GENETIC ANALYSIS of SELECTED PSB RICE VARIETIES and EVALUATION of ELITE BREEDING LINES for RESISTANCE to BACTERIAL BLIGHT
(*Xanthomonas oryzae* pv *oryzae* ex Ishiyama)

MARLOU CADA ABALOS

A thesis submitted to the faculty of the Institute of Graduate Studies, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
Crop Science - Plant Breeding

JUNE 2004

This thesis entitled, **GENETIC ANALYSIS of SELECTED PSB RICE VARIETIES and EVALUATION of ELITE BREEDING LINES for RESISTANCE to BACTERIAL BLIGHT (*Xanthomonas oryzae* pv *oryzae* ex Ishiyama),** prepared and submitted by **MARLOU C. ABALOS**, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in CROP SCIENCE major in Plant Breeding is hereby accepted.

LUN G. MATEO
Member, Advisory Committee

Date signed

RONALDO T. ALBERTO
Member, Advisory Committee

Date signed

QUIRINO D. dela CRUZ
Chairman, Advisory Committee

Date signed

ANTONIO A. ALFONSO
Co-Chairman, Advisory Committee

Date signed

Accepted as partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in CROP SCIENCE major in Plant Breeding.

NEMESIO V. TAMAYO
Chairman, Department of Crop Science

Date signed

CYNTHIA C. DIVINA
Dean

Date signed

Institute of Graduate Studies
Central Luzon State University
Science City of Muñoz, Nueva Ecija

Name: MARLOU CADA ABALOS
Degree: Master of Science in Crop Science
Major: Plant Breeding

Thesis Title: GENETIC ANALYSIS of SELECTED PSB RICE VARIETIES and EVALUATION of ELITE BREEDING LINES for RESISTANCE to BACTERIAL BLIGHT (*Xanthomonas oryzae* pv *oryzae* ex Ishiyama)

Approved:

Dr. Nemesio V. Tamayo
Chairman IGS Representative
Examining Committee

Date signed

Prof. Apolonia L. Lalap
Member IGS Representative
Examining Committee

30 Jan '20
Date signed

English Editor:

Format Editor:

BIOGRAPHICAL SKETCH

The author was born on September 26, 1972 in Umingan, Pangasinan. He is the eldest of the two children of Mrs. Lorenza Cada Abalos and the late Mr. Ernesto H. Abalos.

He completed his elementary education at the Umingan Central Elementary School in 1985 and his secondary education at Immaculate Conception High School in 1989. He obtained his degree of Bachelor of Science in Agricultural Education major in Crop Science at the Central Luzon State University in 1993.

He started his professional career on October 16, 1993 as a Sales Agronomist on hybrid corn at the Pioneer Hi-Bred Agricultural Technologies, Inc.. On February 16, 1998, he was employed as a Research Assistant at the Plant Breeding and Biotechnology Division (PBBD), Philippine Rice Research Institute working on the Development of Inbred Rice Varieties for Rainfed Lowland, and Development of Breeding Lines for Resistance to Bacterial Blight. In 2004, he was given a permanent position as Science Research Specialist I.

He pursued his Master of Science in Crop Science major in Plant Breeding at the Central Luzon State University, on a part-time basis with the consent of his immediate supervisors and the Division Head of PBBD.

The author is happily married to Arlene Ortiz-Abalos. They are blessed with two daughters, Carla Joy and Angela Mae, and are now expecting their third child.

Acknowledgement

The author wishes to extend his heartfelt gratitude and sincere appreciation to the several persons who unselfishly contributed their knowledge and support toward the completion of this study. They are:

Dr. Rodante E. Tabien and Dr. Rico A. Caldo, former breeders and geneticists at PhilRice and immediate supervisors of the author for their unconditional support and shared knowledge that led to the completion of this study;

Dr. Quirino D. dela Cruz and Dr. Antonio A. Alfonso, chair and of co-chair respectively of the Advisory Committee, Dr. Lun G. Mateo and Dr. Ronaldo T. Alberto, members of the Advisory Committee, for their comments and suggestions that improved the manuscript;

Dr. Nemesio V. Tamayo and Prof. Apolonia L. Lalap, Chair and member of the Examining Committee respectively, for their important suggestions to improve the manuscript.

