UNIVERSITI PUTRA MALAYSIA

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

PARISA AZIZI

ITA 2015 2

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

COPYRIGHT

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

By

PARISA AZIZI

April 2015

ABSRACT

Chairman : Professor Mohd Rafii Yusop, PhD
Institute : Tropical Agriculture

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

PARISA AZIZI

April 2015

Pengerusi : Profesor Mohd Rafii Yusop, PhD
Institut : Pertanian Tropika

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.

ACKNOWLEDGEMENTS

Foremost, I would like to express my utmost appreciation to my supervisor, Professor Dr. Mohd Rafii Yusop, who gives me constructive comments and great support.

I am sincerely grateful to my committee members; Professor Dr. Mohamed Hanafi Musa, Professor Dr. Siti Nor Akmar binti Abdullah, Professor Dr. Maziah Mahmood and Dr. Md. Abdul Latif for sharing their truthful and illuminating views on a number of issues related to the project.

I would also like to expree my deepest thanks to my beloved husband, Mahbod, for his endless kindness, support and love, infinite patience and understanding, and kind assistance without which this thesis whould be much harder to finalize.

Last but not least, very special thanks to my loving father and mother for their endless love and support.

I certify that a Thesis Examination Committee has met on 29 April 2015 to conduct the final examination of Parisa Azizi on her thesis entitled "Development of Blast Disease- Resistant Transgenic Rice Variety from MR219 Through Transformation with *PIKH* Gene" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

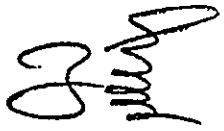
Members of the Thesis Examination Committee were as follows:

Yuzine bin Esa, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Janna Ong binti Abdullah, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Wong Mui Yun, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Stefaan Werbrouck, PhD
Professor
University Ghent
Belgium
(External Examiner)



ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 August 2015

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:

Mohd. Rafii Yusop, PhD
Professor
Institute of Tropical Agriculture
Universiti Putra Malaysia
(Chairman)

Mohamed Hanafi Musa, PhD
Professor
Institute of Tropical Agriculture
Universiti Putra Malaysia
(Member)

Siti Nor Akmar binti Abdullah, PhD
Professor
Institute of Tropical Agriculture
Universiti Putra Malaysia
(Member)

Maziah Mahmood, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Md. Abdul Latif, PhD
Senior Lecturer
Institute of Tropical Agriculture
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

Declaration by graduate student

I here by confirm that:

- this thesis is my original work;
- quotations, illustrationsn and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vicechancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules, or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is unheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No: _____

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Table **Page**

LIST OF ABBREVIATIONS

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.

REFERENCES

Abbruscato, P., Nepusz, T., Mizzi, L., Del Corvo, M., Morandini, P., Fumasoni, I., Michel, C., Paccanaro, A., Guiderdoni, E., and Schaffrath, U. (2012). OsWRKY22, a monocot WRKY gene, plays a role in the resistance response to blast. Molecular Plant Pathology 13 (8), 828-841.

Abbruscato, P., Nepusz, T. s., Mizzi, L., Del Corvo, M., Morandini, P., Fumasoni, I., Michel, C., Paccanaro, A., Guiderdoni, E., and Schaffrath, U. (2012). OsWRKY22, a monocot WRKY gene, plays a role in the resistance response to blast. Molecular Plant Pathology 13 (8), 828-841.

Adam, N. M., Sorooshian, S., Zhang, X. T., Tezara, C., Azizi, P., Sahebi, M., Bande, Y. M., Nurhaiza, S., and Kaiser, A. (2012). Engineering Research Methods. Lulu. com.

Alker, A. P., Mwapasa, V., and Meshnick, S. R. (2004). Rapid real-time PCR genotyping of mutations associated with sulfadoxine-pyrimethamine resistance in Plasmodium falciparum. Antimicrobial Agents and Chemotherapy 48 (8), 2924-2929.

Anbazhagan, M., Rajendran, R., Kalpana, M., Natarajan, V., and Panneerselvam, R. (2009). Agrobacterium mediated transformation of rice, var. Pusa Basmati-1. J Ecobiotechnol 1 (1), 007-011.

Antolin-Llovera, M., Ried, M. K., Binder, A., and Parniske, M. (2012). Receptor kinase signaling pathways in plant-microbe interactions. Annual Review of Phytopathology 50, 451-473.

Ashikari, M., and Matsuoka, M. (2002). Application of rice genomics to plant biology and breeding. Botanical Bulletin of Academia Sinica 43.

Ashikawa, I., Hayashi, N., Yamane, H., Kanamori, H., Wu, J., Matsumoto, T., Ono, K., and Yano, M. (2008). Two adjacent nucleotide-binding siteâ€“leucine-rich repeat class genes are required to confer Pikm-specific rice blast resistance. Genetics 180 (4), 2267-2276.

Ashkani, S. (2011). Molecular dissection and QTL mapping of rice blast disease resistance using simple sequence repeat markers. PhD Thesis. Serdang: Universiti Putra Malaysia.

Atkinson, N. J., and Urwin, P. E. (2012). The interaction of plant biotic and abiotic stresses: from genes to the field. Journal of Experimental Botany 63 (10), 3523-3543.

Azizi, P., Rafii, M. Y., Abdullah, S. N. A., Nejat, N., Maziah, M., Hanafi, M. M., Latif, M. A., and Sahebi, M. (2014). Toward understanding of rice innate immunity against Magnaporthe oryzae. Critical Reviews in Biotechnology (0), 1-10.

Bao, Y., Dharmawardhana, P., Mockler, T. C., and Strauss, S. H. (2009). Genome scale transcriptome analysis of shoot organogenesis in Populus. *BMC Plant Biology* 9 (1), 132.

Barriuso, J., Solano, B. R., and Gutierrez Manero, F. J. (2008). Protection against pathogen and salt stress by four plant growth-promoting rhizobacteria isolated from Pinus sp. on *Arabidopsis thaliana*. *Phytopathology* 98 (6), 666-672.

Bartlett, J. G., Alves, S. C., Smedley, M., Snape, J. W., and Harwood, W. A. (2008). High-throughput *Agrobacterium*-mediated barley transformation. *Plant Methods* 4 (1), 22.

Bastiaans, L. (1991). Ratio between virtual and visual lesion size as a measure to describe reduction in leaf photosynthesis of rice due to leaf blast. *Phytopathology* 81 (6), 611-615.

Bastiaans, L. (1993). Effects of leaf blast on photosynthesis of rice. 1. Leaf photosynthesis. *Netherlands Journal of Plant Pathology* 99 (4), 197-203.

Belkhadir, Y., Subramaniam, R., and Dangl, J. L. (2004). Plant disease resistance protein signaling: NBS-LRR proteins and their partners. *Current Opinion in Plant Biology* 7 (4), 391-399.

Berger, S., Sinha, A. K., and Roitsch, T. (2007). Plant physiology meets phytopathology: plant primary metabolism and plant-pathogen interactions. *Journal of Experimental Botany* 58 (15-16), 4019-4026.

Bhalla, P. L. (2006). Genetic engineering of wheat-current challenges and opportunities. *Trends in Biotechnology* 24 (7), 305-311.

Bhatnagar-Mathur, P., Vadez, V., and Sharma, K. K. (2008). Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. *Plant Cell Reports* 27 (3), 411-424.

Bieche, I., Laurendeau, I., Tozlu, S., Olivi, M., Vidaud, D., Lidereau, R., and Vidaud, M. (1999). Quantitation of *MYC* gene expression in sporadic breast tumors with a real-time reverse transcription-PCR assay. *Cancer Research* 59 (12), 2759-2765.

Bieche, I., Olivi, M., Champeme, M.-H., Vidaud, D., Lidereau, R., and Vidaud, M. (1998). Novel approach to quantitative polymerase chain reaction using real-time detection: Application to the detection of gene amplification in breast cancer. *International Journal of Cancer* 78 (5), 661-666.

Binns, A. N., and Thomashow, M. F. (1988). Cell biology of Agrobacterium infection and transformation of plants. *Annual Reviews in Microbiology* 42 (1), 575-606.

Birch, R. G. (1997). Plant transformation: problems and strategies for practical application. *Annual Review of Plant Biology* 48 (1), 297-326.

Bohnert, H. J., Nelson, D. E., and Jensen, R. G. (1995). Adaptations to environmental stresses. *The Plant Cell* 7 (7), 1099.

Bohnert, H. U., Fudal, I., Dioh, W., Tharreau, D., Notteghem, J.-L., and Lebrun, M.-H. (2004). A putative polyketide synthase/peptide synthetase from Magnaporthe grisea signals pathogen attack to resistant rice. *The Plant Cell Online* 16 (9), 2499-2513.

Boller, T., and Felix, G. (2009). A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annual Review of Plant Biology* 60, 379-406.

Boller, T., and He, S. Y. (2009). Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens. *Science* 324, 742-744.

Bolton, M. D. (2009). Primary metabolism and plant defense-fuel for the fire. *Molecular Plant-Microbe Interactions* 22 (5), 487-497.

Bonardi, V., and Dangl, J. L. (2012). How complex are intracellular immune receptor signaling complexes? *Frontiers in Plant Science* 3.

Bray Speth, E., Lee, Y. N., and He, S. Y. (2007). Pathogen virulence factors as molecular probes of basic plant cellular functions. *Current Opinion in Plant Biology* 10 (6), 580-586.

Bryan, G. T., Wu, K.-S., Farrall, L., Jia, Y., Hershey, H. P., McAdams, S. A., Faulk, K. N., Donaldson, G. K., Tarchini, R., and Valent, B. (2000). A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. *The Plant Cell Online* 12 (11), 2033-2045.

Burch-Smith, T. M., Schiff, M., Caplan, J. L., Tsao, J., Czymmek, K., and Dinesh-Kumar, S. P. (2007). A novel role for the TIR domain in association with pathogen-derived elicitors. *PLoS biology* 5 (3), e68.

Burrell, M. M., and Rees, T. (1974). Carbohydrate metabolism of rice leaves infected by *Pyricularia oryzae*. *Physiological Plant Pathology* 4, 489-496.

Carding, S. R., Lu, D., and Bottomly, K. (1992). A polymerase chain reaction assay for the detection and quantitation of cytokine gene expression in small numbers of cells. *Journal of Immunological Methods* 151 (1), 277-287.

Century, K., Reuber, T. L., and Ratcliffe, O. J. (2008). Regulating the regulators: the future prospects for transcription-factor-based agricultural biotechnology products. *Plant Physiology* 147 (1), 20-29.

Cesari, S., Thilliez, G., Ribot, C., Chalvon, V., Michel, C., Jauneau, A., Rivas, S., Alaux, L., Kanzaki, H., and Okuyama, Y. (2013). The rice resistance protein pair *RGA4/RGA5* recognizes the *Magnaporthe oryzae* effectors AVR-Pia and AVR1-CO39 by direct binding. *The Plant Cell Online* 25 (4), 1463-1481.

Chan, K. L., Ho, C. L., Namasivayam, P., and Napis, S. (2007). A simple and rapid method for RNA isolation from plant tissues with high phenolic compounds and polysaccharides. *Nature Protocol* 184.

Chen, J., Shi, Y., Liu, W., Chai, R., Fu, Y., Zhuang, J., and Wu, J. (2011). A *Pid3* allele from rice cultivar Gumei2 confers resistance to *Magnaporthe oryzae*. *Journal of Genetics and Genomics* 38 (5), 209-216.

Chen, S., Songkumarn, P., Venu, R. C., Gowda, M., Bellizzi, M., Hu, J., Liu, W., Ebbole, D., Meyers, B., and Mitchell, T. (2013). Identification and characterization of in planta-expressed secreted effector proteins from *Magnaporthe oryzae* that induce cell death in rice. *Molecular Plant-Microbe Interactions* 26 (2), 191-202.

Chen, X., and Ronald, P. C. (2011). Innate immunity in rice. *Trends in Plant Science* 16 (8), 451-459.

Chen, X., Shang, J., Chen, D., Lei, C., Zou, Y., Zhai, W., Liu, G., Xu, J., Ling, Z., and Cao, G. (2006). AB-lectin receptor kinase gene conferring rice blast resistance. *The Plant Journal* 46 (5), 794-804.

Chen, X., Shang, J., Chen, D., Lei, C., Zou, Y., Zhai, W., Liu, G., Xu, J., Ling, Z., and Cao, G. (2006). AB-lectin receptor kinase gene conferring rice blast resistance. *The Plant Journal* 46 (5), 794-804.

