The 2nd International Conference on

Bacterial Blight of Rice

Nanjing, China

October 1-3, 2007

The 2nd International Conference on

Bacterial Blight of Rice

Nanjing, China

October 1-3, 2007

Meeting Sponsors

The 111 project (B07030, Nanjing Agricultural University)

Ministry of Education of the People's Republic of China

National Natural Science Foundation of China

Nanjing Agricultural University

Shanghai Bioon Info-Tech. Co., Ltd.

TaKaRa biotechnology (Dalian) Co., Ltd.

Capital Bio Corporation National Engineering Research Center for Beijing Biochip Technology Shanghai Office

Gene Time Co., Ltd.

Chinese Society for Plant Pathology

International Society of Plant Pathology

Organizing Committee

J. S. Wang (Head, NAU, China)

J. E. Leach (Colorado State University, USA)

T. W. Mew (IRRI, Philippines)

H. Leung (IRRI, Philippines)

F. F. White (Kansas State University, USA)

R. Sonti (Center for Cellular and Molecular Biology, India)

H. Kaku (National Institute of Agricultural Science, Japan)

C. Vera Cruz (IRRI, Philippines)

H. S. Zhang (Rice Research Institute of NAU, China)

F. Q. Liu (Department of Plant Pathology, NAU, China)

C. Y. He (Chinese Academy of Agricultural Science, China)

Byoung-Moo Lee (National Institute of Agricultural Biotechnology, Rural Development Administration, Korea)

Local Organizing Committee

Xiaobo Zheng (NAU)

Jinsheng Wang (NAU)

Hongsheng Zhang (NAU)

Congfeng Song (NAU, Secretary)

Fengquan Liu (NAU)

Chengyang He (CAASC)

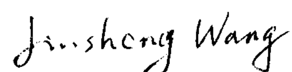
PREFACE

The 2nd International Conference on Bacterial Blight of Rice (ICBB) is held in Nanjing China from October 1 to 3, 2007. As a model disease, the significant progress has been made in past few years on different aspects and levels of research on this disease. The aim of this conference is to present the latest progress made in the research of bacterial blight on rice. Hopefully, by holding this conference, we could provide a good platform for the information exchange and facilitate the communications among the scientists from different countries.

I would like to express my special thanks , on behalf of organization committee, going to our guess speakers and all participants who are from 9 countries: American, Japan, Korea, India, Singapore, Philippine, France, Bangladesh and China for taking time to attend this Conference and give us the benefit of their knowledge. The proceedings contain 68 paper and abstracts. The contents are arranged in topics. It is necessary to emphasize that International Society for Plant Pathology and Chinese Society for Plant Pathology have fully supported this conference.

I would like to thank Chinese Ministry of education, National Natural science foundation of China and Nanjing Agriculture University for their sponsoring this conference and financial supports.

Jinsheng Wang



Chair of the 2nd ICBB

Special Lecture 5
Understanding *Xanthomonas oryzae*/Rice Interactions to Guide Disease Control 6
Creating public genetic and genomic resources for investigation of broad-spectrum disease resistance in rice 7
Topic 1. Resistance genes and functions in interaction between rice-Xoo 8
Dominant and recessive R genes of rice: Revealing host adaptations to transcriptional reprogramming by the pathogen *Xanthomonas oryzae* pv. *oryzae* 9
R gene-mediated bacterial blight resistance is influenced by its expression pattern 9
Localization of XA27 to apoplast of vascular elements for resistance to *Xanthomonas oryzae* pv. *oryzae* depends on N-terminal signal anchor with triple arginine motif 10
High-resolution genetic mapping of bacterial blight resistance gene *Xa10* 11
Identification of resistance sources and mapping of resistance QTLs to African strains of *Xanthomonas oryzae* pv. *oryzae* causing Bacterial Leaf Blight in rice 11
Topic 2. Determinants of pathogenicity and avirulence in Xoo 13
The TAL Effectors Tip the Balance of Resistance and Susceptibility in Bacterial Blight of Rice 14
A genetic analysis of virulence functions in *Xanthomonas oryzae* pv. *oryzae* 14
Regulation of virulence by xrvA in *Xanthomonas oryzae* pv. *oryzae* 15
A genetic analysis of virulence functions in *Xanthomonas oryzae* pv. *oryzae* 17
Insight into bacterial blight from comparative pathogen genomics and host transcriptomics 17
Comparative genomics of *Xanthomonas*: The puzzle of lifestyle 18
Identification and functional analysis of a regulatory pathway leading from Tdrxoo/GacAxoo to motility and virulence in *Xanthomonas oryzae* pv. *oryzae* 20
Identification and functional analysis of a regulatory pathway leading from *Tdrxoo/GacAxoo* to motility and virulence in *Xanthomonas oryzae* pv. *oryzae* 21
DNA microarray analysis of transcriptional profiling of △*fleQxoo*, a deletion mutants of *Xanthomonas oryzae* pv. *oryzae* 21
Transcriptional profiling of △*gacAxoo*, a deletion mutants of *Xanthomonas oryzae* pv. *oryzae* revealed by DNA microarray analysis 22
Functional analysis of the *avrBs3/pthA* gene family in african *Xanthomonas oryzae* pv. *oryzae* strains 23
AvrXa3-mediated recombinants of the *AvrBs3/pthA* gene from *Xanthomonas oryzae* pv.*oryzae* cause pathotypic variation on adult rice lines 23
Molecular identification and characterization of an enolase-phosphatase gene from *Xanthomonas oryzae* pv. *oryzae* 24
The *Xanthomonas oryzae* pv. *oryzae* RaxST tyrosine sulfotransferase is required for AvrXa21 activity 25
The type-III protein hrpA affects colonization of *Xanthomonas oryzae* pv. *oryzae* on rice 26
A conserved *hpa2* gene encoding a protein of lytic activity to bacterial cell wall in phytopathogenic *Xanthomonas oryzae* 26
Analysis of virulence and growth of a purine auxotrophic mutant of *Xanthomonas oryzae* pathovar *oryzae* 27
Zinc uptake regulator (*zur*) gene involved in zinc homeostasis and virulence of *Xanthomonas oryzae* pv. *oryzae* in rice 27

Involvement of gluconeogenic pathway in virulence of *Xanthomonas oryzae* pv. *oryzae* 28
Xanthomonas oryzae pv. *oryzae* twin-arginine-dependent translocation is important for virulence, flagellation, and chemotaxis 29
Induction of crg gene expression *Xanthomonas oryzae* pv. *oryzae* by H2O2 generated during bacterial interactions of rice suspension-cultured cells or applied exogenously...........................29
Characterization and identification of genes involved in biosynthesis of LPS O- antigen and exopolysaccharide from lipopolysaccharide biosynthesis gene cluster in *Xanthomonas oryzae* pv. *oryzae* 30
Expressional regulation of of *metB* and *xomtA*, Lipopolysaccharide O-antigen biosynthesis related genes in *Xanthomonas oryzae* pv. *oryzae* 31
Functional analysis of Type IV pili system related *pilQ* gene of *Xanthomonas oryzae* pv. *oryzae*...31
Development of a RT-Q-PCR targeting *lipAxoo* and *purHxoo* for quantification of bacterial infection process of rice by *Xanthomonas oryzae* pv. *oryzae*..32
Xanthomonas oryzae pv. *oryzae* crg gene expression inducted by H_2O_2 generated during bacterial interactions of rice cells or applied exogenously 33
Preliminary Study on avirulence genes of *avrBs3/pthA* family in PXO99......................................33
Topic 3. Molecular and physiological aspects in rice resistance to bacterial blight 35
Insights into the rice defense response 36
Induction and suppression of host innate immunityin *Xanthomonas*-rice interactions................36
Identification of rice genes differentially-regulated by *Xanthomonas oryzae* pv. *oryzae* by cDNA-AFLP: functional analysis of an induced transcription factor OsBTF3.................................37
Comparative analysis of nitric oxide generation and induction of defense gene expression by *Xanthomonas oryzae* pv. *oryzae* and *X. campestris* pv. *vesicatoria* of rice suspension-cultured cells 38
Nitric oxide generation and defense gene expression induced by *Xanthomonas oryzae* pv. *oryzae* and *X. campestris* pv. *vesicatoria* of rice suspension-cultured cells 38
Topic 4. Population structures and diversity of pathotypes and genotypes of Xoo 40
Genomics and genetic diversity of Korean *Xanthomonas oryzae* pv. *oryzae* 41
Pathotype diversity among Indian isolates of the bacterial leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae*...41
Analysis of pathotypic and genotypic diversity of *Xanthomonas oryzae* pv. *oryzae* in China 42
Molecular analysis unravel new *Xanthomonas oryzae* pv. *oryzae* strains in West-Africa 43
Genetic diversity and change in race frequency of *Xanthomonas oryzae* pv. *oryzae* causing bacterial blight of rice in Punjab, India 44
Screening, phenotypic and physiological structures of *Xanthomonas oryzae* pv. *oryzae* and its races in Bangladesh * 45
Physiological structures of *X. oryzae* pv. *oryzae*..47
Topic 5. Resistance breeding and transgenic techniques...59
Studies on genetics and improvement of resistance to Bacterial Blight in China..........................60
Impact of gene pyramids on *Xanthomonas oryzae* pv. *oryzae* population structures—implications for deployment of *Xa* genes 61
Introgression of effective bacterial blight resistance genes, fragrance genes and QTL mapping for aroma and other grain quality traits in Basmati-derived rice lines 63
[2]International Rice Research Institute, DAPO Box 7777, Metro Manila, The Philippines 63

Table 1. Reaction of F_8 pedigree lines of the cross (IR71730-51-2/IRBB60) to 4 races of *X. oryzae* pv. *oryzae*, their molecular genotypes for....70
BB resistance genes and *fgr genes* along with grain quality data....70
++ = *fgr* present; -- = *fgr* absent....70
Table 3. Means and ranges for various grain quality traits for parents and RILs....71
Bacterial blight resistant hybrid rice parental lines developed via bi-directional marker-aided selec tion....76
Molecular breeding for bacterial blight resistance in japonica rice....77
Three novel bacterial blight resistance genes identified, mapped and transfer to cultivated rice *O. sativa* L.....82
Inheritance of resistance to bacterial blight in 21 cultivars of rice (*Oryza sativa* L.)....84
Bacterial Blight resistance and semidwarfing genes transferred to improve plant architecture and productivity of two traditional basmati varieties....85
Resistant classification of Korean rice varieties to Bacterial Blight....86
Confirmation of *Xa* genes introgressed into elite Basmati derived lines....87
Development of bacterial blight and blast resistant indica rice cultivars by gene pyramiding using marker assisted breeding....88
Transgenic rice expressing a harpin-encoding gene (*hrf1*) exhibits nonspecific resistance to *Magnaporthe grisea*....89
Topic 6. Chemical and biological control strategies against Xoo....104
Structural proteomics of *Xanthomonas oryzae* pv. *oryzae* (Xoo) for anti-bacterial drug development against bacterial blight (BB)....105
Study on the efficacy of rice cultivar mixtures for rice bacterial blight management....105
Miscellous....107
Development of DNA microarray of *Xanthomonas oryzae* pv. *oryzae* and its validity in gene expression profile analysis....108
Design of genome-directed primers (GDPs) for selective labeling of transcripts of *Xanthomonas oryzae* pv. *oryzae* and its application for bacterial expression profiling during early infection revealed by DNA microarray analysis....108
Quantitative *Forecasting Method* for *Xanthomonas oryzae* pv. *oryzae*....109
N-terminal mutants of *Xanthomonas oryzae* harpins that abolish the activity to elicit hypersensitive reaction retain to induce systemic acquired resistance on tobacco....110
Cloning and characterization of a *hrf1* homolog from rice (*Oryza sativa* L.)....110
Generation and Characterization of an Anti-idiotypic Antibody of Harpin (subscript Xoo), a Proteineous Elicitor of *Xanthomonas oryzae* pv. *oryzae*....111
hrpF and *hpa1* Genes of *Xanthomonas oryzae* pv. *oryzicola* Determine the Development of the Leaf Streak Symptom, but Have No Effects on Infection Route in Rice....112
Genome-wide microarray analysis of transgenic cotton expressing harpinxoo from *Xanthomonas oryzae* pv. *oryzae*....112
Cloning and Expression of hpa1 homology sequence from *Triticum aestivum* L.....113

Special Lecture

Understanding *Xanthomonas oryzae*/Rice Interactions to Guide Disease Control

Jan E. Leach[1], Kimberly M. Webb[1,2], Jianfa Bai[2], Isabelita Oña[3], M. Bruce[1], P. Manosalva[1], S. Lee[1], J. Stephens[1], Karen Garrett[2], T.W. Mew[3], Casiana M. Vera Cruz[3]

[1]Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80537-1177, [2]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502; [3]Division of Entomology and Plant Pathology, The International Rice Research Institute, Los Baňos, Philippines.

One strategy to achieve long lasting bacterial blight (BB) resistance in plants is to accumulate single disease resistance (*R*) genes with known durability. However, accurately predicting how long *R* genes will remain effective after deployment is dependent on understanding the factors influencing their durability. One factor previously shown to contribute to durability of the rice BB *R* gene *Xa7* is the cost in pathogenic fitness associated with adaptation of the pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), to virulence. After planting a rice cultivar with *Xa7* (IRBB7) in field sites for 10 years (20 crops), however, the *Xoo* population structure changed to one with an increased proportion of strains virulent to IRBB7 and higher aggressiveness to rice without *Xa7*. Despite these changes, the *Xa7* gene remains highly effective because little disease was observed in fields planted with IRBB7 relative to rice cultivars without *Xa7*. We have found that, in addition to adaptation to virulence, climate influences *R* gene durability. *Xa7* functions to restrict disease more effectively at high than low temperature regimes, whereas activity of other resistance genes either was not affected or the genes were less effective at high temperatures. Of the two rice cropping seasons per year in the Philippines, the one with higher day/night temperatures is characterized by more disease. We suggest that seasonal high temperature effects on *Xa7* function may impose oscillating selection on the *Xoo* population, and thereby positively impact the durability of *Xa7* in the field.
A second strategy to achieve durable resistance to BB is to accumulate quantitative trait loci (QTL) that confer broad spectrum resistance. This approach requires knowing which genes within the QTL are contributing to the disease resistance phenotype so that they can be used as markers in the accumulation of the traits. Using mutant analysis, gene silencing and expression profiling, we have demonstrated that several genes associated with QTL function in disease resistance. Two genes, *OsPal4* and *GF14-e*, which encode phenylalanine ammonia lyase and a 14-3-3 protein, respectively, are associated with chromosome 2 disease resistance QTL. Both genes contribute, albeit differently, to BB resistance. The challenge now is to understand allelic differences in effective and non-effective disease resistance QTL so that the information can be applied, along with information on durability of resistance governed by single *R* genes, to guide breeding programs and stabilize rice production.

Creating public genetic and genomic resources for investigation of broad-spectrum disease resistance in rice

Hei Leung

Plant Breeding, Genetics and Biotechnology, International Rice Research Institute, DAPO Box 7777, Manila, Philippines;

Through collaboration with multiple institutions and laboratories around the world, IRRI strives to develop a range of genetic resources and approaches to fully exploit the available rice genome sequence for gene discovery. In this presentation, several activities that are particularly relevant to understanding the genetic basis of broad-spectrum resistance are discussed.

Mutant collection. A large collection of chemical- and irradiation-induced mutants of IR64 has been established and publicly available for screening for traits of agronomic interests. Both loss- and gain-of-resistance to bacterial blight mutants are found in high frequency, providing a good source of genetic materials to isolate genes conditioning broad-spectrum resistance. A series of double mutants has also been created to facilitate the dissection of disease response pathways.

Genome-wide SNP discovery across diverse varieties. Under the OryzaSNP project (http://www.oryzasnp.org), a set of diverse varieties including representatives from indica, tropical and temperate japonicas, aus, deepwater, and aromatic types of rice was examined for SNP variation in 100 Mb of the genome using array-based re-sequencing technology (Perlegen). Approximately 2.6 SNPs per kb was detected between a pair of varieties, providing high density markers for association and mapping analysis.

Whole-genome genotyping and expression platforms. To relate genotypes to phenotypes, we explore various array platforms to simultaneously determine genotypes and expression patterns under disease stress conditions. We observed that some genomic regions exhibit aggregated or correlated expression that could be important for quantitative disease resistance. Pairwise comparisons of expression signatures and high-resolution genotypes of specialized genetic stocks (mutants and near-isogenic lines) can provide a versatile approach to identify genomic variation responsible for phenotypes of interest.

Through a consortium approach, we will continue our efforts to develop low-cost, universal gene discovery platforms that can be broadly accessible. Community efforts to use these resources and to share results can accelerate the identification of new genes useful for managing multiple rice diseases, including bacterial blight.

Topic 1. Resistance genes and functions in interaction between rice-Xoo

Dominant and recessive R genes of rice: Revealing host adaptations to transcriptional reprogramming by the pathogen *Xanthomonas oryzae* pv. *oryzae*

Frank F. White, Bing Yang[1], Akiko Sugio[2], Ginny Antony, and Zhongchao Yin[3]

Plant Pathology, Kansas State University, Manhattan, KS 66506; 1 Genetics, Development and Cell Biology, Iowa State University, IA; 2 Disease and Stress Biology, John Innes Centre, Norwich, UK; 3 Temasek Life Sciences Laboratory, National University of Singapore, Singapore

Bacterial blight of rice represents both a disease of great agronomic importance as well as a robust system for understanding the interaction and co-adaptation process of a bacterial pathogen and the host. *Xanthomonas oryzae* pv. *oryzae*, like many members of the γ-proteobacteria, depend on a type III secretion system for effective invasion and colonization of the host, in this case, rice. While the bacteria harbor a variety of type III effectors, the substrates of the type III system, related to other plant and animal pathogens, the pathovar is particularly noteworthy for the dependence on one family of type III effector that we have named the transcription activator-like (TAL) effectors. We have further identified by genetic analyses both major and moderate TAL effector genes based on their contribution to the virulence or aggressiveness of a particular strain. In addition, three of the TAL effectors were originally identified based on the recognition of the effectors by the host defense system and the elicitation of a resistance reaction. These effectors are named as avirulence or Avr genes accordingly and include *avrXa10*, *avrXa7* and *avrXa27*. The TAL effectors have three remarkable features that conspire to induce the transcription of specific host genes supporting a model whereby the effectors target specific host functions that facilitate bacterial colonization. At the same time, the dominant host R gene *Xa27*, which was cloned by a now classical chromosome walking strategy, mimicks the intended function of the TAL effectors whereby the end result is the inducement of a hypersensitive response upon expression of *Xa27*. In a sense, the response is akin to a self-induced lesion mimick response. We have used microarray hybridization analyses to identify important host genes that are involved in the interaction of pathogen and host.

R gene-mediated bacterial blight resistance is influenced by its expression pattern

Shiping Wang

National Key Laboratory of Crop Genetic Improvement, National Center of Plant Gene Research (Wuhan), Huazhong Agricultural University, Wuhan 430070, China

Email: swang@mail.hzau.edu.cn

Genetic background and developmental stage influence the function of some disease resistance (*R*) genes. The molecular mechanisms of these modifications remain elusive. Our results show that the two factors are associated with the expression of the *R* gene in rice *Xa3/Xa26*–mediated resistance to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), which in turn influences the expression of defense-responsive genes. The background of *japonica* rice, one of the two major subspecies of Asian cultivated rice, facilitates the function of *Xa3/Xa26* more than the background of *indica* rice, another rice subspecies. *Xa3/Xa26* expression gradually increases from early seedling stage to adult stage. *Japonica* plants carrying *Xa3/Xa26* regulated by the native promoter showed an enlarged resistance spectrum (i.e. resistance to more *Xoo* races), increased resistance level (i.e. further reduced lesion length), and whole-growth-stage resistance compared to the *indica* rice; this enhanced resistance was associated with an increased expression of *Xa3/Xa26* throughout the growth stages in the *japonica* plants, which resulted in enhanced expression of defense-responsive gene *OsWRKY13*, an activator of a salicylic acid-dependent defense pathway. Overexpressing *Xa3/Xa26* with a constitutive strong promoter further enhanced rice resistance due to further increased *Xa3/Xa26* transcripts in both *indica* and *japonica* backgrounds, whereas regulating *Xa3/Xa26* with a pathogen-induced weak promoter impaired rice resistance.

Localization of XA27 to apoplast of vascular elements for resistance to *Xanthomonas oryzae* pv. *oryzae* depends on N-terminal signal anchor with triple arginine motif

Lifang Wu, Mei Ling Goh, and Zhongchao Yin (yinzc@tll.org.sg)

Temasek Life Sciences Laboratory, 1 Research Link, the National University of Singapore, Singapore 117604, Republic of Singapore

The rice gene *Xa27* confers resistance against *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), the causal agent of bacterial blight in rice. *Xa27* encodes a novel protein and the structure analysis of the *Xa27* protein (XA27) provided little or no clues as to the mode-of-action of the protein. Here, we demonstrate that XA27 localizes to apoplast of vascular elements for resistance to bacterial blight. Functional XA27-GFP proteins expressed under *Xa27* native promoter were mainly localized at vascular elements, where the bacterial blight pathogens propagated in host. Immuno-gold electron microscopy showed that XA27-FLAG proteins were localized to apoplast of infected vascular elements, especially xylem vessels. This finding was further verified by observing XA27-GFP on cell wall of roots of transgenic lines after plasmolysis. A signal anchor at the N-terminal region of XA27 with a triple arginine motif is required for XA27 localization to cell wall. The 57-amino acid of XA27 N-terminal region was

sufficient to localize N57-GFP fusion proteins to cell wall. Substitution of the triple arginine motif in N57-GFP, XA27-GFP or XA27 with glycines or lysines blocked their localization to cell wall and abolished their function for resistance to the normally avirulent *Xoo* strain PXO99^{A}. Our results suggest that the N-terminal signal anchor-dependent localization of XA27 to apoplast of vascular elements is essential for its resistance to *Xoo*.

High-resolution genetic mapping of bacterial blight resistance gene *Xa10*

Gu Keyu, Singh Jatinder Sangha and Zhongchao Yin

Temasek Life Sciences Laboratory, 1 Research Link, the National University of Singapore, Singapore 117604

Bacterial blight of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is the most devastating disease of rice (Oryza sativa L). Rice lines that carry resistance (R) gene Xa10 confer race-specific resistance to Xoo strains harboring avirulence (Avr) gene avrXa10. Here we report on genetic study, disease evaluation and fine genetic mapping of the Xa10 gene. The inheritance of Xa10-mediated resistance to PXO99A(pZWavrXa10) didn't follow typical Mendelian inheritance for single dominant gene in F2 population derived from IR24 x IRBB10. A locus might be present in IRBB10 that caused distorted segregation in F2 population. To eliminate this locus, an F3 population (F3-65) was identified, which showed normal Mendelian segregation ratio of 3:1 for resistance and susceptibility. A new near-isogenic line (F3-65-1743) of Xa10 in IR24 genetic background selected from F3-65 showed similar resistance specificity as that of IRBB10 and provided complete resistance to PXO99A(pZWavrXa10) from seedling to adult stages. After linkage analysis using RFLP markers and F2 mapping population, the Xa10 locus was initially mapped to the proximal side of E1981S with genetic distance at 0.93 cM. Five new molecular markers were developed based on the available genomic sequence of Nipponbare and used for fine mapping. Xa10 was finely mapped to the region at genetic distance of 0.28 cM between proximal marker M491 and distal marker M419 and co-segregated with markers S723 and M604. The physical distance between M491 and M419 on Nipponbare genome is 74Kb. The results of this study should be useful in Xa10 cloning and marker-assisted breeding.