Ms. Thelma F. Padolina and Mr. Hilario C. dela Cruz, Jr., Division Head and Senior Consultant of the Plant Breeding and Biotechnology Division, for their compassion and encouragement;

Dr. Leocadio S. Sebastian, Executive Director of the Philippine Rice Research Institute (PRRI), for allowing and recommending the author to pursue his Master's degree at the Institute of Graduate Studies in CLSU;

Dr. Edilberto D. Redoña, Deputy Executive Director for R&D of PRRI, for allowing the author to use the PhilRice facilities in the conduct of his thesis;

Dr. Cynthia C. Divina, Dean of the Institute of Graduate Studies and her staff for their helpful assistance extended to the author;

Dr. Casiana M. Vera Cruz and Dr. Edwin Javier, Pathologist and Breeder respectively of the International Rice Research Institute, for sharing their expertise to the author;

Haizel, Lani, Mac, Bing, Lyn, Beth, Johanna, Raynato, Rusty, Edwin, and Fely of the Molecular Genetics Laboratory and other staff of the Tissue Culture and Genetic Transformation Laboratory of PhilRice for their help and moral support; his colleagues and fellow researchers at the Plant Breeding Division especially to Maricar P. Fernando, Gina M. Babb and Johna C. Duque, who assisted the researcher during the conduct of laboratory activities;

To *Pare* Leo T. Sta. Ines and *Kuya* Lito Dancel, for their support;

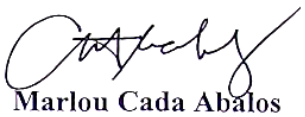
Jose Yapchiongco, Jr., Benjamin Patricio, Jr., Ronaldo de Guzman and Benedicto Barroga, for the assistance in setting up the experiment in the screenhouse;

To his beloved mother, Mrs. Lorenza C. Abalos and stepfather, Mr. Vicente Salacup; his parent in-laws, Mr. Luis P. Ortiz and Mrs. Lucena D. Ortiz, his brother Alex C. Abalos and sister-in-law Vilma L. Abalos; his sister-in-laws, Melanie O. Roldan and Melinda O. La Rosa; his friends, relatives and all those who in one way or another have helped him finished this undertaking;

The author acknowledges his wife, Arlene, for her endless support, patience and understanding, and his children who had to endure the consequences of having a working student father, and whose eternal love and prayers served as inspiration in all the authors' efforts and aspirations in life; and

Above all, to the God Almighty, Who provided the author with strength, wisdom and perseverance to complete his course.

To all of them, this piece of work is sincerely dedicated.



Marlou Cada Abalos

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Abalos Marlou Cada, Institute of Graduate Studies, Central Luzon State University, Science City of Muñoz, Nueva Ecija, 3120, JUNE 2004, **GENETIC ANALYSIS of SELECTED PSB RICE VARIETIES and EVALUATION of ELITE BREEDING LINES for RESISTANCE to BACTERIAL BLIGHT (*Xanthomonas oryzae* pv *oryzae* ex Ishiyama)**

Adviser: Dr. Quirino D. dela Cruz

Bacterial Blight (BB) caused by *Xanthomonas oryzae* pv *oryzae* ex Ishiyama is a serious disease and one of the major constraints in rice production in many Asian countries. Effective chemical-based control measures are not available against BB and the use of resistant varieties remains the most important ecological control method. More than 25 genes are available for resistance(R) to BB in rice including the *Xa-21, Xa-7, Xa-4, xa-8, xa-5, Xa-13, Xa-10* and *Om gene*. It was in this light that this study aimed to analyze BB resistance observed in some PSB rice varieties possibly caused by new resistance genes. It also aimed to evaluate backcross progenies for any presence of the *Xa-21* and *xa-5* R genes by using DNA markers and by analyzing their reaction to *Xoo* race 6, the most virulent strain.