Cheng, J., Zhang, Y., and Li, Q. (2004). Real-time PCR genotyping using displacing probes. *Nucleic Acids Research* 32 (7), e61-e61.

Cheng, X., Sardana, R., Kaplan, H., and Altosaar, I. (1998). *Agrobacterium* transformed rice plants expressing synthetic *cryIAb* and *cyrIAc* genes are highly toxic to striped stem borer and yellow stem borer *Proceedings of the National Academy of Sciences* 95, 2767-1772.

Chomczynski, P., and Sacchi, N. (2006). The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. *Nature Protocols* 1 (2), 581-585.

Christianson, J. A., Dennis, E. S., Llewellyn, D. J., and Wilson, I. W. (2010). ATAF NAC transcription factors: regulators of plant stress signaling. *Plant Signaling & Behavior* 5 (4), 428-432.

Chujo, T., Takai, R., Akimoto-Tomiyama, C., Ando, S., Minami, E., Nagamura, Y., Kaku, H., Shibuya, N., Yasuda, M., and Nakashita, H. (2007). Involvement of the elicitor-induced gene *OsWRKY53* in the expression of defense-related genes in rice. *Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression* 1769 (7), 497-505.

CORREA-VICTORIA, F. J., and Zeigler, R. S. (1993). Pathogenic variability in *Pyricularia grisea* at a rice blast hot spot breeding site in eastern Colombia. *Plant Disease* 77 (10), 1029-1035.

Dai, S., Carcamo, R., Zhang, Z., Chen, S., and Beachy, R. (2001). The bacterial cytosine deaminase gene used as a conditional negative selection marker in transgenic rice plants. *Plant Cell Reports* 20 (8), 738-743.

Dai Yin, C., Yong Hai, L. U. O., Min, S. H. I., Da, L. U. O., and Hong Xuan, L. I. N. (2005). Salt-responsive genes in rice revealed by cDNA microarray analysis. *Cell Research* 15 (10), 796-810.

Dangl, J. L., and Jones, J. D. G. (2001). Plant pathogens and integrated defence responses to infection. *Nature* 411 (6839), 826-833.

Datta, K., Tu, J., Oliva, N., Ona, I., Velazhahan, R., Mew, T. W., Muthukrishnan, S., and Datta, S. K. (2001). Enhanced resistance to sheath blight by constitutive expression of infection-related rice chitinase in transgenic elite indica rice cultivars. *Plant Science* 160 (3), 405-414.

de Wit, P. J. G. M., Mehrabi, R., van den Burg, H. A., and Stergiopoulos, I. (2009). Fungal effector proteins: past, present and future. *Molecular Plant Pathology* 10 (6), 735-747.

Dean, R. A., Talbot, N. J., Ebbole, D. J., Farman, M. L., Mitchell, T. K., Orbach, M. J., Thon, M., Kulkarni, R., Xu, J.-R., and Pan, H. (2005). The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature* 434 (7036), 980-986.

Djamei, A., and Kahmann, R. (2012). Ustilago maydis: dissecting the molecular interface between pathogen and plant. *PLoS Pathogens* 8 (11), e1002955.

Dodds, P. N., and Rathjen, J. P. (2010). Plant immunity: towards an integrated view of plant-pathogen interactions. *Nature Reviews Genetics* 11 (8), 539-548.

FAO (2014). Rice Market Monitor pp. 1-40.

Fleige, S., and Pfaffl, M. W. (2006). RNA integrity and the effect on the real-time qRT-PCR performance. *Molecular Aspects of Medicine* 27 (2), 126-139.

Foley, K. P., Leonard, M. W., and Engel, J. D. (1993). Quantitation of RNA using the polymerase chain reaction. *TRENDS in Genetics* 9 (11), 380-385.

Fredeslinda, C. E., Rhodora, R. A., and Lilian, B. U. (2009). Callusing and regeneration potential of rice (*Oryza sativa* L.) genotypes toward the develpment for salt tolerance. *Philipin Journal of Science* 138 (2), 169-176.

Fukuoka, S., Saka, N., Koga, H., Ono, K., Shimizu, T., Ebana, K., Hayashi, N., Takahashi, A., Hirochika, H., and Okuno, K. (2009). Loss of function of a proline-containing protein confers durable disease resistance in rice. *Science* 325 (5943), 998-1001.

Ge, X., Chu, Z., Lin, Y., and Wang, S. (2006). A tissue culture system for different germplasms of indica rice. *Plant Cell Reports* 25 (5), 392-402.

Gelvin, S. B. (2000). Agrobacterium and plant genes involved in T-DNA transfer and integration. *Annual Reviews of Plant Biology* 51 (1), 223-256.

Gibson, N. J. (2006). The use of real-time PCR methods in DNA sequence variation analysis. *Clinica Chimica Acta* 363 (1), 32-47.

Gibson, U. E., Heid, C. A., and Williams, P. M. (1996). A novel method for real time quantitative RT-PCR. *Genome Research* 6 (10), 995-1001.

Gill, B. S., Appels, R., Botha-Oberholster, A.-M., Buell, C. R., Bennetzen, J. L., Chalhoub, B., Chumley, F., Dvoak, J., Iwanaga, M., and Keller, B. (2004). A workshop report on wheat genome sequencing international genome research on wheat consortium. *Genetics* 168 (2), 1087-1096.

Glaszmann, J. C. (1987). Isozymes and classification of Asian rice varieties. *Theoretical and Applied Genetics* 74 (1), 21-30.

Gnanamanickam, S. S. (2009). *Biological Control of Rice Diseases*. Springer, pp. 13-42.

Gohre, V., Jones, A. M. E., Sklenar, J., Robatzek, S., and Weber, A. P. M. (2012). Molecular crosstalk between PAMP-triggered immunity and photosynthesis. *Molecular Plant-Microbe Interactions* 25 (8), 1083-1092.

Gosal, S. S., and Kang, M. S. (2012). *Plant Tissue Culture and Genetic Transformation for Crop Improvement*. Wiley, pp. 357-397.

Hamer, J. E., Howard, R. J., Chumley, F. G., and Valent, B. (1988). A mechanism for surface attachment in spores of a plant pathogenic fungus. *Science* 239 (4837), 288-290.

Hammond-Kosack, K. E., and Kanyuka, K. (2007). Resistance genes (R genes) in plants. *Encyclopedia Life Science* 40, 1-21.

Hardie, D. G. (2007). AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nature Reviews Molecular Cell Biology* 8 (10), 774-785.

Hayashi, K., and Yoshida, H. (2009). Refunctionalization of the ancient rice blast disease resistance gene *Pit* by the recruitment of a retrotransposon as a promoter. *The Plant Journal* 57 (3), 413-425.

Hayashi, N., Inoue, H., Kato, T., Funao, T., Shirota, M., Shimizu, T., Kanamori, H., Yamane, H., Hayano-Saito, Y., and Matsumoto, T. (2010). Durable panicle blast-resistance gene *Pb1* encodes an atypical CC-NBS-LRR protein and was generated by acquiring a promoter through local genome duplication. *The Plant Journal* 64 (3), 498-510.

Higuchi, R., Dollinger, G., Walsh, P. S., and Griffith, R. (1992). Simultaneous amplification and detection of specific DNA sequences. *Biotechnology* 10 (4), 413-417.

Higuchi, R., Fockler, C., Dollinger, G., and Watson, R. (1993). Kinetic PCR analysis: real-time monitoring of DNA ampliï¬•cation reactions. *Biotechnology* 11, 1026-1030.

Hirose, S., Kawahigashi, H., Ozawa, K., Shiota, N., Inui, H., Ohkawa, H., and Ohkawa, Y. (2005). Transgenic rice containing human CYP2B6 detoxifies various classes of herbicides. *Journal of Agricultural and Food Chemistry* 53 (9), 3461-3467.

Hu, H., Dai, M., Yao, J., Xiao, B., Li, X., Zhang, Q., and Xiong, L. (2006). Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proceedings of the National Academy of Sciences* 103 (35), 12987-12992.

Hu, Y. G., and Xie, C. H. (2011). Effect of toxins from cav on physiological and biochemical indexes of rice. *Hubei Agricultural Sciences* 50, 4-8.

Hu, Z., Wu, Y.-R., Li, W., and Gao, H.-H. (2006). Factors affecting Agrobacterium tumefaciens-mediated genetic transformation of Lycium barbarum L. *In Vitro Cellular & Developmental Biology-Plant* 42 (5), 461-466.

Hua, L., Wu, J., Chen, C., Wu, W., He, X., Lin, F., Wang, L., Ashikawa, I., Matsumoto, T., and Wang, L. (2012). The isolation of *Pi1*, an allele at

the Pik locus which confers broad spectrum resistance to rice blast. *Theoretical and Applied Genetics* 125 (5), 1047-1055.

Hwang, C.-F., Bhakta, A. V., Truesdell, G. M., Pudlo, W. M., and Williamson, V. M. (2000). Evidence for a role of the N terminus and leucine-rich repeat region of the *Mi* gene product in regulation of localized cell death. *The Plant Cell Online* 12 (8), 1319-1329.

Imbeaud, S., Graudens, E., Boulanger, V., Barlet, X., Zaborski, P., Eveno, E., Mueller, O., Schroeder, A., and Auffray, C. (2005). Towards standardization of RNA quality assessment using user-independent classifiers of microcapillary electrophoresis traces. *Nucleic Acids Research* 33 (6), e56-e56.

Ishiga, Y., Uppalapati, S. R., Ishiga, T., Elavarthi, S., Martin, B., and Bender, C. L. (2009). The phytotoxin coronatine induces lightâ€•dependent reactive oxygen species in tomato seedlings. *New Phytologist* 181 (1), 147-160.

Jia, Y., McAdams, S. A., Bryan, G. T., Hershey, H. P., and Valent, B. (2000). Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. *The EMBO Journal* 19 (15), 4004-4014.

Jones, J. D. G., and Dangl, J. L. (2006). The plant immune system. *Nature* 444 (7117), 323-329.

Kaku, H., Nishizawa, Y., Ishii-Minami, N., Akimoto-Tomiyama, C., Dohmae, N., Takio, K., Minami, E., and Shibuya, N. (2006). Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. *Proceedings of the national Academy of Sciences* 103 (29), 11086-11091.

Kang, S., Sweigard, J. A., and Valent, B. (1995). The PWL host specificity gene family in the blast fungus Magnaporthe grisea. *MPMI-Molecular Plant Microbe Interactions* 8 (6), 939-948.

Kankanala, P., Czymmek, K., and Valent, B. (2007). Roles for rice membrane dynamics and plasmodesmata during biotrophic invasion by the blast fungus. *The Plant Cell Online* 19 (2), 706-724.

Kanzaki, H., Nirasawa, S., Saitoh, H., Ito, M., Nishihara, M., Terauchi, R., and Nakamura, I. (2002). Overexpression of the wasabi defensin gene confers enhanced resistance to blast fungus (*Magnaporthe grisea*) in transgenic rice. *Theoretical and Applied Genetics* 105 (6-7), 809-814.

Karimi, M., Inze, D., and Depicker, A. (2002). GATEWAY vectors for Agrobacterium-mediated plant transformation. Trends in Plant Science 7 (5), 193-195.

Kato, H. (2001). Rice blast disease. Pesticide outlook 12 (1), 23-25.

Kawano, Y., Chen, L., and Shimamoto, K. (2010). The function of Rac small GTPase and associated proteins in rice innate immunity. Rice 3 (2-3), 112-121.

Khush, G. S. (2005). What it will take to feed 5.0 billion rice consumers in 2030. Plant Molecular Biology 59 (1), 1-6.

Kiefer, E., Heller, W., and Ernst, D. (2000). A simple and efficient protocol for isolation of functional RNA from plant tissues rich in secondary metabolites. Plant Molecular Biology Report 18 (1), 33-39.

Kim, C. Y., Lee, S.-H., Park, H. C., Bae, C. G., Cheong, Y. H., Choi, Y. J., Han, C.-d., Lee, S. Y., Lim, C. O., and Cho, M. J. (2000). Identification of rice blast fungal elicitor-responsive genes by differential display analysis. Molecular Plant-Microbe Interactions 13 (4), 470-474.