Identification of resistance sources and mapping of resistance QTLs to African strains of *Xanthomonas oryzae* pv. *oryzae* causing Bacterial Leaf Blight in rice

Gustave Djedatin [1,2], Mathias Lorieux [3], Thierry Mathieu [4], Alain Ghesquière [4], Marie-Noëlle Ndjiondjop [1] and Valérie Verdier [4]

1 Biotechnology Laboratory, Africa Rice Center (WARDA) 01BP2031 Cotonou/Bénin

2 Laboratoire de Génétique FAST/Université d'Abomey-Calavi 01BP526 Cotonou/Bénin

3 CIAT-IRD, Biotechnology Unit, AA6713, CALI, Colombia

4 Institut de la Recherche pour le Développement (IRD), Laboratoire Génome et Développement des Plantes, UMR5096, 911Av Agropolis, 34394 Montpellier, France

Rice bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) was reported in Africa in the 80s, since then it is increasing in importance. Breeding strategies for durable BB resistance need to be urgently developed in Africa. The objectives are to identify sources of resistance to *Xoo* in African germplasm and to characterize genes for durable resistance. 28 accessions of African cultivated rice *Oryza glaberrima* (selected for the Generation *iBridges* project) were screened using two African *Xoo* strains MAI1 and BAI3. A reference IR64 x Azucena RI population made of 172 lines was mapped with the same strains. The accessions were grown under controlled conditions in glasshouse. Leaf clipping inoculation was performed on 5 week-old plants. Phenotypic evaluation was performed 3 weeks after inoculation by measuring leaf lesion length. All *O. glaberrima* accessions were susceptible to BAI3 while CG14, TOG6308 and TOG6356 showed a high resistance to MAI1. QTL mapping based on ANOVA evidenced five putative QTLs common to the two *Xoo* strains and are located on chromosomes 1, 4, 7, 10 and 11 respectively with a major QTL on chromosome 7 explaining 29.8 % of the total variance. Two additional QTLs were strain-specific and were located on chromosome 3 and 8 for strain BAI3 and MAI1 respectively. Most of the QTLs detected differ from those previously characterized.

Topic 2. Determinants of pathogenicity and avirulence in Xoo

The TAL Effectors Tip the Balance of Resistance and Susceptibility in Bacterial Blight of Rice

Bing Yang,

Department of Genetics, Development and Cell Biology 1035C Carver Co-Lab Iowa State University Ames, IA 50011 The United States of America
byang@iastate.edu

The infection of rice by its pathogen, *Xanthomonas oryzae* pv. *oryzae* leads to either compatible (susceptible) or incompatible (resistant) interaction, depending on the molecular interactions between the host factors and often the type III effectors of pathogen. Such interactions can be exemplified by the Philippine strain PXO86 and the rice varieties containing resistant (R) or the otherwise susceptible (S) gene. *avrXa7* in PXO86 elicits a resistant reaction in rice containing the corresponding R gene *Xa7*. On the other hand, *avrXa7* is also a major virulence factor for PXO86 to cause disease (bacterial multiplication and disease development) on the otherwise susceptible rice. AvrXa7 is a member of type III effector family characteristic of transcription activator-like (TAL) features and one of the estimated 16-18 TAL effectors in PXO86. The nuclear localization signal motifs at its C-terminus are required for its avirulence function (ability to elicit resistance responses), so is the C-terminal eukaryotic transcription activation domain. The same requirements apply to its virulence activity (ability to induce a state of disease). Our recent work shows that AvrXa7 up-regulates a few rice genes by the action of its transcription activator like features. *Os11N3*, a relative of *Os8N3* and also a member of MtN3/Saliva family, is one of those transcriptionally activated genes by AvrXa7. The knockdown or silencing of *Os11N3* expression by RNA interfering (RNAi) using a fragment of gene specific to *Os11N3* results in transgenic plants that lost the susceptibility to *avrXa7* dependent strains. The *Os11N3*-silenced plants also lost the susceptibility to *pthXo3* dependent strains as PthXo3 also induces the *Os11N3* gene expression similarly to AvrXa7. However, the *Os11N3*-silenced transgenic plants were still susceptible to strains that use the alternative major virulence factors, such as *pthXo1* or *pthXo2*. Our data suggests another example of gene-for-gene susceptibility which is conferred by a dominant virulence gene in pathogen and a dominant S gene in host plant. Our data also indicates that the TAL effectors (AvrXa7 or PthXo1) play the determining roles for the outcomes of interaction between host and its pathogen in the bacterial blight of rice.

A genetic analysis of virulence functions in *Xanthomonas oryzae* pv. *oryzae*

Alok Pandey, Amit Das and Ramesh V. Sonti

Centre for Cellular and Molecular Biology Uppal Road,
Hyderabad-500007, INDIA
e-mail: sonti@ccmb.res.in

I will provide an overview of our work on identification of various virulence functions of *Xanthomonas oryzae* pv. *oryzae* (Xoo). In particular, I will discuss the role of specific iron uptake functions and adhesin like functions in promoting virulence of Xoo.

Iron uptake functions: We have identified the siderophore (low molecule weight ferric chelator) biosynthetic gene cluster of Xoo. Mutations in this gene cluster lead to total abrogation of siderophore production. Siderophore deficient mutants grow poorly on low iron medium but retain wild type levels of virulence. A mutation in the Xoo homolog of feoB, which encodes the major bacterial ferrous transporter, is severely deficient for virulence. This indicates that FeoB mediated ferrous uptake is essential, while siderophore mediated ferric uptake is not essential for virulence.

Adhesin like functions: We have isolated mutations in the genes for three different non-fimbrial adhesin like functions and a putative fimbrial adhesin of Xoo. The role of these functions in leaf attachment/entry as well as later stages of infection will be discussed.

Regulation of virulence by xrvA in *Xanthomonas oryzae* pv. *oryzae*

J. X. Feng, C. Wang, Y. Q. Wu, Z. Z. Song, C. J. Duan, J. L. Tang

Guangxi Key Laboratory of Subtropical Bioresource Conservation and Utilization, The Key Laboratory of Ministry of Education for Microbial and Plant Genetic Engineering, and College of Life Science and Technology, Guangxi University, Nanning, Guangxi, 530004, P. R. China
Contact email: feng@public.nn.gx.cn

Xanthomonas oryzae pv. *oryzae* (*Xoo*) is the causative agent of rice bacterial leaf blight, a serious disease of rice. It produces copious amounts of extracellular polysaccharide (EPS) that is involved in the virulence of the bacterium on rice. A cosmid clone pGXN3400 from the genomic library of *Xoo* Chinese strain 13751 was found to be able to reduce the production of EPS about 50% and virulence of *Xoo* on rice cultivar Guanggui 110 about 50% when pGXN3400 presented in the cells of strain 13751. The growth of *Xoo* 13751 harboring pGXN3400 in planta was not significantly different from that of *Xoo* 13751. The gene(s) responsible for these effects were located on a 1.3 kb DNA region of pGXN3400 by subcloning and Tn5 mutagenesis. Nucleotide sequence and primer extension analysis of this region revealed the presence of a single gene which we named *xrvA* (for *Xanthomonas*

regulator of virulence).

The *xrvA* ORF (GenBank accession No. X97866) is 402 bp in length and may encode a protein with 133 amino acids. The encoded protein is highly conserved in *Xanthomonas oryzae*, *Xanthomonas campestris* and *Xanthomonas axonopodis*. Protein domain analysis using SMART indicated that XrvA possessed an H-NS (histone-like nucleoid –structuring) domain from amino acid 78 to 121 at its C-terminal, however, only partial sequence of the putative H-NS domain (amino acid 78 to 108 of XrvA) showed highest homology with the DNA-binding protein StpA (H-NS homolog StpA) of *E. coli* at 41% identity and 63% similarity.

A nonpolar mutant GXN1280 of strain 13751 *xrvA* gene was constructed by homologous suicide plasmid integration. The plasmid was constructed by cloning the PCR-amplified DNA sequence containing *xrvA* nucleotide 4 to 255 into a suicide vector pKMob18GII. A complemented strain GXC2088 of the mutant GXN1280 was constructed by introducing a recombinant plasmid pLJxrvA, which carried 402 bp the *xrvA* ORF, 264 bp DNA sequence upstream and 235 bp DNA sequence downstream the ORF in cosmid vector pLAFRJ (as pLAFR3 but the *Hin*dIII-*Eco*RI MCS was replaced by that of pUC18), into the mutant strain GXN1280. The plasmid pLJxrvA was also introduced into the wild-type strain 13751 to form an over-expression strain GXO3098.

Virulence assay on hybrid rice cultivar Teyou 63 by leaf clipping method showed that the virulence of mutant GXN1280 was significantly reduced at high (1×10^{8}cfu/ml) and low (1×10^{5}cfu/ml) inoculation concentrations compared to that of the wild-type strain. The length of the lesions caused by mutant GXN1280 was about 70% of that of the lesions caused by the wild-type strain 14 days post inoculation at both inoculation concentrations. The growth of GXN1280 in rice leaves was similar to that of the wild-type strain 13751. The EPS yield produced by GXN1280 in the shake flask fermentation was about half of that of the wild-type strain. The virulence and the production of EPS of the complemented strain GXC2088 were similar to those of the wild-type strain, confirming that only *xrvA* gene was disrupted in the mutant GXN1280. The production of extracellular endoglucanase and xylanase of the mutant GXN1280 was unaltered compared to the wild-type. The above tested phenotypes for the over-expression strain GXO3098 were similar to those of the mutant GXN1280.

Northern hybridization analysis showed that the expression of *xrvA* in modified *hrp*-inducing medium XOM2 was higher than that in rich medium OB, suggesting that the expression of *xrvA* is induced in limited medium.

The expression of *gumB*, *rpfC*, *hrpG* and *hrpX* genes in the wild-type, *xrvA* mutant GXN1280 and *xrvA* over-expression strain GXO3098 was analyzed by Northern hybridization. It seemed that the expression of *gumB*, *hrpG* and *hrpX* in *xrvA* mutant is apparently lower than that of these genes in wild-type strain, indicating that the

reduction in EPS production and virulence of *xrvA* mutant may partially result from the decreased expression of *gumB*, *hrpG* and *hrpX*. The expression of *gumB* and *rpfC* in over-expression strain GXO3098 is obviously lower than that of these genes in wild-type strain, implying that the reduced production of EPS and virulence of over-expression strain may partially result from the decreased expression of *gumB* and *rpfC*. All these results indicate that *xrvA* is an important regulatory gene that can regulate the expression of several key genes required for EPS biosynthesis or virulence of *Xathomonas oryzae* pv. *oryzae*.

A genetic analysis of virulence functions in *Xanthomonas oryzae* pv. *oryzae*

Alok Pandey, Amit Das and Ramesh V. Sonti

Centre for Cellular and Molecular Biology Uppal Road, , Hyderabad- 500007, INDIA
e-mail: sonti@ccmb.res.in

I will provide an overview of our work on identification of various virulence functions of *Xanthomonas oryzae* pv. *oryzae* (Xoo). In particular, I will discuss the role of specific iron uptake functions and adhesin like functions in promoting virulence of Xoo.
Iron uptake functions: We have identified the siderophore (low molecule weight ferric chelator) biosynthetic gene cluster of Xoo. Mutations in this gene cluster lead to total abrogation of siderophore production. Siderophore deficient mutants grow poorly on low iron medium but retain wild type levels of virulence. A mutation in the Xoo homolog of feoB, which encodes the major bacterial ferrous transporter, is severely deficient for virulence. This indicates that FeoB mediated ferrous uptake is essential, while siderophore mediated ferric uptake is not essential for virulence.
Adhesin like functions: We have isolated mutations in the genes for three different non-fimbrial adhesin like functions and a putative fimbrial adhesin of Xoo. The role of these functions in leaf attachment/entry as well as later stages of infection will be discussed.

Insight into bacterial blight from comparative pathogen genomics and host transcriptomics

Adam Bogdanove

Department of Plant Pathology, Iowa State University, Ames, IA 50011 USA

Comparative analysis of *Xanthomonas oryzae* pv. *oryzae* genomes with those of other xanthomonads can reveal adaptations specific to this pathogen that are important in its ability to colonize rice vascular tissues and cause bacterial blight. Comparative transcript profiling of rice plants infected with X. *oryzae* pv. *oryzae* or its non-vascular counterpart, X. *oryzae* pv. *oryzicola*, which causes bacterial leaf streak of rice, can identify genes and processes important in disease susceptibility, and tissue specificity of infection by the two pathovars. Two X. *oryzae* pv. *oryzae* strains have been sequenced. In collaboration with scientists from around the world, we have mapped the genomes of a third, as well as a strain of X. *oryzae* pv. *oryzicola* and a strain of X. campestris pv. armoraciae. X. campestris pv. armoraciae is the non-vascular counterpart of the vascular pathogen of crucifers, X. campestris pv. campestris, for which two genome sequences also are available. These genomes complete a set of paired xylem and mesophyll pathogens of the leading models for plant biology, rice and Arabidopsis thaliana. Comparative genomics centered on this group of pathogens has revealed the likely pattern of acquisition of specific gene clusters during evolution that play essential roles in interactions with plant hosts, and has hinted at the identity of determinants of host- and tissue-specificity, as well as other adaptive factors. A striking example of the latter is lipopolysaccharide (LPS) for which biosynthetic loci vary greatly across genomes. We have confirmed through mutagenesis and complementation studies, coupled with structural analysis, that the O-chain of LPS of X. *oryzae* pv. *oryzicola* is essential for full virulence, and we have uncovered a functional relationship between LPS and type III secretion system–mediated delivery of bacterial effector proteins into host cells. On the host side, also in collaboration with others, we have discovered distinct patterns of gene expression responses in rice to X. *oryzae* pvs. *oryzae* and *oryzicola*, and demonstrated that these changes correlate with susceptibility, and depend on the function of specific, type III secreted, transcription activator–like effector proteins.

Comparative genomics of *Xanthomonas*: The puzzle of lifestyle

Boris Szurek , Valérie Verdier and Ralf Koebnik

UMR 5096 CNRS-UP-IRD, 911 Avenue Agropolis BP 64501, 34394 Montpellier Cedex 5, France;
Ralf.Koebnik@mpl.ird.fr

Introduction

Key questions concerning the lifestyle of *Xanthomonas* are: What determines the host range, mode of infection, and tissue tropism? What makes the difference between vascular (systemic) and non-vascular (mesophyll) pathogens? Which factors are crucial for the life outside the plant (i.e. epiphytic phase, survival in the soil)?

Materials and Methods

To get an idea about candidate factors of crucial importance for the life style, we performed a detailed comparison of eight *Xanthomonas* genomes, using bioinformatics: Entrez (www.ncbi.nlm.nih.gov/sites/ entrez), Comprehensive Microbial Resource (cmr.tigr.org/tigrscripts/CMR/CmrHomePage.cgi), Pfam (pfam.janelia.org/), Artemis (www.sanger.ac.uk/Software/Artemis/), BLAST (www.ncbi.nlm.nih.gov/ Ftp/), FASTA (www.ebi.ac.uk/fasta33/proteomes.html), Gepard (mips.gsf.de/services/analysis/ gepard), HMMER (hmmer.janelia.org/).

Results and Discussion

Candidate type III-secreted effectors and adhesins were predicted, thus deciphering species-, pathovar-, and strain-specific genes. Differences in their repertoires might contribute to host specificity, tissue tropism, and the mode of infection. Adhesins may also play a role outside the plant (e.g. biofilm formation). Similarly, genome-wide dot plots revealed interesting repetitive regions which correspond to species- and pathovar-specific chemotaxis gene glusters. Different repertoires of sensory chemotaxis proteins might as well play a role in tissue tropism and lifestyle outside the plant.

Strain-specific regions of the two *X. oryzae* pv. *oryzae* strains (*Xoo* KACC10331 and MAFF311018) were detected. These regions are typically associated with transposable elements. We also detected multiple copies of VGR (Val-Gly-repeat)-related proteins as well as a strain-specific prophage. The majority of strain-specific genes are annotated as hypothetical genes. Exceptions are a pseudouridin synthase, a predicted lysine exporter, a polymerase V, and a dGTPase. Interestingly, three of the nine *Xoo* MAFF311018-specific regions are in the vicinity of *avrBs3/pthA* genes.

Of special interest is a newly discovered bacterial defense system, consisting of CRISPR (Clustered Regularly Interspaced Short Palindrome Repeats) regions and *cas* (CRISPR-associated) genes (Ref. 2). We show that CRISPR regions and cas genes are present in the two completely sequenced Xoo genomes but are absent in the only sequenced *X. oryzae* pv. *oryzicola* genome. Interestingly, most of the interspersed sequences are related to *Xanthomonas*-specific bacteriophages. A highly related system is present in only one of the other six completely sequenced xanthomonads, namely in *X. axonopodis* pv. *citri*.

Recently, integrons were reported to be present in all 32 *Xanthomonas* strains tested, representing 12 pathovars of *X. campestris* and *X. axonopodis* (Ref. 1). They were exclusively detected next to the *ilvD* gene encoding dihydroxyacid dehydratase. We show that *X. oryzae* pv. *oryzicola* does not carry an integron next to the *ilvD* gene. Moreover, based on whole genome analyses, we suggest that complex integrons are not typical for *Xanthomonas* species, and that a second integron is present in some xanthomonads next to the *secF* gene. The acquisition of diverse gene cassettes (integrons) and CRISPR regions by different species and pathovars has contributed to

the species-genome diversity of the genus. Their role in adaptation and survival outside the plant is currently unknown.

From these analyses, correlations of specific factors with host- or tissue-specificity are not readily apparent. We therefore speculate that lifestyle is a multifactorial decision and/or is determined by subtle differences in specific genes.

References

Gillings *et al.*, 2005, Proc. Natl. Acad. Sci. USA 102: 4419-4424
Makarova *et al.*, 2006, Biology Direct 1: 7

Identification and functional analysis of a regulatory pathway leading from Tdrxoo/GacAxoo to motility and virulence in *Xanthomonas oryzae* pv. *oryzae*

Jingsheng Xu, Maosen Wu and Chenyang He*

*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China. *E-mail:* cyhe@caas.net.cn

To identify the novel genes involved in Xoo virulence, *gacAxoo*, a *gacApst* homologue was cloned from PXO99^{A} with the degenerated primers by PCR. GacAxoo had the conserved domain of LuxR-like proteins. Δ*gacAxoo*, a deletion mutant was generated after a double cross-over recombination event of marker exchange and validated by PCR analysis. Δ*gacAxoo* is characterized by no significant changes in pathogenicity on rice, HR induction on tobacco and extracellular enzyme production compared to PXO99^{A}. However, motility of Δ*gacAxoo* was reduced on semi-solid medium. Therefore, GacAxoo is involved in the regulation of motility. Tdrxoo, a GacAxoo-interacting protein homological to those with TonB-dependent-receptor domain, is identified by Y2H using GacAxoo as the bait. An extracellular loop to recognize the signal and a β-sheet for transmembrane domains exist in Tdrxoo. Tdrxoo is localized in the outer membrane, receiving unidentified extracellular signals. Δ*tdrxoo*, a disrupted mutant was produced after a single cross-over recombination event. Δ*tdrxoo* lost abilities of causing bacterial blight of rice, and producing the extracellular enzymes and EPS. In addition, motility and *in vitro* growth of Δ*tdrxoo* was significantly reduced. Therefore, a novel Tdrxoo/GacAxoo-containing regulatory system was identified from Xoo in this study. Tdrxoo is probably localized in the outer membrane of bacterial cells, recognizing the signals from extracellular environment, and inducing the intracellular signal transduction. Gacxoo is the downstream component regulating the bacterial motility.

Keywords: *Xanthomonas oryzae* pv. *oryzae*; Tdrxoo/GacAxoo; motility; virulence; regulatory pathway

Identification and functional analysis of a regulatory pathway leading from *Tdrxoo/GacAxoo* to motility and virulence in *Xanthomonas oryzae* pv. *oryzae*

Jingsheng Xu, Maosen Wu and Chenyang He

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China. E-mail: cyhe@caas.net.cn

To identify the novel genes involved in Xoo virulence, *gacAxoo*, a *gacApst* homologue was cloned from PXO99^{A} with the degenerated primers by PCR. GacAxoo had the conserved domain of LuxR-like proteins. Δ*gacAxoo*, a deletion mutant was generated after a double cross-over recombination event of marker exchange and validated by PCR analysis. Δ*gacAxoo* is characterized by no significant changes in pathogenicity on rice, HR induction on tobacco and extracellular enzyme production compared to PXO99^{A}. However, motility of Δ*gacAxoo* was reduced on semi-solid medium. Therefore, GacAxoo is involved in the regulation of motility. Tdrxoo, a GacAxoo-interacting protein homological to those with TonB-dependent-receptor domain, is identified by Y2H using GacAxoo as the bait. An extracellular loop to recognize the signal and a β-sheet for transmembrane domains exist in Tdrxoo. Tdrxoo is localized in the outer membrane, receiving unidentified extracellular signals. Δ*tdrxoo*, a disrupted mutant was produced after a single cross-over recombination event. Δ*tdrxoo* lost abilities of causing bacterial blight of rice, and producing the extracellular enzymes and EPS. In addition, motility and *in vitro* growth of Δ*tdrxoo* was significantly reduced. Therefore, a novel Tdrxoo/GacAxoo-containing regulatory system was identified from Xoo in this study. Tdrxoo is probably localized in the outer membrane of bacterial cells, recognizing the signals from extracellular environment, and inducing the intracellular signal transduction. Gacxoo is the downstream component regulating the bacterial motility.

Key words: *Xanthomonas oryzae* pv. *oryzae*；Tdrxoo/GacAxoo；motility；virulence；regulatory pathway

DNA microarray analysis of transcriptional profiling of Δ*fleQxoo*, a deletion mutants of *Xanthomonas oryzae* pv. *oryzae*

Shiqiang Gao, Jing Zhang, Jingsheng Xu, Maosen Wu and Chenyang He*

*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China. *E-mail: cyhe@caas.net.cn*

FleQxoo, a transcriptional regulator has been showed to be involved in regulation of flagellar motility of *Xanthomonas oryzae* pv. *oryzae* (Xoo) in our previous studies. To identify the regulons of FleQxoo, transcriptional profiles of ∆*fleQxoo*, a deletion mutants and PXO99^A, a wild-type strain of Xoo grown in nutrition-rich medium NBY were revealed by DNA microarray analysis. Sixteen differentially-expressed genes (12 up-regulated and 4 down-regulated) of ∆*fleQxoo* cultured in NBY were found in comparison to PXO99^A. Among the up-regulated genes, there are 4 genes encoding T2SS components, one gene encoding T2SS effector (egl), 3 genes encoding extracellular enzymes (pglA, protease, cellulase S), 3 genes of flagella gene cluster, one GGDEF family gene and one O-antigen biosynthesis gene *rbfC*. These genes may be negatively regulated and suppressed by FleQxoo. Four down-regulated genes are *fliE*, *hrpB4,* one pectinesterase gene and one protease gene. RT-PCR analysis confirmed that *fliE* is obviously a gene positively-regulated by FleQxoo. Mostly importantly, both DNA miroarray analysis and RT-PCR assay shows that *fleQxoo* is highly expressed in ∆*fleQxoo*, while expression of *fleQxoo* is relatively low in PXO99^A. Therefore, results in this study suggest that expression of *fleQxoo* may be self-suppressed.

