GENOTYPIC VARIATION AND ESTIMATION OF BLAST RESISTANCE GENES ON BACKCROSS INBRED LINES (US-2*2/MALAY 2) USING RICE BLAST DIFFERENTIAL SYSTEM

KARLA TRISHA FERNANDEZ ALEJANDRO

An undergraduate thesis manuscript submitted to the faculty of the Department of Crop Protection, College of Agriculture, Central Luzon State University
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An undergraduate thesis manuscript presented to the faculty of the Department of Crop Protection, College of Agriculture, in partial fulfillment of the requirements for the degree of Bachelor of Science in Agriculture major in Crop Protection

APPROVED:

RONALDO T. ALBERTO
Adviser
6-13-18
Date Signed

CELYNNE O. PADILLA
Critic
6-13-18
Date Signed

JONATHAN M. NIONES
Co-Adviser
6-13-18
Date Signed

RONALDO T. ALBERTO
Department Research Coordinator
6-13-18
Date Signed

MARITA S. LABE
Department Chairperson
13 June 2018
Date Signed

ACCEPTED:

ERNESTO A. MARTIN
Dean
6-13-18
Date Signed

RECORDED:

RONALDO T. ALBERTO
College Research Coordinator
6-13-18
Date Signed

BIOGRAPHICAL SKETCH

The author, Karla Trisha F. Alejandro was born on December 3, 1998 at their humble home in Barangay Mauban, Balungao, Pangasinan. She is the third among the four children of Engr. James P. Alejandro and Mrs. Marivic F. Alejandro.

She started her primary education at the Mauban Elementary School and graduated as the class Valedictorian in the year 2010. She then took her secondary education at Rosales National High School where she also graduated as the class Valedictorian in the year 2014.

She pursued her dreams and enrolled at the Central Luzon State University (CLSU) where she took Bachelor of Science in Agriculture major in Crop Protection specialized in Plant Pathology.

She received the Certificate of Agricultural Science embracing With Honors Award in the year 2016. And during her college years, she was an academic scholar for several semesters.

She is one of the scholars of the Department of Agriculture - Agriculture Competitiveness Enhancement Fund (DA-ACEF) for her whole college years (2014-2018) and also a Tulong Dunong – Schools, Universities and Colleges (TD-SUC) scholar for a year (2017-2018). She is a proud member of the Pest Management Society (PMS).

Finally, she obtained her degree Bachelor of Science in Agriculture in the year 2018.

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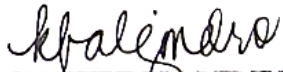

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GENOTYPIC VARIATION AND ESTIMATION OF BLAST RESISTANCE GENES ON BACKCROSS INBRED LINES (US-2*2/MALAY 2) USING RICE BLAST DIFFERENTIAL SYSTEM[1/]

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ABSTRACT

Resistance estimation of gene of the BC_1F_3 mapping population derived from US-2*2/Malay 2 crosses was conducted at PhilRice-CES blast nursery. Two types of evaluation were performed: leaf infection type evaluation conducted at 35 and 60 days after sowing (DAS) and percent severity evaluation conducted at 35 DAS. For the leaf infection type evaluation, 25% of the mapping population were resistant, 64.53% were susceptible. For percent severity evaluation, 6.98% were resistant, 20.93% were intermediate and 72.09% were susceptible. Comparing the disease reaction of US-2*2/Malay 2 mapping population with the reaction of blast differential varieties, five genes, namely: *Pik-m, Pib, Pish, Pi3, Pi19(t)* were estimated to be possibly carried by the population associated to blast resistance. The estimated R genes were validated by inoculating the 15 blast resistant lines with two differential isolates, M101-1-2-9-1 and JMB8401. These two blast isolates have shown differentiating ability against the estimated R genes. Following inoculation with differential isolates, *Pik-m, Pib, Pish* and *Pi3* genes were confirmed to be present in the BC_1F_3 mapping population of US-2*2/Malay 2.