Kim, S.-H., Oikawa, T., Kyozuka, J., Wong, H. L., Umemura, K., Kishi-Kaboshi, M., Takahashi, A., Kawano, Y., Kawasaki, T., and Shimamoto, K. (2012). The *bHLH Rac immunity1 (RAI1)* is activated by *OsRac1* via *OsMAPK3* and *OsMAPK6* in rice immunity. *Plant and Cell Physiology* 53 (4), 740-754.

Kindich, R., Florl, A. R., Jung, V., Engers, R., Muller, M., Schulz, W. A., and Wullich, B. (2005). Application of a modified real-time PCR technique for relative gene copy number quantification to the determination of the relationship between NKX3. 1 loss and MYC gain in prostate cancer. *Clinical chemistry* 51 (3), 649-652.

King, B. C., Waxman, K. D., Nenni, N. V., Walker, L. P., Bergstrom, G. C., and Gibson, D. M. (2011). Arsenal of plant cell wall degrading enzymes reflects host preference among plant pathogenic fungi. *Biotechnol Biofuels* 4 (4).

Kjaersgaard, T., Jensen, M. K., Christiansen, M. W., Gregersen, P., Kragelund, B. B., and Skriver, K. (2011). Senescence-associated barley NAC (NAM, ATAF1, 2, CUC) transcription factor interacts with radical-induced cell death 1 through a disordered regulatory domain. *Journal of Biological Chemistry* 286 (41), 35418-35429.

Kombrink, E., and Schmelzer, E. (2001). The hypersensitive response and its role in local and systemic disease resistance. *European Journal of Plant Pathology* 107 (1), 69-78.

Konigshoff, M., Wilhelm, J., Bohle, R. M., Pingoud, A., and Hahn, M. (2003). HER-2/neu gene copy number quantified by real-time PCR: comparison of gene amplification, heterozygosity, and immunohistochemical status in breast cancer tissue. *Clinical chemistry* 49 (2), 219-229.

Kubista, M., Andrade, J. M., Bengtsson, M., Forootan, A., Jonak, J., Lind, K., Sindelka, R., Sjoback, R., Sjogreen, B., and Strombom, L. (2006). The

real-time polymerase chain reaction. *Molecular Aspects of Medicine* 27 (2), 95-125.

Kyozuka, J., Otoo, E., and Shimamoto, K. (1988). Plant regeneration from protoplasts of indica rice: genotypic differences in culture response. *Theoretical and Applied Genetics* 76 (6), 887-890.

Lampard, G. R., Lukowitz, W., Ellis, B. E., and Bergmann, D. C. (2009). Novel and expanded roles for MAPK signaling in *Arabidopsis* stomatal cell fate revealed by cell type-specific manipulations. *The Plant Cell Online* 21 (11), 3506-3517.

Lee, S.-K., Song, M.-Y., Seo, Y.-S., Kim, H.-K., Ko, S., Cao, P.-J., Suh, J.-P., Yi, G., Roh, J.-H., and Lee, S. (2009). Rice *Pi5*-mediated resistance to *Magnaporthe oryzae* requires the presence of two coiled-coil-nucleotide-binding-leucine-rich repeat genes. *Genetics* 181 (4), 1627-1638.

Leong, S. A. (2008). *Genomics of Disease*. Springer, pp. 199-216.

Leutenegger, C. M., Mislin, C. N., Sigrist, B., Ehrengruber, M. U., Hofmann-Lehmann, R., and Lutz, H. (1999). Quantitative real-time PCR for the measurement of feline cytokine mRNA. *Veterinary Immunology and Immunopathology* 71 (3), 291-305.

Li, C., Wei, J., Lin, Y., and Chen, H. (2012). Gene silencing using the recessive rice bacterial blight resistance gene *xa13* as a new paradigm in plant breeding. *Plant Cell Reports* 31 (5), 851-862.

Li, D., Wang, L., Wang, M., Xu, Y. Y., Luo, W., Liu, Y. J., Xu, Z. H., Li, J., and Chong, K. (2009). Engineering *OsBAK1* gene as a molecular tool to improve rice architecture for high yield. *Plant Biotechnology Journal* 7 (8), 791-806.

Li, H. S. (2006). Modern plant physiology. Higher Education Press, Beijing. pp. 145-147.

Li, T., Liu, B., Spalding, M. H., Weeks, D. P., and Yang, B. (2012). High-efficiency TALEN-based gene editing produces disease-resistant rice. *Nature Biotechnology* 30 (5), 390-392.

Li, X., Duan, X., Jiang, H., Sun, Y., Tang, Y., Yuan, Z., Guo, J., Liang, W., Chen, L., and Yin, J. (2006). Genome-wide analysis of basic/helix-loop-helix transcription factor family in rice and *Arabidopsis*. *Plant physiology* 141 (4), 1167-1184.

Lin, F., Chen, S., Que, Z., Wang, L., Liu, X., and Pan, Q. (2007). The blast resistance gene *Pi37* encodes a nucleotide binding site-leucine-rich

repeat protein and is a member of a resistance gene cluster on rice chromosome 1. *Genetics* 177 (3), 1871-1880.

Lin, R., Zhao, W., Meng, X., Wang, M., and Peng, Y. (2007). Rice gene *OsNAC19* encodes a novel NAC-domain transcription factor and responds to infection by *Magnaporthe grisea*. *Plant Science* 172 (1), 120-130.

Lin, Y. J., and Zhang, Q. (2005). Optimising the tissue culture conditions for high efficiency transformation of indica rice. *Plant Cell Reports* 23 (8), 540-547.

Liss, B. (2002). Improved quantitative real-time RT-PCR for expression profiling of individual cells. *Nucleic Acids Research* 30 (17), e89-e89.

Liu, J., Wang, X., Mitchell, T., Hu, Y., Liu, X., Dai, L., and Wang, G.-L. (2010). Recent progress and understanding of the molecular mechanisms of the rice-*Magnaporthe oryzae* interaction. *Molecular Plant Pathology* 11 (3), 419-427.

Liu, X., Lin, F., Wang, L., and Pan, Q. (2007). The in silico map-based cloning of Pi36, a rice coiled-coil-nucleotide-binding site-leucine-rich repeat gene that confers race-specific resistance to the blast fungus. *Genetics* 176 (4), 2541-2549.

Lu, B.-R., and Snow, A. A. (2005). Gene flow from genetically modified rice and its environmental consequences. *BioScience* 55 (8), 669-678.

Maleszka, R., and Clark-Walker, G. D. (1993). Yeasts have a four-fold variation in ribosomal DNA copy number. *Yeast* 9 (1), 53-58.

MARDI (2009). MR 219, a new high-yielding rice variety with yields of more than 10 mt/ha. FFTC: An international information center for smallscale farmers in the Asian and Pacific region, Taipei.

MARDI (2013). Proceeding Book of Persidangan Padi. *Sunway Convention Centre Pulau*. Pinang.

Marsh, E., Alvarez, S., Hicks, L. M., Barbazuk, W. B., Qiu, W., Kovacs, L., and Schachtman, D. (2010). Changes in protein abundance during powdery mildew infection of leaf tissues of Cabernet Sauvignon grapevine (Vitis vinifera L.). *Proteomics* 10 (10), 2057-2064.

May, G. D., Afza, R., Mason, H. S., Wiecko, A., Novak, F. J., and Arntzen, C. J. (1995). Generation of transgenic banana (*Musa acuminata*) plants via *Agrobacterium*-mediated transformation. *Nat Biotechnol* 13 (5), 486-492.

Mentlak, T. A., Kombrink, A., Shinya, T., Ryder, L. S., Otomo, I., Saitoh, H., Terauchi, R., Nishizawa, Y., Shibuya, N., and Thomma, B. P. H. J.

(2012). Effector-mediated suppression of chitin-triggered immunity by *Magnaporthe oryzae* is necessary for rice blast disease. *The Plant Cell Online* 24 (1), 322-335.

Miah, G., Rafii, M. Y., Ismail, M. R., Puteh, A. B., Rahim, H. A., Asfaliza, R., and Latif, M. A. (2013). Blast resistance in rice: a review of conventional breeding to molecular approaches. *Molecular Biology Reports* 40 (3), 2369-2388.

Miki, S., Matsui, K., Kito, H., Otsuka, K., Ashizawa, T., Yasuda, N., Fukiya, S., Sato, J., Hirayae, K., and Fujita, Y. (2009). Molecular cloning and characterization of the AVR-Pia locus from a Japanese field isolate of *Magnaporthe oryzae*. *Molecular Plant Pathology* 10 (3), 361-374.

Monaghan, J., and Zipfel, C. (2012). Plant pattern recognition receptor complexes at the plasma membrane. *Current Opinion in Plant Biology* 15 (4), 349-357.

Mosquera, G., Giraldo, M. C., Khang, C. H., Coughlan, S., and Valent, B. (2009). Interaction transcriptome analysis identifies *Magnaporthe oryzae* BAS1-4 as biotrophy-associated secreted proteins in rice blast disease. *The Plant Cell Online* 21 (4), 1273-1290.

Nakamura, H., Hakata, M., Amano, K., Miyao, A., Toki, N., Kajikawa, M., Pang, J., Higashi, N., Ando, S., and Toki, S. (2007). A genome-wide gain-of-function analysis of rice genes using the FOX-hunting system. *Plant Molecular Biology* 65 (4), 357-371.

Nakashima, A., Chen, L., Thao, N. P., Fujiwara, M., Wong, H. L., Kuwano, M., Umemura, K., Shirasu, K., Kawasaki, T., and Shimamoto, K. (2008). RACK1 functions in rice innate immunity by interacting with the Rac1 immune complex. *The Plant Cell Online* 20 (8), 2265-2279.

Nakashima, K., Tran, L.-S. P., Van Nguyen, D., Fujita, M., Maruyama, K., Todaka, D., Ito, Y., Hayashi, N., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2007). Functional analysis of a NAC-type transcription factor *OsNAC6* involved in abiotic and biotic stress-responsive gene expression in rice. *The Plant Journal* 51 (4), 617-630.

Narusaka, Y., Narusaka, M., Yamasaki, S., and Iwabuchi, M. (2012). Methods to Transfer Foreign Genes to Plants.

Nicaise, V., Roux, M., and Zipfel, C. (2009). Recent advances in PAMP-triggered immunity against bacteria: pattern recognition receptors watch over and raise the alarm. *Plant Physiology* 150 (4), 1638-1647.

Nishizawa, Y., Nishio, Z., Nakazono, K., Soma, M., Nakajima, E., Ugaki, M., and Hibi, T. (1999). Enhanced resistance to blast (*Magnaporthe grisea*) in transgenic Japonica rice by constitutive expression of rice chitinase. *Theoretical and Applied Genetics* 99 (3-4), 383-390.

Nurnberger, T., and Kemmerling, B. (2009). Pathogen-associated molecular patterns (PAMP) and PAMP-triggered immunity. *Annual Plant Reviews* 34, 16-47.

Nuruzzaman, M., Manimekalai, R., Sharoni, A. M., Satoh, K., Kondoh, H., Ooka, H., and Kikuchi, S. (2010). Genome-wide analysis of NAC transcription factor family in rice. *Gene* 465 (1), 30-44.

O'Garra, A., and Vieira, P. (1992). Polymerase chain reaction for detection of cytokine gene expression. *Current Opinion in Immunology* 4 (2), 211-215.

Oka, H. I. (2012). *Origin of Cultivated Rice*. Elsevier, Tokyo.

Okuyama, Y., Kanzaki, H., Abe, A., Yoshida, K., Tamiru, M., Saitoh, H., Fujibe, T., Matsumura, H., Shenton, M., and Galam, D. C. (2011). A multifaceted genomics approach allows the isolation of the rice Pia-blast resistance gene consisting of two adjacent NBS-LRR protein genes. *The Plant Journal* 66 (3), 467-479.

Olsen, A. N., Ernst, H. A., Leggio, L. L., and Skriver, K. (2005). NAC transcription factors: structurally distinct, functionally diverse. *Trends in Plant Science* 10 (2), 79-87.

Orbach, M. J., Farrall, L., Sweigard, J. A., Chumley, F. G., and Valent, B. (2000). A telomeric avirulence gene determines efficacy for the rice blast resistance gene Pi-ta. *The Plant Cell Online* 12 (11), 2019-2032.

Ou, S. H. (1985). *Rice Diseases*. IRRI.

Ow, D. W. (2007). GM maize from site-specific recombination technology, what next? *Current Opinion in Biotechnology* 18 (2), 115-120.