Keywords: *Xanthomonas oryzae* pv. *oryzae*; ∆*fleQxoo*; transcriptional profiling; regulons; DNA microarray analysis

Transcriptional profiling of ∆*gacAxoo*, a deletion mutants of *Xanthomonas oryzae* pv. *oryzae* revealed by DNA microarray analysis

Shiqiang Gao, Jingsheng Xu, Maosen Wu and Chenyang He*

*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China. *E-mail: cyhe@caas.net.cn*

GacAxoo, a response regulator of two-component system (TCS) of Xoo has been identified in our previous study. To identify the regulons of GacAxoo, transcriptional profiles of ∆*gacAxoo*, a deletion mutants and PXO99^A, a wild-type strain grown in nutrition-rich medium NBY and *hrp*-inducing medium XOM2 were revealed by DNA microarray analysis. Seventeen differentially-expressed genes of PXO99^A grown in XOM2 were identified compared to NBY (10 up-regulated and 7 down-regulated). Seven of up-regulated genes are *hrp* genes (*hrpB*, *hrpB1*, *hrpB3*, *hrpD6* and *hrpF*) and effector genes (*hpa1* and *hpaB*). *hpa1* is one of the most up-regulated genes with an over expression of 13 folds. Twenty-night genes of ∆*gac*Axoo were found to be differentially expressed when grown in XOM2 compared with PXO99^A (17 up-regulated and 12 down-regulated). Seven of up-regulated genes encode hypothetical proteins and one is *vieA* gene. Among 12 down-regulated genes, there are one gene for GGDEF family protein, one T3SS effector gene *hpa1*, 4 genes in flagella gene cluster (*flgC*、*fliC*、*fliO*、*cheA*). Therefore, GacAxoo might influence the bacterial pathogenicity through regulation of *hpa1* and influence motility through

induction of flagella gene expression. Furthermore, GacAxoo may influence the metabolisms of c-di-GMP through regulation of *vieA* and GGDEF family genes, thus modify the phenotypes of Xoo.

Keyword: *Xanthomonas oryzae* pv. *oryzae*; ∆*gacA*xoo; DNA microarray analsis; transcriptional profiling; regulons

Functional analysis of the *avrBs3/pthA* gene family in african *Xanthomonas oryzae* pv. *oryzae* strains

Yanhua Yu1,2, Boris Szurek1,Thierry Mathieu1, Jiaxun Feng2 and Valérie Verdier1
1UMR5096 CNRS/IRD/Université de Perpignan, IRD Montpellier, BP 64501, 34394 Montpellier cedex 5, France.
2Guangxi Key Laboratory for Subtropical Bio-Resource Conservation and Utilization, Nanning, Guangxi, China.

avrBs3/pthA effector genes are unique to the *Xanthomonas* genus, with one exception found in Ralstonia solanacearum. Their copy number is fairly variable, from no gene in some strains up to 28 paralogs in some rice-pathogenic xanthomonads. *AvrBs3/pthA* (also called TAL for Transcription Activator-Like) proteins contain eukaryotic nuclear localization signals and an acidic activation domain at the C terminus. They are thought to facilitate host colonization by altering the host transcriptome. The *avrBs3/pthA* gene family contributes significantly to the pathogenicity of some pathovars, as it is the case for *Xanthomonas oryzae* pv. *oryzae* (Xoo), which carries up to 16 paralogs. Recently, new Xoo races originating from West Africa were characterized, highlighting substantial differences between Asian and African Xoo genomes. Among other specific features, African strains were shown to harbour a reduced set of *avrBs3/pthA*-like genes. A systematic mutagenesis approach has been initiated aiming at deciphering the contribution of each of the 8 paralogs of the African Xoo to its life style and pathogenicity. 16 candidates were characterized, one of which was found to be highly affected in its capacity to lead to typical leaf lesion symptoms.

AvrXa3-mediated recombinants of the *AvrBs3/pthA* gene from *Xanthomonas oryzae* pv.*oryzae* cause pathotypic variation on adult rice lines

G.Y. Zhang, J.Y. Long, R.X. Duan, Y. Zhang, J.H. Zhang, X.Y. Wang, C.F. Song, J.Sh. Wang *
The key Laboratory of Monitoring and Management of Plant Diseases and Insects of Chinese Ministry of Agriculture;Department of Plant Pathology, Nanjing Agriculture University, Nanjing

210095, China.
* Corresponding author: wangjsh@njau.edu.cn

The *avrXa3* comes from JxoIII and belongs to the *avrBs3/pthA* gene family, featured 8.5 copies of 102bp repeat sequence with dual-functions, expressing avirulence on Wase Aikoku 3 (Xa3) and virulence on Cas209 (Xa10) [Li et al. 2004].Complementation of the *avrXa3* gene into PXO99^A, or its defective mutant, PXO99Δ*avr*, produced two genetic recombinants, PXO99/*avrXa3* and PXO99Δ*avr*/*avrXa3*. PXO99Δ*avr* was created by knocking out *avrBs3/pthA* family members from PXO99^A using a transformation unit consisting of pKNG101 and *avrXa3*. Mutant PXO99Δ*avr* had five missing bands 2.9kb, 3.2kb, 3.5kb, 3.9kb and 4.2kb in length that represented the five *avr* genes as *Bp*2.9, *Bp*3.2, *Bp*3.5, *Bp*3.9and *Bp*4.2. Complementation with *avrXa3* did not return PXO99Δ*avr* to the wild-type phenotype. The pathotypes of PXO99Δ*avr*, PXO99/*avrXa3*, and PXO99Δ*avr/avrXa3* were compared to PXO99^A by measuring lesion length on 36 near isogenic lines (NILs) and rice cultivars with *Xoo R* genes. It showed that the virulence of PXO99^A was strengthened by *avrXa3* gene on the rice cultivar Java14,Tetep,IRBB203,Wase Aikoku 3, and reduced on IRBB1, IRBB2, IRBB3, IRBB4, IRBB5, IRBB7, IRBB10, IRBB11, IRBB14, IRBB205, IRBB210, TN-1, Cas209. .*AvrXa*3 is avirulence on adult rice lines with *Xa3*, while some of *Bp*2.9, *Bp*3.2, *Bp*3.5, *Bp*3.9and *Bp*4.2 function as virulence factors. Therefore, *avrXa3* and the five knocked out genes, *Bp*2.9, *Bp*3.2, *Bp*3.5, *Bp*3.9and *Bp*4.2, interfere with each other in PXO99^A and its derivatives. The interaction between PXO99^A and various rice lines indicates that the suite of type III effectors in a given strain may exert functional overlap or redundancy, and individual effector changes may subtly alter bacterial pathogenicity. Many avirulence genes have dual functions, having a role in virulence as well as avirulence.

Molecular identification and characterization of an enolase-phosphatase gene from *Xanthomonas oryzae* pv. *oryzae*

Y. Zhang, G. Y. Zhang, J. H. Zhang, X. Y. Wang, C. F. Song, and J. S. Wang

The key Laboratory of Monitoring and Management of Plant Diseases and Insects of Chinese Ministry of Agriculture;Department of Plant Pathology, Nanjing Agriculture University, Nanjing 210095, China.

Functional analysis of genes in genomic DNA fragment notablely decreasing bacterial virulence is an interesting task. A 4.5-kb DNA fragment of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strain JXOШ ,when introduced into *Xoo* strain PXO99^A, resulted in shorter lesion length on rice leaves. A gene encoding homolog of enolase-phosphatase (E1) was located in this 4.5-kb region, which was named as *xep*. Function of *xep* gene remains unclear. A *xep* mutant, Mxep10, led to 2-3cm decrease of lesion length when inoculated on rice plants leaves, indicating *xep* was dispensable for bacterial virulence. It was proved that xep gene was required for bacterial growth

using MTA as the sole sulfur source. This demonstrated *xep* acted in downstream of MTA in the methionine salvage pathway. Mxep10 decreased the expression of *raxP* gene by 0.64 fold, as shown by real-time quantitative PCR. We hypothesize that the impact of *xep* mutation on expression of *raxP* gene might due to alteration of ultimate amount of upstream metabolites of 1, 2-dihydroxy-3-keto-5-methylthiopentene anion. Phylogenetic analysis indicated that *xep* and its homologs could be divided into three groups. Complexity of species in group B and characteristics of members in small clades implied that its evolution was closely related to environmental nutrients and innate metabolisms. Our works ,in part, excluded the possibility that *xep* gene are main contributor for bacterial virulence in this fragment. The real components confered $PXO99^A$ weak virulence need to be further investigated. However, it still remains possible that *xep* are co-contributor for reducing virulence of $PXO99^A$.

The *Xanthomonas oryzae* pv. *oryzae* RaxST tyrosine sulfotransferase is required for AvrXa21 activity

Sang-Wook HAN, Sang-Won LEE, and Pamela, C. RONALD

Dept. of Plant Pathology, University of California, Davis, CA 95616 USA. swhan@ucdavis.edu

The pathogen recognition receptor XA21 confers immunity against strains of *Xanthomonas oryzae* pv. *oryzae* (Xoo) carrying the AvrXa21 molecule. In previous studies, we identified eight genes that are required for AvrXa21 activity (rax) in Xoo. Expression of these rax genes depends on population density and other functioning rax genes (Figure 1; Lee, et. al., 2006). These data suggest that AvrXa21 is involved in quorum sensing, and that AvrXa21 represents a previously uncharacterized class of Gram-negative bacterial signaling molecules. To determine the molecular basis of the AvrXa21/XA21 interaction and to further understand the role of AvrXa21 in quorum sensing, we have characterized raxP, raxQ, and raxST genes encoding proteins involved in the sulfation pathway (Goes da silva, et. al., 2004). Here, we show that AvrXa21 activity in a raxQ knockout (KO) strain is complemented by exogenous PAPS and that the raxST gene or its ortholog is required for AvrXa21 activity in X. campestris pv. campestris (Xcc) and X. c. pv. vesicatoria (Xcv). We also demonstrate, using 35S-Na2SO4, that the sulfated molecule(s) is secreted via the RaxABC Type I secretion system. To monitor enzymatic activity of the raxST product, we developed an enzyme coupling assay. Using this assay, we demonstrate that the RaxST protein has tyrosine sulfotransferase activity and can utilize a synthetic peptide, previously shown to be a substrate for human tyrosine sulfotransferases. This is the first report of a bacterial sulfotransferase with tyrosine sulfotransferase activity. Furthermore, our data suggest that Xoo RaxST utilizes the PAPS produced by RaxP and RaxQ to

transfer a sulfuryl group to a target molecule, possibly the AvrXa21 PAM.

The type-III protein hrpA affects colonization of *Xanthomonas oryzae* pv. *oryzae* on rice

H. Liu, T. Song, D. Wang, D. Chen, F. Liu and H. Dong

Department of plant pathology, College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China

HrpA and HrpF are important type-III proteins in *Xanthomonas oryzae* pv. *oryzae* (Xoo). Mutagenesis in both genes was done with the wild-type (WT) Xoo strain PXO99 by single recombination, generating two mutants, AOS and FOS, which both diminished pathogenicity on rice. Complementing AOS and FOS with the pUFR034 vector containing WT *hrpA* and *hrpF*, respectively, produced complementary strains cAOS and cFOS. They partially restored AOS and FOS pathogenicity on rice. The *hrpA* mutant AOS completely lost virulence to the isogenic rice variety IR24 and failed to induce the hypersensitive response (HR) in tobacco. The virulence of FOS to IR24 also was markedly decreased, but the induction of HR was not affected evidently. Then, the PXO99, AOS, FOS, cAOS, and cFOS were labeled by GFP to visualize bacterial localization in the plant. Inoculation tests showed that the virulence of these GFP strains was similar to that of unlabeled strains. Fluorescence microscopy and electron microscopy revealed that AOS cells dispersed over epidermal surfaces, but PXO99 and cAOS multiplied and congregated over the aquaporin on IR24 leaves. Based on these results, *hrpA* plays an important role in localization of rice by the pathogen. Inversely, *hrpF* gene do not obviously affect bacterial congregation on the plant.

A conserved *hpa2* gene encoding a protein of lytic activity to bacterial cell wall in phytopathogenic *Xanthomonas oryzae*

Jiahuan Zhang [a], Xiaoyu Wang [a],Yan Zhang [a], Guiying Zhang [a], Congfeng Song [a], Jinsheng Wang [a, *]

[a] *Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China*

The *hpa2* is a gene of *hrp* cluster that function is associated with hypersensitive response and pathogenicity. Five strains all had the 712 bp *hpa2* gene including 148 bp promoter and 564 bp open reading frame in pv.*oryzae* and pv.*oryzicola* of *Xanthomonas oryzae*. Hpa2 protein encoded by a *hpa2* from *Xanthomonas oryzae* pv. *oryzae* PXO99, one of the five resulting strains, was first successfully expressed in *E. coli* BL21 (DE3). Hpa2 contained putative 187 amino acids, and was putative molecular weight of 20.8 kDa. Content of general acid-base catalysis residue in the

Hpa2 was close to those in 70 kDa soluble lytic transglycosylase and Enterobacteria phage T4 Lysozyme, respectively. A trusted soluble lytic transglycosylase domain was found between the residue 65 and 177 in Hpa2 protein. After selected bacterial strains were treated by the Hpa2, optical density values of bacterial solutions distinctively decreased, and cell wall of gram-positive bacterium *Micrococcus luteus* dramatically represented rough and waved, even severely disrupted shape. The findings suggest that the Hpa2 protein display lytic activity, and might help assembly of the type III secretion system by enlarging gaps in the peptidoglycan meshwork.

Analysis of virulence and growth of a purine auxotrophic mutant of *Xanthomonas oryzae* pathovar *oryzae*

Young-Jin Park1, Eun-Sung Song1, Yeong-Tae Kim1, Tae-Hwan Noh2, Hee-Wan Kang3 & Byoung-Moo Lee1

1National Institute of Agricultural Biotechnology, Rural Development Administration, Suwon, Korea; 2Honam Agricultural Research Institute, Rural Development Administration, Iksan, Korea; and 3Graduate School of Bio & Information Technology, Hankyong National University, Ansung, Korea

Xanthomonas oryzae pv. *oryzae* (Xoo) causes bacterial blight of rice. A random insertional mutant library of Xoo KACC10331 was constructed using a Tn5-derived transposon, and the virulence of the mutants against the susceptible rice cultivar IR24 was assayed. After the virulence assay, the M793 (purD::Tn5) mutant that had reduced virulence against the rice plants was isolated. Thermal asymmetric interlaced-PCR and sequence analysis revealed that the transposon was inserted into the purD gene (encodes a phosphoribosylamine-glycine ligase) of the M793 mutant. The reverse transcriptase-PCR assay revealed that the mutation of the purD gene did not affect the expression of other purine biosynthesis genes. However, the M793 mutant required exogenous purines and thiamine for growth in minimal media. These results indicate that the purD gene plays a crucial role in the growth and virulence of Xoo.

Zinc uptake regulator (*zur*) gene involved in zinc homeostasis and virulence of *Xanthomonas oryzae* pv. *oryzae* in rice

Wanfeng Yang[a,b,1], Yan Liu[a,1], Lei Chen[a], Tongchun Gao[b], Baishi Hu[a], Dongfang Zhang[b], Fengquan Liu[a]

[a] *Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, Republic of China*
[b] *Institute of Plant Protection, Anhui Academy Agricultural Sciences, Hefei 230031, Republic of China*

[1] *These authors contributed equally to this work.*

Correspondence to: F.Q. Liu; *email:* fqliu20011@yahoo.com.cn

Abstract *Xanthomonas oryzae* pv. *oryzae* causes bacterial leaf blight, one of the most widespread and destructive bacterial diseases in rice. In order to understand the gene of zinc uptake regulator (*zur*) involving in virulence of the pathogen in rice, we generated a mutant OSZRM by homologous suicide plasmid integration. The mutant failed to grow in NYGB medium supplemented with Zn^{2+} or Fe^{3+} at a concentration of 500 µM or 6 mM, whereas the wild-type strain grew well at the same conditions. The *zur* mutant was hypersensitive to hydrogen peroxide and exhibited reduction catalase activity and the production of extracellular polysaccharide (EPS). Interestingly, the mutant showed a reduction in virulence on rice but still kept triggering hypersensitive response (HR) in tobacoo. When the mutant was complemented with the *zur* gene, the response was recovered to wild-type. These results suggested that *zur* gene is a functional member of the Zur regulator family that controls zinc and iron homeostasis, oxidative stress, and EPS production, which is necessary for virulence in *X. oryzae* pv. *oryzae*.

Involvement of gluconeogenic pathway in virulence of *Xanthomonas oryzae* pv. *oryzae*

Wanfeng Yang[a], Yan Liu[a], Lei Chen, Guoliang Qian, Hongxia Liu, Baishi Hu, Fengquan Liu

[a]These authors contributed equally to this work

Authors' address: Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China (correspondence to Fengquan Liu. E-mail: fqliu20011@yahoo.com.cn)

Wanfeng Yang. E-mail: ywf307@163.com; Yan Liu. E-mail: 2004102044@njau.edu.cn

Keywords: gluconeogenesis, phosphoenolpyruvate synthase, virulence, *Xanthomonas oryzae* pv. *oryzae*

Abstract
Xanthomonas oryzae pv. *oryzae* causes bacterial leaf blight, one of the most widespread and destructive bacterial diseases of rice. A phosphoenolpyruvate synthase (*ppsA*) disrupted mutant OSPAM was generated by homologous suicide plasmid integration. The mutant was unable to grow in medium with pyruvate or

C_4-dicarboxylates as the sole carbon source, compared to wild-type, indicating a disruption in *ppsA* function. The mutant showed a reduction in virulence on rice but still induced a hypersensitive response in tobacoo. When the mutant was complemented, the response was recovered to wild-type. These results suggested that *X. oryzae* pv. *oryzae* possesses only PPSA route in gluconeogenesis, which is necessary for virulence.

Xanthomonas oryzae pv. *oryzae* twin-arginine-dependent translocation is important for virulence, flagellation, and chemotaxis

L.Chen, W.F.Yang, G.L.Qian, B.Han, B.S.Hu and F.Q.Liu*

Lab of Immunology & Quarantine, Department of plant pathology, College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China

This study characterized the contribution of the twin-arginine translocation (TAT) pathway to growth, motility, and virulence of the phytopathogen *Xanthomonas oryzae* pv. *oryzae*. *tatB* and *tatC* disruption mutants were successfully generated by a single cross-over event, and confirmed by PCR and Southern blotting. In contrast to wild-type strain PX099, the *tatB* and *tatC* null mutant failed to export the green fluorescent protein fused to the trimethylamine *N*-oxide reductase (TorA) signal sequence. The *tat* mutants displayed defects in growth rate when grown in minimal medium. PX099 cells were highly motile in both rich and minimal media. By contrast, the *tatB* and *tatC* mutations impaired motility. *tatB* and *tatC* mutant cells lacked detectable flagella and *tat* mutant cells failed to exhibit chemotaxis toward sugars under aerobic conditions. *X. oryzae* pv. *oryzae* PX099 *tatC* mutant showed a reduction in ability to elicit the hypersensitive response (HR) when compared with the wild type. Our findings establish that, in addition to its role in secretion of folded cofactor-bound enzymes functioning in alternative respiration, the TAT system of *X. oryzae* pv. *oryzae* is an important virulence determinant. Furthermore, this secretion pathway contributes to flagellar biogenesis and chemotactic responses.

Induction of crg gene expression *Xanthomonas oryzae* pv. *oryzae* by H2O2 generated during bacterial interactions of rice suspension-cultured cells or applied exogenously

Jianbo Zhou[1, 2], Maosen Wu[1], Jun Hu[2] and Chenyang He[1]

[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094 China; [2]Department of Plant Protection, Neimenggu Agricultural University, Huhehaote 010019, China. E-mail: cyhe@caas.net.cn

To elucidate the roles of hydrogen peroxide (H_2O_2) produced during the interaction of rice suspension-cultured cells with *Xanthomonas oryzae* pv. *oryzae* (Xoo) or applied exogenously in inducing expression of bacterial catalase-related gene (*crg*), H_2O_2 production and *crg* expression during the rice–Xoo interactions, in which either H_2O_2 scavenge catalase (CAT) was exogenously added or not, was quantitatively analyzed. *In vitro* growth and *crg* expression of Xoo exposed by exogenously-applied H_2O_2 was quantitatively examined as well. Significant increases in H_2O_2 content and *crg* expression was observed during the interaction, while reduction in H_2O_2 concentration and *crg* expression was obviously found when CAT was exogenously added to the rice–Xoo interacting system. *In vitro* growth was inhibited by exogenously-applied H_2O_2 in a dosage manner, which strongly induced the expression of Xoo *catB* and *srpA*. H_2O_2 production was resulted from the rice–Xoo interaction, and Xoo *crg* expression was significantly induced by H_2O_2 either produced during the interaction or added exogenously. Therefore, results in this study suggest that Xoo possess one of novel pathogenic pathways, through which the pathogen can degrade H_2O_2 and suppress H_2O_2-mediated hypersensitive response in rice.

Keywords: *Xanthomonas oryzae* pv. *orzae*; H_2O_2; catalase-related gene; quantitative analysis

Characterization and identification of genes involved in biosynthesis of LPS O- antigen and exopolysaccharide from lipopolysaccharide biosynthesis gene cluster in *Xanthomonas oryzae* pv. *oryzae*

Ji-Chun Wang [1], Byeong-Ho So [1], Byoung-Moo Lee [2], Young -Jin Park [2] and Hee-Wan Kang[1] *

1. Graduate School of Biotechnology and Information Technology, Hankyong National University, Ansung 456-749, Korea

2. National Institute of Agricultural Biotechnology, Suwon 441-707, Korea

*Corresponding author: kanghw2@hknu.ac.kr

Abstract

Virulence deficient mutants on rice have been isolated from a transposon mutant library of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) KACC 10331. Mutant strains with ransposon insertion in *cys*B-*met*B-*smt*A-*wxo*ABCD of LPS gene cluster were isolated and characterized. Mutant strains of the *cysB-metB-smtA-wxoABC* genes excepting *cysB* and *wxoD* were virulence deficient on susceptible rice leaves. Southern blot hybridization and PCR analysis showed that the genes were mostly present in a number of *Xoo* strains, but not in some *Xoo* strains and *X. oryzae*.pv. *oryzicola* strain. The smtA that is homologous to methytransferase FkbM,and rfbT proteins is designated as *xomt*A and its function has been remained to be elucidated. In addition, *cysB-metB-xomtA* genes shows high homology to gene related to methionine synthesis.

The *metB-xomtA-wxoABC* mutant strains shows reduced mucoidy colony morphology and relatively reduced EPS amount in comparison to that of the wild type strain. LPS bands of high molecular that could potentially be identified as O-antigen on SDS-PAGE are lost in *metB-womtA-wxoABC* mutant strains, when compared to wild-type strains.

