Sequence Analyses of the Cloned Products with Available Sequences in the Genbank	46
Identification of Disease Resistant Related Motifs in RGCs	50
Induction of Sporulation and Germination of <i>Fusarium oxysporum</i> <i>Cubense</i> Race 4 (Focr4)	52
Time-Course Study of Plant-Pathogen Model ('Jari Buaya'-Focr4)	56
Phenylalanine Ammonia Lyase (PAL) as Marker for Wounding Caused by Focr4	60
Gene Expression Studies with RT-PCR Analyses	62
Optimization of the RT-PCR Technique	63
Analysis of Resistant Gene Candidates (RGCs) Expression	66
Gene Expression of The RGC1, RGC2 and RGC3 Sequences	67
Gene Expression of The RGC4, RGC5 and RGC6 Sequences	71
Gene Expression of The RGC1 Under Abiotic Water Stress	73
Southern Analysis	73
Conclusions	75

<b>IV BIOCHEMICAL AND CELLULAR ANALYSES OF THE PASSIVE DEFENSE SYSTEM IN THE DEVELOPMENT OF FUSARIUMWILT RESISTANT IN THE CULTIVAR, 'JARI BUAYA'</b>	<b>76</b>
Introductions	76
Materials and Methods	77
Extraction of Free and Wall Bound Phenolics	77
Total Phenolic Content Determination: The Folin-Ciocalteau Assay	78



Histological Study: Detection of Lignin	79
Results and Discussions	80
Biochemical Characterizations of Focr4 Resistant (‘Jari Buaya’) and Susceptible (‘Rastali’) Banana Cultivars	
Intracellular and Wall Bound Total Phenolic Content	80
Total Phenolic Content in Liquid Media (Root Exudates)	85
PAL Enzyme Activity	88
Histological Studies for Lignin Detection: Phloroglucinol-HCl Staining	90
Conclusions	93
<b>V   GENERAL CONCLUSIONS AND SUGGESTIONS</b>	<b>94</b>
General Conclusions and Suggestions for Future Work as a Continuation in This Study	94
<b>BIBLIOGRAPHY</b>	<b>98</b>
<b>APPENDICES</b>	<b>106</b>
<b>BIODATA OF THE AUTHOR</b>	<b>118</b>



## LIST OF TABLES

Table		Page
1	Five classes of resistant gene.	7
2	Summary of the conserved domains in various classes of <i>R</i> -gene.	9
3	Comparison of the LRR consensus sequence of LRR-containing proteins.	11
4	Methodologies in identification and characterization of putative <i>R</i> -genes and their analogues in the research field of plant defense system	24
5	Summary of tailor-made degenerate primers.	33
6	Touch down PCR for NBS-LRR and Kinase classes of RGC.	34
7	Summary of BLASTN results for NBS-LRR and kinase classes RGCs.	49
8	Summary of the RGC related motifs for isolated RGCs from ‘Jari Buaya’ with ‘MOTIF SEARCH’ site ( <a href="http://motif.genome.jp">http://motif.genome.jp</a> ).	51
9	Summary of all the Focr4 infected or non-infected root segments, plating on PDA agar.	57
10	The ANOVA test shows the differences in intracellular total phenolic compounds in roots at various sampling time points.	82
11	The ANOVA test shows the differences in wall bound total phenolic compounds in roots at various sampling time points.	84
12	The ANOVA test shows the differences in exudates total phenolic compounds in roots at various sampling time points.	87
13	The ANOVA test shows the differences in PAL activity in roots at various sampling time points	89



## LIST OF FIGURES

<b>Figure</b>		<b>Page</b>
1	Schematic representation of NBS-LRR class protein sequence.	8
2	Schematic representation of kinase class protein sequence.	10
3	Schematic representation of LRR-TMS-Kinase class protein sequence.	10
4	Schematic representation of LRR-TMS class protein sequence.	10
5	Schematic representation of LRR region of plant resistant genes.	11
6	Diagram of defense features that plant possess based on existing (constitutive) or induced (active) defenses.	13
7	Host-pathogen gene-for-gene specificity model.	17
8	Propose biochemical models of the <i>RRS1-R-PopP2</i> interaction. <i>RRS1-R</i> might perceive <i>PopP2</i> by direct interaction (a). Alternatively, the interaction is mediated by importin- $\alpha$ (b) or SUMO (c). Direct or indirect interaction of <i>RRS1-R</i> and <i>PopP2</i> leads to nuclear import of <i>RRS1-R</i> and activation of defense related genes.	19
9	The complexity of pathogen defense in plants. Major components of the signal transduction chain from elicitor perception to gene activation.	21
10	Sense (NBS-F) and antisense (NBS-R) primer pairs for NBS-LRR class protein sequence.	33
11	Sense (K-F) and antisense (k-R) primer pairs for kinase class protein sequence.	33
12	PCR products amplified with the degenerate primers in lanes 1 – 4 are NBS-F1/R1, NBS-F2/R2, K-F1/R1 and K-F2/R2, respectively.	45
13	<i>EcoRI</i> and <i>HindIII</i> double digestion of pNEB205A from white colonies to confirm the correct inserted size for NBS-LRR (A) and kinase classes' RGCs (B).	47





