CENTRAL LUZON STATE UNIVERSITY

EVALUATION OF BACTERIAL BLIGHT RESISTANCE IN MARKER ASSISTED SELECTION (MAS) - BRED RICE LINES AGAINST PHILIPPINES ISOLATES OF *Xanthomonas oryzae* pv. *oryzae*

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APPROVAL SHEET

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"Commit your works to the Lord and your plans will be established."
-Proverbs 16:3

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ABSTRACT

AGUILAR, RONEL JR. T., Bachelor of Science in Biology, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, May 2016. **EVALUATION OF BACTERIAL BLIGHT RESISTANCE IN MARKER-ASSISTED SELECTION (MAS)-BRED RICE LINES AGAINST PHILIPINES ISOLATES OF *Xanthomonas oryzae* pv. *oryzae***

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Among rice diseases, bacterial blight (BB) was named as one of the most destructive causing yield losses ranging from 74-81% in severe condition (Srinivasan and Gnanamanickam, 2005). *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), the causative agent of BB is a gram-negative bacterial pathogen infecting the xylem of the rice, causing lesions and eventually leading to plant death (Liu et al., 2006). One of the major goals of rice improvement programs has been to develop rice cultivars with stable BB resistance. Through Marker-Assisted Selection (MAS), rice breeders in the Philippine Rice Research Institute (PhilRice) had generated several elite lines introgressed with 2-4 BB resistance genes in various combinations. In this study, thirteen promising MAS-bred rice lines were evaluated for reactions to BB using three most virulent *Xoo* races (*Pxo-99, Pxo-79* and *Pxo-347*) under controlled conditions. The absorbance at 600 nanometer ($Å_{600}$) of each inoculum suspension used was adjusted to 2.0 using UV-VIS spectrophotometer. IR24 and IRBB64 were used as susceptible and resistant checks, respectively. Data

LITERATURE CITED

BABUJEE, L. AND S.S. GNANAMICKAM. (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.

BASABDATTA, D., SENGUPTA, S., PRASAD, M. AND T. GHOSE. (2014). Genetic diversity of the conserved motifs of six bacterial leaf blight resistance genes in a set of rice landraces. BMC Genetics, 15:82.

BHARANI, M., NAGARAJANA, P., RABINDRANB, R., SARASWATHIC, R., BALASUBRAMANIANA, P. AND J. RAMALINGAMA. (2010). Bacterial leaf blight resistance genes (*Xa21, xa13* and *xa5*) pyramiding through molecular marker assisted selection into rice cultivars. Archives of Phytopathology and Plant Protection, 43 (10): 1032–1043.

BRADBURY, J.F. (1984). Genus II. Xanthomonas Dowson. In: Bergey's Manual of Systematic Bacteriology (Krieg, N.R. and Holt, J.G., eds), 199–210. Baltimore: Williams & Wilkins.

CHEEMA, K., GREWA, N., VIKAL, Y., SHARMA, R., LORE, JS., DAS, A., BHATIA, D., MAHAJAN, R., GUPTA, V., BHARAJ, TS. AND K. SINGH. (2008). A novel bacterial blight resistance gene from *Oryza nivara* mapped to 38 kb region on chromosome 4 L and transferred to *Oryza sativa* L. Genet Res., 90:397–407.

CHEN, H., WANG, S. AND Q. ZHANG. (2002). New gene for bacterial blight resistance in rice located on chromosome 12 identified from Minghui 63, an elite restorer line. Phytopahtology, 92: 750-754.

CHU, Z., YUAN, M., YAO, J., GE, X., YUAN, B., XU, C., LI, X., FU, B., LI, Z., BENNETZEN, J.L., ZHANG, Q. AND S. WANG. (2006) Promoter mutations of an essential gene for pollen development result in disease resistance in rice. Genes Dev., 20: 1250–1255.

COLLARD, B. AND J. MACKILL. (2008). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. DOI: 10.1098/rstb.2007.2170.

DATTA, K., BAISAKH, N., THET, K.M., TU, J. AND S.K. DATTA. (2002). Pyramiding transgenes for multiple resistance in rice against bacterial blight, yellow stem borer and sheath blight. Theor. Appl. Genet., 106:1–8.