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LITERATURE CITED

BABUJEE, L. and S. S. GNANAMANICKAM. 2000. Molecular Tools for Characterization of Rice Blast Pathogen (*Magnaporthe grisea*) Population and Molecular Marker-Assisted Breeding for Disease Resistance. Curr. Sci. 78 : 248–257

BENT, A. F. and D. MACKEY. 2007. Elicitors, Effectors, and R Genes: The New Paradigm and a Lifetime Supply of Questions. Annual Review of Phytopathology, 45: 399-436.

BIOLOGY DISCUSSION. 2016. Rice Blast: Meaning, Symptoms and Disease Predication | Plant Disease. Article Shared by Shivani, R. Retrieved on May 29, 2017

BLISS, F. A. 1981. Utilization of Vegetable Germplasm. Hort.Science. 16 : 129–132.

BUSINESS WORLD ONLINE. 2017. World Bank Sees Philippine Rice Output Rising 2%. Retrieved on May 29, 2017 from http://www.bworldonline.com/content.php?section=Economy&title=world-bank-sees-philippine-rice-output-rising-2&37&id=139671

BUTRUILLE, D. V., R. P. GURIES and T. C. OSBORN. 1999. Linkage Analysis of Molecular Markers and Quantitative Trait Loci in Populations of Inbred Backcross Lines of *Brassica napus* L. Genetics. 153 : 949–964.

CASTILLA, N., S. SAVARY, C. M. VERA CRUZ and H. LEUNG. 2010. Rice Blast. Retrieved on may 29, 2017 from Rice Knowledge Bank: http://www.knowledge bank.irri.org.

DELA PEÑA, F. A. and M. C. PASCUAL. 2013. Resistance of Popular Rice Varieties to *Magnaporthe grisea* H., The Rice Blast Fungus from Different Rice Growing Areas in the Philippines. Philippine Rice R and D Highlights 2013. 7-8.

DE WIT, P. J., R. MEHRABI, H. VAN DEN BURG and I. STERGIOPOULOS. 2009. Fungal Effector Proteins: Past, Present and Future. Molecular Plant Pathology, 10(6): 735-747.

FOOD AND AGRICULTURE ORGANIZATION (FAO) 2004. Rice is Life. Italy: FAO. http:// www.fao.org/newsroom/en/focus/200436887/ index.html.

FUKUTA, Y., I. KOGA, T. UNG, K. SATHYA, A. KAWASAKI-TANAKA, Y. KOIDE, N. KOBAYASHI, M. OBARA, H. YADANA and N. HAYASHI. 2014. Pathogenicity of Rice Blast (*Pyricularia oryzae* C.) Isolates from Cambodia. JARQ. 48 : 155 - 166

GALHANO, R. and N. J. TALBOT. 2011. The Biology of Blast: Understanding How *Magnaporthe oryzae* Invades Rice Plants. Fungal Biology Reviews, 25(1): 61-76.

GNANAMANICKAM, S. 2009. Biological Control of Rice Diseases (Vol. 8). Dordrecht, The Netherlands: Springer Science and Business Media, 53-65.

HARTMAN, J. B. and D. A. ST. CLAIR. 1999. Combining Ability for Beet Armyworm (*Spodoptera exigua*) Resistance and Horticultural Traits of Selected *Lycopersicon pennellii*-derived Inbred Backcross Lines of Tomato. Plant Breeding. 118 : 523–530.

HAYASHI, N. and Y. FUKUTA. 2009. Proposal for A New International System of Differentiating Races of Blast (*Pyricularia oryzae* C.) by Using LTH Monogenic Lines in Rice (*Oryza sativa* L.). Japan International Research Center for Agricultural Sciences. 63: 11-15.

HOGENHOUT, S. A., R. VAN DER HOORN, R. TERAUCHI and S. KAMOUN. 2009. Emerging Concepts in Effector Biology of Plant-Associated Organisms. Molecular Plant-Microbe Interactions, 22(2): 115-122.