14	<i>F. oxysporum</i> cubense race 4 conidia under 40x magnification.	53
15	Growth of <i>F. oxysporum</i> cubense race 4 in PDA medium.	53
16	Time-course of <i>F. oxysporum</i> cubense race 4 total spores production (A) and spore viability (B).	55
17	Time-course study of banana ‘Jari Buaya’- <i>F. oxysporum</i> cubense race 4.	59
18	PAL specific enzyme activity in banana ‘Jari Buaya’ roots after treatment with FOCR4 conidia suspension.	61
19	Determination of linear range in RT-PCR.	64
20	Amplicons of internal control, $\beta$ -actin, for PCR and RT-PCR products.	66
21	Identification of the amplified RT-PCR product through BLASTN analyses in GenBank database.	66
22	RT-PCR amplification of mRNA for RGC1 [I], RGC2 [II] and RGC3 [III].	68
23	RT-PCR amplification of mRNA for RGC4 [I], RGC5 [II] and RGC6 [III].	72
24	RT-PCR amplification of mRNA for RGC1 under water stress.	74
25	Hybridization patterns obtained with the RGC1 probe, using ‘Jari Buaya’ genomic DNA.	75
26	Intracellular free phenolic compounds in the roots of the banana cultivars ‘Jari Buaya’ and ‘Rastali’.	82
27	Wall bound phenolic compounds in the roots of the banana cultivars ‘Jari Buaya’ and ‘Rastali’.	84
28	Total phenolic compounds secreted by roots of the banana cultivars ‘Jari Buaya’ and ‘Rastali’.	87
29	PAL specific enzyme activity in the roots of ‘Jari Buaya’ and ‘Rastali’	89
30	Cytochemical localization of lignin detection through positive purple-red reaction to phloroglucinol-HCl treatment in roots of resistant (‘Jari Buaya’) and susceptible (‘Rastali’) banana cultivars at 40x magnification.	91



31	Cytochemical localization of lignin detection through positive purple-red reaction to phloroglucinol-HCl treatment in roots of resistant ('Jari Buaya') and susceptible ('Rastali') banana cultivars at 100x magnification.	92
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## LIST OF ABBREVIATIONS

BAC	Baterial artificial chromosomes
bp	basepair
BSA	Bovine serum albumin
CBB	Coomassie Brilliant Blue
CC	Coiled-coil
Ct	Cortex
CTAB	hexadecyltrimethylammonium bromide
DNA	Deoxyribonucleic acid
dNTP	deoxynocitnamide triphosphate
Ed	Endodermis
EDTA	Ethylenediaminetetraacetic acid
Ex	Exodermis
FAA	Formalin / acetic acid / ethanol
Focr4	<i>Furasium oxysporum</i> cubense race 4
GAE	Gallic acid equivalents
HCl	Hydrochloric acid
HR	Hypersensitivity response
IPA	Isopropyl alcohol
IRD	Infected root density
JA	Jasmonic acid
kb	kilo basepair
LAR	Localized acquire resistant
LB	Luria-Bertani



LRR	Leucine-rich repeat
LZ	Leucine zipper
MAPKs	Mitogen-activated protein kinases
MgCl <sub>2</sub>	Magnesium chloride
Min	Minute
MS	Murashige and Skoog
NBS	Nucleotide binding side
NLB	Nuclear lysis buffer
NLS	Nuclear localization signals
NO	Nitric oxide
PAL	Phenylalanine ammonia lyase
PDA	Potato dextrose agar
pK	Kinase plasmid
P-loop	Phosphate binding site
pNBS	Nucleotide binding site plasmid
pNEB205A	Cloning vector
PR	Pathogenesis-related
RFLP	Restriction fragment length polymorphism
RGCs	Resistant gene candidates
RNA	Ribonucleic acid
ROI	Reactive oxygen intermediates
RT-PCR	Reverse transcription-polymerase chain reaction
SA	Salicylic acid
SAR	Systemic acquired resistant



SDS	Sodium dodecyl sulphate
SP	Signal peptide
SSH	Supression subtraction hybridization
SUMO	Small ubiquitin related modifier
TIR	Toll and interleukin-1 receptor
TMS	Transmembrane
TMV	Tobacco mosaic virus
Tris-Cl	Tris-chloride
VIGS	Virus-induced gene silencing
Vs	Vascular system