Expressional regulation of of *metB* and *xomtA*, Lipopolysaccharide O-antigen biosynthesis related genes in *Xanthomonas oryzae* pv. *oryzae*

Ji-Chun Wang [(1)], Byeong-Ho So [(1)], Byoung-Moo Lee [(2)], Young -Jin Park [(2)] and Hee-Wan Kang[(1)] *

1. Graduate School of Biotechnology and Information Technology, Hankyong National University, Ansung 456-749, Korea

2. National Institute of Agricultural Biotechnology, Suwon 441-707, Korea

*Corresponding author: kanghw2@hknu.ac.kr

It was demonstrated that the *met*B and *xomt*A genes were involved in the biosynthesis of LPS O-antigen and exopolysaccharide production, and located in upstream the related LPS synthesis genes. In this study, the regulations of *metB* and *xomtA* genes were investigated at transcriptional and translational levels. The Reverse transcriptase polymerase chain reaction (RT-PCR) results showed *wxoABCD* genes were regulated by the *xomt*A-*met*B expression. Transcriptional expression level of *wxoABCD* in *xomtA* mutant were relatively reduced in comparison to wild type strain, but not in LPS related genes such as *wzt, wzm* and *etfA*， which were located in the same region. The *xomt*A-*met*B genes were overexpressed in *E. coli* system using pET-15b vector. The fused MetB and XomtA proteins were purified and used for raising polyclonal antibodies in rabbit. Western blot analysis using anti-XomtA-antibody identified a 28kDa protein in cytoplasmic and membrane fraction from wild type strain, indicating feature of transmembrane protein, but not in them from *xomt*A and *met*B mutant. In addition, Anti-wxoC-antibody detected the 45kDa protein band in wild type strain, but not in *xomt*A and *met*B mutants, suggesting xomtA and metB regulate the expression of wxoC protein.

Functional analysis of Type IV pili system related *pilQ* gene of *Xanthomonas oryzae* pv. *oryzae*

[1]Byoung-Ho So, [1]Sun-Hwa Lim, [1]Ji-Chun Wang 2Byoung-Moo Lee, and [2]Young-Jin Park,[1]Hee-Wan Kang*.

1Graduate School of Biotechnology and Information Technology,

Hankyong National University, Ansung 456-749, Korea,
2National Institute of Agricultural Biotechnology, Suwon 441-707, Korea.
* Corresponding author (kanghw2@hknu.ac.kr)

Bacterial blight (BB) of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is the most devastating bacterial disease on rice. Type IV pili are flexible, filamentous structure protruding from the cell surface of gram-negative bacteria and twenty seven pili genes were identified in different regions of *Xoo* genome. A virulence deficient *pilQ* mutant strain, KACC10331Δ*pil*Q was selected from a mutant library constructed by transposon insertion. The PilQ has been known as a multimeric outer membrane protein that formes gated pores, through which the pilus is excluded. In *Xoo*-*pil*Q mutant analysis, it was revealed that the *pi*l*Q* gene was associated with cell aggregation, biofilm formation and twitching motility. The *pilQ* encoding protein showed high homology of more than 70% to *pilQ* genes from two *Xanthomonas* species, but was low sequence homology of 35% to other bacterial species. Total RNAs was extracted from wild type and KACC10331Δ*pil*Q. Transcriptional expression profiles of *pilQ* regulated genes were analyzed on *Xoo*-DNA microarray. The *pilQ* is a member of gene cluster *pilMNOPQ* in *Xoo* genome. The pili gene cluster region was cloned, mutated by transposon and marker exchanged to wild type strain (KACC10859) and mutant strains of the pili genes were tested for pathogenecity.

Development of a RT-Q-PCR targeting *lipAxoo* and *purHxoo* for quantification of bacterial infection process of rice by *Xanthomonas oryzae* pv. *oryzae*

Lei Sun, Maosen Wu and Chenyang He*

*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China. *E-mail:* cyhe@caas.net.cn

To establish the novel molecular quantitative assays for quantification of bacterial population of *Xanthomonas oryzae* pv. *oryzae* (Xoo) *in planta*, real-time quantitative polymerase chain reaction (RT-Q-PCR) assay based on SYBR Green I technology was developed to target *lipAxoo* and *purHxoo* for the quantification of *in planta* growth of Xoo. The changes in bacterial population density *in planta* measured by RT-Q-PCR assay is similar to those assessed by bacterial plate counting. There is no significant difference between the two primer sets evaluated for RT-Q-PCR. Bacterial accumulation within rice showing no disease symptoms was observed at 3 d post-inoculation (dpi), then the bacterial density within rice increased significantly 5 dpi with rising bacterial leaf blight, and bacterial numbers reached a peak and maintained a high population 9-14 dpi when the plants displayed severe disease symptoms. Such a relation between bacterial population density *in planta* and host plant disease progression might be associated with quorum sensing of the pathogen.

The results of this study illustrate that RT-Q-PCR can be successfully used to directly and accurately quantify Xoo within leaf tissues of rice. Furthermore, "bacterial target gene copies—total DNA amount—bacterial population—host disease progression", a model of the pathogen assessment has been proposed for accurate monitoring of bacterial infection process of rice by Xoo, which might be applicable to the molecular quantification of other bacterial and fungal diseases of rice.

Keywords: *Xanthomonas oryzae* pv. *oryzae*; rice; *lipAxoo*; *purHxoo*; real-time quantitative PCR; bacterial population *in planta*

Xanthomonas oryzae pv. *oryzae* crg gene expression inducted by H_2O_2 generated during bacterial interactions of rice cells or applied exogenously

Jianbo Zhou[1, 2], Maosen Wu[1], Jun Hu[2] and Chenyang He[1]*

[1]*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094 China;* [2]*Department of Plant Protection, Neimenggu Agricultural University, Huhehaote 010019, China. *E-mail: cyhe@caas.net.cn*

To elucidate the roles of hydrogen peroxide (H_2O_2) produced during the interaction of rice suspension-cultured cells with *Xanthomonas oryzae* pv. *oryzae* (Xoo) or applied exogenously in inducing expression of bacterial catalase-related gene (*crg*), H_2O_2 production and *crg* expression during the rice–Xoo interactions, in which either H_2O_2 scavenge catalase (CAT) was exogenously added or not, was quantitatively analyzed. *In vitro* growth and *crg* expression of Xoo exposed by exogenously-applied H_2O_2 was quantitatively examined as well. Significant increases in H_2O_2 content and *crg* expression was observed during the interaction, while reduction in H_2O_2 concentration and *crg* expression was obviously found when CAT was exogenously added to the rice–Xoo interacting system. *In vitro* growth was inhibited by exogenously-applied H_2O_2 in a dosage manner, which strongly induced the expression of Xoo *catB* and *srpA*. H_2O_2 production was resulted from the rice–Xoo interaction, and Xoo *crg* expression was significantly induced by H_2O_2 either produced during the interaction or added exogenously. Therefore, results in this study suggest that Xoo possess one of novel pathogenic pathways, through which the pathogen can degrade H_2O_2 and suppress H_2O_2-mediated hypersensitive response in rice.

Keywords: *Xanthomonas oryzae* pv. *orzae*; H_2O_2; catalase-related gene; quantitative analysis

Preliminary Study on avirulence genes of *avrBs3/pthA* family in PXO99

Ruixu Duan; Juying Long; Congfeng Song, Jinsheng Wang,
[a]*Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China*
*Corresponding author

The interactions between *Xanthomonas oryzae* pv. *Oryzae* (Xoo) and rice are controlled in a gene-for-gene manner. In this study, a 2.67kb- *avrXa*3 gene fragment from *Xoo* JXOIII was used as a probe to screen the genomic library of the PXO99^{A} strain of Xoo. The results demonstrated that diverse members of the *avrBs3/pthA* family exist in the form of isolated individual portions or clusters in PXO99^{A} genome. Clone P15.5 was selected for further analysis according to the Southern blot results. It located outside the *avrBs3/pthA* cluster. Sequencing analysis showed, it contains15.5 copies of the 102 bp repeat, which is same with that of *avrXa10* isolated from PXO86. The 12th and 13th amino acids in variable region differed from each other. The further functional analysis of P15.5 are going on.

A deletion mutant PXO△avr was created by inducing Chloramphenicol resistance gene inserted *avrXa3* into PXO99^{A}. Southern blot results showed PXO△avr differed from PXO99^{A} with a 2.9kb,3.3kb, 3.5kb,4.1kb,4.3kb band disappeard. Each of these deleted 5 genes has the same size with that in the previously identified gene cluster correspondingly which containing avrXa27 from PXO99A (Gu et al 2005; Yang et al 2004). By screening the genomic library of PXO99^{A}, we obtained clone P5 which contained the deleted 5 gene cluster. It will be interesting to characterize these genes and their function.

Topic 3. Molecular and physiological aspects in rice resistance to bacterial blight

Insights into the rice defense response

P. Ronald

Genome Center, GBSF, 5th floor (5212A),UC Davis ， One Shields Avenue, Davis, CA 95616

Innate immunity provides a first line of defense against pathogen attack and is activated rapidly following infection. The plant innate immune system uses a set of defined receptors for pathogen recognition. While it is now widely appreciated that these receptors play a key role in innate immunity in plants and animals, very little is known about the downstream signal transduction cascade or about the bacterial molecules recognized by such receptors. A long-term goal of my laboratory is to elucidate the role of bacterial and plant signaling molecules governing the rice/ *Xanthomonas oryzae* pv. *oryzae* interaction.

We have shown that the rice XA21 receptor, detects a pathogen associated molecule (AvrXa21) produced by *Xanthomonas oryzae* pv. *oryzae*.

We have also shown that AvrXa21 activity requires the presence of *Xoo rax* (required for AvrXa21 activity) genes. Expression of *rax* genes is dependent on population density, two component regulatory systems, virulence and other functioning *rax* genes. These data suggest a model where XA21 has evolved to recognize AvrXa21, a bacterial signaling molecule critical for cell-cell communication.

Induction and suppression of host innate immunityin *Xanthomonas*-rice interactions

Gopaljee Jha, Hitendra Kumar Patel, R. Rajeshwari and Ramesh V. Sonti,

Centre for Cellular and Molecular Biology，Uppal Road, Hyderabad-500007，INDIA
e-mail: sonti@ccmb.res.in

Xanthomonas oryzae pv. *oryzae* (Xoo) uses a type II secretion system (TIIS) to secrete hydrolytic enzymes such as a cellulase, cellobiosidase and lipase/esterase. The TIIS as well as the individual secreted enzymes are important for Xoo virulence, presumably because they are needed to degrade rice cell walls. Conversely, these cell wall degrading enzymes induce several host defense responses including a hypersensitive response (HR) like reaction. The defense responses that are induced by the cell wall degrading enzymes are so potent that prior treatment with these enzymes will immunize rice against subsequent Xoo infection (Jha et al 2007). The suppression

of these innate immune responses of the host is crucial for the ability of Xoo to cause disease. It accomplishes this task using effectors that are secreted through the Type III secretion system (TIIIS).We are interested in understanding how cell wall degrading enzymes induce rice innate immunity and in identifying the TIIIS effectors that suppress this innate immunity. Results from microarray analysis will be presented which suggest that the jasmonic acid mediated pathway is involved in elaborating the HR like response that is induced by cell wall degrading enzymes.

Jha et al 2007. Molecular Plant-Microbe Interactions. 20: 31-40

Identification of rice genes differentially-regulated by *Xanthomonas oryzae* pv. *oryzae* by cDNA-AFLP: functional analysis of an induced transcription factor OsBTF3

Maosen Wu, Jing Wu, Weizhi Liu, Yanan Bai, Guangxu Li and Chenyang He*

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China.
**E-mail:* cyhe@caas.net.cn

To elucidate the gene expression response of rice to bacterial inoculation with *Xanthomonas oryzae* pv. *oryzae* (Xoo) at the genomic levels, cDNA-AFLP was used to analyze the gene expression patterns of mock- and Xoo-inoculated rice suspension cultured cells. Three hundred and sixty-three (9.1%) from approximately 4,000 cDNA fragments analyzed were differentially regulated (295 up-regulated and 68 down-regulated) after co-cultivation with Xoo. Seven gene expression patterns of the differential fragments were identified. Ten of 31 sequenced fragments showed homology to genes with known or putative functions involved in metabolism, pathogen response and signaling. 21 others did not show any homology to sequences with known functions. cDNA-AFLP differential patterns for 5 selected genes were confirmed via real-time reverse transcription PCR analysis. Thus, a set of differentially-expressed genes of rice was identified from the transcriptional profiling in response to Xoo via cDNA-AFLP analysis.

OsBTF3 is one of the up-regulated genes by Xoo both in the suspension-cultured cells and plants of rice. *OsBTF3* is located in the chromosome 3 with 5 exsons and 4 introns, encoding a 175 aa protein with a NAC domain. *OsBTF3* is amplified by RT-PCR, cloned and sequenced. The subcellular localization of OsBTF3 using the GFP fusion indicates it is mainly present in the nuclei in the onion epidermal cells. The over-expression and RNAi constructs of *OsBTF3* were transformed into rice (cv. Nipponbare) callus via Agrobacterium-mediated gene transformation. The plantlets resistant to hygromycin B were regenerated to be T_0 generation of transgenic plants, which is confirmed by PCR and GUS assay. T_0 transgenic plants with over-expression constructs of *OsBTF3* is higher, while those with RNAi constructs is shorter than the non-transgenic plants, indicating the growth and

development of rice might be influenced by OsBTF3.

Keywords: rice; *Xanthomonas oryzae* pv. *oryzae*; differentially-expressed genes; OsBTF3; functional analysis

Comparative analysis of nitric oxide generation and induction of defense gene expression by *Xanthomonas oryzae* pv. *oryzae* and *X. campestris* pv. *vesicatoria* of rice suspension-cultured cells

Wei Meng[1, 2], Jingzhi Wen[2], Maosen Wu[1] and Chenyang He[1]

[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094 China; [2]Department of Plant Protection, Northeastern Agricultural University, Haerbin 150030, China. E-mail: cyhe@caas.net.cn

To elucidate a key role for nitric oxide (NO) played during rice-*Xanthomonas* spp. interactions by triggering hypersensitive cell death (HCD) and inducing defense genes, NO production and defense gene expression in rice suspension-cultured cells was compared after inoculations with *X. oryzae* pv. *oryzae* (Xoo), the bacterial blight pathogen and *X. campestris* pv. *vesicatoria* (Xcv), a non-host bacterial pathogen of rice. NO burst, activation of nitric oxide synthase (NOS), expression of NO synthesis-related genes (*nos* and *nr*) and defense-related genes (*pal*, *pox* and *gst*) was found to be significantly induced by Xcv, not by Xoo. Application of NO scavenger PTIO or co-inoculation of Xcv with Xoo resulted in the significant suppression of Xcv-induced gene expression. In conclusion, Xcv induces defense gene expression and HCD of rice suspension cultures through a NO-dependent signal pathway, whereas Xoo inhibits NO-mediated defense gene expression and HCD by suppressing NO production and detoxifying NO.

Keywords: rice; *Xanthomonas* spp.; nitric oxide; defense gene expression; hypersensitive cell death

Nitric oxide generation and defense gene expression induced by *Xanthomonas oryzae* pv. *oryzae* and *X. campestris* pv. *vesicatoria* of rice suspension-cultured cells

Wei Meng[1, 2], Jingzhi Wen[2], Maosen Wu[1] and Chenyang He[1]*

[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094 China; [2]Department of Plant

*Protection, Northeastern Agricultural University, Haerbin 150030, China. *E-mail: cyhe@caas.net.cn*

To elucidate a key role for nitric oxide (NO) played during rice-*Xanthomonas* spp interactions by triggering hypersensitive cell death (HCD) and inducing defense genes, NO production and defense gene expression in rice suspension-cultured cells was compared after inoculations with *X. oryzae* pv. *oryzae* (Xoo), the bacterial blight pathogen and *X. campestris* pv. *vesicatoria* (Xcv), a non-host bacterial pathogen of rice. NO burst, activation of nitric oxide synthase (NOS), expression of NO synthesis-related genes (*nos* and *nr*) and defense-related genes (*pal*, *pox* and *gst*) was found to be significantly induced by Xcv, not by Xoo. Application of NO scavenger PTIO or co-inoculation of Xcv with Xoo resulted in the significant suppression of Xcv-induced gene expression. In conclusion, Xcv induces defense gene expression and HCD of rice suspension cultures through a NO-dependent signal pathway, whereas Xoo inhibits NO-mediated defense gene expression and HCD by suppressing NO production and detoxifying NO.

Keywords: rice; *Xanthomonas* spp.; nitric oxide; defense gene expression; hypersensitive cell death

Topic 4. Population structures and diversity of pathotypes and genotypes of Xoo

Genomics and genetic diversity of Korean *Xanthomonas oryzae* pv. *oryzae*

Lee, Byoung-Moo[1], Young-Jin Park[1], Eun-Sung Song[1] and Tae-Hwan Noh[2]

[1]*National Institute of Agricultural and Biotechnology, RDA, Suwon, 441-707, Korea.*
[2]*Honam Agricultural Research Institute, Iksan, 570-080, Korea*

Xanthomonas oryzae pv. *oryzae* (*Xoo*) causes bacterial blight (BB) in rice. The strains of *Xoo* have been classified into many races based on their pathogenic type. Each country has its own differential system of Xoo. Therefore, the differential cultivars used for race differentiation are different depending on the country. Korean *X. oryzae* pv. *oryzae* are classified into five races based on race differential system..

Recently, a new Xoo race is isolated from faddy field in Korea. And this new race is not included in Korean Xoo race differential system. Furthermore, the genetic analysis of Korean Xoo revealed that Xoo race classified by old differential system and their genetic patterns did not correspond. Therefore, we are now trying to set up a new monogenic line and to find new genetic markers including avr and IS elements for Korean Xoo race differential system.

Pathotype diversity among Indian isolates of the bacterial leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae*

Deo Mishra[1], Kotilingam Konda[1], Yog Raj[1], Ninh Thuan Nguyen[2], and Ed Roumen[2]
[1]Bayer CropScience, Hyderabad, INDIA, and [2]Bayer CropScience, SINGAPORE

Address of presenting author
8-1-39, Qutub Shahi Tombs Road, Tolichowki, Hyderabad -08 (AP)
e-mail: deos.mishra@bayercropscience.com

Abstract

Developing durably resistant cultivars to bacterial leaf blight (BLB) requires an understanding of both the virulence diversity within the pathogen and its dynamics, and the genetics of host resistance. We report here the responses of BLB differential cultivars carrying a number of well known resistance (R) genes and R-gene combinations against Indian isolates of *Xanthomonas oryzae* pv. *oryzae* (Xoo). A total of 350 isolates obtained from leaf samples collected from 18 major rice growing states between 2004 and 2006, were inoculated onto nine IR24 based near isogenic lines (NILs) (IRBB-1, 3, 4, 5, 7, 8, 10, 13, & 21) and seven gene stacks (IRBB-52, 54, 55, 57, 58, 59 & 60) at 3 different plant growth stages. Analysis of the resulting infection types revealed the presence of at least 10 major pathotypes, defined as being

distinct and repeatable virulence patterns. The results further revealed that isolates obtained from any particular location in any given year typically represented a single pathotype in more than 90% of cases. Considering the individual R-genes, xa13 appeared as the most broadly effective, conferring resistance against 84% of the isolates, regardless of plant growth stage, followed in decreasing order by Xa21(50%), xa8 (42%), xa5 (24%), Xa7 (12%) and Xa4 (7%). Further, Xa21 and xa8 displayed a strong residual effect when defeated, the magnitude of which also varied with plant stage. To part of the pathotypes, but not all, a lesser degree of residual resistance was also observed for Xa4 and xa5. No residual effects whatsoever were observed for Xa1, Xa3, Xa7, Xa10 and xa13. Considering R-gene combinations, a synergistic effect towards increased resistance was observed when Xa4 or xa5 was combined with Xa21. Whereas, a combination of xa13 and Xa21 showed such synergy only when xa13 was resistant. The gene combination xa5+xa13+Xa21 provided complete resistance against all isolates tested thus far.

Analysis of pathotypic and genotypic diversity of *Xanthomonas oryzae* pv. *oryzae* in China

Li Gang, Congfeng Song, Xiaomei Pan, Yang Yue, Jinsheng Wang*
The key Laboratory of Monitoring and Management of Plant Diseases and Insects of Chinese Ministry of Agriculture; College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, JiangSu, China. Telephone: +86-25-843-96178; Fax: +86-25-843-96178;
*Corresponding author; Jinsheng Wang; E-mail address: wangjsh@njau.edu.cn

Diversity of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) based on pathotype and haplotype, were analyzed of isolates collected from major rice-growing provinces in China. A total of 103 isolates were classified into 61 pathotypes based on their virulence on the 12 near-isogenic rice lines and IR24 as susceptible check, each containing one known resistance (*R*) gene. There were 42 haplotypes revealed using a probe derived from 1376 bp *Sph*I-digested fragment from *avrXa3*, a member of the *AvrBs3/pthA* gene family, to detect homologous copies in genomic DNA by Southern hybridization among 52 Chinese strains of the 103 China *Xoo*. No Clusters (for pathotype) or Lineages (for haplotype) were supported by bootstrap value greater than 30%. Interesting, we found that some isolates with different pathotypes shared the same haplotype, other tested isolates with different haplotypes harbored identical pathotype. There was a weak correlation between DNA fingerprint similarities and Pathotypes. Furthermore, our results indicated that *Xa21*, *Xa10*, and *xa5* have the broader resistance than others to the number of pathotypes, and IRBB54 has the best two *R* gene combinations. Among the cultivars, DV85, BJ1 and IRBB54 are resistant to all the Chinese strains tested.

Molecular analysis unravel new *Xanthomonas oryzae* pv. *oryzae* strains in West-Africa

Gonzalez C., Yu Y., Szurek B., Soto M., Mathieu T., **Verdier V.**

Laboratoire Génome et Développement des Plantes, Institut de la Recherche pour le Développement, UMR5096, 911Av Agropolis, 34394 Montpellier, France
email: verdier@mpl.ird.fr

Rice bacterial blight (BB) was reported in Africa in the 80s, since then it is increasing in importance. The use of varietal resistance has proven to be very efficient in controlling the disease in Asia, but breeding strategies for durable BB resistance in Africa need to be urgently developed. As a prerequisite, informations on the biology and diversity of the pathogen in Africa as well as the characterization of microbial genes involved in the host-pathogen interaction are needed.
DNA polymorphism analysis and pathogenicity assays were used to characterize strains of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) collected in West-Africa. African *Xoo* strains showed a reduction of IS elements in their genome: IS*1112* content is fairly reduced and there is no copy of *TnX1* (IS*1113)*, an insertion sequence found to be conserved in all Asian *Xoo* strains. Interestingly, African *Xoo* strains also showed a reduction of *avrBs3/pthA* effector genes (8 copies while up to 16 are detected in Asian strains), some of them are known to be crucial pathogenicity factors. A systematic mutagenesis approach was therefore initiated aiming at identifying and characterizing major *avrBs3/pthA* virulence genes in African *Xoo* strains. 16 mutants were obtained and characterized, one of them showing to be highly affected in its capacity to lead to typical leaf lesion symptoms. Most recent progress on the detailed analysis of B3-1-1 mutant will be presented.
While no differences between African and Asian strains could be detected with respect to their capacity to provoke disease symptoms on susceptible lines, pathogenicity assays performed on NILs revealed three new races among African *Xoo* strains. Race A1 strains showed *avrXa4*, *avrxa5* and *avrXa7* activities on matching *Xa4*, *xa5* and *Xa7*-containing lines while races A2 and A3 are incompatible on almost all tested lines. Intriguingly, all *Xoo* strains originating from Africa induced a non-host HR in tobacco leaves, while Asian ones did not.
Much remains to be learned about the genes that are responsible for the differences between African and Asian *Xoo* strains. To that end, we recently developed suppressive subtractive hybridization (SSH) approaches and identified a pool of African *Xoo* strains-specific DNA sequences. Identification of such genes may provide us with clues about the intriguing origin and evolution of these new *Xoo* strains originating from Africa.