Seven BB isolates were used in the inoculation using a clipping method for introducing the bacteria into the plant leaves. Six modern varieties were subjected to genetic analysis for resistance to BB. These included the PSB Rc2, PSB Rc34, PSB Rc56, PSB Rc64, PSB Rc80 and PSB Rc82 varieties. The PSB Rc2 variety was found to contain the *Xa-4* gene; PSB Rc34 had 4 genes effective against races 1, 6 and 9; PSB Rc56 had *Xa-4, Xa-14* and an unknown gene found to be very effective for race 8; PSB

Rc64 had *Xa-4, Xa-14, Xa-7* and a putative new gene that was effective against race 6; PSB Rc80 contained *Xa-4* gene found effective against races 1 and 7; and PSB Rc82 had at least nine different genes that made it resistant to *Xoo* races 1, 6, 7, 8 and 9 based on the analysis of two populations (PSB Rc82 x IR24 & PSB Rc82 x IRBB5). Out of the 56 backcross-derived lines from General Yield Trial and Preliminary Yield Trial tested, only 23 showed consistent resistant reaction to *Xoo* race 6 at 21 days after inoculation (DAI). A DNA marker analysis using the *Xa-21* and RG556a primers, revealed that 13 lines had both the *Xa-21* and *xa-5* genes, respectively. Twelve lines had *Xa-21* gene while five lines had *xa-5* alone. Knowledge on the properties of BB resistance genes, their effectiveness against specific races and the R genes present in certain commercial varieties are useful in the deployment of suitable varieties for locations where specific *Xoo* races are prevalent.

LITERATURE CITED

ALDRICK, S.J., I.W. BUDDENHAGEN, and A.P.K. REDDY. 1973. The occurrence of bacterial leaf blight in wild and cultivated rice in Northern Australia. Australian Journal of Agricultural Research 24:219-227

BHAPKAR, D.G., N.B. KULKARNI, and V.M. CHAVAN. 1960. Bacterial blight of paddy. Poona Agricultural College Magazine (51) 36:46

BLAIR MW, SR McCOUCH. Microsatellite and sequence tagged site markers diagnostic for the rice bacterial leaf blight resistance gene Xa-5. Theor Appl Genet 1997;252:597–607.

BRADBURY, J.F., GOTO, M., A.C., LELLIOT, R.A. and M.N. SCHROTIF. 1980. International Standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. Review of Plant Pathology. 59: 153-168.

CHAKRAVARTI, B.P. and M. RANGARAJAN. 1967. A virulent strain *Xanthomonas oryzae* from rice seeds in India. Phytopathology 57: 688-690.

CHOI, Y.C., CHO, E.K. and C.K. PARK. 1972. Experiments on the resistance of Tongil variety to major disease. pp 46-58.

DEVEDATH, S., 1983. Upward translocation of antibiotics and their role in controlling the bacterial blight of rice. Rico. 22: 33-37.

DYE, D.N., 1978. Genus IX *Xanthomonas* Dowson (1939). New Zealand Journal of Agricultural Research 21: 162-166.

EXCONDE, O.R. (1973) Yield losses due to bacterial leaf blight of rice. Philippines Agriculture 57, 128-140.

FANG, C.T., REN, H.C., CHEN, T.Y., CHU, Y.K., FAAN, H.C. and S.C. WU. 1957. A comparison of the rice bacterial leaf blight organism with the bacterial leaf streak organism of rice and Leersia hexandra Swartz. 3: 99-124.

GOTO, K., FUKTATZU, R. and K. OKATA. 1953. Overwintering of the causal bacteria of rice blight in rice plant and grasses. (Preliminary Report). Agriculture and Horticulture, Japan 28: 207-208.

_____. 1964. Kresek and pale yellow leaf systemic symptoms of bacterial leaf blight of rice caused by *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson. Plant Disease Reporter 48: 858-861.

HIFNI, H.R., NISHIYAMA, K. and A. EZUKA. 1975. Bacteriological characteristics of some isolates of *Xanthomonas oryzae* different in their pathogenecity and locality. Contributions of the Central Research Institute of Agriculture Bogor No. 16. pp 18.

HORINO, O. 1973. Ultrastructure of *Xanthomonas oryzae* and its morphological changes by chemical treatment. Annals of the Phytopathological Society of Japan 39: 14-26.

HUANG N, ANGELS ER, DOMINGO J, MANGPANTAY G, SINGH S, ZHANG G, KUMAR N, VADIVEL BJ, KHUSH GS. Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theor Appl Genet 1997;95:313–20.

IRRI. 1964. Annual Reports for 1964-1969.

IRRI. 1983. Field Problems of Tropical Rice. Pp 60-63.

ISHIYAMA, S. 1922. Studies on the white leaf disease of rice plants. Report of the Agricultural Experiment Station, Tokyo. 45: 233-251.