HUANG, J., W. SI, Q. DENG, P. LI and S. YANG. 2014. Rapid Evolution of Avirulence Genes in Rice Blast Fungus *Magnaporthe oryzae*. BioMed Central Genetics. 15(1): 45.

JIA, Y., S. A. MCADAMS, G. T. BRYAN, H. T. HERSHEY and B. VALENT. 2000. Direct Interaction of Resistance Gene and Avirulence Gene Products. The European Molecular Biology Organization Journal, 19(15): 4004-4014.

KOBAYASHI, N., MJ. TELEBANCO-YANORIA, H. TSUNEMATSU, H. KATO, T. IMBE and Y. FUKUTA. 2007. Development of New Sets of International Standard Differential Varieties for Blast Resistance in Rice (*Oryza sativa* L.). JARQ. 41 (1) : 31 – 37

LANOISELE, V. 2009. Rice Blast Disease. Agriculture and Food. http://www.agric.wa. gov.au/rice/rice-blast-disease

LIN, S. Y., T. SASAKI and M. YANO. 1998. Mapping Quantitative Trait Loci Controlling Seed Dormancy and Heading Date in Rice, *Oryza sativa* L., Using Backcross Inbred Lines. Theoretical and Applied Genetics. 96 : 997–1003.

NALLEY, L., F. TSIBOE, A. D. MORAT, A. SHEW and G. THOMA. 2016. Economic and Environmental Impact of Rice Blast Pathogen (*Magnaporthe oryzae*) Alleviation in the United States. PLoS ONE 11.

NIONES, J. M., J. DOMINGO and N. DESAMERO. 2006. Rice Variety Development for Rainfed Lowland Drought-submergence and Waterlog-Prone Sub-Ecosystems in Mindanao. Philippine Rice RandD Highlights 2006. pp.1

NIONES, J. M., T. E. MANANGHAYA, L. M. PEREZ, J. T. NIONES, J. P. RILLON, M. L. PALMA and T. F. PADOLINA. 2015. Finding Durable and Novel Blast Resistance Genes Effective Against Philippine's *Magnaporthe grisea* Pathogen Population. Plant Breeding and Biotechnology Division. pp. 10-15

OU, S. H. (1980). Pathogen Variability and Host Resitance in Rice Blast Disease. Annual Review of Phytopathology. 18(1) : 167-187.

PHILIPPINE RICE RESEARCH INSTITUTE (PhilRice) (2003). National Cooperative Testing Manual for Rice. Guidelines and Policies.

PIERCY, K. 2000. Blast, Biotech and Big Business, Implications of Corporate Strategies on Rice Research in Asia. Retrieved on May 25, 2017 from http://www.grain.org/publications/reports/blast.htm.

RICEPEDIA. 2012. Philippines. IRRI Rice Almanac. Retrieved on May 29, 2017 from http://ricepedia.org/philippines.

RICEPLUS MAGAZINE. 2017. World Rice Production 2016/2017. Retrieved on May 29, 2017 from http://riceplusmagazine.blogspot.com/2017/04/13th-april2017-daily-global regional-and.html

ROBBINS, M. D., M. D. CASLER and J. E. STAUB. 2008. Pyramiding QTL for Multiple Lateral Branching in Cucumber using Inbred Backcross Lines. Molecular Breeding. 22 : 131–139.

SALEH, D., J. MILAZZO, H. ADREIT, E. FOURNIER and D. THARREAU. 2014. South-East Asia is the Center of Origin, Diversity and Dispersion of the Rice Blast Fungus, *Magnaporthe oryzae.* New Phytologist. 201(4) : 1440-1456

SALEH, D., J. MILAZZO, H. ANDREIT, D. THARREAU and E. FOURNIER. 2012. Asexual Reproduction Induces A Rapid and Permanent Loss of Sexual Reproduction Capacity in the Rice Fungal Pathogen *Magnaporthe oryzae:* Results of In-vitro Experimental Assays. BMC Evolutionary Biology. 12 : 48

SCHEUERMANN, K. K., J. V. RAIMONDI, R. MARSCHALEK, A. DE ANDRADE and E. WICKERT. 2012. *Magnaporthe oryzae* Genetic Diversity and Its Outcomes on the Search for Durable Resistance. The Molecular Basis of Plant Genetic Diversity. 374

STAR, VERSION 2.0.1 2014. Biometrics and Breeding Informatics, PBGB Division, International Rice Research Institute, Los Baños, Laguna.