Panahi, M., Alli, Z., Cheng, X., Belbaraka, L., Belgoudi, J., Sardana, R., Phipps, J., and Altosaar, I. (2004). Recombinant protein expression plasmids optimized for industrial E. coli fermentation and plant systems produce biologically active human insulin-like growth factor-1 in transgenic rice and tobacco plants. *Transgenic Research* 13 (3), 245-259.

Park, C.-H., Chen, S., Shirsekar, G., Zhou, B., Khang, C. H., Songkumarn, P., Afzal, A. J., Ning, Y., Wang, R., and Bellizzi, M. (2012). The *Magnaporthe oryzae* effector Avr *Piz-t* targets the RING E3 Ubiquitin Ligase APIP6 to suppress pathogen-associated molecular pattern-triggered immunity in rice. *The Plant Cell Online* 24 (11), 4748-4762.

Parker, D., Beckmann, M., Enot, D. P., Overy, D. P., Rios, Z. C., Gilbert, M., Talbot, N., and Draper, J. (2008). Rice blast infection of Brachypodium distachyon as a model system to study dynamic host/pathogen interactions. *Nature Protocols* 3 (3), 435-445.

Peccoud, J., and Jacob, C. (1998). *Gene Quantification*. Springer, pp. 111-128.

Peng, X.-x., Tang, X.-k., Zhou, P.-l., Hu, Y.-j., Deng, X.-b., He, Y., and Wang, H.-h. (2011). Isolation and expression patterns of rice *WRKY82* transcription factor gene responsive to both biotic and abiotic stresses. *Agricultural Sciences in China* 10 (6), 893-901.

Perfect, S. E., and Green, J. R. (2001). Infection structures of biotrophic and hemibiotrophic fungal plant pathogens. *Molecular Plant Pathology* 2 (2), 101-108.

Peyyala, R., and Farman, M. L. (2006). Magnaporthe oryzae isolates causing gray leaf spot of perennial ryegrass possess a functional copy of the AVR1-CO39 avirulence gene. *Molecular Plant Pathology* 7 (3), 157-165.

Pineda, M., Sajnani, C., and Baron, M. (2010). Changes induced by the Pepper mild mottle tobamovirus on the chloroplast proteome of *Nicotiana benthamiana*. *Photosynthesis Research* 103 (1), 31-45.

Qi, M., and Yang, Y. (2002). Quantification of *Magnaporthe grisea* during infection of rice plants using real-time polymerase chain reaction and northern blot/phosphoimaging analyses. *Phytopathology* 92 (8), 870-876.

Qian, Q., Huang, L., Yi, R., Wang, S., and Ding, Y. (2014). Enhanced resistance to blast fungus in rice (*Oryza sativa* L.) by expressing the ribosome-inactivating protein alpha-momorcharin. *Plant Science* 217, 1-7.

Qiu, D., Xiao, J., Xie, W., Liu, H., Li, X., Xiong, L., and Wang, S. (2008). Rice gene network inferred from expression profiling of plants overexpressing *OsWRKY13*, a positive regulator of disease resistance. *Molecular Plant* 1 (3), 538-551.

Qiu, Y., and Yu, D. (2009). Over-expression of the stress-induced *OsWRKY45* enhances disease resistance and drought tolerance in *Arabidopsis*. *Environmental and Experimental Botany* 65 (1), 35-47.

Qu, S., Liu, G., Zhou, B., Bellizzi, M., Zeng, L., Dai, L., Han, B., and Wang, G.-L. (2006). The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site-leucine-rich repeat protein and is a member of a multigene family in rice. *Genetics* 172 (3), 1901-1914.

Ramamoorthy, R., Jiang, S.-Y., Kumar, N., Venkatesh, P. N., and Ramachandran, S. (2008). A comprehensive transcriptional profiling of the WRKY gene family in rice under various abiotic and phytohormone treatments. *Plant and Cell Physiology* 49 (6), 865-879.

Raman, R., Chahal, G., and Dhaliwal, H. (1994). Screening of genotype for callus induction and plant regeneration in rice. *Crop Improvment* 21, 1-2.

Ramkumar, G., Biswal, A. K., Mohan, K. M., Sakthivel, K., Sivaranjani, A. K. P., Neeraja, C. N., Ram, T., Balachandran, S. M., Sundaram, R. M., and Prasad, M. S. (2010). Identifying novel alleles of rice blast resistance genes *Pikh* and *Pita* through allele mining. *Plant breeding*.

Repellin, A., Baga, M., Jauhar, P. P., and Chibbar, R. N. (2001). Genetic enrichment of cereal crops via alien gene transfer: new challenges. *Plant Cell, Tissue and Organ Culture* 64 (2-3), 159-183.

Ribot, C., Cesari, S., Abidi, I., Chalvon, V., Bournaud, C., Vallet, J., Lebrun, M. H., Morel, J. B., and Kroj, T. (2013). The *Magnaporthe oryzae* effector AVR1–CO39 is translocated into rice cells independently of a fungal-derived machinery. *The Plant Journal* 74 (1), 1–12.

Rice, I. N. f. G. E. o. (1996). *Standard Evaluation System for Rice*. IRRI, International Rice Research Institute.

Rubio-Pina, J. A., and Zapata-Perez, O. (2011). Isolation of total RNA from tissues rich in polyphenols and polysaccharides of mangrove plants. *Electronic Journal of Biotechnology* 14 (5), 11-11.

Sahebi, M., Hanafi, M. M., Siti Nor, A. A., Nejat, N., Rafii, M. Y., and Azizi, P. (2013). Extraction of total RNA from mangrove plants to identify different genes involved in its adaptability to the variety of stresses. *Pakistan Journal of Agricultural Sciences* 50 (4), 529-536.

Sahoo, K. K., Tripathi, A. K., Pareek, A., Sopory, S. K., and Singla-Pareek, S. L. (2011). An improved protocol for efficient transformation and regeneration of diverse indica rice cultivars. *Plant Methods* 7 (1), 49.

Saitoh, H., Fujisawa, S., Mitsuoka, C., Ito, A., Hirabuchi, A., Ikeda, K., Irieda, H., Yoshino, K., Yoshida, K., and Matsumura, H. (2012). Large-scale gene disruption in *Magnaporthe oryzae* identifies *MC69*, a secreted protein required for infection by monocot and dicot fungal pathogens. *PLoS Pathogens* 8 (5), e1002711.

Sandhu, G. S., Kline, B. C., Stockman, L., and Roberts, G. D. (1995). Molecular probes for diagnosis of fungal infections. *Journal of Clinical Microbiology* 33 (11), 2913-2919.

Schoonbeek, H.-j., Wang, H.-H., Stefanato, F. L., Craze, M., Bowden, S., Wallington, E., Zipfel, C., and Ridout, C. J. (2015). *Arabidopsis EF-Tu* receptor enhances bacterial disease resistance in transgenic wheat. *New Phytologist* 206 (2), 606-613.

Seo, Y.-S., Chern, M., Bartley, L. E., Han, M., Jung, K.-H., Lee, I., Walia, H., Richter, T., Xu, X., and Cao, P. (2011). Towards establishment of a rice stress response interactome. *PLoS Genetics* 7 (4), e1002020.

Sesma, A., and Osbourn, A. E. (2004). The rice leaf blast pathogen undergoes developmental processes typical of root-infecting fungi. *Nature* 431 (7008), 582-586.

Shang, J., Tao, Y., Chen, X., Zou, Y., Lei, C., Wang, J., Li, X., Zhao, X., Zhang, M., and Lu, Z. (2009). Identification of a new rice blast resistance gene, *Pid3*, by genomewide comparison of paired nucleotide-binding siteâ€“leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes. *Genetics* 182 (4), 1303-1311.

Sharma, T., Madhav, M., Singh, B., Shanker, P., Jana, T., Dalal, V., Pandit, A., Singh, A., Gaikwad, K., and Upreti, H. (2005). High-resolution mapping, cloning and molecular characterization of the *Pi-k h* gene of rice, which confers resistance to *Magnaporthe grisea*. *Molecular Genetics and Genomics* 274 (6), 569-578.

Sharma, T. R., Rai, A. K., Gupta, S. K., and Singh, N. K. (2011). Broad-spectrum Blast Resistance Gene *Pi-k h* Cloned from Rice Line Tetep Designated as Pi54. *Journal of Plant Biochemistry and Biotechnology* 19 (1), 87-89.

Sharma, T. R., Rai, A. K., Gupta, S. K., Vijayan, J., Devanna, B. N., and Ray, S. (2012). Rice blast management through host-plant resistance: retrospect and prospects. *Agricultural Research* 1 (1), 37-52.

Sharma, T. R., Shanker, P., Singh, B. K., Jana, T. K., Madhav, M. S., Gaikwad, K., Singh, N. K., Plaha, P., and Rathour, R. (2005). Molecular mapping of rice blast resistance gene *Pi-k h* in the rice variety Tetep. *Journal of Plant Biochemistry and Biotechnology* 14 (2), 127-133.

Shavindra, J., and Amitabh, M. (2005). Advanced in rice biotechnology in the new millennium. *Plant Biotechnology Journal* 3, 275-307.

Shibuya, N., and Minami, E. (2001). Oligosaccharide signalling for defence responses in plant. *Physiological and Molecular Plant Pathology* 59 (5), 223-233.

Shimada, T. L., Shimada, T., and Hara-Nishimura, I. (2010). A rapid and non-destructive screenable marker, FAST, for identifying transformed seeds of *Arabidopsis thaliana*. *The Plant Journal* 61 (3), 519-528.

Shimizu, T., Nakano, T., Takamizawa, D., Desaki, Y., Ishiiâ€ • Minami, N., Nishizawa, Y., Minami, E., Okada, K., Yamane, H., and Kaku, H. (2010). Two LysM receptor molecules, *CEBiP* and *OsCERK1*, cooperatively regulate chitin elicitor signaling in rice. *The Plant Journal* 64 (2), 204-214.

Shimono, M., Koga, H., Akagi, A. Y. A., Hayashi, N., Goto, S., Sawada, M., Kurihara, T., Matsushita, A., Sugano, S., and Jiang, C. J. (2012). Rice *WRKY45* plays important roles in fungal and bacterial disease resistance. *Molecular Plant Pathology* 13 (1), 83-94.

Shimono, M., Sugano, S., Nakayama, A., Jiang, C.-J., Ono, K., Toki, S., and Takatsuji, H. (2007). Rice *WRKY45* plays a crucial role in benzothiadiazole-inducible blast resistance. *The Plant Cell Online* 19 (6), 2064-2076.

Shiu, S.-H., and Bleecker, A. B. (2001). Receptor-like kinases from *Arabidopsis* form a monophyletic gene family related to animal receptor kinases. *Proceedings of the national Academy of Sciences* 98 (19), 10763-10768.

Silva, J., Scheffler, B., Sanabria, Y., De Guzman, C., Galam, D., Farmer, A., Woodward, J., May, G., and Oard, J. (2012). Identification of candidate genes in rice for resistance to sheath blight disease by whole genome sequencing. *Theoretical and Applied Genetics* 124 (1), 63-74.

Simms, D., Cizdziel, P. E., and Chomczynski, P. (1993). TRIzol: A new reagent for optimal single-step isolation of RNA. *Focus* 15 (4), 532-535.

Skamnioti, P., and Gurr, S. J. (2009). Against the grain: safeguarding rice from rice blast disease. *Trends in Biotechnology* 27 (3), 141-150.

Smedegaard-Petersen, V., Wood, F. R. S., and Jellis, G. J. (1984). The role of respiration and energy generation in diseased and disease resistant plants. *Plant diseases: infection, damage and loss. Oxford, UK: Blackwell Science Ltd*.

Sridevi, G., Sabapathi, N., Meena, P., Nandakumar, R., Samiyappan, R., Muthukrishnan, S., and Veluthambi, K. (2003). Transgenic indica rice variety Pusa Basmati 1 constitutively expressing a rice chitinase gene exhibits enhanced resistance to Rhizoctonia solani. *Journal of Plant Biochemistry and Biotechnology* 12 (2), 93-101.

Sun, H.-J., Uchii, S., Watanabe, S., and Ezura, H. (2006). A highly efficient transformation protocol for Micro-Tom, a model cultivar for tomato functional genomics. *Plant Cell Physiol* 47 (3), 426-431.

