UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF BLAST DISEASE-RESISTANT TRANSGENIC RICE VARIETY FROM MR219 THROUGH TRANSFORMATION WITH PIKH GENE

PARISA AZIZI

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DEVELOPMENT OF BLAST DISEASE-RESISTANT TRANSGENIC RICE VARIETY FROM MR219 THROUGH TRANSFORMATION WITH *PIKH* GENE

By

PARISA AZIZI

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

April 2015

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DEDICATED TO

My Father Nazar Ali Azizi;
My Mother Manijeh Zarasvand Asadi;
My beloved housband Mahbod Sahebi;
My Brothers and sisters

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for Doctor of Philosophy

DEVELOPMENT OF BLAST DISEASE-RESISTANT TRANSGENIC RICE VARIETY FROM MR219 THROUGH TRANSFORMATION WITH *PIKH* GENE

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PARISA AZIZI

April 2015

ABSRACT

Chairman : Professor Mohd Rafii Yusop, PhD
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Food security has become a concern of global importance, and major price spike of staple food crops, such as rice has occurred in recent years. These price spikes are partly due to the brunt of plant disease. *Magnaporthe oryzae*, rice blast fungus, is a plant pathogen causes a serious rice disease and therefore poses a threat to the world's important food security crop. Plant transformation technology has become an adaptable system for cultivar development and also for functional analysis of gene in plants. The objectives of this study were (i) to screen Malaysian rice varieties phenotypically, physiologically and genetically against blast disease (ii) to isolate *Pikh* gene from leaves of resistant rice variety (PH9), (iii) to construct the over-expression vector carrying CDS of *Pikh*gene, and (iv) to determine the effects of (over-expression)of *Pikh* in MR219 rice variety. Sixteen important Malaysian rice varieties were screened phenotypically (by scoring), physiologically (measuring photosynthesis and its components) and genetically (gene expression analysis using Real-Time PCR). Specific primer was designed to isolate full Coding DNA Sequence (CDS) of *Pikh* gene from PH9 rice variety. Entry and the expression vectors were constructed using the Gateway Technology. *Agrobacterium*-mediated transformation technology was used to introduce *Pikh* gene to the callus of MR219. Transgenic plants were evaluated from DNA to protein stages by polymerase chain reaction (PCR), Semi-quantitative RT-PCR, Real-Time PCR, high performance liquid chromatography. Transgenic plants also were compared to the control plants using Real-Time quantification technique (to quantify pathogen population) and also challenging of transgenic and control plants with the local most virulent *M. oryzae*pathotype, P7.2. The TRIzol method was quick and reliable method for RNA extraction from leaves of rice. Ten out of 16 varieties (MR159, PH9, MR84, MR185, MR253, MR269, MRQ74, MRQ50, Pulut Siding and Pongsu Seribu 2) demonstrated high degree of resistance to pathotype P7.2 rice leaf blast. Photosynthesis and chlorophyll contents were decreased significantly among treated susceptible varieties compared to the controls. There were absent of differences in photosynthesis and its components between inoculated and non-inoculated resistant varieties. Hence, it seems that energy sources are provided

for both resistant and susceptible plants, but the expression of defence-associated genes restricts the pathogens accessibility in the resistant varieties.Our findings provide evidences that the expression profiling of *Pikh, Pi9, Pi21*, and *Osw45* genes is involved in the defense responses in the leaves of rice 31 h after inoculation of plants by *M. oryzae*. Full CDS of*Pikh*gene with 1206 bp length was obtained through amplification of the cDNA template using specific primer. *Pikh* gene was up-regulated in the transgenic plants in comparison to the control plants. The amount of leucine amino acid of transgenic rice plants has increased significantly from 17.131 in the wild-type to 47.865 mg g^{-1}. The *M.oryzae* population was constant at 31, 48 and 72h after inoculation in transgenic plants while itincreased in inoculated control plants. This study successfully clarified that over-expression of *Pikh* gene in the transgenic plants is able to improve the resistance of rice against *M.oryzae*pathotype P7.2.

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

PEMBANGUNAN VARIETI PADI TRANSGENIK RINTANG PENYAKIT KARAH DARI MR219 MELALUI TRANSFORMASI GENE *PIKH*

Oleh

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Isu keselamatan makanan kini telah menjadi antara isu penting peringkat global dan kenaikan harga tanaman makanan ruji, seperti beras telah berlaku pada tahun kebelakangan ini. Lonjakan harga ini adalah sebahagiannya disebabkan oleh kemusnahan disebabkan penyakit tanaman. *Magnaporthe oryzae*, kulat penyakit karah padi, adalah patogen tanaman yang mengakibatkan penyakit padi yang serius dan boleh mengancam pengeluaran tanaman yang penting ini dalam jaminan keselamatan makanan dunia. Teknologi transformasi tumbuhan telah menjadi satu sistem yang telah disesuaikan untuk pembangunan kultivar dan juga untuk analisis fungsi gen dalam tanaman. Objektif kajian ini ialah (i) untuk menyaring varieti padi dari Malaysia berdasarkan ciri fenotip, fisiologi dan genetik terhadap penyakit karah (ii) untuk mengasingkan *Pikh* gen dari daun bagi varieti (PH9) padi resistan penyakit karah, (iii) untuk membina vektor yang membawa pengekspresan-ketara CDS gen *Pikh*, dan (iv) untuk menentukan kesan (pegekspresan-ketara) gen *Pikh* dalam varieti padi MR219. Enam belas varieti utama padi Malaysia telah disaring secara fenotip (kaedah skor), fisiologi (mengukur fotosintesis dan komponennya), dan secara genetik (analisis gen menggunakan *Real-Time* PCR). Primer asas yang spesifik telah direkabentuk untuk isolasi pengkodan jujukan DNA (CDS) *Pikh* gen sepenuhnya dari varieti padi PH9. *Entry* dan pengekspresan vektor telah dibina menggunakan *Gateway Technology*. Teknologi transformasi *Agrobacterium*-pengantara telah digunakan untuk memasukkan gen *Pikh* ke dalam kalus MR219. Pokok transgenik yang terhasil telah dinilai dari peringkat DNA sehingga ke peringkat protein dengan oleh tindakbalas berantai polimerase (PCR), Semi-kuantitatif RT-PCR, *Real-Time* PCR, kromatografi cecair berprestasi tinggi. Tumbuhan transgenik juga telah dibandingkan dengan tumbuhan kawalan menggunakan teknik kuantifikasi *Real-Time* (untuk menganggarkan populasi patogen) dan juga menguji kerintangan tumbuhan transgenik dan tumbuhan kawalan terhadap patotip *M. oryzae* yang paling virulans, P7.2. Kaedah TRIzol merupakan kaedah yang cepat dan berkesan untuk mengekstrak RNA dari daun pokok padi. Sepuluh daripada 16 varieti (MR159, PH9, MR84, MR185, MR253, MR269, MRQ74, MRQ50, Pulut

Siding dan Pongsu Seribu 2) menunjukkan tahap kerintangan yang tinggi terhadap penyakit karah daun padi, patotip P7.2. Fotosintesis dan kandungan klorofil telah menurun secara ketara di kalangan varieti yang rentan penyakit berbanding dengan varieti kawalan. Bagi kadar fotosintesis dan komponennya, tidak wujud perbezaan di antara varieti tahan penyakit yang diinokulasi dengan yang tidak diinokulasi. Ini menunjukkan bahawa sumber tenaga dibekalkan untuk pokok tahan penyakit dan pokok yang rentan penyakit, tetapi pengekpresan gen kerintangan penyakit yang terlibat telah menyekat kemasukan patogen ke dalam varieti yang tahan penyakit. Berdasarkan penemuan ini, membuktikan terdapat profil pengekspresan gen *Pikh*, *Pi9, Pi21*, dan *Osw45* yang terlibat dalam tindakbalas kerintangan pada daun padi pada 31 jam (h) selepas inokulasi *M. oryzae.* CDS lengkap *Pikh* gen dengan kepanjangan 1206 bp telah diperolehi secara keseluruhannya melalui amplifikasi templat cDNA malalui penggunaan primer spesifik. *Pikh* gen menunjukkan pengekspresan secara menaik dalam pokok transgenik berbanding dengan pokok kawalan. Jumlah asid amino leucine pokok padi transgenik telah meningkat dengan ketaranya dari 17.131 dalam jenis pokok asal berbanding 47.865 mg g^{-1} dalam pokok transgenik. Populasi *M. oryzae* adalah kekal pada 31, 48 dan 72 h selepas inokulasi ke pokok transgenik tetapi ianya meningkat bagi pokok kawalan. Kajian ini telah berjaya membuktikan pengekspresan ketara gen *Pikh* pada pokok trangenik telah meningkatkan kerintangan pokok padi terhadap *M. oryzae* patotip P7.2.