## CHAPTER I

### INTRODUCTION

Banana is recognized as one of the important food sources in the tropics with global sales of five billion (US) dollars per annum (Jalil *et al.*, 2003). Malaysia has 50 types of banana cultivars that may serve as sources of genetic variability for agronomic and disease improvement programs (Jalil *et al.*, 2003). However, *Fusarium oxysporum* (Fo) that is the causative agent of Fusarium wilt disease in many banana species is considered a major problem to the banana industry in Malaysia and worldwide. The management of Fusarium wilt is fastidious and it contributes to a substantial proportion of the overall production cost (Lheureux *et al.*, 2003). To date, 4 races of pathogen have been identified: races 1, 2, 3 and 4. *F. oxysporum cubense* race 4 (Focr4) is the most destructive in Malaysia, infecting almost all banana cultivars especially ‘Rastali’ is the most susceptible one, except for a few such as ‘Jari Buaya’, and ‘Intan’. (Jeger *et al.*, 1996). The fungus infects the roots during early growth stages of banana plant and gradually colonizes the vascular system which leads to total collapse of the plant.

Today, an integrated disease management is employed in areas where Fusarium wilt is a major problem in order to minimize heavy usage of pesticides and chemicals. This approach relies on good agricultural practices with limited usage of chemicals, and enhances of banana to infection of Focr4 and other major diseases to produce a more sustainable banana industry in Malaysia. The development of a long term disease control strategy remains as the top priority. With the advances of plant genetic engineering technology, cultivars with good disease properties have been exploited by breeders to



develop new planting materials with enhanced resistant to selected plant pathogens including the major ones. This has been the most economical and effective alternative of combating the disease. However, genetic improvement of banana for resistant to Focr4 infection is very difficult using conventional breeding methods due to the complex polyploidy genetic composition of banana. Thus, a better understanding of banana genomic structure and molecular characterization of disease resistant genes are crucial and of high priority (Wang *et al.*, 1999). Studies on the interaction between host plant and pathogen at molecular level for nearly two decades have revealed a specific gene-for-gene interaction existed between the host and the pathogen. The resistant gene (*R*) protein from cultivar interacts with the avirulence gene (*avr*) protein from pathogen triggering a defense response in the plant to combat the pathogen. The genetic engineering of this specific disease resistant gene from cultivar to susceptible cultivar was widely used in many plant species such as tomato, tobacco, *Arabidopsis* to produce a disease tolerant or trait (Ellis *et al.*, 2000; Rommens and Kishore, 2000; Rivas *et al.*, 2004).

Here, similar approach was used to isolate a set of gene candidates (RGCs) from 'Jari Buaya' which has been recognized as a cultivar to Focr4 (Bink *et al.*, 1997), using degenerate primers targeting the nucleotide binding site (NBS) and kinase subdomain consensus domains of *R*-genes. This prompted the examination of gene expression of the RGCs with RT-PCR in 'Jari Buaya'-Focr4 interaction, to gain insights into gene expression of active plant defense mechanism. Passive defense (lignification) was speculated to be involved in plant defense response along with the active defense, thus, comparative studies on the fundamental of physiological, biochemical and cellular



aspects involving the ('Jari Buaya') and susceptible ('Rastali') banana cultivars were also carried out.

These overall objectives of this study were:

1. To identify the putative disease candidates (RGCs) from 'Jari Buaya' that may be involved in active defense for specific 'Jari Buaya'-Focr4 interaction.
2. To study the biochemical properties in terms of total phenolic compounds, phenylalanine ammonia lyase (PAL) activity and lignin deposition in the root tissue of 'Jari Buaya' in comparison with 'Rastali, in order to reflect its promising passive defense properties.



## CHAPTER II

### LITERATURE REVIEW

#### Banana ‘Jari Buaya’ Cultivar Genotype

Banana is one of the most important crops in tropical regions. Majority of the cultivars are highly sterile, polyploid and derived from two major species: *Musa acuminata* and *Musa balbisiana* (Baurens *et al.*, 1996). However, the genetic composition of cultivated *Musa* is complex. Their genetic composition is usually represented with the letter codes A and B, representing the two wild species, respectively. The cultivars can be diploid (AA, BB) or tetraploid (AAAB, AABB, ABBB) but majority are triploid (AAA, AAB, ABB) (Geering *et al.*, 2001). Banana ‘Jari Buaya’ cultivar, which belongs to the group of *Musa acuminata*, is the most promising due to its disease resistant properties (Binks *et al.*, 1997).

#### Resistant (*R*) Genes in Combating Disease Problems in Banana Industry

The management of disease and pest in banana plantations covers not only problems associated with fungi (Fusarium wilt), but also viruses (banana streak virus), bacteria (bacterial blight disease), nematodes and various environmental disorders. Several diseases cause significant losses in the production of banana industry such as the banana streak disease caused by banana streak virus, black sigatoka, Fusarium wilt caused by *Fusarium oxysporum* (Fo) and so on (Jeger *et al.*, 1996). However, Fo, which is the