KAMESWARA RAO K., M. LAKSHMINARASU, K.K. JENA. (2002) DNA markers and marker-assisted breeding for durable resistance to bacterial blight disease in rice. Biotechnology Advances 20 pp 33–47

KARGANILLA, A., M. PARIS-NATURAL, and S.H. Ou. 1973. A comparative study of culture media for *Xanthomonas oryzae* Philippine Agriculturist 57: 141-152.

KAUFFMAN, H.E., A.P.K. REDDY, S.P.Y. HSIEH and S.D. NERCA. 1973. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae* Plant Disease Reporter 57: 537-541.

KIM, J.H., and Y.S. CHO. 1970. Effects of NPK fertilizer levels on the development of bacterial leaf blight in rice plant. Kor. J. Pl. Prot. 3: 7-13.

KHUSH GS, DJ MACKILL, GS SIDHU. Breeding rice for resistance to bacterial blight. Bacterial Blight of Rice. Proceedings of the International Workshop on Bacterial Blight Rice, IRRI, Manila, Philippines. 1989;207–17.

LEE, K.H. and H.W. CHUNG. 1965. Relationships between the seasonal change of bacterial leaf blight.

_____., Y.C. CHOI, E.K. CHO, B.J. CHUNG, and H.A. LEMEY. 1973. An investigation on losses and epidemic outbreak on bacterial leaf blight of rice. Sym. Pl. Env. Res. Commemoration of Dr. Kim's birthday. pp 79-89.

MEW, T.W., I.C. MEW, and J.S. HUANG. 1984. Scanning electron microscopy of virulent and avirulent strains of *Xanthomonas campestris* pv *oryzae* (Xco) on rice leaves. Phytopathology 74: 635-641.

_____. 1987. Current Status and future projects of reseach on Bacterial blight of rice. Phytopathology 25: 359-382.

MIZUKAMI, T. 1961. Studies on the ecological properties of *Xanthomonas Oryzae* (Uyeda et Ishiyama) Dowson, the causal organism of bacterial leaf blight of rice plant. 13: 1-85.

_____. 1964. Occurrence of bacterial leaf blight disease in India. Journal of Plant Protection 18: 179-181.

MUKO, H. and K. YOSHIDA. 1951. A needle inoculation method for bacterial leaf blight disease of rice. 15: pp 179.

_____. , and M. WATANABE. 1958. Studies on nutritional physiology of the organism causing bacterial leaf blight of rice. (2) Carbon and Nitrogen sources in relation to growth. 23 pp 9.

_____., and M. ISAKA. 1964. Re-examination of some physiological characteristics of *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson. Annals of the Phytopathological Society of Japan. 29: 13-19.

OU, S.H., 1972. Rice Diseases. The cambrian News (Aberstuyth) Ltd. Pp 61-96.

PADMANABHAN, S.Y. (1983) Integrated control of bacterial blight of rice. Oryza 20, 188-194.

REDDY, A.P.K., and H.E. KAUFFMAN. 1974. Population studies of mixed inoculum of *Xanthomonas oryzae* in susceptible and resistant varieties of rice. Annals of the Phytopathological Society of Japan 40: 93-97

REDDY, O.R. and S.H. OU. 1976. Characterization of *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson, the bacterial blight pathogen of rice. Annals of the Phytopathological Society of Japan 42: 124-130.

REITSMA, J. and P.S.J. SCHURE. 1950. 'Kresek', a bacterial disease of rice. Contributions of the Central Agricultural Research Station, Bogor No. 117: pp17.

RONALD PC. 1997 Molecular basis of disease resistance. Plant Mol Biol 35:179–86.

RONALD PC, B ALBANO, R TABIEN, L ABENES, K WU, SR McCOUCH, SD TANKSLEY. Genetic and physical analysis of the bacterial blight disease resistance locus, *Xa-21*. Mol Gen Genet 1992;236:113–20.

SANCHEZ, A. C. Brar, D. S. Huang, N. Li, Z. Khush, G. S. 2000. Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. Crop Sci. 40(3): 792-797.

SANCHEZ AC, B FU, D YANG, GS KHUSH, Z LI. Isolation and sequence analysis of candidate cDNA clones for the *Xa-5* gene in rice. International Congress on Plant Molecular Biology (ICPMB) June 18–24, at Quebec, Canada. 2000. Abstracts published by ICPMB.