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This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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A. *tumefaciens*	*Agrobacterium. tumefaciens*
Asp	Aspartic acid
Arg	Arginine
Ala	Alanine
aa	Amino acid
Bp	Base Pairs
cDNA	Complementary DNA
CTAB	Hexacetyltrimethyl ammonium bromide
DEPC	Diethyl pyrocarbonate
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
$dNTP_S$	deoxynucleotides
Ds	Double-stranded
EtBr	Ethidium bromide
G	Gram
Glu	Glutamic acid
Gly	Glycine
His	Histidine
h	Hour
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
Ile	Isoleucine
kb	Kilo base-pair
L	Liter
LB	Luria-bertani
LiCl	Lithium chloride
Lys	Lysine
Leu	Leucine
M	Molar
min	Minure
Met	Methionine
mg	Milligram
mg g^{-1}	Miligram per gram
mL	Milliliter
mM	Millimolar
mRNA	Massenger RNA
NaCl	Sodium chloride
NCBI	National Center for Biotechnology Information
ng	Nanogram
OD	Optical density
ORF	Open reading frame
PCR	Polymerase chain reactions
PVP	Polyvinylpyrrolidone
Pro	Proline
Phe	Phenylalanine
RNA	Ribonucleic acid
RT	Room Tempreture

RT-PCR	Reverse transcriptase polymerase chain reaction
RNase	Ribonuclease
g (rcf)	Gravity
ROS	Reactive oxygen species
SDS	Sodium dodecyl sulphate
Sec	Second
Ser	Serine
ss	Single-stranded
spp	Species
TAE	Tris acetate EDTA
TBE	Tris borate EDTA
TE	Tris-EDTA
T-DNA	Transfer DNA
Thr	Threonine
Tyr	Tyrosine
Val	Valine
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside
µg μL^{-1}	Microgram per microlitre
µL	Microliter
µg	Microgram
˚C	Degree Centigrade
%	Percentage

CHAPTER 1

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa* L.) is the second most cultivated crop in the world. It belongs to the genus *Oryza*, family Gramineae (Poaceae) and tribe Oryzeae and comprised of two subspecies, indica and japonica(Oka, 2012). Rice production should be increased up to 40% by 2030 owing to the growing global population (Khush, 2005). Hence, the production of rice varieties with higher potency and stable yield is indispensable to overcome the grain yield reduction and arable land limitation.

Both biotic and abiotic stresses cause huge yield losses in rice. Plant diseases as the main source of these yield losses are a constant threat to global food security. *Magnaporthe oryzae*, rice blast fungus, is a plant pathogen causes a serious disease and consequently poses a threat to the world's most important food security crop (Talbot, 2003). Rice blast is one of the most calamitous diseases, which reduce about 10 to 30% of annual grain yield in rice growing countries (Bastiaans, 1993). It infects different parts of a plant such as leaf, collar, node, neck and panicle by production of spores and penetration of infection hyphae (Hajime, 2001). Blast disease also demonstrates destructive effects on physiological growth in rice. Leaf blast disease decreases photosynthesis through a reduction in the green leaf area and effects on photosynthesis of green leaf region surrounding the lesions (Bastiaans, 1993).

Wide ranges of control strategies have been applied to control blast disease included burning of diseased tissues, fertilizer management, chemical control, using resistant cultivars and forecasting systems. Diseased straw can become inoculum source for the future crop seasons, so they must be burned and composted. Intensive application of chemicals creates severe environmental pollutions, causes hazards to the health of consumers.

The disease could be efficiently managed using rice resistant varieties (Huang *et al.*, 2002). In this way, genetic transformation has become an important means in the crop improvement strategies (Rahman *et al.*, 2011).

Genetic engineering technology offers an alternative possibility for the plant improvement with increased disease resistance (Zheng *et al.*, 2009). However, among the ways to improve plants against diseases, conventional breeding is laborious, time consuming, and highly depends on environmental conditions (Miah *et al.*, 2013).

One of the most important traits in rice is blast resistance which has been purposed to improve in breeding programs over the past decades. Three ways to achieve this objective were suggested by breeders; using of the field resistance to blast disease carried by native varieties, gene transformation and development of high-level field resistance varieties (Nishizawa *et al.*, 1999). The goal of this

particular research is to develop a new rice variety which shows broad-spectrum resistance against blast.
This study was carried out with the following objectives:

1. To screen Malaysian rice varieties phenotypically, physiologically and genetically against blast disease.

2. To isolate CDS of *Pikh* gene from resistant rice variety (PH9).

3. To construct the over-expression vector carrying CDS of *Pikh* gene.

4. To determine the effects of over-expression transcript of *Pikh* in transgenic variety.

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APPENDICES

APPENDIX A

Formulation for media and solution

Table A.1. MS Medium (Modified)

Name of stock solutions	Components	Quantity (g/L)	Stock vol for 1 L medium (mL)	Final concentration (mg/L)
MS1	NH_4NO_3	82.5	20	1650.0
	KNO_3	95.0		1900.0
MS2	$MgSO_4 \cdot 7H_2O$	37.0	10	370.0
	$MnSO_4 \cdot 4H_2O$	2.23		22.3
	$ZnSO_4$	1.058		10.6
	$CuSO_4 \cdot 5H_2O$	0.0025		0.025
MS3	$CaCl_2 \cdot H_2O$	44.0	10	440.0
	KI	0.083		0.83
	$CoCl_2 \cdot 6H_2O$	0.0025		0.025
MS4	KH_2PO_4	17.0	10	170.0
	H_3BO_3	0.62		6.2
	$Na_2MoO_4 \cdot 2H_2O$	0.025		0.25
MS5	$FeSO_4 \cdot 7H_2O$	2.785	10	27.85
	$Na_2EDTA \cdot 2H_2O$	3.725		37.25

Continued Table A.1:

Name of stock solutions	Components	Quantity (g/L)	Stock vol for 1 L medium (mL)	Final concentration (mg/L)
Vitamins	Nicotinic acid	10.0 mg/100 mL	5	0.5
	Pyridoxine HCl	10.0 mg/100 mL		0.5
	Thiamine HCl	20.0 mg/100 mL		1.0
	Glycine	40.0 mg/100 mL		2.0
Hormones	2,4-D	10 mg/100 mL	20	10.0
Myoinositol				100.0
Sucrose				30,000.0
Gelrite				2800.0
pH	5.6–5.8			

Table A.2. N6 Medium (Modified)