Sun, L., Zhang, H., Li, D., Huang, L., Hong, Y., Ding, X. S., Nelson, R. S., Zhou, X., and Song, F. (2013). Functions of rice NAC transcriptional factors, *ONAC122* and *ONAC131*, in defense responses against Magnaporthe grisea. *Plant Molecular Biology* 81 (1-2), 41-56.

Swarbrick, P. J., Schulze-Lefert, P., and Scholes, J. D. (2006). Metabolic consequences of susceptibility and resistance (race-specific and broad-spectrum) in barley leaves challenged with powdery mildew. *Plant, Cell & Environment* 29 (6), 1061-1076.

Sweigard, J. A., Carroll, A. M., Kang, S., Farrall, L., Chumley, F. G., and Valent, B. (1995). Identification, cloning, and characterization of *PWL2*, a gene for host species specificity in the rice blast fungus. *The Plant Cell Online* 7 (8), 1221-1233.

Takahashi, A., Hayashi, N., Miyao, A., and Hirochika, H. (2010). Unique features of the rice blast resistance *Pish* locus revealed by large scale retrotransposon-tagging. *BMC Plant Biology* 10 (1), 175.

Talbot, N., Ebbole, D., and Hamer, J. (1993). Identification and characterisation of *MPG1*, a gene involved in pathogenicity from the rice blast fungus *Magnaporthe grisea*. *Plant Cell* 5, 1575–1590.

Talbot, N. J. (1995). Having a blast: exploring the pathogenicity of *Magnaporthe grisea*. *Trends in Microbiology* 3 (1), 9-16.

Talbot, N. J. (2003). On the trail of a cereal killer: exploring the biology of Magnaporthe grisea. *Annual Reviews in Microbiology* 57 (1), 177-202.

Talbot, N. J., Salch, Y. P., Ma, M., and Hamer, J. E. (1993). Karyotypic variation within clonal lineages of the rice blast fungus, Magnaporthe grisea. *Applied and Environmental Microbiology* 59 (2), 585-593.

Tameling, W. I. L., and Baulcombe, D. C. (2007). Physical association of the NB-LRR resistance protein Rx with a Ran GTPase-activating protein is required for extreme resistance to Potato virus X. *The Plant Cell Online* 19 (5), 1682-1694.

Tao, Z., Liu, H., Qiu, D., Zhou, Y., Li, X., Xu, C., and Wang, S. (2009). A pair of allelic *WRKY* genes play opposite roles in rice-bacteria interactions. *Plant Physiology* 151 (2), 936-948.

Temin, H. M. (1974). On the origin of RNA tumor viruses. *Annual Review of Genetics* 8 (1), 155-177.

Tena, G., Boudsocq, M., and Sheen, J. (2011). Protein kinase signaling networks in plant innate immunity. *Current Opinion in Plant Biology* 14 (5), 519-529.

Toki, S. (1997). Rapid and efficient Agrobacterium-mediated transformation in rice. *Plant Molecular Biology Reporter* 15 (1), 16-21.

Toyoda, S., and Suzuki, N. (1957). Histochemical studies on the lesions of rice blast caused by Pyricularia oryzae Cav. III. Changes in the respiration of infected tissues. *Annals of the Phytopathological Society of Japan* 22, 173-177.

Tzfira, T., Jensen, C. S., Wang, W., Zuker, A., Vinocur, B., Altman, A., and Vainstein, A. (1997). Transgenic Populus tremula: a step-by-step protocol for its Agrobacterium-mediated transformation. *Plant Molecular Biology Repors* 15 (3), 219-235.

Tzfira, T., Li, J., Lacroix, B. t., and Citovsky, V. (2004). *Agrobacterium* T-DNA integration: molecules and models. *Trends in Genetics* 20 (8), 375-383.

Uehara, T., Arase, S., Honda, Y., Park, P., and Nozu, M. (1997). Primary effects of *Magnaporthe grisea* Toxin (s) on mitochondria of rice [Oryza sativa] leaf cells. *Annals of the Phytopathological Society of Japan (Japan)*.

URI, Genomics and Sequencing Center of University of Rhode Island.

Valasek, M. A., and Repa, J. J. (2005). The power of real-time PCR. *Advances in Physiology Education* 29 (3), 151-159.

Valent, B., and Chumley, F. G. (1991). Molecular genetic analysis of the rice blast fungus, Magnaporthe grisea. *Annual Review of Phytopathology* 29 (1), 443-467.

Valent, B., and Khang, C. H. (2010). Recent advances in rice blast effector research. *Current Opinion in Plant Biology* 13 (4), 434-441.

Vasconcelos, M., Datta, K., Oliva, N., Khalekuzzaman, M., Torrizo, L., Krishnan, S., Oliveira, M., Goto, F., and Datta, S. K. (2003). Enhanced iron and zinc accumulation in transgenic rice with the *ferritin* gene. *Plant Science* 164 (3), 371-378.

Vega, R., Vasquez, N., Espinoza, A. M., Gatica, A. M., and Valdez-Melara, M. (2009). Histology of somatic embryogenesis in rice (*Oryza sativa* cv. 5272). *Revista de Biologia Tropical* 57, 141-150.

Veluthambi, K., Gupta, A. K., and Sharma, A. (2003). The current status of plant transformation technologies. *Current science* 84 (3), 368-380.

Veneault-Fourrey, C., Barooah, M., Egan, M., Wakley, G., and Talbot, N. J. (2006). Autophagic fungal cell death is necessary for infection by the rice blast fungus. *Science* 312 (5773), 580-583.

Vila, L., Quilis, J., Meynard, D., Breitler, J. C., Marfa, V., Murillo, I., Vassal, J. M., Messeguer, J., Guiderdoni, E., and San Segundo, B. (2005). Expression of the *maize proteinase inhibitor (mpi)* gene in rice plants enhances resistance against the striped stem borer (*Chilo suppressalis*): effects on larval growth and insect gut proteinases. *Plant Biotechnology Journal* 3 (2), 187-202.

Wang, H., Hao, J., Chen, X., Hao, Z., Wang, X., Lou, Y., Peng, Y., and Guo, Z. (2007). Overexpression of rice *WRKY89* enhances ultraviolet B tolerance and disease resistance in rice plants. *Plant Molecular Biology* 65 (6), 799-815.

Wang, Z. X., Yano, M., Yamanouchi, U., Iwamoto, M., Monna, L., Hayasaka, H., Katayose, Y., and Sasaki, T. (1999). The *Pib* gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. *The Plant Journal* 19 (1), 55-64.

Wang, Z. X., Yano, M., Yamanouchi, U., Iwamoto, M., Monna, L., Hayasaka, H., Katayose, Y., and Sasaki, T. (1999). The *Pib* gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. *The Plant Journal* 19 (1), 55-64.

Wen, N., Chu, Z., and Wang, S. (2003). Three types of defense-responsive genes are involved in resistance to bacterial blight and fungal blast diseases in rice. *Molecular Genetics and Genomics* 269 (3), 331-339.

Wirthmueller, L., Zhang, Y., Jones, J. D. G., and Parker, J. E. (2007). Nuclear Accumulation of the *Arabidopsis* Immune Receptor RPS4 Is Necessary for Triggering EDS1-Dependent Defense. *Current Biology* 17 (23), 2023-2029.

Witham, F. H., Blaydes, D. F., and Devlin, R. M. (1971). D Van Nostrand, New York, pp. 55-58.

Wu, S.-C., Halley, J. E., Luttig, C., Fernekes, L. M., Gutierrez-Sanchez, G., Darvill, A. G., and Albersheim, P. (2006). Identification of an endo-β-1, 4-D-xylanase from *Magnaporthe grisea* by gene knockout analysis, purification, and heterologous expression. *Applied and Environmental Microbiology* 72 (2), 986-993.

Xia, Q. P., Gao, H. B., and Li, J. R. (2011). Effects of γ-aminobutyric acid on the photosynthesis and chlorophyll fluorescence parameters of muskmelon seedlings under hypoxia stress. *Chinese Journal of Applied Ecology* 22 (4), 999-1006.

Xiang, X., Qiu, D., Hegele, R. D., and Tan, W. C. (2001). Comparison of different methods of total RNA extraction for viral detection in sputum. *Journal of Virological Methods* 94 (1), 129-135.

Xiao, J., Cheng, H., Li, X., Xiao, J., Xu, C., and Wang, S. (2013). Rice *WRKY13* regulates cross talk between abiotic and biotic stress signaling pathways by selective binding to different cis-elements. *Plant Physiology* 163 (4), 1868-1882.

Xiong, L., and Yang, Y. (2003). Disease resistance and abiotic stress tolerance in rice are inversely modulated by an abscisic acid-inducible mitogen-activated protein kinase. *The Plant Cell Online* 15 (3), 745-759.

Yang, H., Huang, Y., Zhi, H., and Yu, D. (2011). Proteomics-based analysis of novel genes involved in response toward soybean mosaic virus infection. *Molecular Biology Reports* 38 (1), 511-521.

Yarasi, B., Sadumpati, V., Immanni, C. P., Vudem, D. R., and Khareedu, V. R. (2008). Transgenic rice expressing Allium sativum leaf agglutinin (ASAL) exhibits high-level resistance against major sap-sucking pests. *BMC Plant Biology* 8 (1), 102.

Yi, J., Hu, Y., Zhang, L., Yang, G., and Tang, L. (2013). The Effect of Magnaporthe oryzae Conidia on Photosystem in Rice. *International Journal of Environmental Science & Development* 4 (5).

Yokotani, N., Sato, Y., Tanabe, S., Chujo, T., Shimizu, T., Okada, K., Yamane, H., Shimono, M., Sugano, S., and Takatsuji, H. (2013). WRKY76 is a rice transcriptional repressor playing opposite roles in blast disease resistance and cold stress tolerance. *Journal of Experimental Botany* 64 (16), 5085-5097.

Yoshida, K., Saitoh, H., Fujisawa, S., Kanzaki, H., Matsumura, H., Yoshida, K., Tosa, Y., Chuma, I., Takano, Y., and Win, J. (2009). Association genetics reveals three novel avirulence genes from the rice blast fungal pathogen Magnaporthe oryzae. *The Plant Cell Online* 21 (5), 1573-1591.

Yuan, B., Zhai, C., Wang, W., Zeng, X., Xu, X., Hu, H., Lin, F., Wang, L., and Pan, Q. (2011). The *Pik-p* resistance to Magnaporthe oryzae in rice is mediated by a pair of closely linked CC-NBS-LRR genes. *Theoretical and Applied Genetics* 122 (5), 1017-1028.

Yun, K.-Y., Park, M. R., Mohanty, B., Herath, V., Xu, F., Mauleon, R., Wijaya, E., Bajic, V. B., Bruskiewich, R., and de los Reyes, B. G. (2010). Transcriptional regulatory network triggered by oxidative signals configures the early response mechanisms of japonica rice to chilling stress. *BMC Plant Biology* 10 (1), 16.

Zeigler, R. S., Leong, S. A., and Teng, P. S. (1994). *Rice Blast Disease*. Int. Rice Res. Inst.

Zhai, C., Lin, F., Dong, Z., He, X., Yuan, B., Zeng, X., Wang, L., and Pan, Q. (2011). The isolation and characterization of *Pik*, a rice blast resistance gene which emerged after rice domestication. *New Phytologist* 189 (1), 321-334.

Zhang, J., Peng, Y., and Guo, Z. (2008). Constitutive expression of pathogen-inducible *OsWRKY31* enhances disease resistance and affects root growth and auxin response in transgenic rice plants. *Cell Research* 18 (4), 508-521.

Zhang, Q., Maroof, M. A. S., Lu, T. Y., and Shen, B. Z. (1992). Genetic diversity and differentiation of indica and japonica rice detected by RFLP analysis. *Theoretical and Applied Genetics* 83 (4), 495-499.

Zhang, S., and Xu, J.-R. (2014). Effectors and Effector Delivery in *Magnaporthe oryzae*. *PLoS Pathogens* 10 (1), e1003826.

Zheng, H., Lin, S., Zhang, Q., Lei, Y., and Zhang, Z. (2009). Functional analysis of 5-untranslated region of a TIR-NBS-encoding gene from triploid white poplar. *Molecular Genetics and Genomics* 282 (4), 381-394.

Zhong, R., Lee, C., and Ye, Z.-H. (2010). Global analysis of direct targets of secondary wall *NAC* master switches in *Arabidopsis*. *Molecular Plant* 3 (6), 1087-1103.