SAKAGUCHI S. Linkage studies on resistance to bacterial leaf blight, *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson, in rice. Bull Natl Inst Agric Sci Ser 1967;16:1–18.

SCHURE, P.S.J. 1953. Attempts to control the kresek disease of rice by chemical treatment of seedlings. Contributions of the Central Agricultural Research Station, Bogor No. 136: pp 17.

SHEKHAWAT, G.S. and D.N. SRIVASTAVA. 1968. Variability in Indian isolates of *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson, the incitant bacterial leaf blight of rice. Annals of the Phytopathological Society of Japan 34: 289-297.

SINGH, S. SIDHU, J. S. HUANG, N. VIKAL, Y. LI, Z. BRAR, D. S. DHALIWAL, H. S. KHUSH, G. S. 2001. Pyramiding three bacterial blight resistance genes (*xa5, xa13* and *Xa21*) using marker-assisted selection into indica rice cultivar PR106. Theor. Appl. Genet. 102(6/7): 1011-1015

SRIVASTAVA, D.N. and Y.P. RAO. 1964. Paddy farmers should beware of bacterial blight. Indian Farming 19: 32-33.

_____. 1967. Epidemiology and control of bacterial blight of rice in India. Proceedings of a symposium on Rice Diseases and their Control by Growing Resistant varieties and other Measures pp 11-18.

SRINIVASAN, M.C., M.J. THIRUMALACHAR, and M.K. PATEL. 1959. Bacterial blight disease of rice. Current Science 28: 469-470.

SWINGS, J., M. VANDEN MOOTER, L. VAUTERIN, B. HOSTE, M. GILLIS, T.W. MEW, and K. KERSTERS. 1980. Reclassification of the causal agents

of bacterial blight (*Xanthomonas campestris* pv *oryzae*) and bacterial leaf streak (*Xanthomonas campestris* pv *oryzicola*) of rice as pathovars of *Xanthomonas oryzae* (ex Ishiyama) sp. Nov., nom. Rev. International J. Systematic Bacteriology 40: 309-311.

TABIEN RE, MC ABALOS, MG PADILLA, RC SAN GABRIEL, YA DIMAANO, JBA DULDULAO, MJC CASAYURAN, LS Sebastian. 2001a. Transplanted lowland rice with *xa-5* and *Xa-21* genes for bacterial blight pathogen, *Xathomonas oryzae* pv *Oryzae*. Poster presented at the Asian Agriculture Congress, Manila, Philippines, April 24-27,2001.

TABIEN RE, MC ABALOS, MG PADILLA, EBR TADLY. 2001b. Improving resistance of PSB rice varieties to bacterial blight. Poster presented at the 14th National Rice R&D Review and Planning Workshop, March 7-9, 2001. PhilRice, Maligaya, Science City of Munoz, Philippines.

TABIEN RE, MC ABALOS, MG PADILLA, EBR TADLY. 2001c. Use of DNA Marker Technology in Rice Improvement, In Philippine Rice R&D Highlights 2001. Philippine Rice Research Institute. pp 35-37.

TAGAMI, Y. and T. MIZUKAMI. 1962. Historical review of the researches on bacterial leaf blight of rice caused by *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson. Special report of the Plant Disease and Insect Pests Forecasting Service No. 10: pp 112.

TANAKA, 1963. Studies on the nutritional physiology of *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson. I. On the nitrogen sources. II. On the carbon sources and a new synthetic medium. Science bulletin of the Faculty of Agriculture, Kyushu Univ. 20: 151-155.

U.S.D.A. 1976. Foreign Agricultural Service, Foreign Agricultural Circular pp 1-76.

YOSHIMURA S, U YAMANOUCHI, Y KATAYOSE, S TOKI, ZX WANG, I KANO, N KURATA, M YANO, N IWATA, T SASAKI. Expression of *Xa-1*, a bacterial blight resistance gene in rice, is induced by bacterial inoculation. Proc Natl Acad Sci USA 1998;95:1663–8.

ZHANG G, ER ANGELES, MLP ABENES, GS KHUSH, N HUANG. RAPD and RFLP mapping of the bacterial blight resistance gene *Xa-13* in rice. Theor Appl Genet 1996b;93:65–70.