Name of stock solutions	Components	Quantity (g/L)	Stock vol for 1 L medium (mL)	Final concentration (mg/L)
N61	KNO_3	141.50	20	2830.0
N62	$MgSO_4 \cdot 7H_2O$	18.5	10	185.0
	$MnSO_4 \cdot 4H_2O$	0.44		4.4
	$ZnSO_4$	0.15		1.5
	$(NH_4)_2SO_4$	46.3		463.0
N63	$CaCl_2 \cdot H_2O$	16.6	10	166.0
N64	KI	0.08		0.8
	KH_2PO_4	40	10	400
	H_3BO_3	0.16		1.6
N65	$FeSO_4 \cdot 7H_2O$	2.785	10	27.85
	$Na_2EDTA \cdot 2H_2O$	3.725		37.25
Vitamins	Nicotinic acid	10.0 mg/100 mL	5	0.5
	Pyridoxine HCl	10.0 mg/100 mL		0.5
	Thiamine HCl	20.0 mg/100 mL		1.0
	Glycine	40.0 mg/100 mL		2.0
Hormones	2,4-D	10 mg/100 mL	20	10.0
Myoinositol				100.0
Sucrose				30,000.0
Gelrite				2800.0
pH	5.6–5.8			

Table A.3. Media for Bacterial growth

LB Agar (1L)		**LB Broth (1L)**	
Agar	15 g	Tryptone	10 g
Tryptone	10 g	NaCl	10 g
NaCl	10 g	Yeast extract	5 g
Yeast extract	5 g		

Add distilled water to final volume of 1L; adjust the pH to 7.0, and autoclave.

Table A.4. Yoshida Culture Solution

Component	Stock concentration Component (g/10 L)
$MgSO_4 \cdot 7H_2O$	3240.0
NH_4NO_3	914.0
$CaCl_2 \cdot 2H_2O$	886.0
K_2SO_4	714.0
$NaH_2PO_4 \cdot 2H_2O$	403.0
H_3BO_3	300.0
$FeCl_3 \cdot 6H_2O$	77.0
$MnCl_2 \cdot 4H_2O$	15.0
$ZnSO_4 \cdot 7H_2O$	0.35
$CuSO_4 \cdot 5H_2O$	0.31
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.74

Table A.5.Low salt LB (1L)

Tryptone	10 g
NaCl	5 g
Yeast extract	5 g

Add distilled water to final volume of 1L; adjust the pH to 7.0, and autoclave.

1. Antibiotics

Ampicillin (100 mg mL^{-1}). Dissolved 1000 mg in 10 mL of sterile water, filter sterilized, and stored at -20˚C.

Rifampicin. 50 mg mL^{-1}, dissolved in DMSO, added sterile water up to 1 mL, filter sterilized, and stored at -20˚C.

Zeocin (50mg/mL). Dissolved 500 mg in 10 mLof sterile water, filter sterilized, and stored at -20˚C.

Streptomycin (100 µg/mL): Dissolved 0.0001 g in 1mL of sterile water, filter sterilized, and stored at -20˚C.

2. Other solutions

TE buffer
Tris-Hcl 10 Mm
EDTA 1 Mm (pH 8.0)

TBE buffer (1X)
Tris base 10.8 g
Boric acid 5.5 g
EDTA 0.37 g
Add sterile water to a final volume of 1 L
.

TAE buffer (1X)
Tris base 4.84 g
Glacial acetic acid 1.142 mL
$Na_2EDTA.2H_2O$ 0.74 g
Add sterile water to a final volume of 1 L.

PCI,CI
Phenol: chloroform: isoamyl alcohol (25: 24: 1)
Chloroform: isoamyl alcohol (24: 1)

Sodium acetate 3M (100mL). Dissolved 40.8 g sodium acetate in sterile water and bring the volume to 100 mL.

APPENDIX B
Figures of vectors map

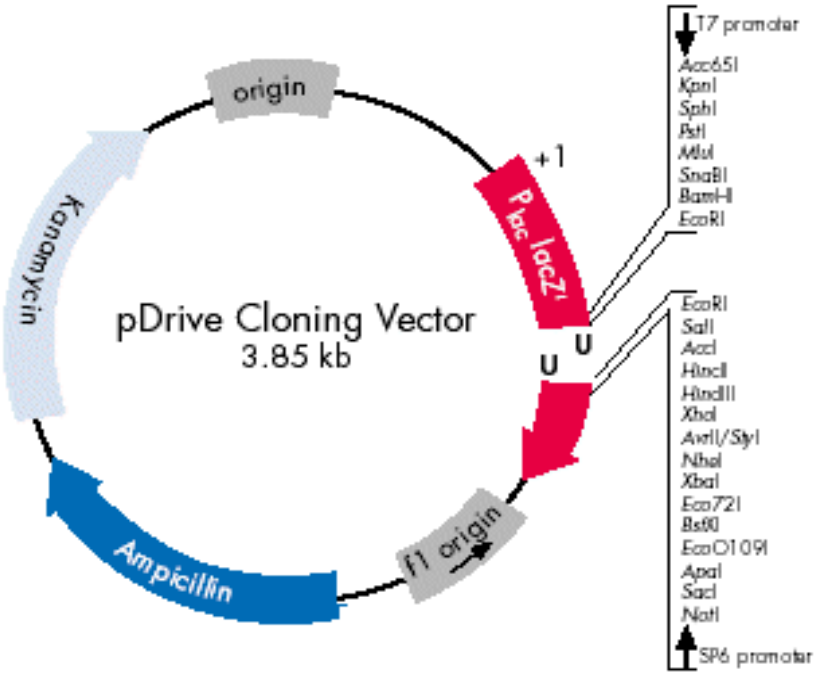


Figure B.1. Plasmid Map of pDrive cloning vector (Qiagen, Germany).

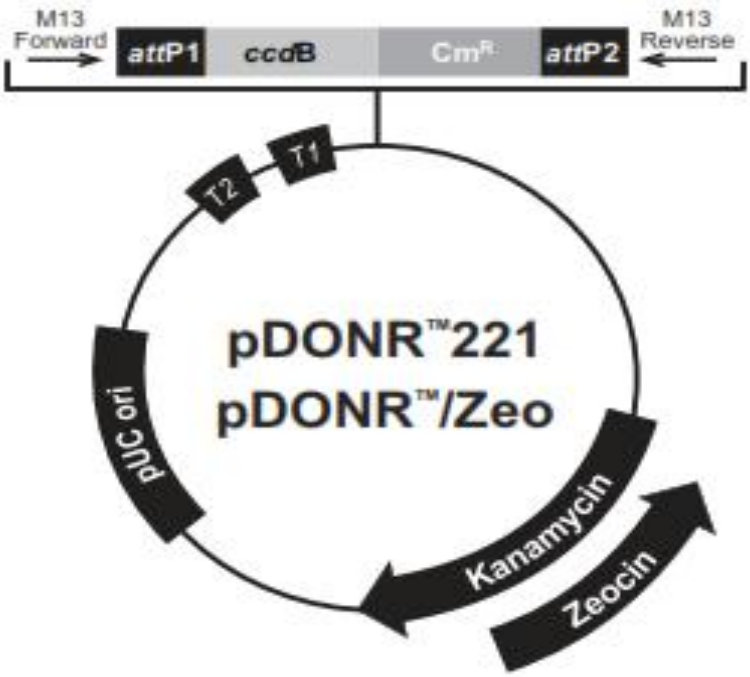


Figure B.2. Plasmid map of pDONOR/Zeo vector (Invitrogen, CA, USA).