Zhou, B., Qu, S., Liu, G., Dolan, M., Sakai, H., Lu, G., Bellizzi, M., and Wang, G.-L. (2006). The eight amino-acid differences within three leucine-rich repeats between *Pi2* and *Piz-t* resistance proteins determine the resistance specificity to Magnaporthe grisea. *Molecular Plant-Microbe Interactions* 19 (11), 1216-1228.

Zipfel, C. (2008). Pattern-recognition receptors in plant innate immunity. *Current Opinion in Immunology* 20 (1), 10-16.

Zipfel, C. (2009). Early molecular events in PAMP-triggered immunity. *Current Opinion in Plant Biology* 12 (4), 414-420.

Zupan, J. R., and Zambryski, P. (1995). Transfer of T-DNA from *Agrobacterium* to the plant cell. *Plant Physiology* 107 (4), 1041.

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

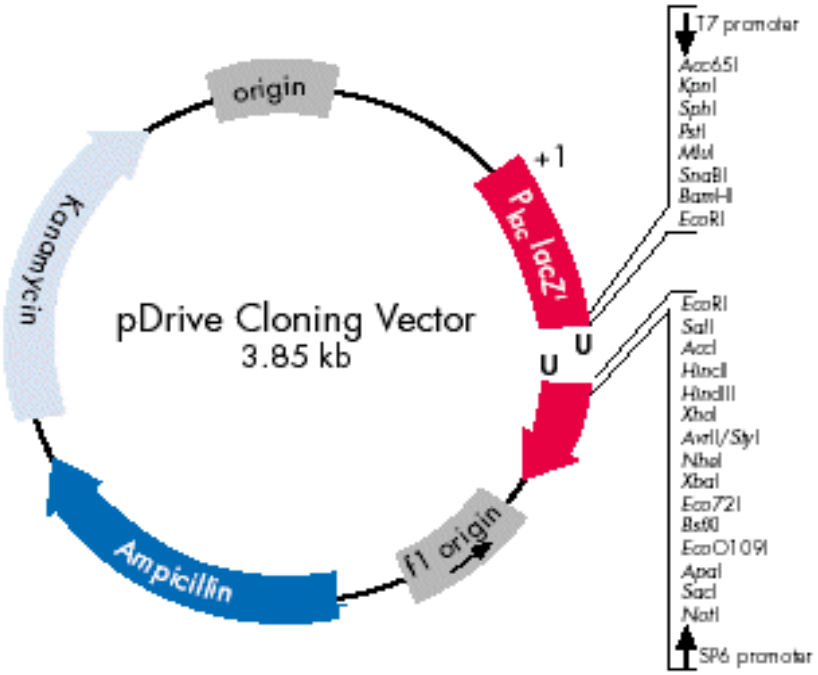


Figure B.1. Plasmid Map of pDrive cloning vector (Qiagen, Germany).

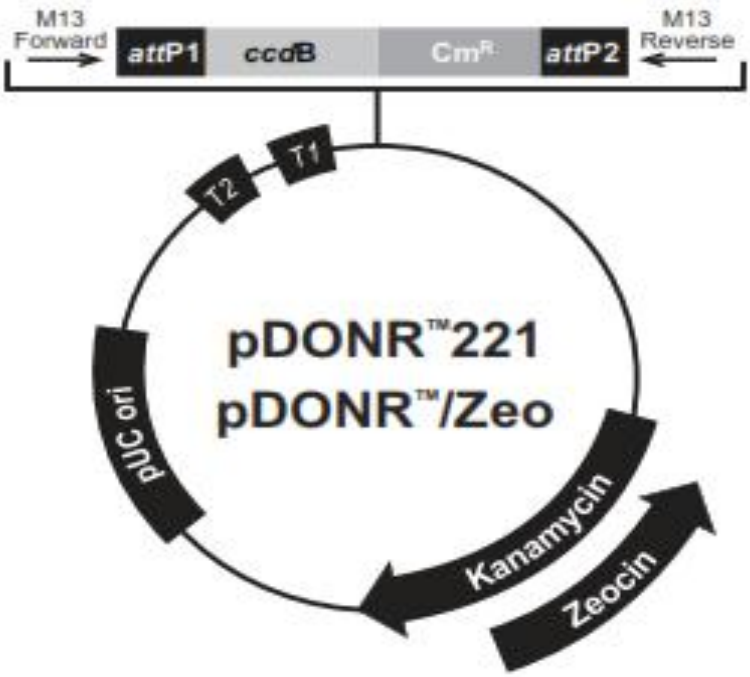


Figure B.2. Plasmid map of pDONOR/Zeo vector (Invitrogen, CA, USA).

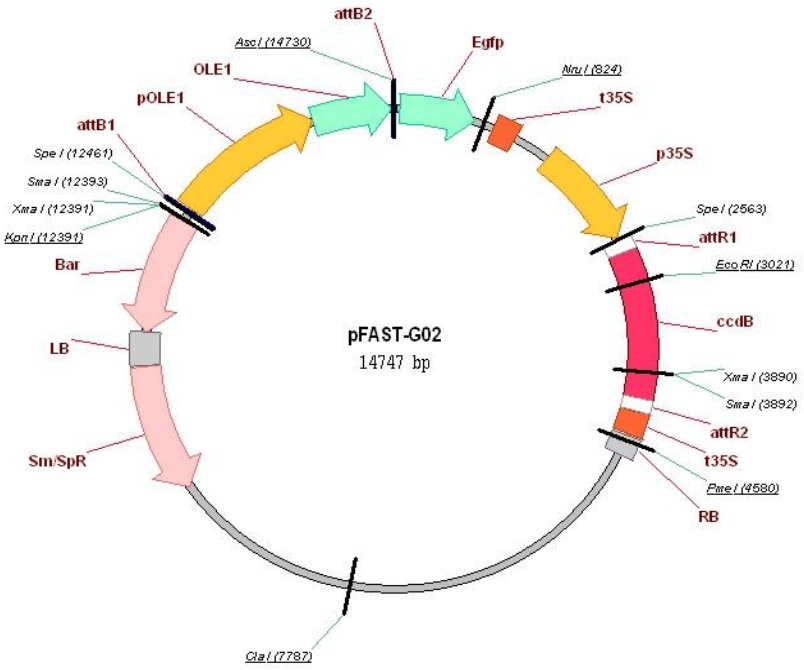
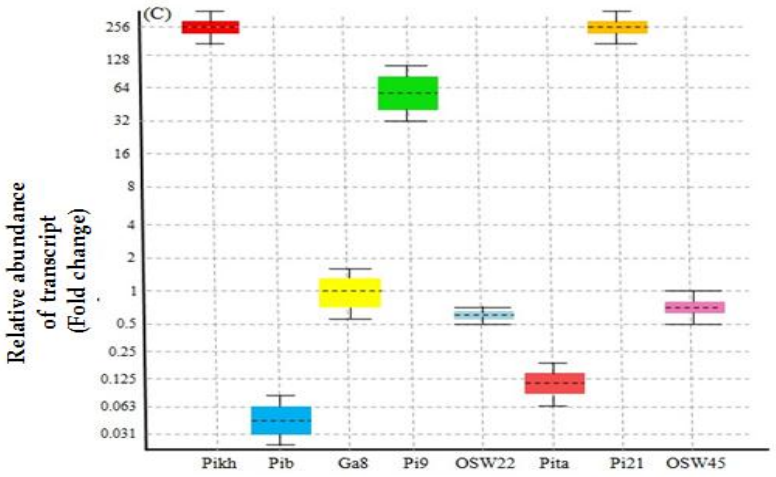


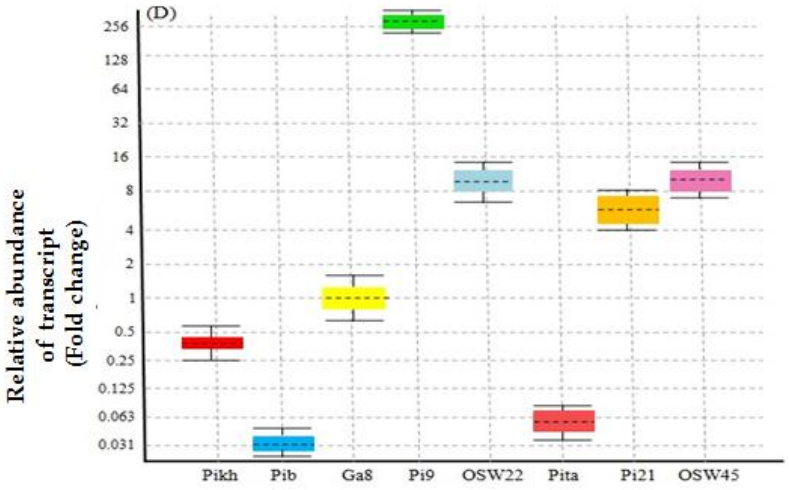
Figure B.3. Plasmid map of pFAST-G02 vector (Shimada *et al.*, 2010).

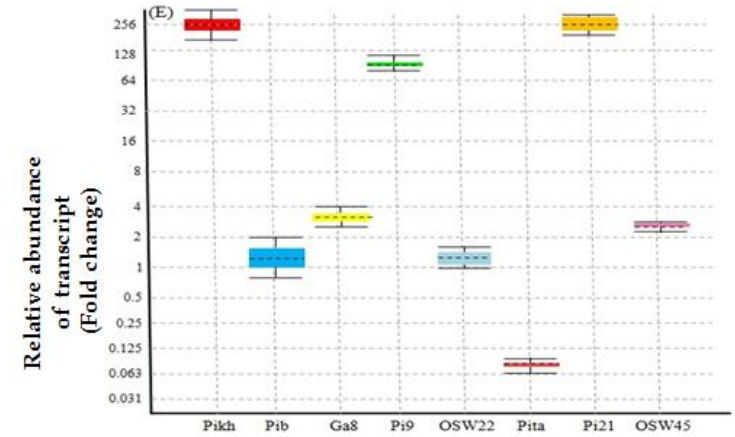
APPENDIX C

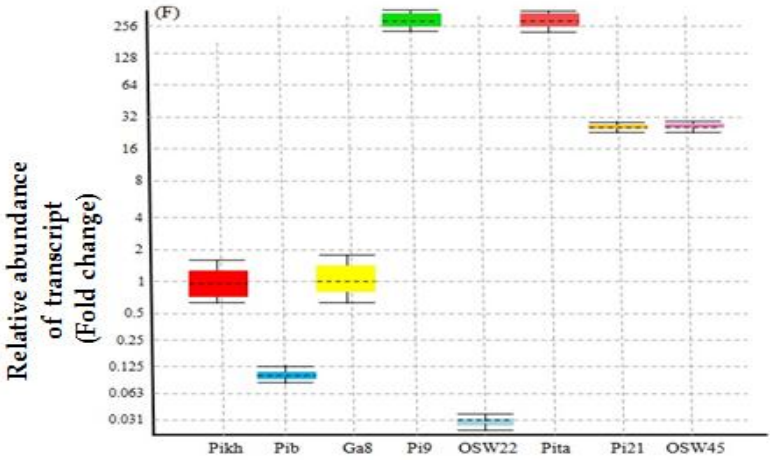
Figure C.1. Relative expression levels of *Pikh*, *Pib*, Os11gRGA8 *(GA8)*, *OsWRKY22 (Osw22)*, *Pita*, *Pi21*, and *OsWRKY45 (Osw45)* genes calibrated using *18sRNA/tubulin* reference genes in infected and control rice plants by relative quantitative real-time PCR. Expression levels of 8 genes in 2 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.