FRANKEL, O.H. (1984). Genetic perspective of germplasm conservation. In Genetic Manipulations: Impact on Man and Society. Edited by Arber W, Llimensee K, Peacock WJ, Stralinger P. Cambridge: Cambridge University Press:161–170.

GARRIS, A.J., MCCOUCH, S.R., AND S. KRESOVICH. (2003). Population structure and its effect on haplotype diversity and linkage disequilibrium surrounding the xa5 locus of rice (*Oryza sativa* L.). Genetics, 165:759–769.

GLASZMANN, J.C., KILIAN, B., UPADHYAYA, H.D. AND R.K. VARSHNEY. (2010). Accessing genetic diversity for crop improvement. Curr Opin Plant Biol., 13:1–7.

HAMMOND-KOSACK, K.E. AND J.D.G. JONES. (1997). Plant disease resistance genes. Annu. Rev. Plant Physiol. Plant Mol. Biol., 48:575–607.

HUANG, N., ANGELES, E.R., DOMINGO, J., MAGPANTAY, G., SINGH, S., ZHANG, G., KUMARAVADIVEL, N., BENNETT, J. AND G.S. KHUSH. (1997). Pyramiding bacterial blight resistance genes in rice: Marker assisted selection using RFLP and PCR. Theor Appl Genet., 95:313–320.

HULBERT, S.H., WEBB, C.A., SMITH, S.M. AND Q. SUN. (2001). Resistance gene complexes: evolution and utilization. Annual Review on Phytopathology 39: 285–312. DOI: 10.1146/annurev.phyto.39.1.285.

IRRI (1996). Standard evaluation system for rice. 4th Edition, IRRI, The Philippines.

IYER, A.S. AND S.R. MCCOUCH. (2004). The rice bacterial blight resistance gene xa5 encodes a novel form of disease resistance. Mol Plant-Microbe Interact., 17:1348–1354.

JENA, K.K. AND D.J. MACKILL. (2008). Molecular markers and their use in marker-assisted selection in rice. Crop Sci., 48:1266–1276.

KELLY, J.D. (1995). Use of random amplified polymorphic DNA markers in breeding for major gene resistance to plant pathogens. HortScience, 30: 461–465.

KHAN, M.A., NAEEM, M. AND M. IQBAL. (2014). Breeding approaches for bacterial leaf blight resistance in rice (*Oryza sativa* L.), current status and future directions. Eur J Plant Pathol., 139:27–37.

KHOSHKDAMAN, M., EBADY, A.A AND D. KAHRIZI (2012). Evaluation of pathogenicity and race classification of Xanthomonas oryzae pv. oryzae in Guilan Province- Iran. Agricultural Science, 4:557-561.

KIM, K.Y., SHIN, M.S., KIM, W.J., MO, Y.J., NAM, J.K., NOH, T.H., KIM, B.K. AND J.K. KO. (2009). Effective combination of resistance genes against rice bacterial blight pathogen. Korean J Breed Sci., 41(3):244-251.

KORINSAK, S., SRIPRAKHON, S., SIRITHANYA, P., JAIRIN., KORINSAK, S., VANAVICHIT, A. AND T. TOOJINDA. (2009). Identification of microsatellite markers (SSR) linked to a new bacterial blight resistance gene xa33(t) in rice cultivar 'Ba70. Maejo Intl J Sci Technol., 3:235–247.

KUMAR, J.R. AND N. SANGHAMITRA. (2010). Gene pyramiding-A broad spectrum technique for developing durable stress resistance in crops. Biotechnology and Molecular Biology Review ISSN 1538-2273.

KUMAR, P.S., NAYAK, D.K., PANDIT, E., BARIK, S.R., MOHANTY, S.P., ANANDAN, A. AND J.N. REDDY. (2015). Characterization of morpho-quality traits and validation of bacterial blight resistance in pyramided rice genotypes under various hotspots of India AJCS, 9(2):127-134.