Genetic diversity and change in race frequency of *Xanthomonas oryzae* pv. *oryzae* causing bacterial blight of rice in Punjab, India

J.S.Lore, R.K.Goel, G.L.Raina, M.S.Hunjan, T.S.Bharaj, Y.Vikal and K.Singh

Department of Plant Breeding, Genetics and Biotechnology, Punjab Agricultural University, Ludhiana-141 004, India

ABSTRACT

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) limits rice yields in all major low land irrigated rice growing regions of the world, including the rice belt comprising Punjab and the other adjoining north-western states in India. Development and deployment of the host resistance is the only effective means of BB management. Globally, 29 major *Xa/xa* genes conferring resistance to one/more races of the bacterium have been identified and utilized in rice breeding programs. However, shift in race frequency caused by the large scale and long term cultivation of specific BB resistant cultivars results in resistance breakdown. At the Punjab Agricultural University, isolates of *Xoo* from samples collected from farmers' fields in the different districts of the Punjab state have been regularly analyzed for their virulence spectrum on near isogenic lines (NILs)in the background of IR 24 (IRBB lines), international differential cultivars with known BB resistance genes and local bacterial blight resistant cultivars during the last three decades. Before 1999, a vast majority (>91 %) of the isolates showed resistant/moderately resistant reaction to PI 231129, DV85 and Patong 32 (now known to carry BB resistance genes *xa*8, *xa*5+*Xa*7 and *Xa*4 respectively) which has been designated as Race I. These isolates were clubbed together as "Punjab races". Other races were encountered in very low frequencies. After the commercial release of BB resistant cultivars PR109 in 1986, PR110 in 1992, PR114 in 1999, PR116 in 2000 and PR 118 in 2003. Majority of the isolates (50-68%) collected during the period 1999-2006 were observed to be virulent on cultivar DV85 and all the known *Xa* genes except *xa*13 as well as local bacterial blight resistant cultivars viz. PR 114, PR 116 and PR 118.

Currently, *Xoo* population from the state could be classified into eight distinct races on the basis of their differential reaction. IRBB-1 (*Xa*1), IRBB-3 (*Xa*3), IRBB-10 (*Xa*10), IRBB-11 (*Xa*11), IRBB-14 (*Xa*14), IR24 (*Xa*18) and TN1 (*Xa*14) were susceptible/ moderately susceptible whereas IRBB-13 (*xa*13) whereas IRBB-51 (*Xa*4 + *xa*13), IRBB-53 (*xa5* + *xa*13), IRBB-55 ((*Xa*13 + *Xa*21), IRBB-56 (*Xa*4 + *xa*5 + *xa*13), IRBB-58 (*Xa*4 + *xa*13 +*Xa*21), IRBB-59 (*xa*5 + *xa*13 +*Xa*21) and IRBB-60 (*Xa*4 + *xa*5 + *xa*13 +*Xa*21), the cultivar Ajay, better known as IET 8585 (IET4141/CR98-7216) were resistant/moderately resistant to all the races of *Xoo* analysed. None of these pathotypes except Race IV and Race VIII could infect *Xa*21. Race VIII (frequency-1.2%) gave moderately susceptible reaction to pyramid lines

IRBB-52 (*Xa*4 + *Xa*21), IRBB-54 (*xa*5 + *Xa*21), IRBB-57 (*Xa*4 + *xa*5 + *Xa*21). Likewise, *xa*8 was effective against all the races except Race V and Race VII. Out of nine BB resistant commercial rice cultivars released for cultivation in Punjab so far, PR109 (IR19660-73-4/IR2415-90-90-4-3-2//IR54), PR111 (IR54/PR106), PR113 (IR8//RP2151-173-1-8/IR8*4), PR115 (RP2151-173-1-8/PR103*3) and PAU 201 (PR103/PAU1126-47-2-2) showed resistant/moderately reaction to all the eight races whereas PR110 (TN1/Patong32//PR106*6), PR114 (TN1/Patong32//PR106*4///IR8), PR116 (PR108///TN1/Patong32//PR106*6////PR108) and PR118 (Pusa44/PR110//Pusa44*3) have already succumbed to one or more of these races. In view of an extremely high degree of variability and a high rate of evoluation for increased virulence in the pathogen, pyramiding of two/more partially effective known *Xa* genes and/or search for new disease resistance genes effective against the wider Xoo population appear to be the most appropriate approaches for BB management in the near future in the north-western India, a region highly important for the food security of this country.

Screening, phenotypic and physiological structures of *Xanthomonas oryzae* pv. *oryzae* and its races in Bangladesh *

M. S. Kabir[1], S. Akter[1], P. S. Biswas[2], M. A. Latif[3] and M. A. Hossain[4]

[1]Senior Scientific Officer, [3]Pincipal Scientific Officer, [4]Chief Scientific Officer and Head
Plant Pathology Division,
[2]Senior Scientific Officer, Plant Breeding Division
Bangladesh Rice Research Institute, Gazipur-1701, Bangladesh

Contact address: Phone +880-2-9257401-5, 9257501-5 Extn 431 (Off.); Fax +880-2-9261110;
email: shahjahankabir@hotmail.com

Abstract

To exploit bacterial blight (BB) resistant sources, a total of 14,000 entries were screened from 1980 to 2006 in Bangladesh Rice Research Institute (BRRI) of which 2,241 entries were found resistance to moderately resistance to BB. Seven entries was found consistently resistant and twenty entries was reconfirmed that could serve as a donor source of developing BB resistance variety. In the selection of segregating population against BB, 142 and 79 moderately resistant progenies were selected from F_2 and F_3 generation respectively in Boro 2007, 142 progenies from F_2 populations in T. Aman 2006. Among the screening in Near Isogenic Lines (NILs), IRBB21 showed moderately resistant and stable reactions in IRBB lines. In phenotypic analyses of *Xanthomonas oryzae* pv. *oryzae* based on 66 morphological, biochemical and physiological traits of 56 strains, no variation was found among the isolates and showed existence of homogeneous populations. On the other hand, most of the isolates showed different groupings in different varieties based on the incubation period and it

did not correspond with the place of origin except a few avirulent strains. It was recorded that incubation period was always negatively correlated with the disease development. Variable number of races was noticed tested on differential varieties. Nine races were found during 1988-1989 using nine differential varieties and using fifty isolates with eleven differential varieties it raised to eleven. A program was undertaken to identify races of *X. oryzae* pv. *oryzae* developed against BB in Bangladesh using molecular techniques and differential varieties (NILs).

1. Introduction

Rice is the staple food crop, grown in 13.9 million hectares and feeds about 140 million people of Bangladesh. It is grown through out the year and covers about 77% of the total cropped area. It contributes 17% to the national GDP (BBS, 2003 and 2004; BRRI 2004), 71% of the total calories and 51% of the protein in the average daily typical diet of the people (Chowdhury, 1999; BBS, 2003 and 2004).

Rice plant infected by 32 diseases of which ten are major in Bangladesh at present. Among the diseases three bacterial diseases are frequently occured in Bangladesh. Diseases are bacterial blight (BB), bacterial leaf streak (BLS) and bacterial foot rot (BFR). Among the three diseases, BB caused by *X. oryzae* pv. *oryzae* considered as a major disease as the disease occurs in all Agro Ecological Zones (AEZ) of Bangladesh and in three rice growing seasons namely, Aus (March-April to June-July), Aman (June-July to November-December) and Boro (November-December to April-May) (Miah 1973; Miah *et al.* 1985) and cause considerable yield loss. It is also an important disease in most of the South and Southeast Asian countries (Sharma, 1991).

In Bangladesh, the intensity and severity of the disease is different in different parts of the country, mostly because of the geographical and climatic variations. Since the introduction of high yielding varieties in the country, the disease became one of the major constraints to rice production in irrigated, rainfed lowland and deep-water rice areas. BB has also intensified due to intensive cultivation of high-yielding susceptible cultivars with reduced genetic variability, higher plant population per unit area, high doses of fertilizer, staggered sowing and planting.

In pathological point of view, various aspects of research was conducted on the causal organism of *X. oryzae pv. oryzae*. This paper will highlight the current research activities and findings about exploration of resistant sources, phenotypic and physiological structures of *X. oryzae* pv. *oryzae* and its variability in Bangladesh.

2. Materials and Methods:

Screening of BB resistant varieties/lines:

Artificial screening was conducted on locally collected rice germplasm, INGER materials, NILs, breeding lines advanced breeding lines and F_2 population for developing BB resistant varieties. These materials were grown in one meter lines with a moderately resistant check (BR14) and a susceptible check (Purbachi). About 3-4 hills were inoculated by clipping method with 10^{8-10} cfu B*Xo*9 (a virulent isolate collected from BRRI farm). Data were collected after fourteen days of inoculation.

These materials were grown at least twice to confirm the disease reaction.

In screening of F_2 population, after crossing with donor parent by the breeder, plants were grown in the field in lines. All hills were inoculated following the same methods as described earlier at panicle initiation to booting stage. Pathologists selected the resistant to moderately resistant plant after fourteen days of inoculation in the field. Finally, breeder selected the plants based on disease resistance and high yielding components from the plants tagged by the pathologists.

In addition to artificial screening, natural screening is also a continuous process to make a database against BB disease of rice. These type of screenings were done in observational trial (OT), primary yield trial (PYT), secondary yield trial (SYT), regional yield trial (RYT) plots *etc*.

Phenotypic analyses of *X. oryzae* pv. *oryzae*

Phenotypic analyses of *X. o.* pv. *oryzae* were performed based on the description of Bradbury (1986) including morphological, cultural, physiological and biochemical characters of the isolates. Fifty-six isolates including six avirulent strains were collected from different AEZs of Bangladesh. Fifty representative isolates from ten groups based on the incubation period on BR9 were selected for these studies. Each test was performed with a minimum of three replications. Bacterilogical tests included morphology and gram reaction, cultural characters, requirements of oxygen, KOH solubility test, effect of NaCl, effect of temperature, oxidase test, catalyse test, reducing substances from sucrose, gas from d-glucose, levan production, methyl red test, acetyl methyl carbinol production (Voges proskauer test), indole production, urease production ammonia production, hydrogen sulphide production, reduction of nitrate, action in milk, gelatin hydrolysis, hydrolysis of starch, hydrolysis of aesculin, casein hydrolysis, hydrolysis of Tween 20 and Tween 80, reduction of tetrazolium chloride (2,3,5-triphenyl-tetrazolium chloride = TZC), action on egg yolk (Lecithinase activity), phosphatase activity, arginine dihydrolase test, utilization of asparagine as a sole source of carbon and nitrogen, phenylalanine deaminase test, sensitivity to Cupric nitrate $Cu(NO_3)_2$, acid production from carbohydrates, utilization of organic acids, growth in Amino-acids, sensitivity to antibiotics, hypersensitivity reaction on Cowpea, Potato maceration test *etc*. Results obtained from the standard morphological, physiological and biochemical tests were used for the computer-assisted numerical taxonomy and multivariate analysis. All tests were scored as either positive or negative. Both positive and negative matches were determined in the calculation by unweighted average pair-group methods (Sneath and Sokal 1973). Similarity coefficients (Ssm) were calculated and data were transposed into a dendrogram using NTSYS-pc. program (Rohlf 1997).

Physiological structures of ***X. oryzae* pv. *oryzae***

Researchs were performed to find out the relationship between BB disease development and its incubation period in three rice varieties namely; BR9, IR8 and Purbachi (Hossain 2001). The isolates of *Xanthomonas oryzae* pv *oryzae* were collected from 29 AEZs of Bangladesh during Boro, Aus and Aman seasons. This study was conducted using 300

isolates as described by Hossain (2001).

Xanthomonas oryzae. pv. *oryzae* was isolated from the advancing lesion of the collected leaves. Surface sterilization was done by soaking the leaf pieces in 70% ethyl alcohol followed by dipping in 5% Chlorox for one minute (Vera Cruz, 1984). The surface sterilized pieces were kept for 30 minutes in sterile water to release the bacterium. Finally, a full loop of the suspension was streaked on a plate of MgFe medium [$Ca(NO_3)_2.4H_2O$ 0.5g, Na_2HPO_4 0.82g,$MgSO_4.4H_2O$ 0.2g, Peptone 5.0g, Na-glutamate 1.0 g, Sucrose 20g, $FeSO_4.7H_2O$ 0.05 g, Agar (Difco) 12.0g, Distilled water 1.0 . pH 7.2]. Observation was made after 72 hours after streaking to see the appearance of the *X. o. pv. oryzae* colonies. After five days, the bright yellow bigger and slimy colonies were selected. The selected colonies were re-streaked on the MgFe medium. Finally, pure single colony was selected and designated as individual isolates. Pathogenicity of three hundred and thirty eight isolates was tested in the green house at BRRI, Gazipur by growing Purbachi as a test plant in earthen pot. Twenty-day-old seedlings of all three varieties were transplanted in cemented tray with three replications.

BB isolates were revived in the PSA medium. Forty five-day-old plants were inoculated with the test isolates with approximately 10^{8-10} cfu /ml *X o* pv *oryzae* suspension. For each strain, 20 leaves were inoculated. The first day of symptom appearance (incubation period) for each strain was recorded starting from 24 hour after inoculation up to a final reading on 15th day. The visual percent leaf area infection of ten leaves for each strain was recorded. Experiment was conducted following factorial in Completely Randomized Block Design (CRBD). The correlation between incubation period and disease development was determined.

Identification of races of *X. oryzae* pv. *oryzae*: BRRI (1992)

An experiment was conducted to find the existing races of *X. oryzae* pv. *oryzae* in Boro, Aus and Aman seasons during 1988-1989 using thirteen differential varieties. Four isolates (B*Xo*5, B*Xo*9, B*Xo*10, B*Xo*89) was used to find the races. Hossain (2001) repeated the same experiment with two sets of differential varieties using fifty isolates. Details methodology was described by Hossain (2001) and BRRI (1992). Differentials used in those experiments are listed in Table 1.

Table 1. Differential varieties used for identification of races.

Sl	Differentials used by BRRI, 1992	Differentials used by Hossain, 2001	
		Set 1	Set 2
1	IR222082-41-2	Kalimekri 77-5	IRBB1
2	IR32822-94-3-3-22	AC19-1-1	IRBB2
3	C732046	Akhnisail	IRBB3
4	IR13155-60-3-1-2-1	BJ1	IRBB4
5	DV85	BR161-2B-25	IRBB5
6	RP2151-33-2	BR171-2B-8	IRBB7
7	Nancy P A	DV85	IRBB 8
8	C721313	Gasmal#89	IRBB10

9	Kuntlan	Hijoli	IRBB11
10	IR35353-94-2-1-3	Camor	IRBB13
11	Kogyoku	Tetep	IRBB14
12	Kalimekri 77-5	Bazail#414	
13	Java 14	DZ192	

3. Results and Discussion:

Screening of BB resistant varieties/lines:

Screening is the main tools for developing resistant varieties and making a database. As of now, 14,000 entries were screened from 1980 to 2006 of which 2,241 entries were found resistance to moderately resistance to BB (Table 2).

Table 2. Screening of entries against BB form BRRI germplasm, IRTN, INGER and breeding materials from 1980 to 2006.

Year	No. of tested entries	No. of resistant to MR* entries
1980-1985	3715	971
1986-1990	670	26
1991-1995	6273	608
1996-2000	762	95
2001-2006	2580	541
Total	**14,000**	**2,241**

*MR= moderately resistant

Mondal and Hossain (1997) found consistent reaction of seven entries showing resistant reaction for five years. The entries are a good donor source of BB resistant rice varieties. The entries are BR808-10-3-3 (BG90-2/IR2863-39-2), BR4611-14-4-1-5 (BR8/IR7676-12-1-2), BR4611-172-1-1-2 (BR8/IR7676-12-1-2), BR4656-16-4-3-2 (BG376-4/IR4744-295-2-3), BR4661-21-1-4-2-5 (BR319-1-HR24/BR161-2B-28), BR4689-17-1-5 (BR161-2B-58/BR319-1-HR11),BR4829-28-2-4 (IR196660-11/BR1081-9-2-5-1). According to BRRI (1997) six entries were found resistant to BB and the entries were CAMOR (Acc. 17366), CISADANE, IR-BB21, IR321120-138-2-1-1-2, IR32822-94-3-3-2-2, IR4442-46-3-3-3 supplied by INGER. Among the entries only IR-BB21 showed moderately resistance to BB during 2005 and 2006. The recently tested (consecutively in T. Aman 05 and 06) moderately resistant materials were AC19-1-1 (ACC32753), IR71676-34-1-1, IR72158-16-3-3, IR72470-19-2-2-3, IR72887-34-2-1-3, SASANG BARUNG (ACC18690) and SPRLR84184-9-5-2-13 that were supplied by INGER. Along with these entries BRRI ACC1437, 1593, 1600 and IR70175-4-1-1-2-3-HR2 were also found moderately reaction to BB in two consequent years. These materials were handed over to Plant Breeding Divisions as donor parent. Continuous screening has been conducting on the native germplasm to find out resistance sources and to make a database for BB resistance.

Besides the screening, selection on F_2 and F_3 population is also important aspect of plant pathologist to develop a resistant variety. In screening of F_3 population against BB, BRRI crosses BR7805, BR7806, BR7807 were screened for Boro 2007;

while that of BR7960, BR7961, BR7962, BR7963, BR7964, BR7965, BR7966 is under screening in Aman 2007. In addition, selection on the crosses BR7984, BR7985, BR7986 were successfully completed in F_2 for Boro 2007 (Table 3). As vertical resistance is less durable; as a result, BRRI released varieties sometimes are not showing the reaction as like the donor parent. Therefore, it needs gene pyramiding to confer more durable resistance. With keeping the concept, breeder already confirmed F_1 with the donor IRBB60 that contained *Xa*4, *Xa*5, *Xa*13, *Xa*21 genes and will be screened in early of next year.

Table 3. Screening of F_2 generation by virulent isolate B*Xo*9.

Sl	Cross no.	Parents reaction against BB	Lines selected by pathologist	Lines selected by breeder	Remarks
1	BR7805	Moderately resistant	22	10	Completed F_3
2	BR7806	Moderately resistant	55	55	Completed F_3
3	BR7807	Moderately resistant	18	6	Completed F_3
4	BR7808	Moderately resistant	19	8	Completed F_3
5	BR7960	Resistant	50	34	F_3 started
6	BR7961	Resistant	39	13	F_3 started
7	BR7962	Resistant	60	24	F_3 started
8	BR7963	Moderately resistant	55	6	F_3 started
9	BR7964	Moderately resistant	47	21	F_3 started
10	BR7965	Moderately resistant	39	19	F_3 started
11	BR7966	Resistant	50	35	F_3 started
12	BR7984	Moderately resistant	106	43	Completed F_2
13	BR7985	Moderately resistant	77	52	Completed F_2
14	BR7986	Moderately resistant	108	49	Completed F_2

In natural screening process, score on BB disease was also taken from the entries of OT, PYT, SYT, RYT to make the database. The recent three years (2004-2006) reaction was included on the mentioned entries in Table 4.

Table 4. Disease scores of OT, PYT, SYT, RYT based on natural field infection against BB at BRRI Gazipur during 2004-2006.

Year	Sources	Total entries	No. of entries	
			Resistant	Moderately resistant
2004	OT, PYT, SYT, RYT	228	4	6
2005	OT1, OT2, PYT1, PYT2	99	37	49
2006	OT, PYT, IIRON, Parent, PVT	279	70	86

As of now, BRRI released 48 rice varieties of which 25 percent rice varieties were moderately resistant to BB (BRRI, 2004).

Phenotypic analyses of *X. oryzae* pv. *oryzae*

The tested morphological, biochemical and physiological traits showed the existence of homogeneous populations of the bacterium (Figure 1) regardless of their place of origin, date of isolation, host and geographic location as described by Hossain (2001).

Physiological structures of *X. oryzae* **pv**. *oryzae*

Incubation period and disease development

The results presented in the Table 5 showed that the incubation period varied from 3-15 days. The collected isolates were grouped into 10, based on the incubation period on BR9. Different groups required different days to show disease symptom. GroupI (GI) was highly virulent and G VIII was the least virulent. However, G X did not produce any symptom even up to the 15th day of inoculation. This group was considered avirulent. On 6th day, symptoms were visible on the highest number of isolates (45.0%) followed by 3rd (15.0%), 5th (13.0%) and 4th (12.7%) days of inoculation respectively. Starting from 7th (5.7%) day onward, the number of isolates showing symptoms gradually decreased with increasing time. Therefore, isolates were divided into ten groups, based on their incubation period. GI showed symptoms three days after inoculation. Subsequently, isolates produced symptoms during 4 to 10 days, these fell into groups, GII-GVIII respectively, whereas, GIX produced symptoms on 12th day of inoculation. The disease development (percent leaf area infection) varied widely within the group. The ranges of the percent leaf area infection were recorded in GI-GIX, these were 24.4 - 85.6%, 16.6 - 87.2%, 26.7 - 80.0%, 8.1 -78.3, 24.4 - 82.2%, 12.6 -27.2%, 10.25 - 25.6%, 9.2 - 22.4% and 8.7 - 11.4% respectively. The highest percent leaf area infection was 87.0% recorded in GII with only four days of incubation. The regression analysis showed that the correlation between percent leaf area infection and incubation period was negative (Figure 2) indicating that as the incubation period increased, the disease development decreased.

Table 5. Grouping of *X. o.* pv. *oryzae* strains based on incubation period on BR9

Strain groups	Percent isolates showing symptoms on different days after inoculation									
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**12**	**15***
G I		15.0									
G II			12.7								
G III				13.0							
G IV					45.3						
G V						5.7					
G VI							2.0				
G VII								1.3			
G VIII									1.0		
G IX										1.3	
G X											2.7

* No symptoms produced

The incubation period in IR8, a BB highly susceptible variety, showed lower variation than that of BR9. The isolates were divided into six groups (Table 6). In this case, GI isolates (0.7%) produced symptoms on 3rd day after inoculation. The largest groups were GII and GIII having the maximum number of the isolates. The highest percent leaf area infection (91.1%) was recorded in GII after a 4 day incubation period. However, 4.7% isolates did not produce any symptoms on 15th day after inoculation. The incubation period and disease development was also negatively correlated. This was similar to BR9 (Figure 3). The ranges of percent leaf area infection did not vary as in BR9. The ranges of the percent leaf area infection were 53.3 - 57.2%, 25.0 - 91.1%, 15.6 - 65.0%, 14.4 - 60.0% in GI – GV respectively.