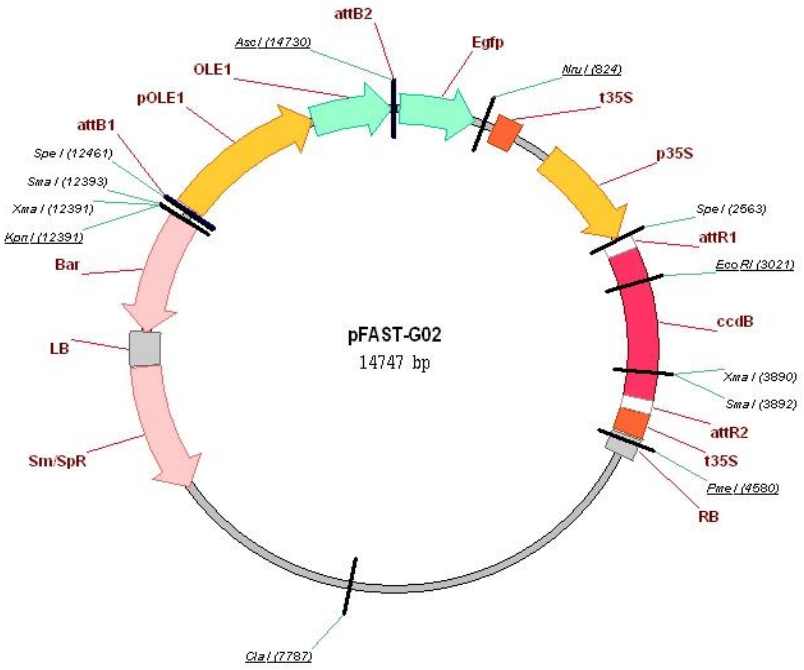
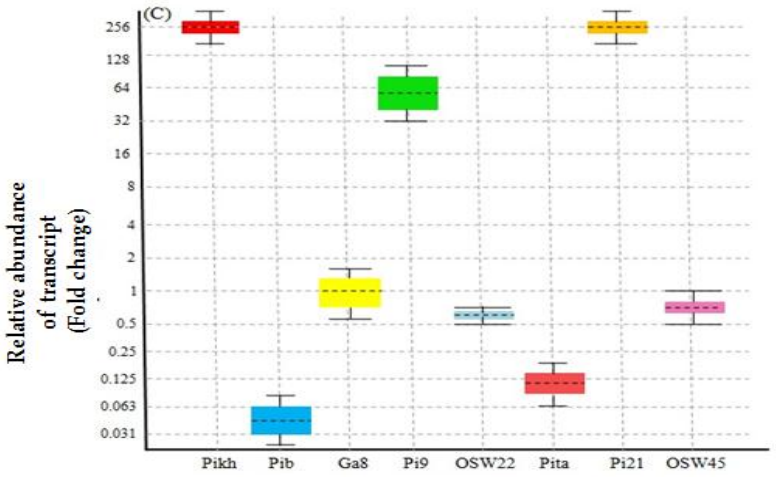


Figure B.3. Plasmid map of pFAST-G02 vector (Shimada *et al.*, 2010).

APPENDIX C

Figure C.1. Relative expression levels of *Pikh*, *Pib*, Os11gRGA8 *(GA8)*, *OsWRKY22 (Osw22)*, *Pita*, *Pi21*, and *OsWRKY45 (Osw45)* genes calibrated using *18sRNA/tubulin* reference genes in infected and control rice plants by relative quantitative real-time PCR. Expression levels of 8 genes in 2 infected varieties (C): MR159; (D): MR84; (E): MR185; (F): MR232; (H): MR253; (L): MR263; (G): MR269; (M): MRQ74; (N): Q50; (O): MR220; (P): MR211; (Q): Pulut siding; (R):.MR1; (S): Pongsu seribu 2.



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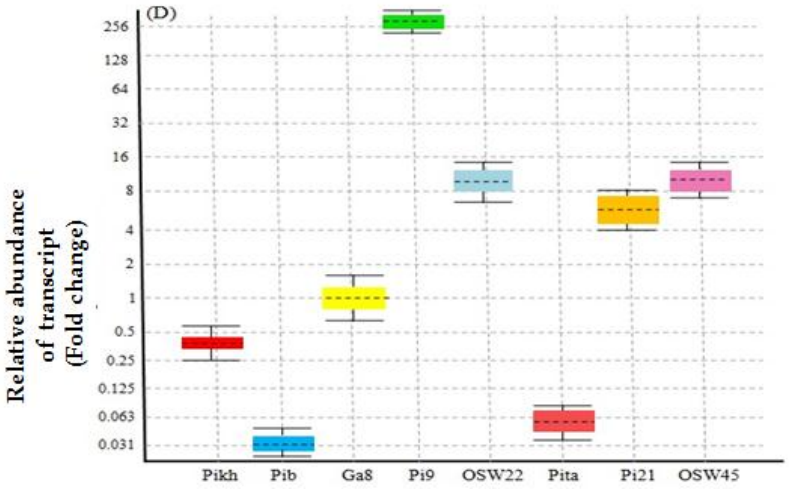
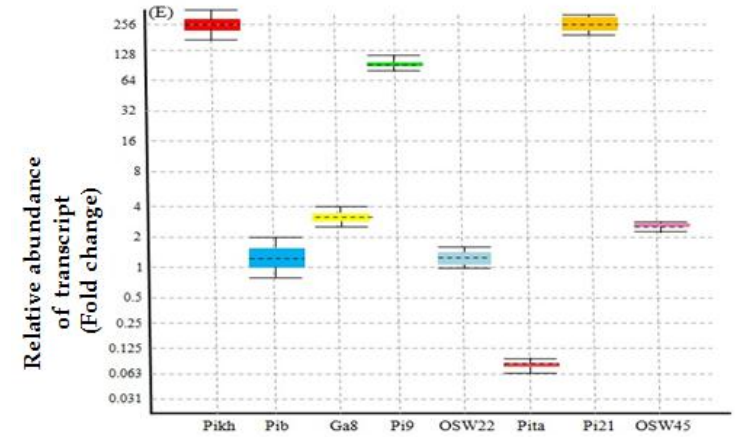
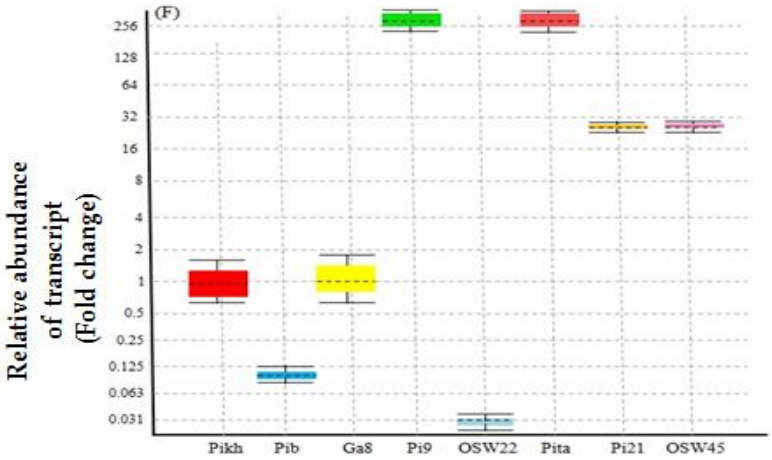
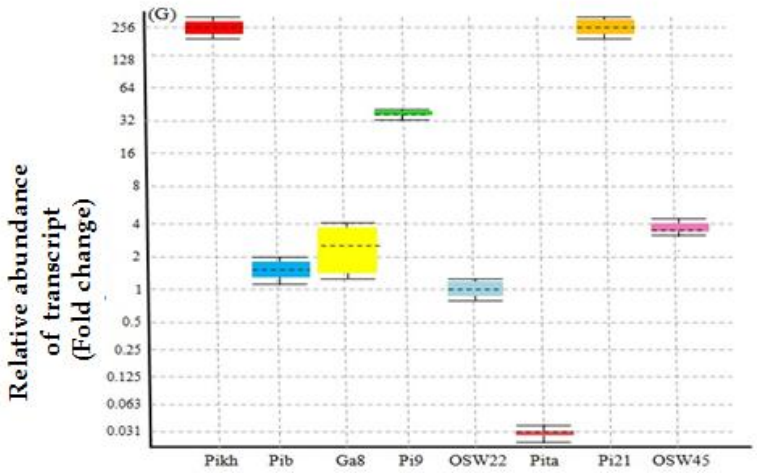
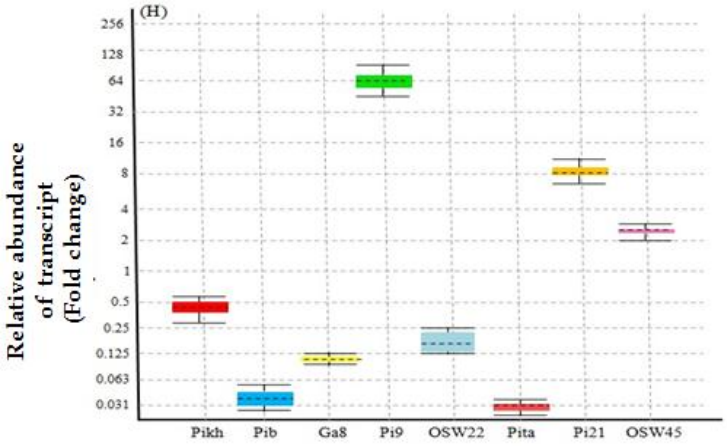
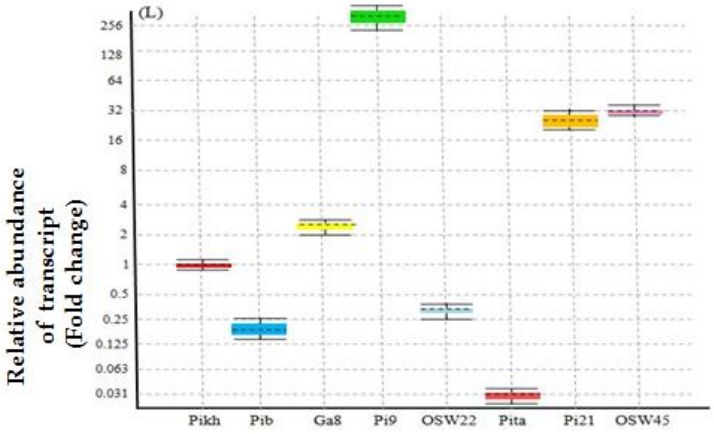