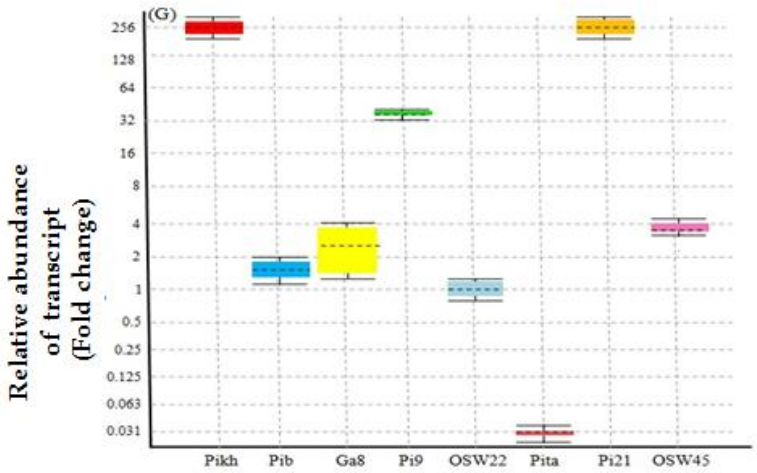


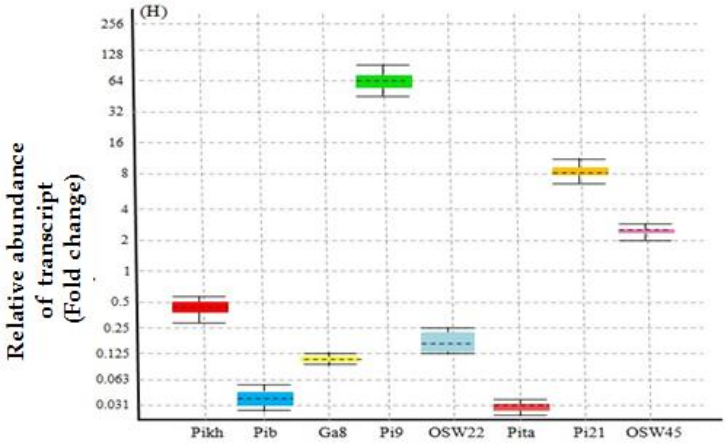
.

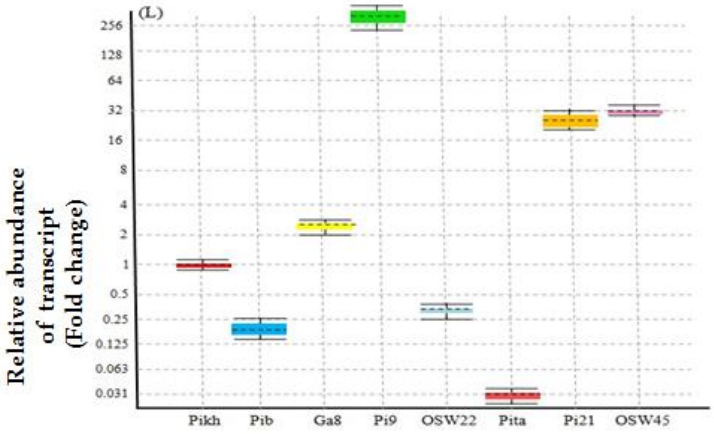


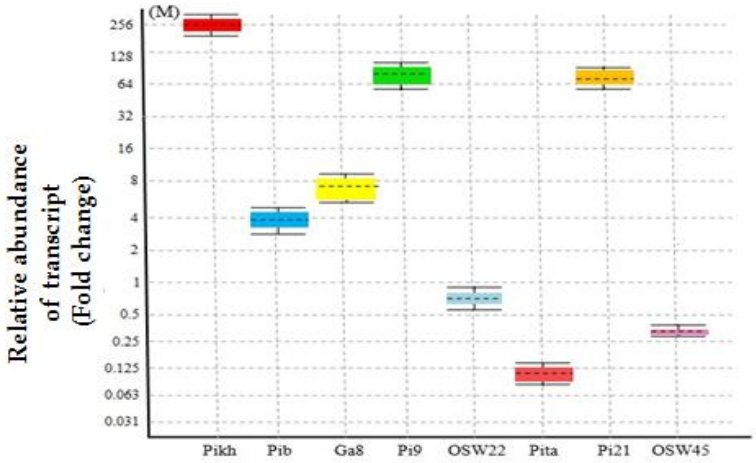


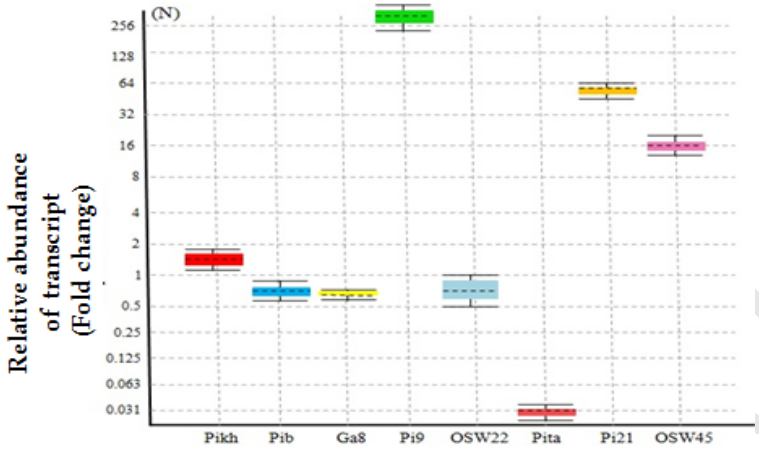


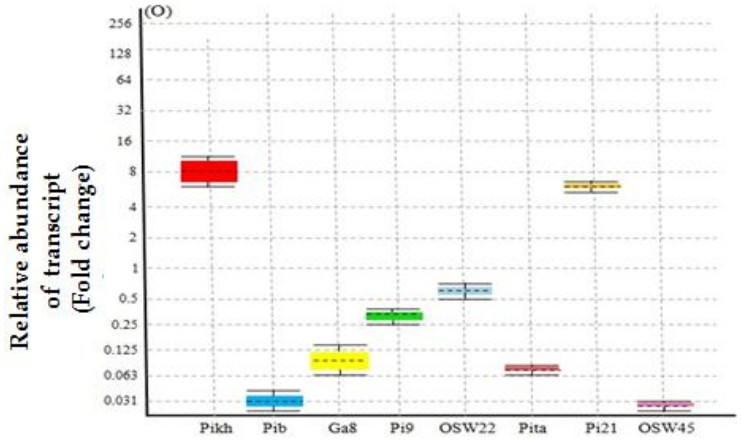


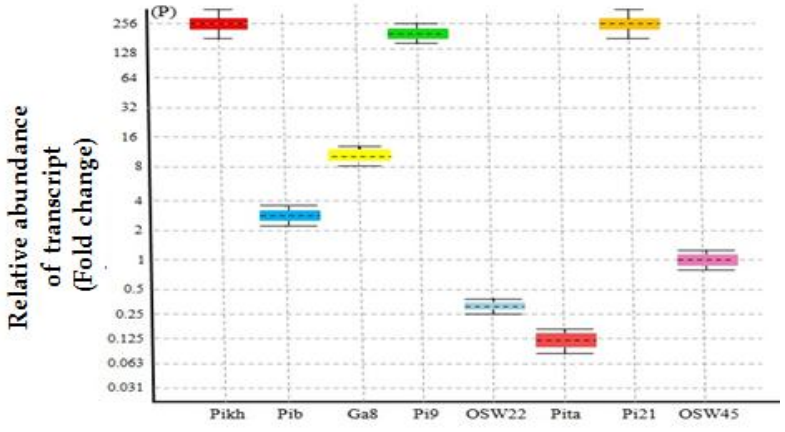

(H)
Relative abundance of transcript (Fold change)
256
128
64
32
16
8
4
2
1
0.5
0.25
0.125
0.063
0.031
Pikh
Pib
Ga8
Pi9
OSW22
Pita
Pi21
OSW45


(L)
Relative abundance of transcript (Fold change)
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


(M)
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


(N)
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


(O)
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


(P)
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

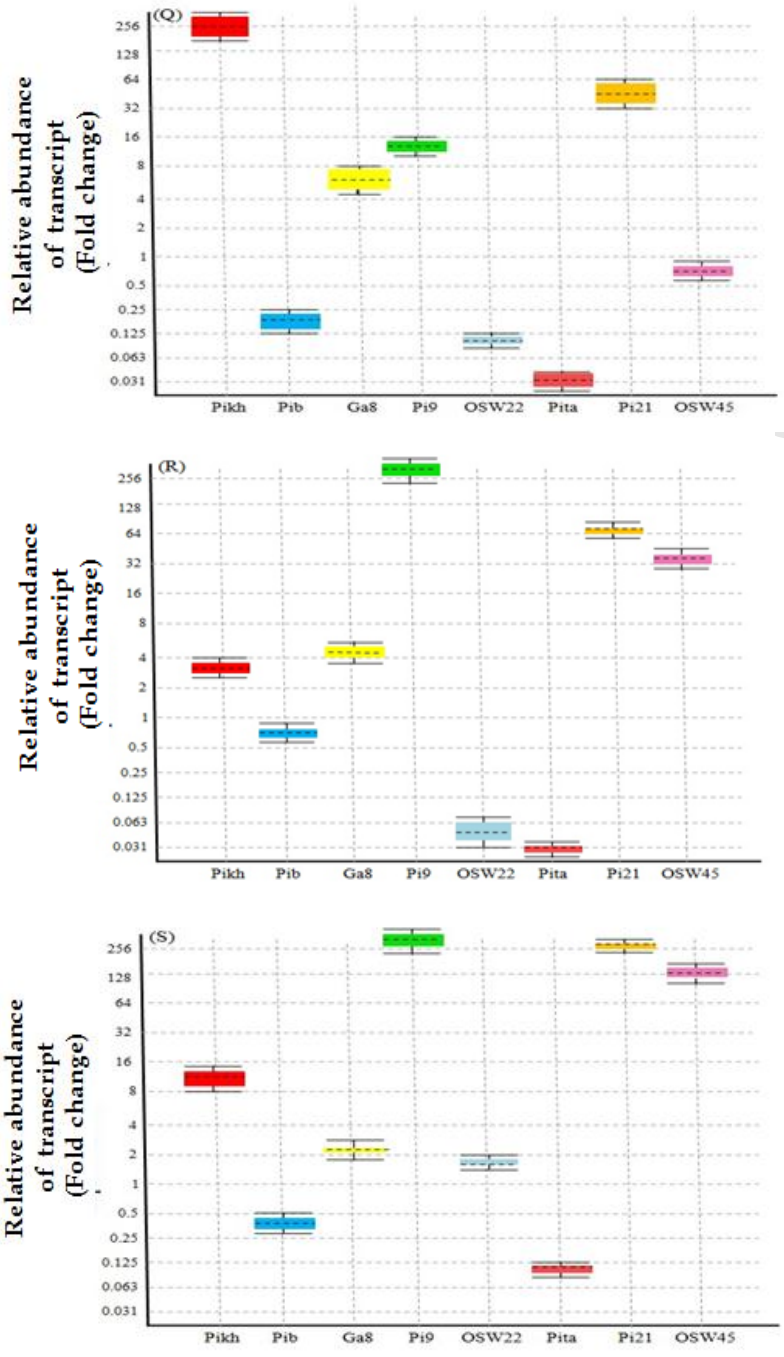


Figure C.2. Melting curve analysis of 10 genes (8 blast resistant genes and 2 reference genes).

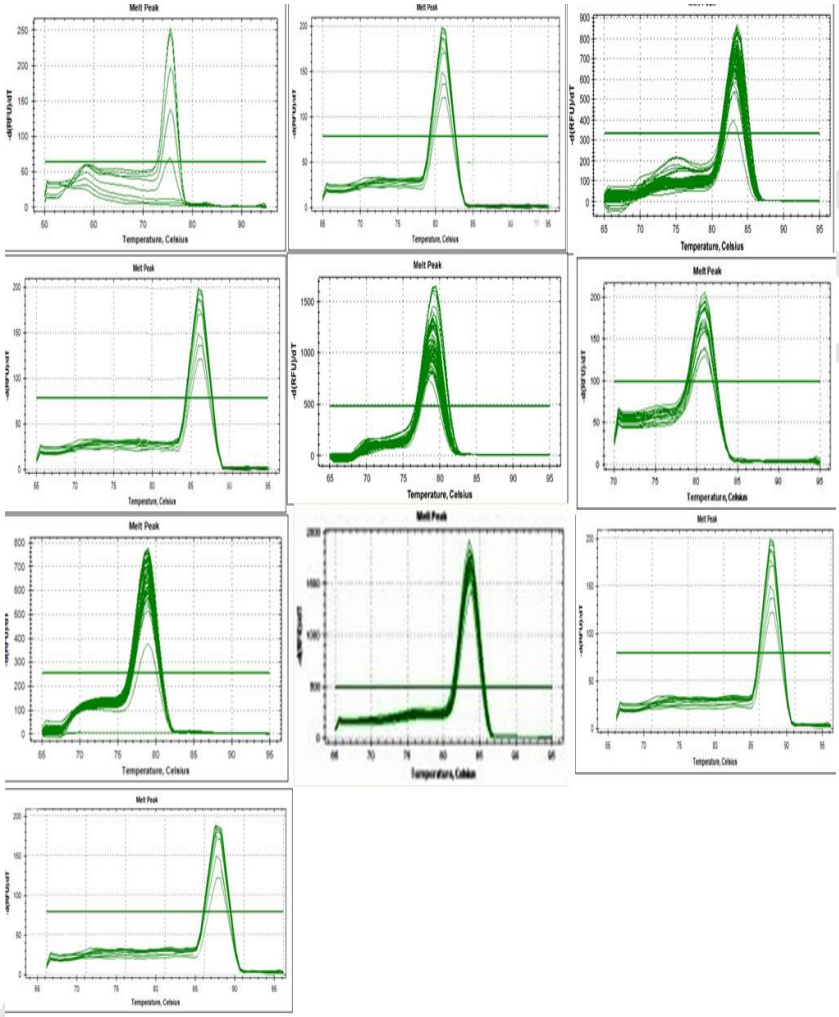


Table C.1. Relative expression patterns and *P*-value of 8 genes and *18SrRNA/tubulin* reference genes in sixteen rice varieties.