Table 6. Grouping of *X. o.* pv. *oryzae* based on incubation period on IR8

Strain groups	**Percent isolates showing symptoms on different days after inoculation**					
	2	**3**	**4**	**5**	**6**	**7**	**15***
G I		0.7					
G II			47				
G III				38.7			
G IV					5.7		
G V						3.3	
G VI							4.7

* No symptoms produced

In the case of Purbachi, a highly BB susceptible variety showed only eight groups of isolates based on the incubation period (Table 7). The GII isolates (17.3%) showed symptoms on 4th day after inoculation. In this case, the highest number (45.0%) of isolates in GII showed symptom on 5th day after inoculation and subsequently, the number of the isolates in each group was reduced from 20.0% to 0.7% (6th to 10th day after inoculation) with the longer incubation period respectively. The percent leaf area infection was similar to BR9 which varied widely within the groups (Figure 3). The range of the percent leaf area infection was 45.6 -100.0%, 13.3 -100.0%, 38.9 - 93.3%, 33.3 - 88.9%, 32.2 -100.0%, 43.3 -85.6%, and 14.4 - 26.7% for GI - GVII respectively. A few isolates (3.6%) did not produce any symptoms even on 15th day after inoculation. The percent leaf area infection was affected due to longer incubation period as for BR9 and IR8. Similarly it showed a negative correlation between incubation period and percent leaf area infection (Figure 4).

Table 7. Grouping of *X. o.* pv. *oryzae* based on incubation period on Purbachi.

Strain groups	**Percent isolates showing symptoms ondifferent days after inoculation**									
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**15***

G I	17.3							
G II		45.0						
G III			20.0					
G IV				7.7				
G V					4.0			
G VI						0.7		
G VII							0.7	
G VIII								3.6

* No symptoms produced

Based on the incubation period, the isolates were grouped into 6, 8 and 10 in IR8, Purbachi and BR9 respectively. The grouping based on the incubation period in one variety differed from that in another variety. There were similarities among the few isolates, BXo303 (AEZ # 7), BXo304 (AEZ # 7), BXo305 (AEZ # 7), BXo307 (AEZ # 7), BXo360 (AEZ # 27), BXo371 (AEZ # 1), and BXo394 (AEZ # 27), these were found to be avirulent in all the three varieties. Some of the isolates produced less disease in 4-6 day incubation period within the same group, which might be due to antagonism by other diseases like leaf scald (a fungal disease) that caused suppression of BB infection (BRRI, 1988) or insect pests like mites and thrips. The maximum isolates produced symptoms 3-6 days after inoculation or after a 3-6 day incubation period resulting in a higher percent leaf area infection. However, incubation period varied between varieties and did not correspond to the origin of collection (AEZ), although most of the avirulent isolates were collected from AEZ # 7 and 27. Whether the virulent isolates showed symptoms within 3-6 days probably depended upon the host genotypes. Virulence may be defined as the degree of ability of a pathogen to cause disease (Agrios, 1988) but Shaner *et al.,* (1992) defined virulence as the relative disease-evoking capacity of an isolate compared to other isolates. However, Japanese workers, Watanabe *et al.* (1976), Nakanishi and Watanabe (1977) found that the *X. o.* pv. *oryzae* produced symptoms within 5 days of inoculation. This indirectly supports our findings. Mew (1993) also reported that ooze inoculation produced symptoms within a week. In the case of bacteria, it is better to consider incubation period rather than latent period because unlike fungi it produces ooze after the appearance of symptoms and depends on many factors like temperature, humidity and varietal genotypes. It is, therefore, very difficult to correlate appearance of symptoms with the development of the disease and the latent period (Koch 1991). In this connection, Ahmed *et al.*, 1997 reported that infection efficiency and latent period may be important determinants of the rate of epidemic development, and are not necessarily accounted for by lesion length measurements. However, the present finding indicated that four to six days incubation period resulted in a higher percent leaf area infection. When the incubation period was more than six days, the disease development was low and most of the time ooze production (visual indication of the presence of bacteria) was observed after the expression of

symptom. Though sometimes, it was noticed few days after the appearance of symptom. Most of the isolates showed different groupings in different varieties based on the incubation period and it did not correspond with the place of origin (AEZ) except in a few avirulent isolates. The present study suggested that the incubation period was directly related to the epidemiology of this disease rather than the latent period.

Races of *X. oryzae* pv. *oryzae*:

Nine races of *X. oryzae* pv. *oryzae* were found using thirteen isolates during the period 1988-1989. Later on, by using two sets of differentials, eleven races of *X. oryzae* pv. *oryzae* were found (Table 8) by Hossain (2001). Research is continuing to collect isolates from all AEZs to find existing races of the pathogen against more known genes available in IRRI.

Table 8. Races of *Xanthomonas oryzae* pv. *oryzae* in two different times in Bangladesh

Grouping during 1988-89		Grouping by Hossain (2001)	
Group	Resistant to	Group	Resistant to
1	B*Xo*5	IRBB1	B*Xo*415, B*Xo*223, B*Xo*294,
2	B*Xo*9	IRBB2	B*Xo*9, B*Xo*415, B*Xo*223
3	B*Xo*10	IRBB3	None
4	B*Xo*89	IRBB4	B*Xo*415, B*Xo*223
5	B*Xo*5 and B*Xo*89	IRBB5	B*Xo*471, B*Xo*415, B*Xo*102, B*Xo*223
6	B*Xo*9 and B*Xo*9	IRBB7	B*Xo*471, B*Xo*415, B*Xo*338, B*Xo*102, B*Xo*223
7	B*Xo*10 and B*Xo*89	IRBB8	B*Xo*424, B*Xo*108, B*Xo*210, B*Xo*415, B*Xo*265, B*Xo*267, B*Xo*223
8	All 4 B*Xo*	IRBB10	B*Xo*268, B*Xo* 415, B*Xo* 223
9	All but B*Xo*10	IRBB11	B*Xo*374, B*Xo* 424, B*Xo* 415, B*Xo* 435, B*Xo* 223
10		IRBB13	B*Xo*424, B*Xo* 415, B*Xo* 223
11		IRBB14	B*Xo*415, B*Xo* 223

Group1 = IR222082-41-2, 2 = IR32822-94-3-3-22, 3 = C732046, 4 = IR13155-60-3-1-2-1, 5 = DV85, RP2151-33-2, 6 = Nancy P A, C721313, Kuntlan, 7 = IR35353-94-2-1-3, 8 = Kogyoku, 9 = Kalimekri 77-5, Java 14

Conclusion:

Bangladesh Rice Research Institute has conducted various research works on bacterial blight of which this paper only highlighted the effort on developing bacterial blight resistant rice varieties by screening, phenotypic and physiological characteristics of the causal organism *Xanthomonas oryzae* pv. *oryzae* and its variability. Exploitation of resistance sources is routine work of plant pathology division. Progenies from F_2

and F_3 has already selected from the crosses of moderately resistant donor parent in T. Aman and Boro seasons. However, research needs to be marker assisted selection along with conventional selection methods that has not been initiated at BRRI. In phenotypic analysis analysis, no variation was found among the isolates and showed existence of homogeneous populations. However, in physiological characteristics, groups were identified based on incubation period and it was found that incubation period is always negatively correlated with disease development. Besides these, researches was undertaken to find out the variability of the causal organism around all over the Bangladesh by more differential varieties and by molecular techniques.

References

Agrios G. N. 1988. Plant Pathology, 3rd Edn. Academic Press, Inc., New York, U S A.

Ahmed H. U., M. R. Finckh, F. Alfonso and C. C. Mundt. 1997. Epidemiological effect of gene deployment strategies on bacterial blight of rice. Pytopathology 87: 66-70.

BBS 2003. Statistical Pocket-book of Bangladesh. Bangladesh Bureau of Statistics, Planning Division, Ministry of Planning, Government of the people's Republic of Bangladesh, Dhaka, Bangladesh p 493

BBS 2004. Statistical Pocket-book of Bangladesh. Bangladesh Bureau of Statistics, Planning Division, Ministry of Planning, Government of the people's Republic of Bangladesh, Dhaka, Bangladesh p 838

Bradbury J F 1986. Guide to Plant Pathogenic Bacteria. CAB International Mycological Institute, Ferry Lane, Kew, Surrey, England. pp.332.

BRRI 1988. Annual Report for 1988. Publication no 98. Bangladesh Rice Research Institute, Gazipur 1701.

BRRI 1992. Annual Report for 1989. Publication no 98. Bangladesh Rice Research Institute, Gazipur 1701. pp 116-117

BRRI 1997. Annual Report for 1997. Publication no 134. Bangladesh Rice Research Institute, Gazipur 1701. p 111

BRRI 2004. Adhunik Dhaner Chas (Bangla Version). Publication no 5, 12th Edition Published by Bangladesh Rice Research Institute, Gazipur 1701. 60P.

Chowdhury, M. S. U. 1999. Rice Research in Bangladesh: Preparing for the 21th century. In: Increasing rice production in Bangladesh- Challenge and Strategies. (Eds. Bhuiyan, S.I. and Reazul Karim, A.N.M.). Bangladesh Rice Research Institute, Gazipur 1701 and Intrenational Rice Research Institute.

Hossain, M. A . 2001. A PhD dissertation submitted to the Department of Botany, University of Dhaka in March 2001.

Koch, M. F. and J. B. Parlevliet. 1991. Genetic analysis of, and selection for, factors affecting quantitative resistance to *Xanthomonas* campestris pv. *oryzae* in rice. Euphytica 53: 235 -245.

Mew, T. W. 1993. *Xanthomonas oryzae* pathovars on rice: cause bacterial blight and bacterial leaf streak. In: *Xanthomonas*. J G Swings and E L Civerolo. (eds.) Chapman & Hall, London. pp.30-40.

Miah, S. A. 1973. Recent research results on rice diseases in Bangladesh. A paper presented at the Annual International Rice Conference held at IRRI, Los Baños, Philippines, during April 1973.

Miah, S. A., A. K. M Shahjahan, M. A. Hossain and N. R. Sharma. 1985. A survey of rice diseases in Bangladesh. Tropical Pest Management 31 (3): 208-213.

Mondal, A.H and M. A. Hossain. 1997. Screening for Resistance against Rice Bacterial blight in Bangladesh. Bangladesh J. Bot. 26 (1):25-29.

Nakanishi, K and M. Watanabe. 1977. Studies on the mechanism of resistance of rice plants against *Xanthomonas oryzae*. III. Relationship between the rate of production of antibacterial substances and of multiplication of pathogenic bacteria in infected leaves of resistant and susceptible varieties. IV. Extraction and partial purification of antibacterial substances from infected leaves. Ann. Phytopath. Soc. Jpn. 4: 265-269 ; 449-454 (Ja, en).

Rohlf, F. J. 1997. NTSYS-pc. Numerical Taxonomy and Multivariate Analysis system. Version 2.0 Exeter Software, 100 North country Rd., Building B Setauket, New York 11733. pp.7-24.

Shaner, G., E. L. Strombrg, G. H. Lacy, K. R. Barker and T. P. Pirone. 1992. Nomenclature and concepts of pathogenicity and virulence. Annu. Rev. Phytopathol. 30: 47-66.

Sharma, N. R., M. A. Hossain, M. A. Haque, A. H. Mondal and M. M. Rahman. 1991.Effect of rate and split application of nitrogen on bacterial leaf blight incidence in rice. Bangladesh J.Plant Pathology.7 (1 & 2):17-20.

Sneath P H A and R R Sokal 1973. Numerical Taxonomy. The Principles and Practice of Numerical Classification. Freeman, San Francisco, California. pp.573.

Vera Cruz, C. M. 1984. Bacteriological and pathological variation of *Xanthomonas* campestris pv. *oryzae* (Ishiyama 1922) Dye 1978, the pathogen of bacterial blight of rice. M.S. Thesis, University of the Philippines at Los Baños, Laguna, Philippines. pp.151.

Watanabe, M and K. Nakanishi. 1976. Studies of the mechanism of resistance of rice plant against *Xanthomonas oryzae*. II. Production of an inhibitor in infected leaves. Ann. Phytopath. Soc. Jpn. 42: 578-582 (Ja, en).

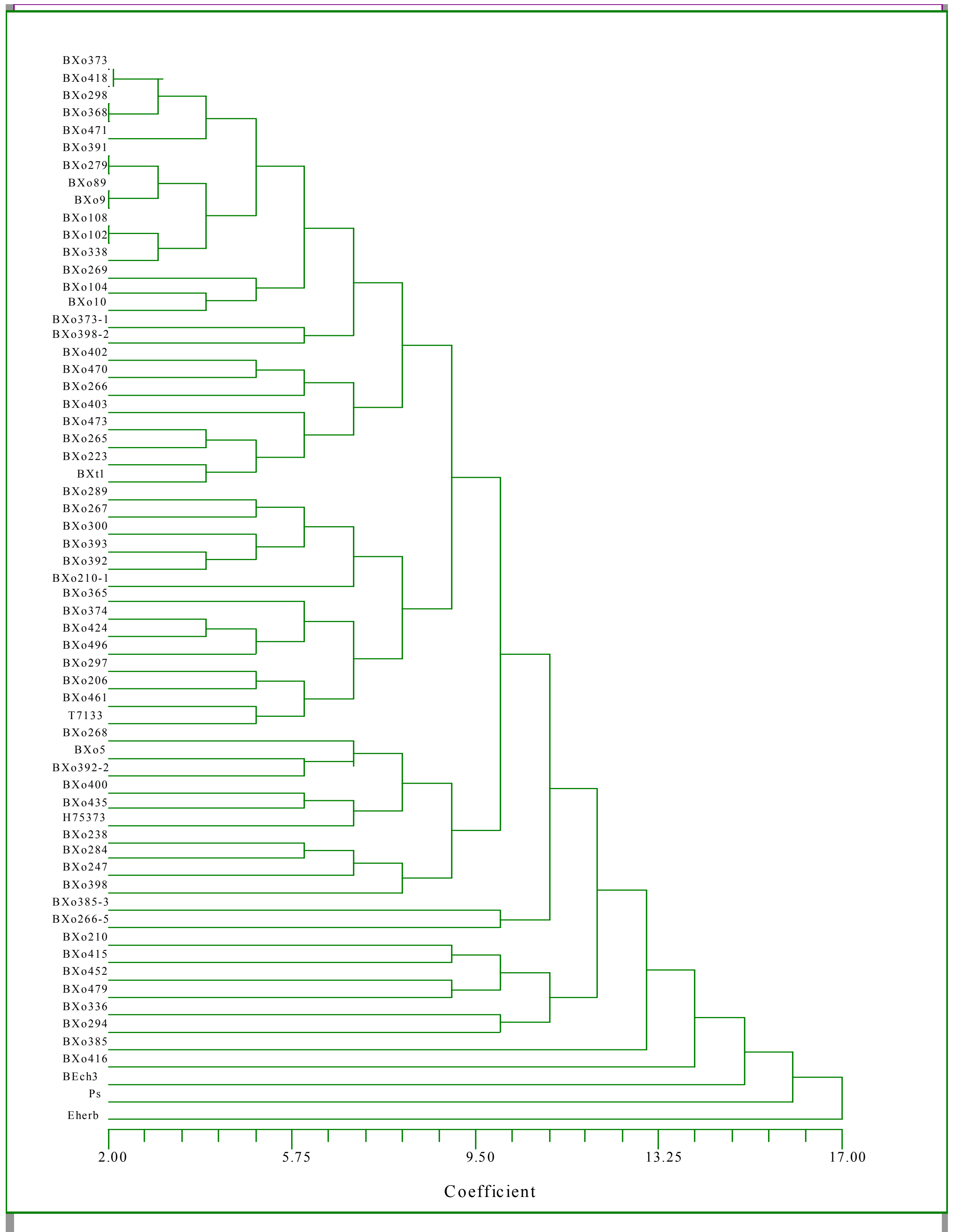


Fig 1 . Dendrogram showing phenotypic relationships based on bacteriological tests of 56 strains of *X. oryzae* pv. *oryzae* of Bangladesh.

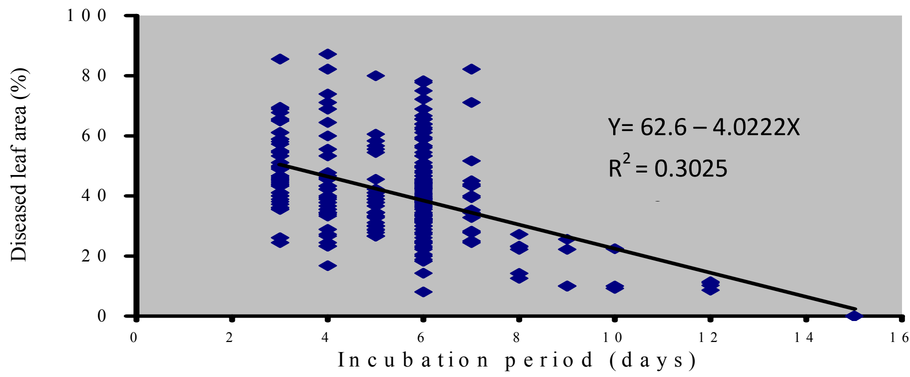


Figure 2. Relationship between disease development and incubation period on BR9

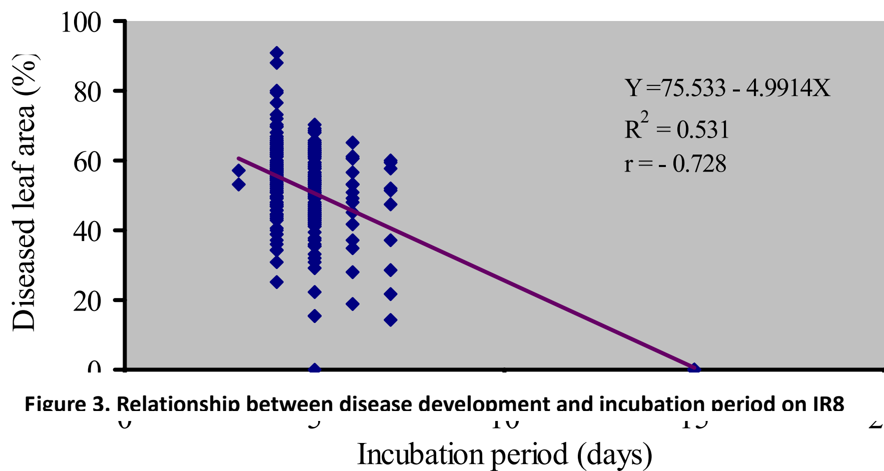


Figure 3. Relationship between disease development and incubation period on IR8

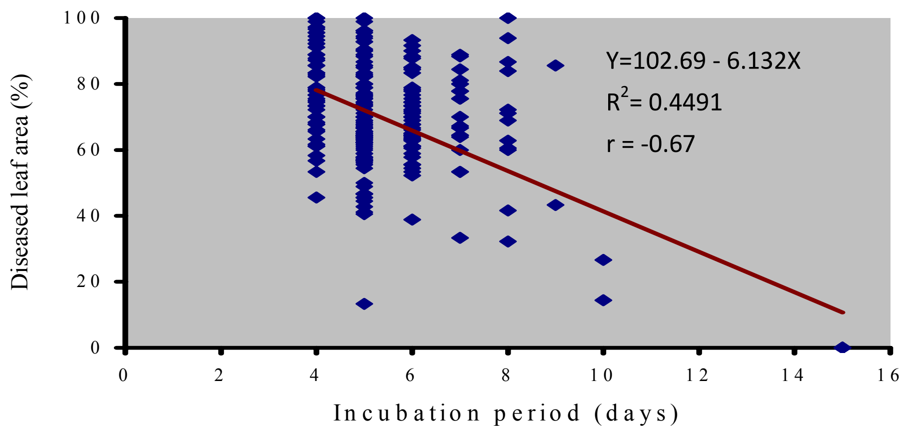


Figure 4. Relationship between disease development and incubation period on Purbachi

Topic 5. Resistance breeding and transgenic techniques

Studies on genetics and improvement of resistance to Bacterial Blight in China

Qi ZHANG [1] and LAM Hon-Ming[2]

[1]Chinese Academy of Agricultural Sciences, Beijing, China, 100081,
E-mail:zhangqi@mail.caas.net.cn
[2]Department of Biology and Program of Molecular Biotechnology, The Chinese University of Hong Kong
E-mail: honming@cuhk.edu.hk

Bacterial blight(*Xanthomonas oryzae* pv. *oryzae*, BB) is one of the most devastating rice diseases just next to blast in China. It was first reported in China in 1930s and has become widely spread nationwide since late 1950s. The late renowned Chinese plant pathologist Prof. Z.D. Fang initiated the study of BB in China in 1950s, by examining its causal organism, symptom, physiology, disease cycle and chemical control. Building on this foundation, extensive, comprehensive, and integrated investigations of rice resistance to BB have been conducted for more than half a century.

Since the Ministry of Agriculture (PRC) launched the rice breeding program targeting at high yield, disease resistance, and good quality in early 1970s, the nationwide studies on the evaluation and identification of germplasms for BB resistance were commenced, and the researches of BB resistance rice breeding have been rapidly and extensively progressing since then. There were 911 indica and 351 japonica new cultivars developed in the period 1960～1986, representing an annual acreage of more than 6,667 hm^2. Among those, 192 (21.1%) out of 911 indica, and 90 (25.6%) out of 351 japonica cultivars were resistant to BB. Before 1980s, 31 (35.6%) out of 87 indica three-line hybrid rice combinations were developed with moderate resistance toward BB. The combinations of Weiyou 64, Weiyou 6, and Shanyou 6 had reached an annual acreage of 1.333 M hm^2. Up to 2005, some combinations exhibiting high yield, good quality and resistance to both BB and fungal blast have accumulated a large acreage, such as Shanyou 77 (2.497 M hm^2 in 1996～2005) , IIyou 084 (0.757 M hm^2 in 2001～2005), etc. For two-line hybrid rice, up to 2006, 6 out of 21 combinations with high yielding , good quality were resistant to both BB and fungal blast, such as Liangyou-peijiou, Peiza-shuangqi, and 70 you 9 (japonica). Up to 2005, the accumulated acreage of Liangyou-peijiou had reached 4.681 M hm^2, representing 50% of the total acreage of two-line hybrid rice in China.

The GEU concept from IRRI was introduced to China by the late Chinese rice breeder Prof. S.C. Lin. This effort brought China into the international collaborative network to share information and materials on BB resistance. In the rice cultivation field of China, the genetic sources of BB resistance are very narrow. Most indica and japonica rice depend on the resistance conferred by *Xa4* and *Xa3*. There is a urgent need to identify new and broad spectrum *Xa* genes. To perform extensive screening of BB resistance rice germplasms, Chinese scientists first established a

standardized and stable evaluation system for BB. Until early 1990s, scientists evaluated approximately 70,424 rice accessions (from local cultivars of different ecological regions, wild *Oryza* species, improved cultivars, and foreign introductions) for BB resistance. For example, Huazhong Agricultural University and Yunnan Academy of Agricultural Sciences collaboratively identified *Xa22^t* by investigated local BB resistant cultivars in Yunnan province. *Xa22^t*, which mapped on chromosome 11 causes resistance to Chinese pathotypes I, II, IV, and VII, most of the pathotypes of Yunnan province, Philippine races 1~ 6, as well as Japan races at the booting stage. Wild *Oryza* species is an important source for BB resistance genes. Two wild *Oryza* species including: *O. rufipogon, and O. officinalis* were evaluated and characterized in these germplasms and led to the discovery of *Xa23* , and *Xa29^t* respectively in China, in which *Xa23* confers the broadest spectrum BB resistance among all *Xa* gene identified thus far. Elite germplasms and cultivars with broad-spectrum resistance to BB have been adopted for rice cultivation and breeding programs. By the end of 1990s, the broad-spectrum resistance genes, *Xa21, Xa7*, *xa5* and *Xa23,* have been widely used to overcome the dependence of limited genetic resources. The cloning of the dominant broad-spectrum R gene *Xa21* and the identification of the complete dominance R gene *Xa23* identified provided more reliable and durable resources of genes for improvement of BB resistance in hybrid rice in China. Classical and molecular studies of Mendelian and quantitative genetics of BB resistance in rice were also performed. The genetic behaviors and resistance phenotypes in hybrid progenies were investigated and used as a guidance for breeding. For instance, the genotypes of the R parents, such as the number of genes, dominant and recessive relationships, resistance in different growth stages might differ, depending on the interaction between host (R gene) and pathogen races (virulence gene).