(H)
Relative abundance of transcript (Fold change)
256
128
64
32
16
8
4
2
1
0.5
0.25
0.125
0.063
0.031
Pikh
Pib
Ga8
Pi9
OSW22
Pita
Pi21
OSW45


(L)
Relative abundance of transcript (Fold change)
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16
8
4
2
1
0.5
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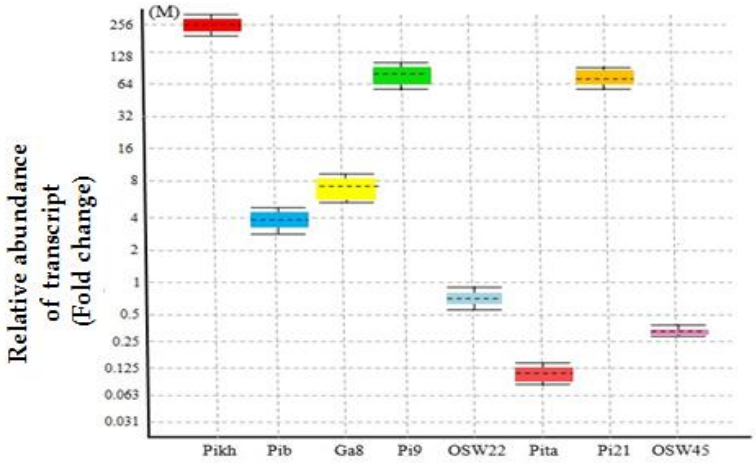
0.125
0.063
0.031
Pikh
Pib
Ga8
Pi9
OSW22
Pita
Pi21
OSW45


(M)
Relative abundance of transcript (Fold change)
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2
1
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Pib
Ga8
Pi9
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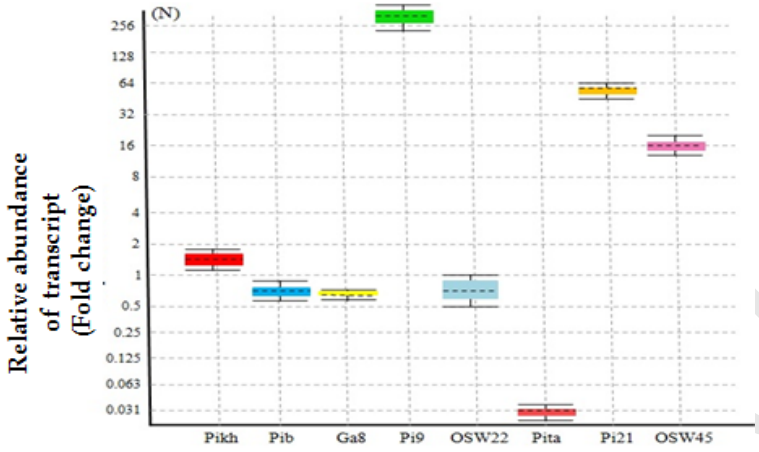
Pita
Pi21
OSW45


(N)
Relative abundance of transcript (Fold change)
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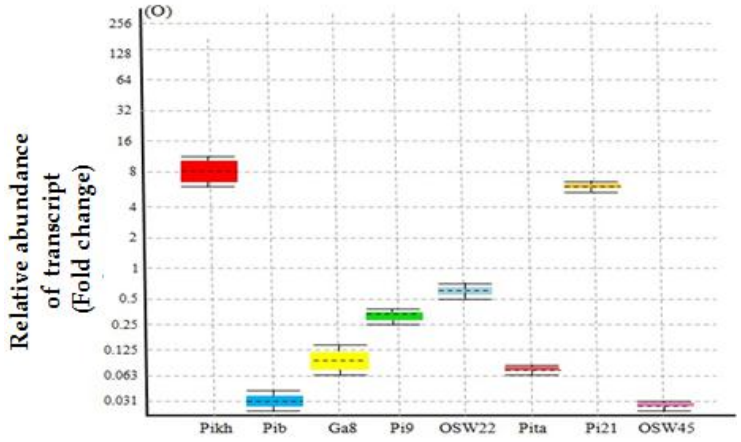
Pikh
Pib
Ga8
Pi9
OSW22
Pita
Pi21
OSW45


(O)
Relative abundance of transcript (Fold change)
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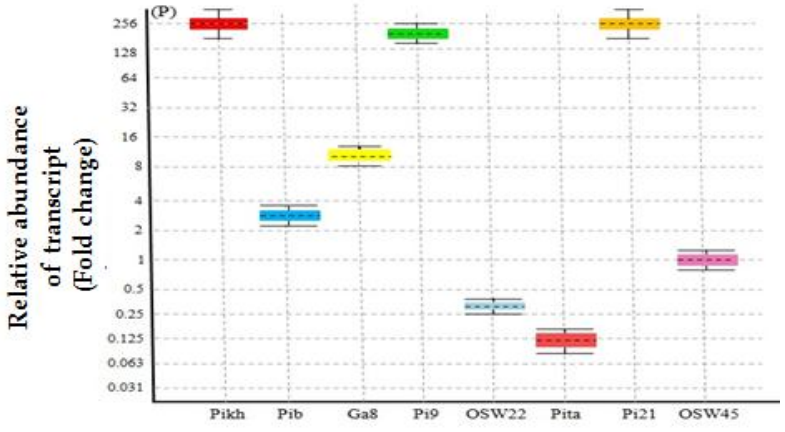
32
16
8
4
2
1
0.5
0.25
0.125
0.063
0.031
Pikh
Pib
Ga8
Pi9
OSW22
Pita
Pi21
OSW45