LEACH, J.E., DAVIDSON, R., LIU, B., MANOSALVA, P., MAULEON, R., CARRILLO, G., BRUCE, M., STEPHENS, J., DIAZ, M.G., NELSON, R., VERA CRUZ, C. AND H. LEUNG (2007). Understanding broad-spectrum durable resistance in rice. Rice Genet V., 191–207.

LEE, K.S. AND GS. KHUSH (2000) Genetic analysis of resistance to bacterial blight, *Xanthomonas oryzae* pv. *oryzae*, Rice. Rice Genet. Newslett., 17: 72.

LIU, Q.Q.. LI, Q.F., CAI, X.L., WANG, H.M., TANG, S.Z., YU, H.X., WANG, Z.Y. AND MH, G.U. (2006). Molecular marker-assisted selection for improved cooking and eating quality of two elite parents of hybrid rice. Crop Sciences, 46: 2354–2360. DOI: 10.2135/cropsci2006.03.0180.

MACKILL, D.J. (2007). Molecular markers and marker-assisted selection in rice. Genomics assisted crop improvement, 2: 147–168.

MAYA, M.M., RANI, D. AND M.M. MAGAR. (2014). Marker Assisted Selection For Bacterial Leaf Blight Resistance In Segregating Populations Of Cottondora Sannalu. Int J Appl Sci Biotechnol., 2(3): 229-237 DOI: 10.3126/ijasbt.v2i3.10570.

MELCHINGER, A.E. (1990). Use of molecular markers in breeding for oligogenic disease resistance, Pl Breeding, 104(1): 1- 19.

MEW, T.W. (1987). Current status and future prospects of research on bacterial blight of rice. Annu. Rev. Phytopathol., 25: 359–382.

MEW, T.W., ALVAREZ, A.M., LEACH, J.E. AND J. SWINGS (1993). Focus on bacterial blight of rice. Plant Disease, 77: 5-12. doi:10.1094/PD-77-0005

MEW, T.W., VERA CRUZ C.M. AND E.S. MEDALLA (1992). Changes in race frequency of *Xanthomonas oryzae* pv. *oryzae* in response to the planting of rice cultivars in the Philippines. Plant Disease, 76: 1029-1032.

MIKLAS, P.N., JOHNSON, E., STONE, V., BEAVER, J.S., MONTOYA, C. AND M. ZAPATA (1996). Selective mapping of QTL conditioning disease resistance in common bean. Crop Sci., 36: 1344–1351.

MISHRA, J., SINGH, M., PALNI, L. AND S. NANDI (2011). Assessment of genetic fidelity of encapsulated microshoots of Picrorhizakurrooa. Plant Cell, Tissue and Organ Culture (PCTOC), 104(2): 181-186.

MUNDT, C.C. (1990). Probability of mutation to multiple virulence and durability of resistance gene pyramids. Phytopathology, 80: 221-223.

NATRAJKUMAR, P., SUJATHA, K., LAHA, G.S., SRINIVASARAO, K., MISHRA, B., VIRAKTAMATH, B.C., HARI, Y., REDDY, C.S., BALACHANDRAN, S.M., RAM, T., SHESHUMADHAV, M., SHOBHARANI, N., NEERAJA, C.N., ASHOKREDDY, G., SHAIK, H. AND R.M. SUNDARAM. (2012). Identification and fine-mapping of Xa33, a novel gene for resistance to *Xanthomonas oryzae* pv. *oryzae*. Phytopathol., 102:222–228.

NGUYEN, T.L., LUY, T.T., KHUYEU, B.T.D. AND B.C. BUU. (2008). Genetics And Breeding For Blast And Bacterial Leaf Blight. Omonrice, 16: 41-49.

NODA, T. AND H. KAKU. (1999). Growth of *Xanthomonas oryzae* pv. *oryzae* in planta and in guttation fluid of rice. Ann. Phytopathol., Soc. Japan, 65: 9–14.

NORDBORG, M. AND D. WEIGEL. (2008). Next-generation genetics in plants. Nature, 456:11.

OU, S.H. (1985). Bacterial leaf blight. In Bacterial Diseases, Rice Disease 2nd edition, 61-96.