In addition, 6 japonica rice NILs were constructed using SN1033 as the recurrent susceptible parent, named CBB- (carrying *Xa2*, *Xa3*, *Xa4*, *Xa12*, *Xa14* and one unnamed *Xa* gene, respectively). Two other pairs NILs (CBB23 and CBB23B) were also built for *Xa23* using JG30 (indica) and IR24 (indica) as the recurrent susceptible parents in China. In the future, a combined strategy composed of traditional breeding, molecular marker-assisted selection, and transgenic technology should bring a new era to the BB resistance rice breeding program in China.

Impact of gene pyramids on *Xanthomonas oryzae* pv. *oryzae* population structures—implications for deployment of *Xa* genes

C.M. Vera Cruz[1], I. Oña[1], M.Y. Reveche[1], K.M. Webb[1,2], G. Carrillo[1], N. Dac Khoa[1,3], L. Quiatchon[1], P. Virk[1], M. Bustamam[4], J. Agarcio[5], J. Wu[6], K. Singh[7], K.K. Jena[8], T.W. Mew[1], J.E. Leach[9]

[1]Plant Breeding, Genetics and Biotechnology, International Rice Research Institute, DAPO Box 7777, Manila, Philippines; [2]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502; [3]Department of Plant Protection, Cantho University, Cantho, Vietnam;

[4]ICABIOGRAD, Bogor, Indonesia; [5]Philippine Rice Research Institute, Nueva Ecija, Philippines; [6]CNRRI, Hangzhou, China; [7]Punjab Agricultural University, Ludhiana, India; [8]Rural Development Administration, Suwon, Korea; [9]Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80537-1177

Previous studies of resistance gene *Xa7* suggested that *Xa7* could impact on the fitness of strains of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) adapted to rice lines containing *Xa7*. It is not known, however, how *Xa7* in combination with other *Xa* genes may affect pathogen fitness and population structure. We tested the performance of near-isogenic lines IRBB4, IRBB5, IRBB7, IRBB21 and pyramid lines IRBB61 (*Xa4+xa5+Xa7*), IRBB62 (*Xa4+Xa7+Xa21*), and IRBB64 (*Xa4+xa5+Xa7+Xa21*) for 3 years (2002-2004) at two sites in the Philippines where *Xa7* had been exposed to local pathogen populations for 7 years. We determined the genetic diversity and virulence spectrum of the *Xoo* populations from these lines. The majority of the populations belong to Lineage C (races 3C and 9a,b,c) while a very few strains of Lineage B (race 2) were detected. The pathogen populations from single gene NILs were genetically less diverse than those collected from pyramid lines. Interestingly, the *Xoo* populations collected from 3-gene pyramids with *Xa4+xa5+Xa21* and *Xa4+Xa7+Xa21* were more diverse than those from a 4-gene pyramid containing *Xa4+xa5+Xa7+Xa21.* In the 4-gene pyramid, haplotypes C7 and C12 representing a majority of race 9b dominated the population at both sites over 2 years. Race 9b showed a continuous variation in virulence to *Xa7*, ranging from moderately to highly virulent. Strains of race 9c were highly adaptive to *Xa7* but found in lower frequency relative to those strains of 9b, suggesting a fitness cost in race 9c associated with adaptation to *Xa7*. Since *Xa7* is present in both 3- and 4-gene pyramids, the different effects of the pyramids on pathogen diversity appear to result from interaction of the *Xa* gene combinations.

In breeding programs, the common practice has been to combine single genes that have been shown effective in a region. Knowing how the gene combinations affect pathogen population structures could have predictive value on the effectiveness of the resistance gene combinations prior to deployment. With markers, it is possible to create different gene combination efficiently. In collaboration with breeders at IRRI and national program partners in Asia, we have applied markers to develop gene combinations in conventional inbreds and new plant types, and in parents of hybrid rice. Some of these lines carrying either single, two or three-gene combinations in Indonesia, the Philippines, China, India and Korea have been released as varieties or used as donors while others are in the advanced lines of testing in their breeding program.

Introgression of effective bacterial blight resistance genes, fragrance genes and QTL mapping for aroma and other grain quality traits in Basmati-derived rice lines

S.N. Begum[1*], P.S. Virk[2] and C.M. Vera Cruz[2]

[1]Bangladesh Institute of Nuclear Agriculture, P.O.Box 4, Mymensingh 2200, Bangladesh;

[2]International Rice Research Institute, DAPO Box 7777, Metro Manila, The Philippines

*Correspondence

Abstract

Basmati rice is a premium class of aromatic rice grown traditionally in Pakistan and India, but is susceptible to bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Molecular markers linked to BB resistance genes (*Xa* genes) and genes for aroma (*fgr genes*) were utilized in a marker-aided selection program to develop elite breeding lines with broad-spectrum resistance to bacterial blight and aroma. Sequence tagged site (STS) and simple sequence repeat (SSR) markers were used to detect the genes for BB and aroma, respectively. A cross was made between a Basmati-derived line IR 71730-51-2 and IRBB60 (a pyramided line containing *Xa4, xa5, xa13* and *Xa21 genes)* at Plant Breeding, Genetics and Biotechnology Division of the International Rice Research Institute (IRRI), Philippines. Phenotypic selection was practiced until F_8 when genotyping for *Xa* genes and *fgr genes* was performed. While one gene might mask the expression of another gene, markers linked to resistance genes helped the pyramiding of target genes. The combination of *Xa* genes provided a wider spectrum of resistance to *Xoo* in the Philippines; singly, *Xa21* was the most effective, followed by *xa5*. Two, three and four gene pyramids of bacterial blight resistance genes had been introgressed from IRBB60 donor to five Basmati-derived rice lines (three lines having *Xa4* and *Xa21*, one line having *Xa4, xa5,* and *xa13* and one line having *Xa4, xa5, xa13,* and *Xa21 genes*). Among them, two lines possessed *fgr genes* for aroma. A set of 200 recombinant inbred lines (RILs) was also developed and was evaluated for BB resistance, six agronomic traits, six physico-chemical characteristics and QTL analysis of grain quality traits. Elite lines that were identified with genes for aroma and BB along with good agronomic performance and other grain quality traits were evaluated in large replicated yield trials. Three QTLs for aroma in chromosome 8, two QTLs in chromosome 6 and one in chromosome 7 for amylose, two QTLs in chromosome 6 for gelatinization temperature, and one QTL each in chromosome 7 for grain length, grain width and grain length-width-ratio were detected from these 200 Basmati-derived RILs population. These elite lines could be readily used in breeding programs to transfer BB and aroma genes and other grain quality traits into aromatic rices.

Key words: Basmati rice, BLB, QTL mapping, aroma, grain quality, STS and SSR markers.

Introduction:

Basmati is the most popular variety with a high demand in the international market for its pleasant aroma, fine long slender grains with superior cooking and eating qualities. The traditional Basmati varieties are tall, photosensitive and susceptible to various diseases like bacterial blight (BB) and insect pests resulting in low grain yield. Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* is the second most important disease of rice in southeast Asia resulting an average of 20-30% yield loss. In some areas of Asia rice yield losses are recorded to be up to 50%. Agarwal *et al.* (2005) reported that in the Basmati rice, yield loss can reach up to 100%. Moreover, there is no resistant source of Basmati rice variety known for its quality and aroma. Durability of resistance is of great concern with bacterial blight. Large scale and long term cultivation of varieties with single genes may enable the pathogen to overcome BB resistance. This can be delayed by pyramiding multiple resistance genes into rice cultivars. Pyramiding of resistance genes in a variety confers resistance against two or more than 2 pathogen races. The pyramid lines showed a wider and higher level of resistance than lines with only a single gene (Yoshimura *et al*., 1996; Huang *et al*., 1997; Singh *et al*., 2001). Basmati rice however, is difficult to improve by conventional breeding method due to genetic differentiation between Basmati and Indica rice varieties; polygenic nature of aroma and grain quality traits and loss of quality characteristics upon hybridization. Thus marker-aided selection can be useful in introgressing desirable traits into elite Basmati lines.

The present study was carried out to introgress bacterial blight resistance genes and fragrance genes into Basmati-derived lines and to map the QTL alleles for aroma and other grain quality traits into Basmati-derived rice lines.

Materials and Methods:

Plant materials

A Basmati-derived rice line, IR71730-51-2 in the background of Basmati-370 was used as recurrent parent for crossing with IRBB60, a pyramid line with *Xa4, xa5, xa13* and *Xa21* genes for bacterial blight resistance at Plant Breeding, Genetics and Biotechnology Division of the International Rice Research Institute (IRRI), Philippines. The line, IR71730-51-2 was shorter, aromatic and agronomically superior to the traditional Basmati variety but lacks resistance to bacterial blight disease. F_1 plants were selfed and 250 F_2 plants were selected and advanced up to F8 generation. Simultaneously, a population of recombinant inbred lines (RILs) was also developed from the cross, IR71730-51-2/IRBB60-1 to map QTLs for aroma and other grain quality traits. F_1 seeds were produced and the cross was named as IR77542 and advanced to F_8 (200 lines). Selection for BB resistance was based on molecular analysis and phenotype evaluation from F_2 to F_8 generation.

Phenotypic evaluation

Selected plants were inoculated with Xoo races 1 and 2 at maximum tillering stage of the plant. Disease reaction was scored 14 days after inoculation by measuring

lesion length following the method of Kauffman *et al*., 1973. At F_8 generation, ninety five pedigree families and two hundred recombinant inbred lines along with their parents simultaneously were evaluated in separate experiment in randomized complete block design with three replications. Both plant to plant and row to row distances were 25 cm. Three plants from each of the progenies were evaluated for plant height, number of tillers and panicle length. Data on days to 50% flowering and days to maturity and grain yield (g) were taken from each plot.

Harvested seeds from individual progenies were analyzed for physico-chemical characters, such as grain size, grain shape, aroma, amylose content and gelatinization temperature. Grains were categorized into different types based on their dimension. For testing aroma, powdered milled rice grains were soaked in 10 ml of 1.7% KOH solution for 1 hour and evaluated by the panel. Grounded powder of rice grains was analyzed with autoanalyzer for amylose content. For estimation of gelatinization temperature (test for ASV), whole milled rice grains were incubated in 10 ml of 1.7% KOH solution for 23 hours for scoring.

Marker genotype determination

To pyramid BB resistance genes into a breeding line, 250 selected F_2 plants were analyzed with four STS markers. Genotyping for *Xa* genes was practiced in F_2, F_5, F_7 and F_8 generations. Phenotyping of BB was done in F_2 to F_7 generation with race 2 and F_8 with race1, race2, race 6 and race10 in the IRRI field and screen house, respectively and susceptible plants were discarded. DNA markers were used to identify homozygotes for each of the genes in every generation. Plants with banding pattern identical to that in IRBB4 showed the presence of *Xa4* gene. The presence of *xa5* and *xa13* genes in the 113 Basmati derived rice lines was determined through PCR analysis using RG556 and RG136 STS markers generated from RFLP markers closely linked to *xa5* and *xa13*, respectively (Huang *et al* 1997), The lines which possessed *xa5* resistant allele showed approximately 450 bp band while 1000 bp band corresponded to the susceptible allele. For *Xa21* gene, the first resistance gene in rice that was cloned by Song *et al*. (1995), polymorphism was detected between the recipient and donor parents. A polymorphic band approximately 1.4 kb represents the resistant allele and the slightly smaller band represents the susceptible allele.

DNA isolation was carried out using mini prep CTAB method. Starting from the F_2 onward, polymerase chain reaction (PCR)–based molecular markers linked to BB resistance genes *Xa4, xa5, xa13* and *Xa21* genes (STS markers) and *fgr genes* (SSR markers) for aroma were used to analyze the plants (Table 1). Lines having 2, 3 and 4 homozygous genes for BB resistance and homozygous for *fgr genes* were selected.

For QTL analysis, a total of 330 SSR markers were screened to detect polymorphism at the 12 chromosomes. Polymorphism in parents and marker genotypes of the RIL population were determined by resolution of PCR amplified product on 8% polyacrylamide gel electrophoresis.

Table 1. Markers linked to BB resistance genes and fragrance genes used in this

study.

Marker	Linked Gene	Primer/probe sequence	Chromosome
MP	**Xa4**	MP1 5'ATCGATCGATCTTCACGAGG3'	11
		Mp2 5'dTGCTATAAAAGGCATTCGGG3'	
RG556	*xa5*	F 5'TAGCTGCTGCCGTGCTGTGC3'	5
		R 5'AATATTTCAGTGTGCATCTC3'	
RG136	*xa13*	**F 5'TCCCAGAAAGCTACTACAGC3'**	8
		R 5'GCAGACTCCAGTTTGACTTC3'	
Xa21	*Xa21*	F 5'ATAGCAACTGATTGCTTTGC3'	11
		R 5'CGATCGGTATAACAGCAAAAC3'	
RM223	*fgr*	F 5'GAGTGAGCTTGGGCTGAAAC	8
		R 5'GAAGGCAAGTCTTGGCACTG	

Data analysis

Single marker analysis was followed for tagging QTLs for aroma and other grain quality traits i.e., grain size, grain shape, aroma, amylose content and gelatinization temperature. One-way ANOVA using SAS (PROC GLM) was performed for each marker/trait combination. This analysis involved SSR marker profile and the phenotypic means for aroma and other grain quality traits scored on F_8 RILs. The proportion of the total phenotypic variation explained by each marker associated with a putative QTL was calculated as R^2 value (R^2 = ratio of the sum of squares explained by the marker locus to the total sum of squares). The statistical significance of each marker was determined from probability values.

Results and Discussion

Of the 250 F_2 plants 113 possessed at least one *Xa* gene. Seven plants possessed four resistance (R) gene combinations (*Xa4, xa5, xa13* and *Xa21*) in homozygous condition, 25 plants carried three *R* genes, 43 plants with two *R* genes and 38 plants with a single *R* gene only either in homozygous or heterozygous condition. A set of selected 113 F_2 plants were also analyzed for fragrance gene through genotypic analysis using SSR markers. Nine of the 113 F_2 plants did not produce any band. Out of 104, 23 plants possessed *fgr genes*.

A set of 113 $F_{3,}$ 68 $F_{4,}$ 39 $F_{5,}$ 23 F_6 progenies were grown in the field and genotyped using molecular markers for four *Xa* genes and *fgr gene*s. Banding pattern of four resistance genes and fragrance genes is shown in Fig. 1(a-d) & 2. Ten progenies in F_7 generation were selected on the basis of grain quality, agronomic performance and BB resistance.

Each of the 10 F_7 progenies were represented by 3 sister lines. STS markers were used to identify homozygotes for each of the *Xa* gene in the F_7 population. Disease severity (%) was very low for all the lines. Finally five promising lines were selected and evaluated in the IRRI field. The *Xa* genes introgressed into the selected F_8 Basmati-derived rice lines and their reaction to four diagnostic races of *X. oryzae* pv *oryzae* and physico-chemical analysis is shown in Table 2.

Xa4 and *Xa21* genes combination were introgressed into three rice lines. Three genes (*Xa4*, *xa5* and *xa13*) were successfully incorporated in IR77542-290-3-2-2-1 line. One line IR77542-234-3-1-1-1 homozygous for the alleles of all resistance genes (*Xa4*, *xa5*, *xa13* and *Xa21*) was detected. Combining the resistance genes *Xa4* and *xa5* or *xa5* and *Xa10* provided a higher level of resistance to *Xoo* than that was found in the sum of the individual genes at the parental level (Yoshimura *et al.*, 1995). Huang *et al.* (1997) reported that lines carrying Xa4 and xa13 showed wider level of resistance. Madamba (2000) found that pyramid lines with *Xa4, xa5, xa13* and *Xa21* enhanced resistance to contemporary races of *X. oryzae* pv. *oryzae* relative to individual genes.

Line IR77542-270-3-2-1-1 containing two genes (*Xa4* and *Xa21*) showed resistance to PXO61 (lesions-0.5cm), PXO86 (lesions-2.29cm), PXO99 (lesions-4.5cm) and PXO341 (lesions-2.03cm) and the lesions were much shorter compared to other two lines (IR77542-155-2-2-3-1, IR77542-220-2-2-3-1) containing the same gene. Lines containing three gene- and four gene-combination (*Xa4*, *xa5*, *xa13* and *Xa21*) showed a reduction in lesion length to all four races diagnostic for the three and four individual genes, while the recipient parent with *Xa4* gene alone showed long lesions to PXO86 (xx cm) and PXO99 (xx cm). Because Basmati 370 does not contain any of the known *Xa* gene, it showed longest lesion to all four races. Based on molecular analysis of fragrance gene (*fgr*), two lines, namely IR77542-270-3-2-1-1 and IR77542-290-3-2-2-1 possessed the *fgr genes* for aroma. On the basis of physico-chemical traits these lines carried strong aroma, 16-24% amylose content and intermediate or low gelatinization temperature. Considering this package of traits, IR77542-270-3-2-1-1 and IR77542-290-3-2-2-1 serve as valuable genetic resources that can be used in future breeding programs.

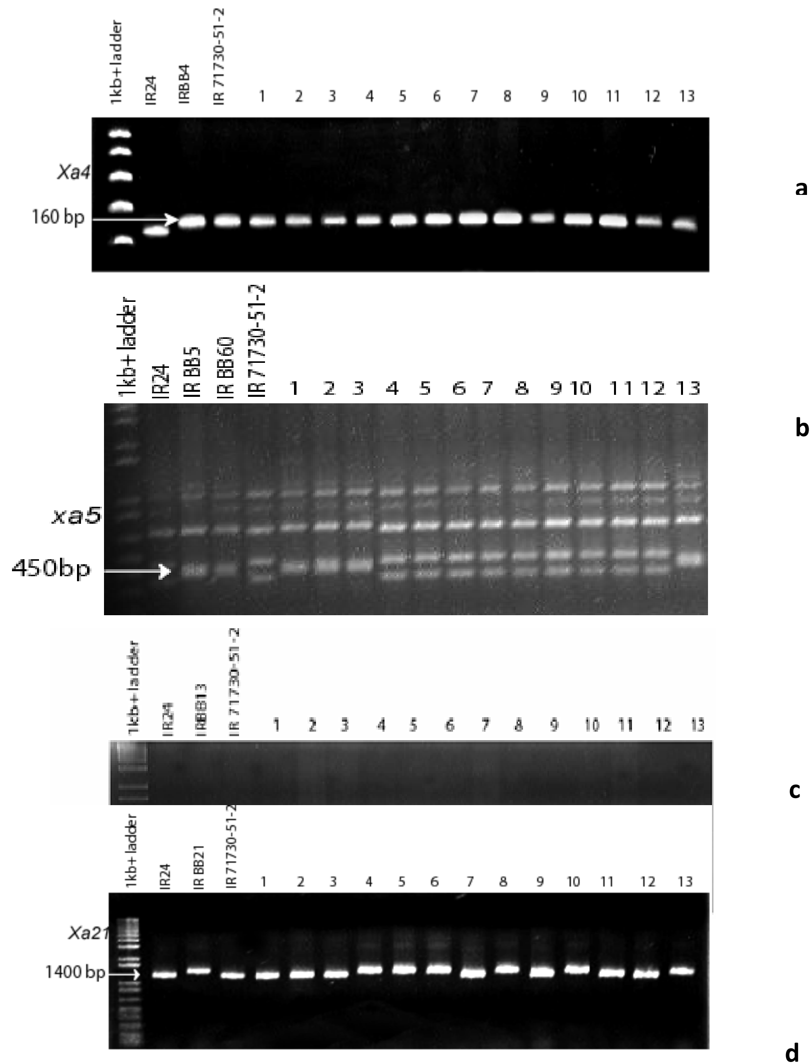


Fig.1(a-d).PCR analysis of 4 marker loci. DNA amplified with MP (linked with *Xa4* gene), RG556 (*xa5* gene), RG136 (*xa13* gene) and Xa21 (*Xa21* gene) primers

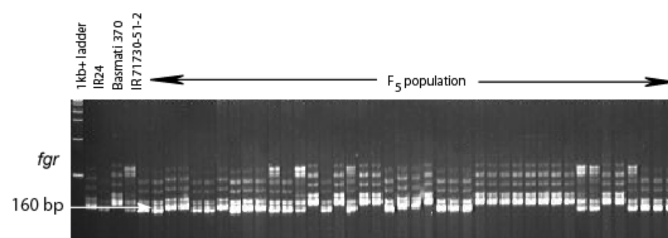


Fig. 2. Banding pattern of parents and segregating progenies for RM223. Aromatic allele co-segregates with a band of approximately 160 bp length

The results indicated that these gene combinations were more effective than a single gene. When different genes are combined, the resistance is expected to be more durable. These wider spectra or higher levels of resistance in the pyramids might be due to interaction and/or complementation between the resistance genes. Multilocation testing of different gene combinations and monitoring the population of the pathogen over several crop cycles would be useful in identifying durable resistance in Basmati derived rice lines against the BB pathogen.

Analysis of the recombinant inbred line population for the *Xa* genes and *fgr genes*

STS and SSR markers for *Xa* and *fgr genes*, respectively, were used to analyze 200 RILs along with the parents (IR71730-51-2 and IRBB60-1) to determine the presence of BB resistance gene and fragrance gene alleles. Twenty five progenies were homozygous or heterozygous for alleles of the four resistance (*R*) genes (*Xa4, xa5, xa13* and *Xa21*), 65 had three *R* genes, 85 had two *R* genes and 25 progenies carried a single *Xa* gene only. Ninety six lines possessed the *fgr genes*.

Based on the presence of *Xa* genes, *fgr genes*, grain quality traits and lesion length with good grain yield (ranging from x-x t/ha or kgs/plot???), 10 recombinant inbred lines were selected. All these lines possessed either four *Xa* genes with *fgr genes* or three *Xa* genes with *fgr genes* or four *Xa* genes with minimum Basmati grain quality standard (Singh, 2000). Resistant to bacterial blight, these lines also showed superior agronomic performance.