(P)
Relative abundance of transcript (Fold change)
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64
32
16
8
4
2
1
0.5
0.25
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0.031
Pikh
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OSW22
Pita
Pi21
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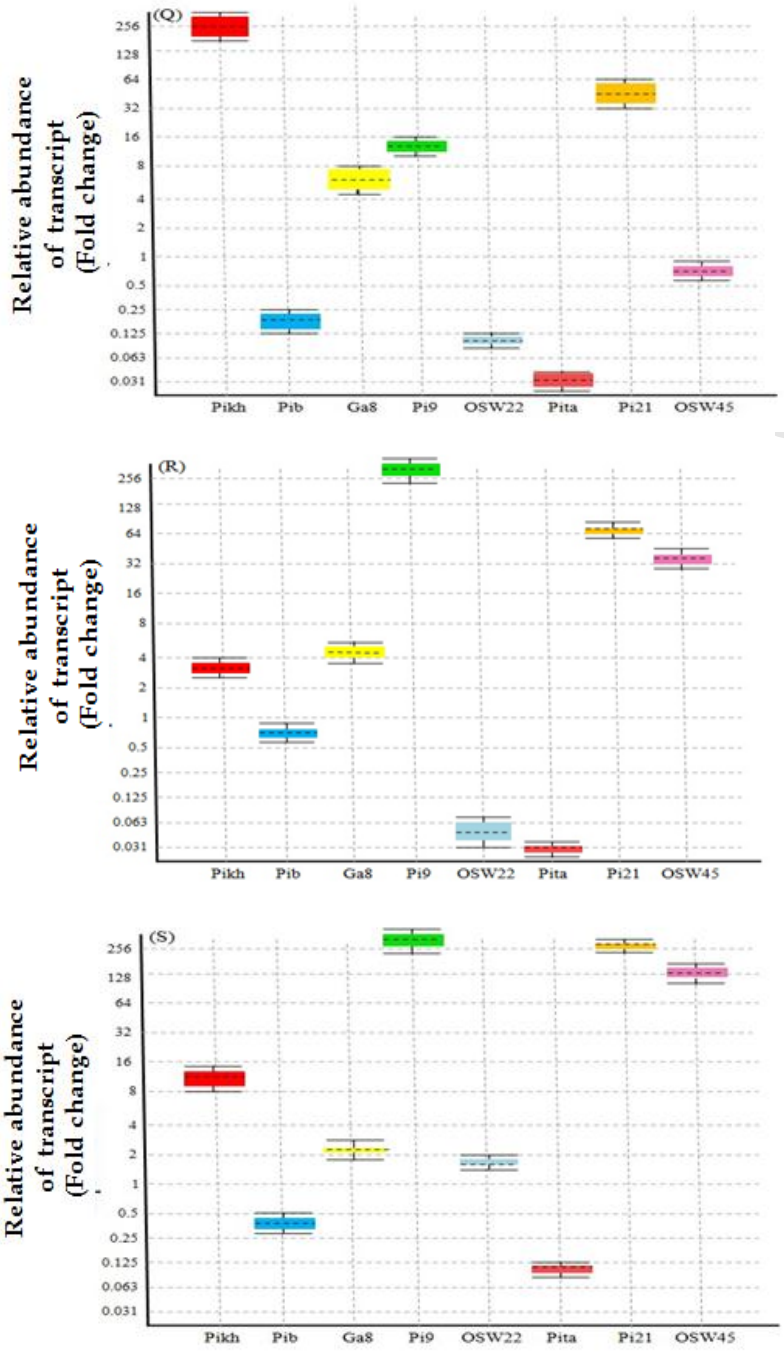


Figure C.2. Melting curve analysis of 10 genes (8 blast resistant genes and 2 reference genes).

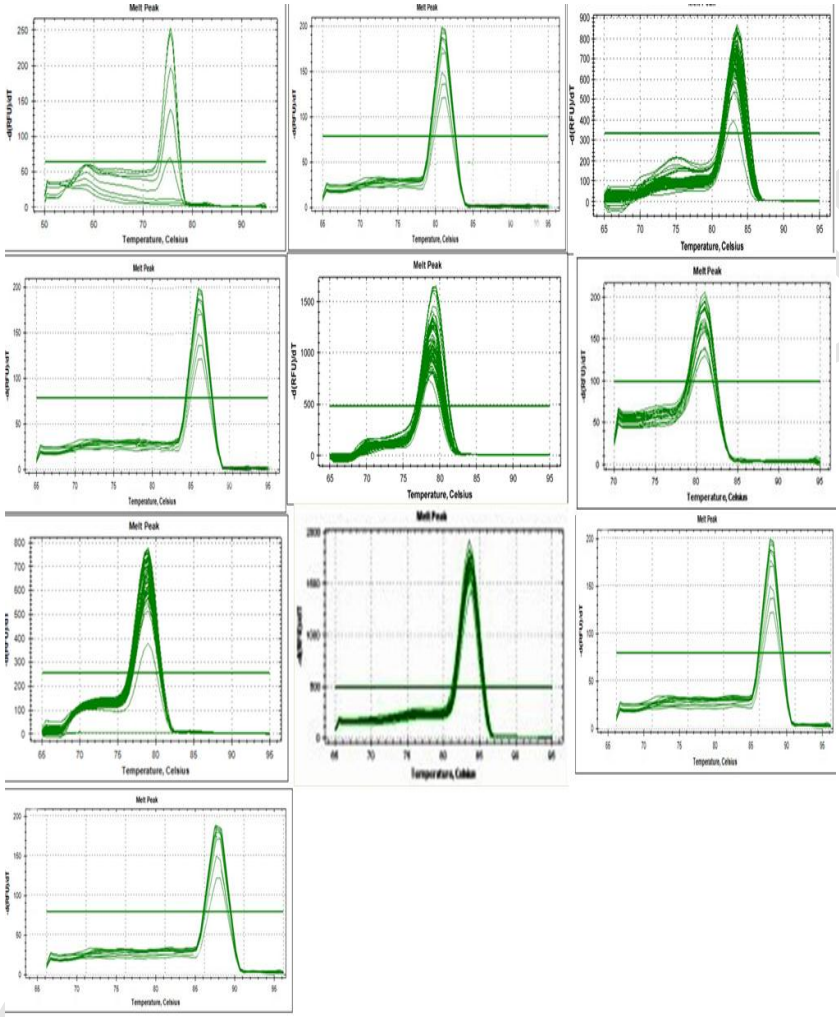


Table C.1. Relative expression patterns and *P*-value of 8 genes and *18SrRNA/tubulin* reference genes in sixteen rice varieties.