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

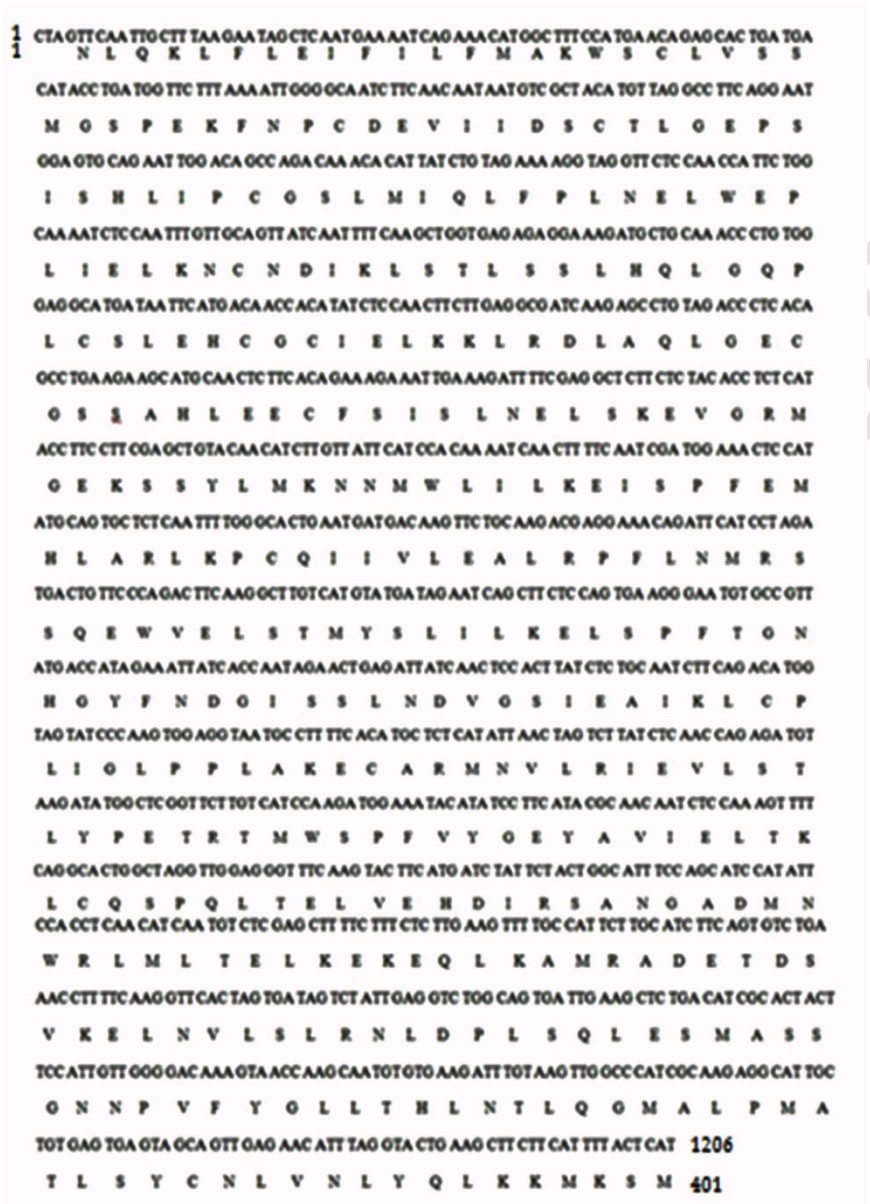


Figure C.3.The 1206 bp nucleotide and deduced amino sequence (401) of Nucleotide binding site leucine rich repeat protein gene (*Pikh*) (The translation of a nucleotide sequence to a protein sequence was done using ExPASY translate tool.

APPENDIX D

Figure D.1. Plant inoculation procedure.

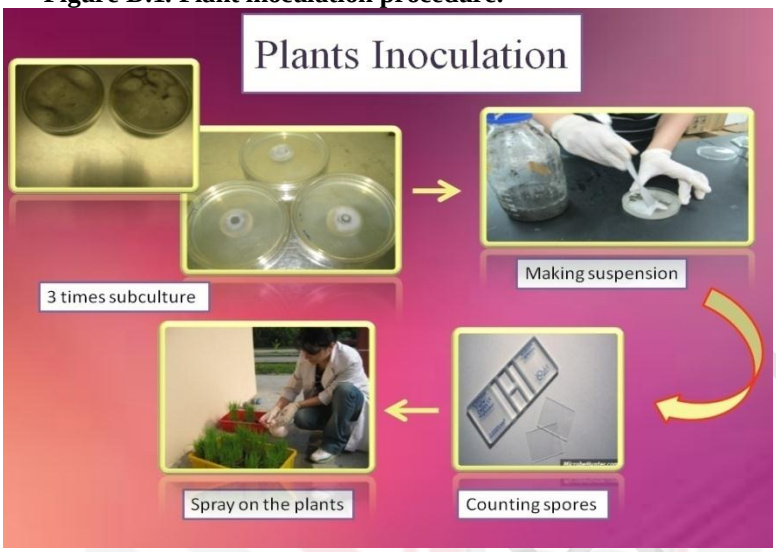
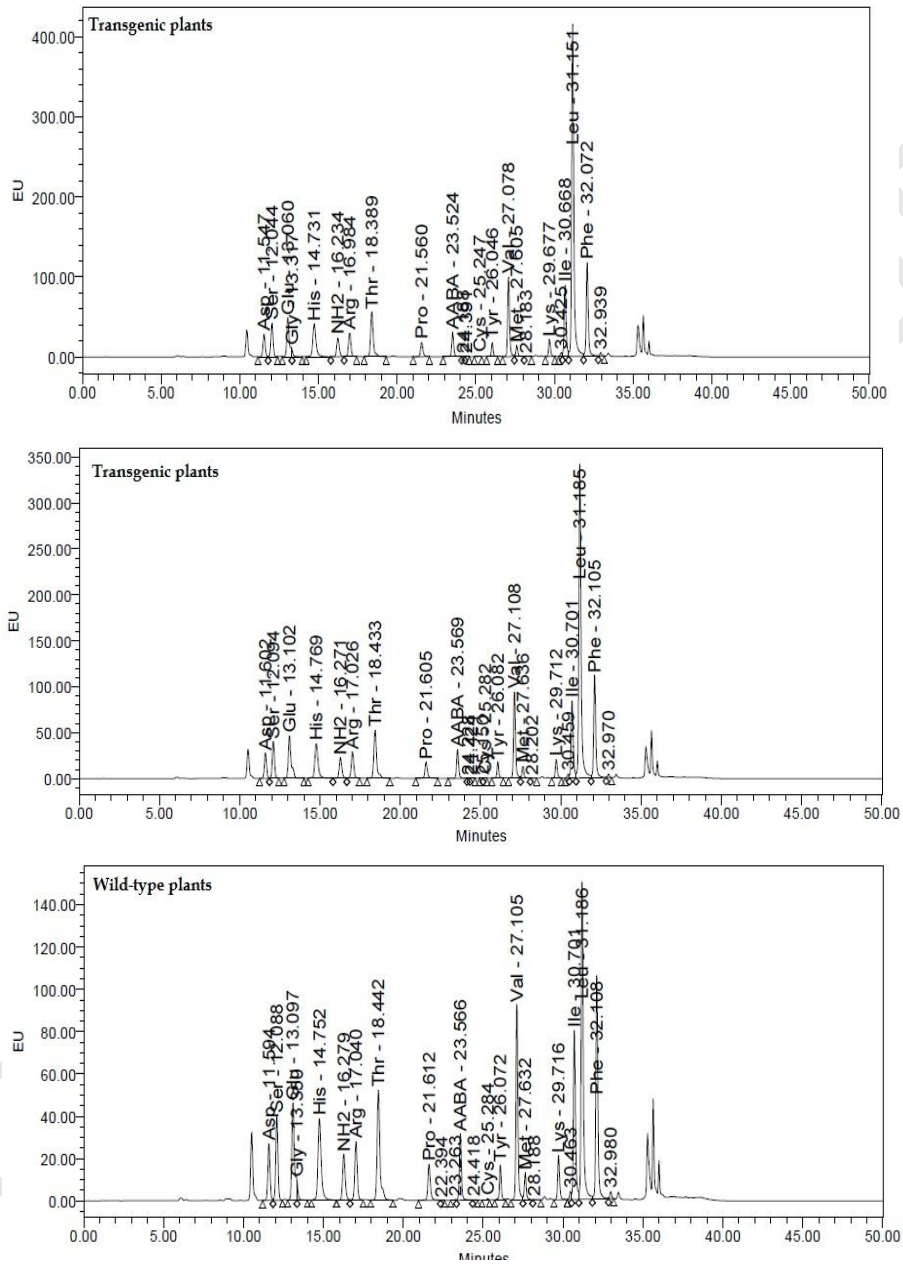


Figure D. 2. Symptomes observed on the leaves.

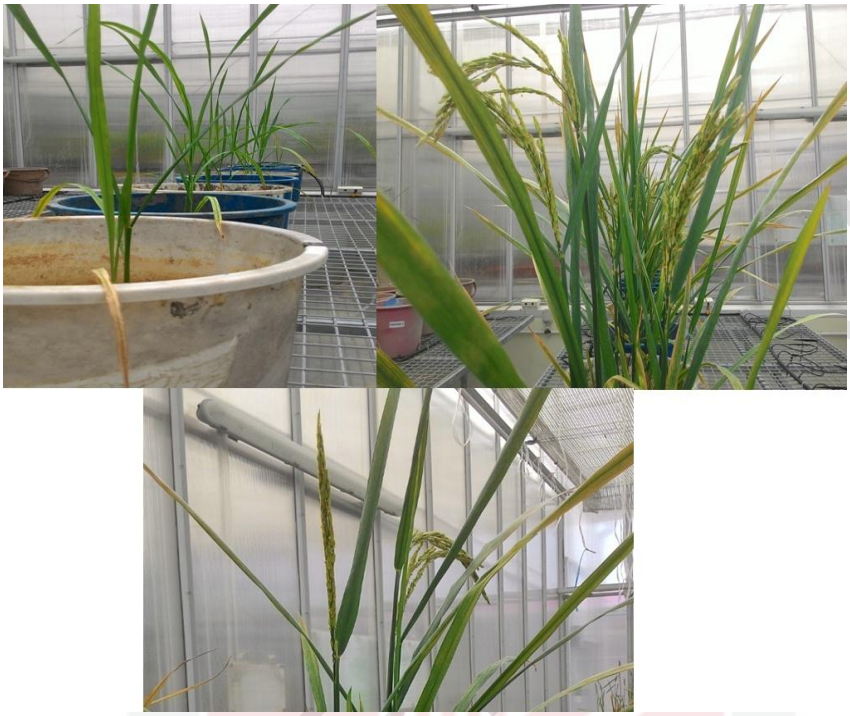
APPENDIX E

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.

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION : _____

TITLE OF THESIS / PROJECT REPORT :

_____

_____

_____

NAME OF STUDENT : _____

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as :

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official Secret Act 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by the organization/institution where research was done).
☐	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for :

☐ **PATENT** Embargo from _____ (date) until _____ (date)

Approved by:

_____
(Signature of Student)
New IC No/ Passport No.:

Date :

_____
(Signature of Chairman of Supervisory Committee)
Name:

Date :

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]