SULLIVAN, J. G. and F. A. BLISS. 1983. Expression of Enhanced Seed Protein Content in Inbred Backcross Lines of Common Bean. Journal of American Society of Horticultural Science. 108 : 787–791.

TABELIN, E., J. NIONES, S. ABDULA, E. ZABATE and N. DESAMERO. 2005. Development and Adaptation of Upland Rice Varieties in Philippine Rice R and D Highlights. Philippine Rice Research Institute, Maligaya Science City of Muñoz 3119. Retrieved on June 26, 2017 from http://dbmp2.philrice.gov.ph/RandD/src/dynamic/article_year.php?co_author=JM%20Niones,%20SE%20Abdula,%20E%20Zabate,%20and%20NV%20Desameroandauthor=EB%20Tabelin%20(PhilRice%20CES)andkeyword=2005andstudy=Development%20and%20Adaptation%20of%20Upland%20Rice%20Varie.

TALUKDER, Z. I., D. THARREAU and A. H. PRICE. 2004. Quantitative Trait Loci Analysis Suggests that Partial Resistance to Rice Blast is Mostly Determined By Race-Specific Interactions. New Phytologist, 162(1): 197-209.

TEBEEST, D. O., C. GUERBER and M. DITMORE. 2007. Rice Blast. The Plant Health Instructor. Retrieved on May 17, 2017 from https://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/RiceBlast.aspx

TELEBANCO-YANORIA, M. J., T. IMBE, H. KATO, H. TSUNEMATSU, L. A. EBRON and C. M. VERA CRUZ. 2008. A Set of Differential Blast Isolates (*Magnaporthe grisea* H.) From the Philippines for Rice (*Oryza sativa* L.) Resistance. Japan Agricultural Research Quarterly. 42 : 23-34.

TELEBANCO-YANORIA, M. J., Y. KOIDE, Y. FUKUTA, T. IMBE, H. TSUNEMATSU and H. KATO. 2011. A Set of Near-Isogenic Lines of Indica-type Rice Variety CO39 as Differential Varieties for Blast Resistance. Molecular Breeding. 27 : 357-353.

TSUNEMATSU, H., MJ. YANORIA, L. EBRON, N. HAYASHI, I. ANDO, H. KATO, T. IMBE and G. KHUSH. 2000. Development of Monogenic Lines of Rice for Blast Resistance. Breeding Science. 50 : 229-234

VASUDEVAN, K., C. M. VERA CRUZ, W. GRUIESSEM and N. K. BHULLAR. 2014. Large Scale Germplasm Screening for Identification of Novel Rice Blast Resistance Sources. Frontiers in Plant Science, 5(505): 1-9.

WEHRHAHN, C. and R. W. ALLARD. 1965. Detection and Measurement of the Effects of Individual Genes Involved in the Inheritance of a Quantitative Character in Wheat. Genetics. 31: 109–119.

WEIHUAI, W., L. WANG, S. ZHANG, Z. LI, Y. ZHANG and F. LIN. 2014. Stepwise Arms Race Between *AvrPik* and *Pik* Alleles in the Rice Blast Pathosystem. Molecular Plant-Microbe Interactions, 27(8): 759-769.

WILSON, R. A. and N. J. TALBOT. 2009. Under Pressure: Investigating the Biology of Plant Infection by *Magnaporthe oryzae*. Nature Reviews Microbiology. 7 : 185-195

ZEIGLER, R. S. and F. J. CORREA. 2000. Applying *Magnaporthe grisea* Analyses for Durable Rice Blast Resistance. doi:10.1094/APSnetFeature-2000-0700A.