Gene	MR159	PH9	MR84	MR185	MR232	MR253	MR219	MR263	MR269
Pikh	UP	UP	DOWN	UP	-	DOWN	-	-	UP
P-value	0.000	0.000	0.000	0.000	0.650	0.000	0.161	0.822	0.000
Pib	DOWN	-	DOWN	-	DOWN	DOWN	-	DOWN	UP
P-value	0.000	0.170	0.000	0.499	0.000	0.000	0.501	0.000	0.000
GA8	-	-	-	UP	-	DOWN	UP	-	-
P-value	0.661	0.169	0.834	0.000	0.650	0.000	0.000	0.162	0.154
Pi9	UP	UP	UP	UP	UP	UP	UP	UP	UP
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Osw22	-	-	UP	-	DOWN	DOWN	-	DOWN	DOWN
P-value	0.169	0.169	0.000	0.827	0.000	0.000	0.502	0.000	0.000
Pita	DOWN	-	DOWN	DOWN	DOWN	DOWN	DOWN	-	DOWN
P-value	0.000	0.169	0.000	0.000	0.000	0.000	0.000	0.162	0.000
Pi21	UP	UP	UP	UP	UP	UP	UP	UP	UP
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Osw45	-	-	-	UP	UP	-	UP	UP	DOWN
P-value	0.339	0.169	0.164	0.000	0.000	0.177	0.000	0.000	0.000

Continued Table C.1:

Gene	MRQ74	MR220	Pulut Siding	MR211	MR1	MRQ50	Pongsu Seribu 2
Pikh	UP	UP	UP	UP	-	-	UP
P-value	0.000	0.000	0.000	0.000	0.078	0.159	0.000
Pib	UP	DOWN	DOWN	UP	-	-	DOWN
P-value	0.000	0.000	0.000	0.000	0.649	0.506	0.000
GA8	-	DOWN	UP	UP	UP	DOWN	UP
P-value	0.837	0.000	0.000	0.000	0.000	0.000	0.000
Pi9	UP	DOWN	UP	UP	UP	UP	UP
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Osw22	-	DOWN	DOWN	DOWN	DOWN	-	UP
P-value	0.667	0.000	0.000	0.000	0.000	0.347	0.000
Pita	DOWN	DOWN	DOWN	DOWN	DOWN	DOWN	DOWN
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pi21	UP	UP	UP	UP	UP	-	UP
P-value	0.000	0.000	0.000	0.000	0.000	0.159	0.000
Osw45	UP	DOWN	-	-	UP	UP	UP
P-value	0.000	0.000	0.331	0.841	0.000	0.000	0.000

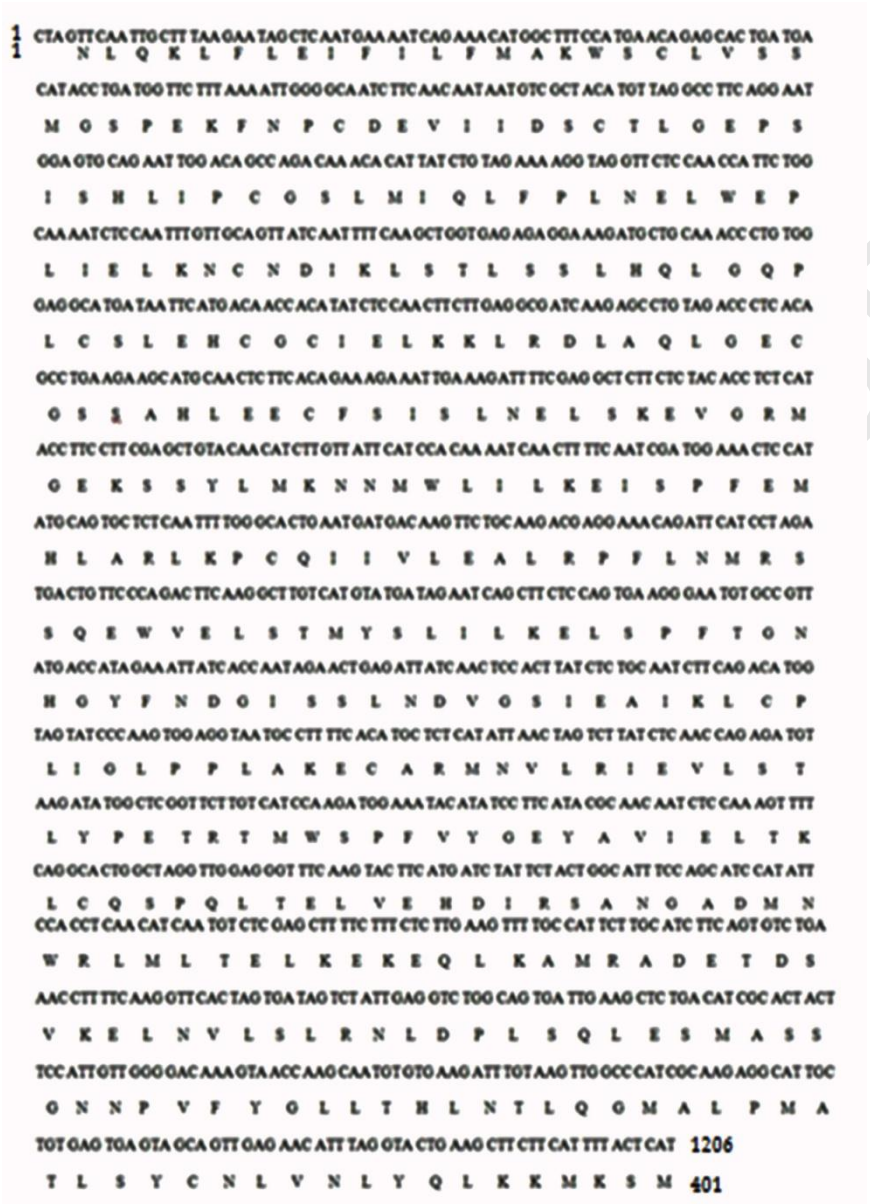


Figure C.3.The 1206 bp nucleotide and deduced amino sequence (401) of Nucleotide binding site leucine rich repeat protein gene (*Pikh*) (The translation of a nucleotide sequence to a protein sequence was done using ExPASY translate tool.

APPENDIX D

Figure D.1. Plant inoculation procedure.