PORTER, B.W., CHITTOOR, J.M., YANO, M., SASAKI, T. AND FF. WHITE. (2003). Development and mapping of markers linked to the rice bacterial blight resistance gene *Xa7*. Crop Sci., 43: 1484–1492.

RAO, K.K., LAKSHMINARASU, M. AND K.K. JENA (2002). Dna markers and marker- assisted breeding for durable resiatance to bacterial blight disease in rice. Biotechnology Advance, 20:33-47.

SATTARI, A., FAKHERI, M., NOROOZI. AND K.H. MOAZAMI GUDARZI. (2014). Leaf blight resistance in rice: a review of breeding and biotechnology. IJFAS Journal-2014-3-8/895-902/ 31. ISSN 2322-4134.

SHANTI, M.L. AND V.V. SHENOY. (2005). Evaluation of resistance genes and their pyramids against rice bacterial leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae*. Oryza, 42: 169-173.

SHIN, M.S., KIM, K.Y., PARK, H.S. AND J.K. KO. (2011). Breeding for resistance to bacterial blight in rice. Korean J Breed, 43:251-261.

SONG, W.Y., WANG, G.L., CHEN, L.L., KIM, H.S., PI, L.Y., HOLSTEN, T., GARDNER, J., WANG, B., ZHAI, W.X., ZHU, L.H., FAUQUET, C. AND P. RONALD. (1995). A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science, 270: 1804–1806.

SONG, W.Y., PI, L.Y., WANG, G.L., GARDNER, J., HOLSTEN, T. AND PC. RONALD. (1997). Evolution of the rice *Xa21* disease resistance gene family. Plant Cell., 9:1279–1287.

SRINIVASAN, B. AND S. GNANAMANICKAM. (2005). Identification of a new source of resistance in wild rice, *Oryzae rufipogon* to bacterial blight of rice caused by Indian strains of *Xanthomonas oryzae* pv.*oryzae*. Current Sciences. 88: 25.

SUH, J.P., JEUNG, J.U., NOH, T.H., CHO, Y.C., PARK, S.H., PARK, H.S., SHIN, M.S., KIM, C.K. AND KK. JENA. (2013). Development of breeding lines with three pyramided resistance genes that confer broad-spectrum bacterial blight resistance and their molecular analysis in rice. Rice, 6:5.

SUN, X., YANG, Z., WANG, S. AND Q. ZHANG. (2003). Identification of a 47 kb DNA fragment containing Xa4, a locus for bacterial blight resistance in rice. Theor Appl Genet., 106:683–687.

SUNDARAM, R.M., VISHNUPRIYA, M.R., BIRADAR, S.K., LAHA, G.S., REDDY, G.A., SHOBA, RANI N., SARMA, N.P. AND R.V. SONTI. (2008). Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. Euphytica, 160: 411-422.

SWAMY, P., PANCHBHAI, A.N., DODIYAN, P., NAIK, V., PANCHBHAI, S.D., ZEHR, U.B., AZHAKANANDAM, K. AND B.R. CHAR. (2006). Evaluation of bacterial blight resistance in rice lines carrying multiple resistance genes and *Xa21* transgenic lines. Curr Sci., 90(6):818–824.

VERA CRUZ, C.M., ARDALES, E.Y., SKINNER, D.Z., TALAG, J., NELSON, R.J., LOUWS, F.J., LEUNG, H., MEW, T.W. AND J.E. LEACH. (1996). Measurement of haplotypic variation in *Xanthomonas oryzae* pv. *oryzae* within a single field by rep-PCR and RFLP analyses. Phytopathology, 86:1352-1359.

WANG, X.Y., CHEN, P.D. AND S.Z. ZHANG. (2001). Pyramiding and marker-assisted selection for powdery mildew resistance genes in common wheat. Acta Genetic Sin., 28(7):640–646.

ZHANG, Q., WANG, C., ZHAO, K., ZHAO, Y., CASLANA, V., ZHU, X.D. AND Q. JIANG. (2001). The effectiveness of advanced rice lines with new resistance gene *Xa23* to rice bacterial blight. Rice Genet. Newslett., 18: 71.