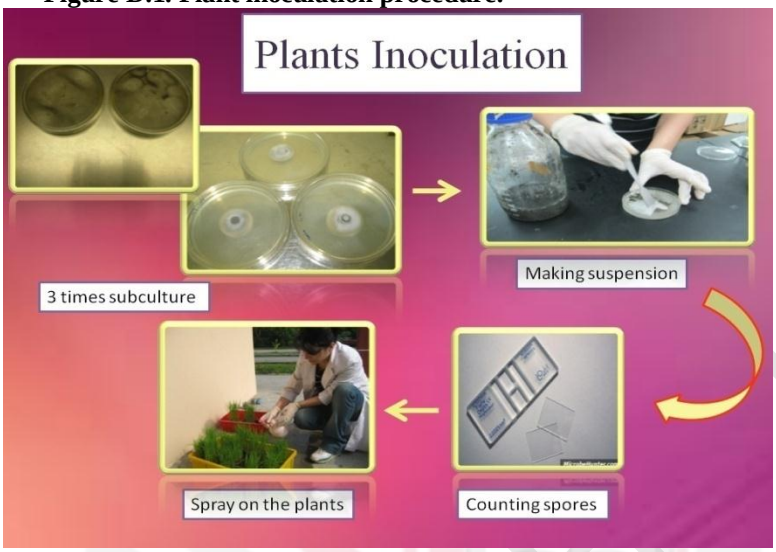
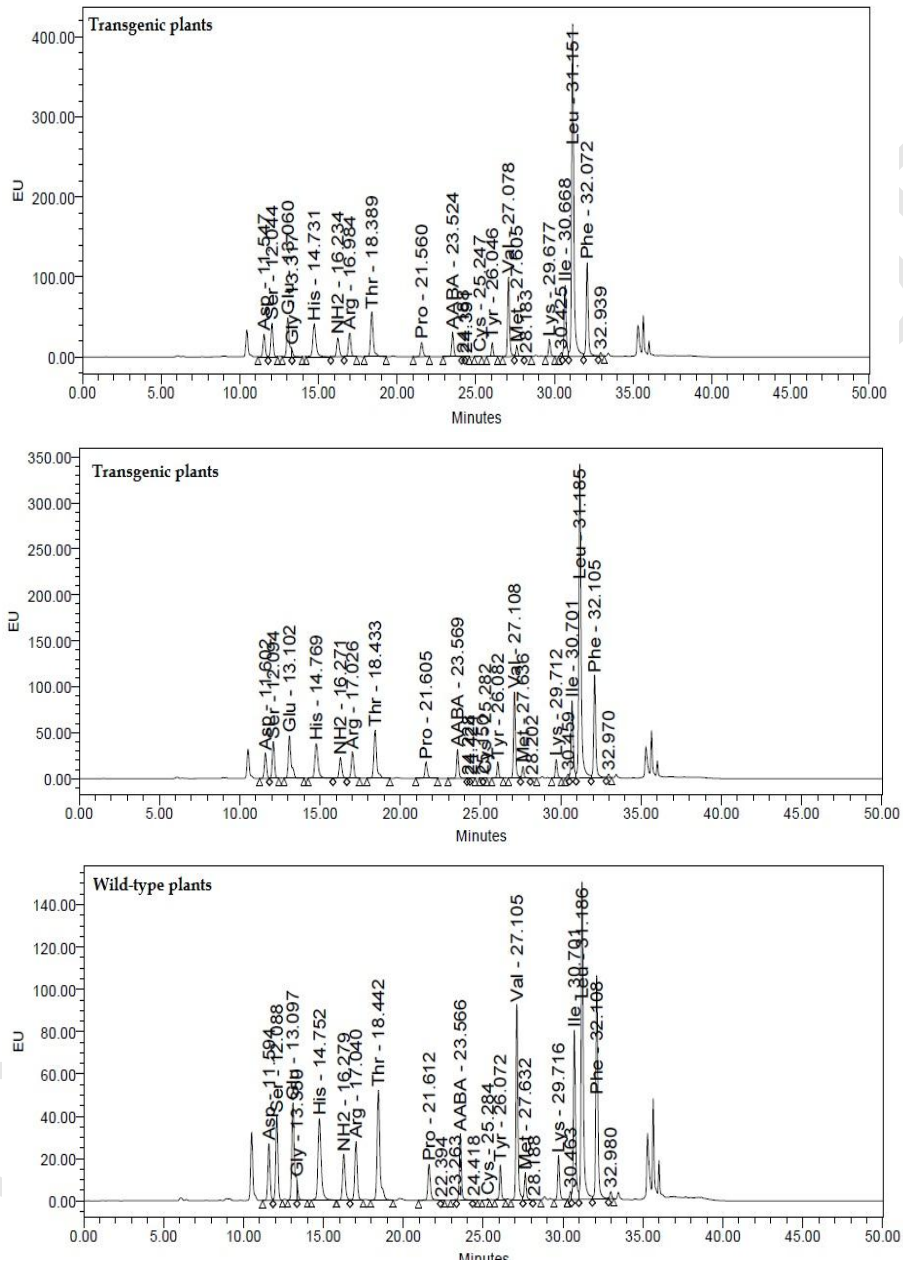


Figure D. 2. Symptomes observed on the leaves.

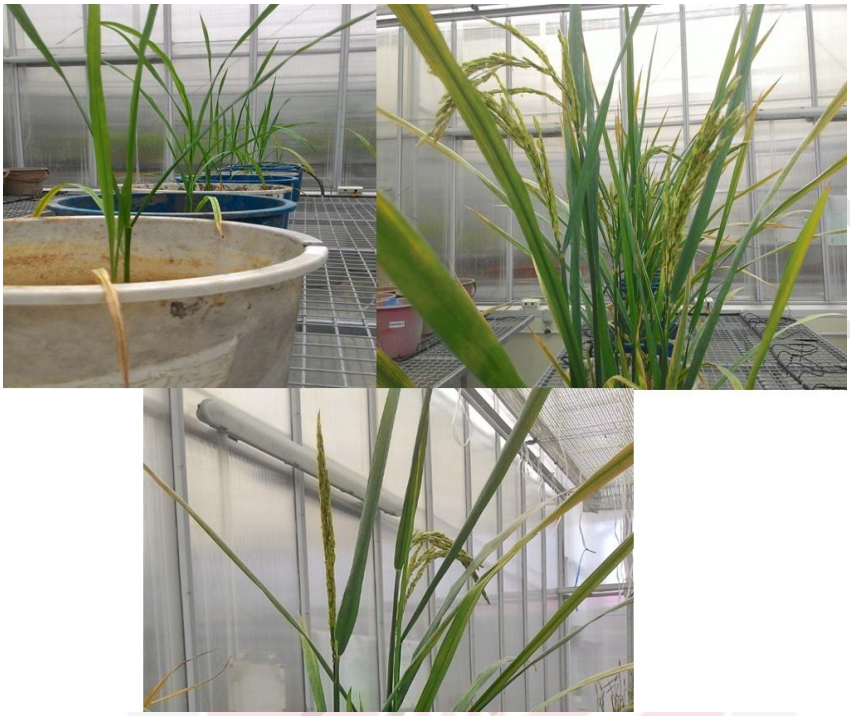
APPENDIX E

Figure E. 1. HPLC chromatogerams of amino acids (Replication 2 and 3 of Transgenic and replication 2 of wild-type plants).



APPENDIX F

Figure F. 1. Transgenic plants in the potes in different stages.



BIODATA OF STUDENT

Parisa Azizi was born on 16 of September 1982 in Ahwaz, Iran. She graduated in 2004 with Bachelor of Science in plant breeding from Islamic Azad University, Shooshtar Division and obtained her Master of science in the field of agricultural engineering, branch of agronomy and plant breeding from Islamic Azad University, Tabriz Division with the average of 16.99; Her project was the top in MSc. The subject of research in master level was: Estimation of broad and narrow sense heritability among single cross hybrids of sunflower. She has worked with Associate Prof. Dr. Rajabi Memari for 2 years in Chamran University since 2008. On September 2011, she started her PhD program on plant biotechnology under supervision of Prof. Dr. Mohd Rafii Yusop in the laboratory of Food crops, Institute of Tropical Agriculture (ITA), Universiti Putra Malaysia. In her post graduate life, he got many opportunities to participate in the several workshops and training programs that were certainly valuable to her research held in Malaysia. She had published two papers in the international journals, one in Critical reviews in biotechnology (IF=7.8) and another one in Mechanism of development (IF=2.2). She also submitted four papers in PLOS ONE, Journal of Plant Interactions and Plant Growth Regulation.

LIST OF PUBLICATIONS

Azizi, P., Rafii, M. Y., Abdullah, S. N. A., Nejat, N., Maziah, M., Hanafi, M. M., Maziah, M, and Sahebi, M. (2014). Toward understanding of rice innate immunity against Magnaporthe oryzae. *Critical Review in Biotechnology* 1-10.

Azizi, P., Rafii, M. Y., Maziah, M., Abdullah, S. N. A., Hanafi, M. M., Latif, M. A., and Sahebi, M. (2015). Understanding the shoot apical meristem regulation: A study of the phytohormones, auxin and cytokinin, in rice. *Mechanism of Development* 135, 1-15.

Azizi, P., Rafii, M. Y., Mahmood, M., Abdullah, S. N., Hanafi, M. M., Nejat, N., Latif, M, and Sahebi, M. (2015). Differential Gene Expression Reflects Morphological Characteristics and Physiological Processes in Rice Immunity against Blast Pathogen *Magnaporthe oryzae*.*PlosONE* 10(5), 1-18.

Azizi, P., Rafii, M. Y., Mahmood, M., Hanafi, M. M., Abdullah, S. N. A., Abiri, R., and Sahebi, M. (2015). Highly efficient protocol for callogenesis, somagenesis and regeneration of Indica rice plants. *Comptes rendus in biologies 338*(7), 463-470.

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