Table 1. Reaction of F_8 pedigree lines of the cross (IR71730-51-2/IRBB60) to 4 races of *X. oryzae* pv. *oryzae*, their molecular genotypes for BB resistance genes and *fgr genes* along with grain quality data

LINE	Resistance genes	Fragrance gene	Lesion length (cm)				Aroma	Amylose content (%)	Gelatinization temperature
			Race1 (PXO61)	Race2 (PXO86)	Race6 (PXO99)	Race10 (PXO341)			
IR77542-155-2-2-3-1	*Xa4/Xa21*	--	0.77	2.80	5.70	3.57	Strong	16	High/Inter
IR77542-220-2-2-3-1	*Xa4/Xa21*	--	1.25	2.56	7.45	4.26	Strong	24	Low
IR77542-234-3-1-1-1	*Xa4/xa5/xa13/Xa21*	--	0.40	1.42	2.34	1.52	Strong	22	High/Inter
IR77542-270-3-2-1-1	*Xa4/Xa21*	++	0.50	2.29	4.50	2.03	Strong	19	Low
IR77542-290-3-2-2-1	*Xa4/xa5/Xa13*	++	1.59	3.91	4.28	0.81	Strong	16	Low
IR24	No Xa gene	--	27.23	26.38	24.98	26.77	None	16	Low
IRBB60	*Xa4/xa5/xa13/Xa21*	--	0.30	0.64	1.53	0.30	None	13	High
Basmati-370	No *Xa* gene	++	33.86	29.12	45.46	28.93	Moderate	19	High
IR71730-51-2	*Xa4*	++	4.92	29.28	32.33	7.34	Moderate	21	Low

++ = *fgr* present; -- = *fgr* absent

Polymorphism of markers

Three hundred and thirty microsatellite (SSR) primers were screened for polymorphism detection between the two parental genotypes (IR71730-51-2 and IRBB60-1). Of these, 37 SSR primers were polymorphic and used to evaluate the RIL population.

The linkage map (Fig. 1) was constructed according to published microsatellites with the IR64/Azucena DH lines from Cornell University developed by Temnykh *et al*. (2001). The construction linkage map from RILs covered only 5 rice chromosomes with a total of 32 SSR markers due to lack of polymorphism.

Phenotypic variation in RILs for grain quality traits

Two hundred recombinant inbred lines and their parents were used to evaluate for various grain quality traits. After harvesting the grains, the grain quality traits were evaluated for aroma, percent amylose for amylose content, alkali digestion test for gelatinization temperature and standard scale for grain shape and size.

A summary of the data for various traits is given in Table 3. Significant differences were observed for all the quality traits between the parents except for grain width. IR71730-51-2 had the higher trait value for aroma, amylose content, gelatinization temperature, grain length and grain length width ratio while IRBB60 had bolder grains. RILs showed higher or lower trait values than both the parents for the aroma, amylose content, grain length, grain width and grain length-width ratio. Using a set of double haploid lines, Arif (2002) found highly significant differences for all the characters between the parents except grain length, and the selected progenies showed higher and lower trait values for grain length, grain width, grain length-width ratio, gelatinization temperature and amylose content. Twenty one BC_1F_3 genotypes of Basmati-derived rice families were subjected to quality analysis by Joseph *et al.* (2004). Most of the selections were found to meet the Basmati grain quality standard.

Table 3. Means and ranges for various grain quality traits for parents and RILs.

Traits	Parents		Selected 200 RILs population		
	Mean values				
	IR 71730-51-2	IRBB60	Mean	SD	Range
Aroma	3.33	1.67	2.49	0.94	1.00-4.00
Amylose content	21.33	13.67	18.46	2.92	13.33-25.33
Gelatinization temperature	7.00	3.00	4.94	1.62	3.00-7.00

Grain length	7.91	6.62	7.18	0.51	6.13-8.95
Grain width	1.86	1.95	1.91	0.06	1.77-2.05
Grain length-width ratio	4.25	3.35	3.76	0.34	3.12-4.53

QTLs affecting grain quality traits

Eleven QTLs affecting grain quality traits were identified in the selected 200 RIL population. These QTLs were mapped to three rice chromosomes (Fig. 3) and collectively explained a significant portion of the phenotypic variation for the measured traits in the selected RIL population.

Identification of QTLs

One way ANOVA using SAS version 8.0 was used to analyze each marker and phenotypic data. The association of molecular markers with phenotypic data was determined and the results are summarized in Table 4. A total of 32 markers were used in the study.

The result revealed that three markers, RM223, RM342A and RM515 on chromosome 8, were strongly associated ($P<0.0001$) with aroma. These markers explained 22.46%, 28.38% and 41.78% of the total phenotypic variation, respectively. Garland *et al.* (2000) reported the usefulness of RM223 in breeding for aroma in rice. Obviously the present results are in agreement with those of Garland *et al.* (2000). Interestingly RM515 which is also mapped at the same position as RM223 which explained more variation for aroma than RM223.

For amylose content, two markers namely RM314 and RM510 on chromosome 6 showed strong association ($P<0.0001$) and explained 8.3%, 15.353% of the phenotypic variation, respectively. These two markers, RM314 and RM510 were also detected to be strongly associated for gelatinization temperature and explained 10.74% and 11.63% phenotypic variation, respectively. These two markers are in the vicinity of *wx* gene which has been mapped in various studies on the short arm of chromosome 6 (www.gramene.org). Ten *et al*. (1999) and Lanceras *et al*. (2000) reported that genes controlling amylose content, gel consistency and gelatinization temperatures are controlled by a single locus coinciding with the '*Wx*' region on chromosome 6. In this study, the amylose content and gelatinization temperature gene were also detected at or near the same. Arif (2002) also found QTL for amylose on chromosome 6. The other QTLs related to the amylose content such as QTLs for amylose content on chromosome 7 could be a different gene.

Three markers RM11, RM234 and RM336 located on chromosome 7 were strongly associated with grain length, grain width and grain length-width ratio. RM234 explained the maximum phenotypic variation for all the three traits. These results are in close agreement with Huang *et al.* (1997) for grain length. Similarly, Arif (2002) identified a QTL for grain length-width ratio on chromosome 7 which coincided with the present study, while Kang *et al.* (1999) found QTLs for grain length-width ratio on chromosome 2, 10 and 12.

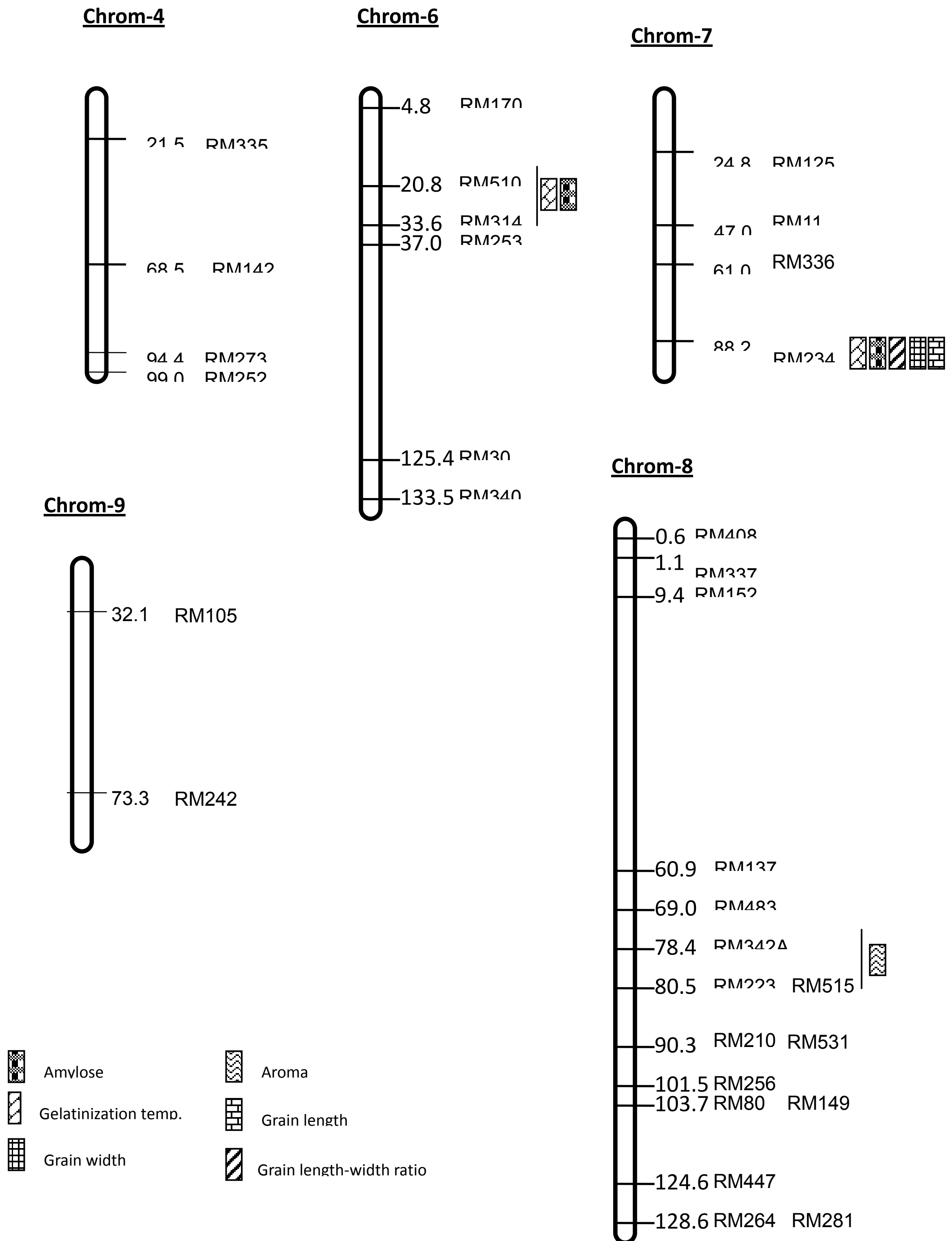


Fig .3. Chromosomal location of QTLs for rice grain quality traits

Table 4. Single marker analysis for grain quality traits in 200 RILs

Sl. No.	Locus	Chromosome	R^2 (%)[1]	F-value	P-value[2]
Aroma:					
1	RM149	8	5.3	11.2	0.001
2	RM210	8	12.33	27.99	0.0001
3	RM223	8	22.46	57.66	0.0001
4	RM256	8	7.79	16.74	0.0001
5	RM342A	8	28.38	75.3	0.0001
6	RM515	8	41.78	143.58	0.0001
Amylose content:					
1	RM170	6	3.6	6.96	0.009
2	RM314	6	8.3	17.27	0.0001
3	RM510	6	15.86	37.53	0.0001
4	RM234	7	7.44	15.6	0.0001
5	RM336	7	2.42	4.68	0.0318
6	RM342A	8	2.46	4.79	0.0298
Gelatinization temperature:					
1	RM170	6	2.51	4.8	0.0297
2	RM314	6	10.74	22.76	0.0001
3	RM510	6	11.63	26.21	0.0001
4	RM234	7	2.63	5.26	0.0229
5	RM210	8	1.83	3.72	0.05
6	RM281	8	3.57	7.19	0.008
7	RM342A	8	3.37	6.64	0.0108
8	RM447	8	4.23	8.8	0.0034
Grain length:					
1	RM273	4	2.25	4.43	0.0365
2	RM314	6	2.16	4.18	0.0423
3	RM11	7	8	17.38	0.0001
4	RM234	7	34.18	100.78	0.0001
5	RM336	7	22.51	54.64	0.0001
6	RM149	8	4.14	8.64	0.0037
7	RM256	8	2.77	5.64	0.0185
Grain width:					

1	RM252	4	2.92	5.87	0.0163
2	RM273	4	3.04	6.03	0.0149
3	RM11	7	10.67	23.65	0.0001
4	RM234	7	32.38	92.9	0.0001
5	RM336	7	26.13	66.52	0.0001
6	RM149	8	1.8	3.67	0.05
7	RM408	8	2.3	4.72	0.0309
Grain length-width ratio:					
1	RM273	4	2.87	5.69	0.018
2	RM314	6	2.32	4.49	0.0354
3	RM11	7	10.25	22.63	0.0001
4	RM234	7	39.52	126.78	0.0001
5	RM336	7	27.75	72.23	0.0001
6	RM149	8	3.87	8.07	0.005
7	RM256	8	2.22	4.5	0.0351

[1]Percent of Variance explained, [2]Probability

Conventional breeding based on phenotypic selection has not been very successful to improve productivity of Basmati. The present approach of marker-assisted introgression shows great promise to improve Basmati rice variety.

References

Agarwal, P.K., G.S. Sidu and S.S. Gosal, 2005. Induction of Bacterial Blight Resistance in Elite Indian Rice (*Oryza Sativa* L.) Cultivars using Gamma Irradiation and Ethyl Methane Sulfonate. Mutation Breeding Newsletter and Reviews. No.1, June 2005 pp. 17-18.

Arif, M. 2002. Molecular mapping of genes/QTLs affecting resistance to *Xanthomonas oryzae* pv. *oryzae* and grain quality traits in rice (*Oryza sativa* L.). Ph.D. Thesis, University of the Philippines Los Baños, College, Laguna, Philippines. 137 p.

Garland, S., L. Lewin, A. Blakeney, R. Reinke, and R. Henry. 2000. PCR-based molecular markers for the fragrance gene in rice (*Oryza sativa* L.). Theor. Appl. Genet. 101:364-371.

Huang, N., E.R. Angeles, J. Domingo, G. Magpantay, S. Singh, G. Zhang, N. Kumaravadivel, J. Bennett and G.S. Khush. 1997. Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theor. Appl. Genet. 95:313-320.

Joseph, M., S. Gopalakrishnan, R.K. Sharma, V.P. Singh, A.K. Singh, N.K. Singh and T. Mohapatra. 2004. Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. Mol. Breed. 00:1-11.

Kang H.J., Y.G. Cho, Y.T. Lee, H.S. Kim, M.Y. Eun and J.U. Shim. 1999. Detection of putative QTL conferring grain size and shape in Rice. Korean J. Breed. 31(4): 330 – 335.

Kauffman, H.E., A.P.K. Reddy, S.P.Y. Hsieh and S.D. Merca. 1973. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. Plant Dis. Rep.

57:537-541.

Lanceras, J.C., Z.L. Huang, O. Naivikul, A.Vanavichit, V. Ruanjaichon and S. Tragoonrung. 2000. Mapping of genes for cooking and eating qualities in Thai Jasmine rice (KDML105). DNA Research 7:93-101.

Madamba, M.R.S.B. 2000. Molecular and phenotypic characterization of *Xanthomonas oryzae* pv. *oryzae* (Ishiyama Dye) swings *et al.* adapted on *Xa21* gene for rice bacterial blight resistance. MS. Thesis. University of the Philippines Los Baños, College, Laguna, Philippines. 74 p.

Singh, R.K., U.S. Singh and G.S. Khush. 2000. Prologue. In: Singh R.K., U.S. Singh and G.S. Khush (eds), Aromatic rices. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India. pp. 1-3.

Singh, S., J.S. Sidhu, N. Huang, Y. Vikal, Z. Li, D.S. Brar, H.S. Dhaliwal and G.S. Khush. 2001. Pyramiding three bacterial blight resistance (*xa5, xa13* and *Xa21*) using marker-assisted selection into indica rice cultivar PR 106. Theor. Appl. Genet. 102:1011-1015.

Tan, Y.F., J.X. Li, S.B. Yu, Y.Z. Xing, C.G. Xu and Q. Zhang. 1999. The three important traits for cooking and eating quality of rice grains are controlled by a single locus. Theor. Appl. Genet. 99:642-648.

Temnykh, S., G. De Clerck, A. Lukashova, L. Lipovich, S. Carthinour and S.R Mccouch. 2001. Computational and experimental analysis microsatellites in rice (*O. sativa L.*): frequency, length variation, transpose associations, and genetic marker potential. Genome Research 11:1441-1452. (http:// www. gramene. org/ microsat/ index. html).

Yoshimura, A., J.X. Lei, T. Malsumoto, H. Tsunematsu, S. Yoshimura, N. Iwata, M.R. Baraoidan, T.W. Mew and R. J. Nelson. 1996. Analysis and pyramiding of bacterial blight resistanmce genes in rice by using DNA markers. pp 577-581. In G.S. Khush (ed): Rice genetics III. Proceedings of the Third International Rice Genetics Symposium. 16-20 Oct. 1995, Shangrila Plaza, Manila Philippines. pp 1011.

Yoshimura, S., A. Yoshimura, N. Iwata, S.R. Mccouch, M.L.Abenes, M.R. Baraoidan, T.W. Mew and R. Nelson. 1995. Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. Mol. Breed.. 1:375-387.

Bacterial blight resistant hybrid rice parental lines developed via bi-direc tional marker-aided selection

Joan Marie S. Agarcio[1§], Lucia M. Borines[2], Samuel A. Ordoñez[1*], Marina P. Natural[3], Brad W. Porter[4†], Frank F. White[4], Hei Leung[5], Casiana M. Vera Cruz[5], Arlen dela Cruz[1], Neil Nemensio Baliuag[1], Fortunato Suratos[1], and Edilberto D. Redoña[5]

[1]*Philippine Rice Research Institute, Muñoz, Nueva Ecija, Philippines;*

[2]*Department of Pest Management, Leyte State University, Visca, 6521-A, Leyte, Philippines;*

[3]*Department of Plant Pathology, University of the Philippines Los Baños, Philippines;* [4]*Department of Plant Pathology, 4024 Throckmorton Plant Science Center, Kansas State University, Manhattan, KS 66506, USA;*
[5]*Plant Breeding, Genetics and Biotechnology Division, International Rice Research Institute, Los Baños, Laguna, Philippines;*

Author for correspondence (e-mail: jmsagarcio@philrice.gov.ph; phone: 540-808-7837
[§]Current address: Crops Soil and Environmental Sciences Department, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA 24060
*Current address: Louisiana State University, Baton Rouge, Louisiana, USA
[†]Current address: University of Hawaii at Manoa, Molecular Biosciences and Bioengineering, Honolulu, HI 96822

The results of the 2006 survey conducted by the Bureau of Agricultural Statistics (BAS) indicate that hybrid rice use doubles harvests and increase income among farmers. The importance of this technology is further exemplified by the tremendous support given by the Philippine Department of Agriculture in the Hybrid Rice Program. However, the success of hybrid rice is constantly threatened by the widespread occurrence of bacterial blight in the farmer's fields thus causing up to 50% yield loss especially during the wet season. In this study, we were able to solve this problem by pyramiding through marker-aided selection at most three bacterial blight resistance genes (Xa4, Xa7, and Xa21) into the commercially released Mestizo1 hybrid parental CMS lines IR58025B and IR58025A. In every backcross generation, linked molecular markers were used to select for plants with only the resistance genes and eliminate plants with the fertility restorer Rf genes. To further confirm presence of the resistance genes, Xoo race inoculation was done. To validate presence of the Rf genes, pollen evaluation (through I2KI staining) was conducted. Percent recovery of the parental types among the advanced progenies was determined through DNA fingerprinting using 13 RGA and 3 SRILS markers and morpho-agronomic tests and results indicate very high similarity of our selections with that of the recurrent parents. Hybrid lines using these improved parents are currently being tested in the field. With the continuously increasing demand for Mestizo1 commercial rice hybrids in farmer's fields, bacterial blight resistant Mestizo1 that will be produced from this study will consequently increase yields of farmers.

Molecular breeding for bacterial blight resistance in japonica rice

K. K. Jena[*,1, 2], J. U. Jeung[2], T. H. Noh[3], S. Heu[5], J. P. Suh[1, 2], K.Y. Kim[3], M. S. Shin[4], M. Y. Reveche[1], A. Pamplona[1], C. M. Vera Cruz[1], Y. G. Kim[2] and D.S. Brar[1]

[1]Plant Breeding Genetics and Biotechnology Division, International Rice Research Institute, Los Baños, Laguna, Philippines
[2]Genetics and Breeding Division, National Institute of Crop Science, Rural Development Administration, Suwon 441-857, Republic of Korea
[3]Honam Agricultural Research Institute, NICS, Rural development Administration, Iksan 570-080, Republic of Korea
[4]Yeongnam Agricultural Research Institute, NICS, Rural development Administration, Milyang, Republic of Korea
[5]Plant Pathology Division, National Institute of Agricultural Science and Technology, RDA, Suwon, Republic of Korea

*Author for correspondence,Email: k.jena@cgiar.org ,Tel: +82 31 290 6871,FAX: +8231294 8185

Abstract
Biotic stresses are major challenges for sustainable production and maintaining superior grain quality of Japonica rice. Bacterial blight (BB) disease of Japonica rice is becoming a major threat due to the dynamic population of the pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Japonica cultivars in Korea mainly possess some major resistance genes which are recently vulnerable to the new BB races. In our study we have assessed the reaction of some major resistance genes and their combinations using BB isolates/strains belonging to five *Xoo* races of Korea as well as 16 field isolates. We have identified IRBB lines possessing three-gene pyramids (*Xa4+xa5+Xa21*) that expressed resistance to all races including the new race K3a. The broad-spectrum resistance in the pyramided line is attributed to quantitative complementation of R genes. We have used conventional as well as molecular-marker assisted selection strategies for incorporation of three R genes into the Japonica cultivar Mangeumbyeo. Advanced backcross derived progenies reconstituted with recurrent Japonica parent genome possessing pyramided genes expressed strong resistance to BB races including the K3a in Korea as well as races 1 and 2 of the Philippines. Evaluation of agronomic traits and selection of promising breeding lines with resistance genes will broaden the genetic base of Japonica cultivars and provide opportunities toward development of broad-spectrum BB resistance in rice.

Introduction
Bacterial leaf blight (BB), a vascular disease of rice caused by the bacterial pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a major agronomic problem worldwide. The disease is prevalent in temperate as well as tropical rice growing areas of irrigated rice ecosystems in Asia and also reported in Australia, Latin America and Carribean (Mew et al. 1993). The disease occurs in seedling, vegetative and reproductive stages, but BB infection at maximum tillering stage causes severe blighting of leaves and yield loss up to 75% has been reported depending on weather, location and rice cultivar used (Ou 1985). Temperate Japonica rice is cultivated in approximately 10% of the areas in temperate rice growing environment in the world. Japonica rice cultivars

exhibit high susceptibility to BB disease due to narrow genetic diversity. In Korea, the southern coastal plain areas are hotspots for BB pathogen evolution and new variants of the pathogen spread into the mid-northern plains causing BB disease in Japonica rice cultivars. In 2003, a serious BB epidemic occurred in the south-western coastal plains accounting for a significant yield loss due to the emergence of a new BB race, K3a (Noh et al. 2003). Genetics of BB resistance has been well reported (Khush 2001). To date 25 BB resistance (R) genes have been identified and all the R genes follow a Mendelian pattern of major gene inheritance and express resistance to a diverse group of *Xoo* pathogens. Of the 25 R genes, three are physically mapped (*Xa2, Xa4 and Xa7*), and six (*Xa1, xa5*, *xa13, Xa21, Xa26 (Xa3*) and *Xa27*) are cloned (Liu et al. 2006). Three genes such as *Xa1, Xa26 and Xa7* are genetically linked to other R genes for BB resistance (He et al. 2006; Lee et al. 2000; Sun et al. 2004). Breeding and deployment of resistant cultivars carrying major R genes have been the most effective strategy to control the BB disease (Khush 2001). In this paper we discussed a novel strategy of incorporating three major resistance genes into a high yielding BB susceptible Japonica cultivar, Mangeumbyeo and conversion of advanced backcross progenies possessing R genes using Marker-assisted background selection approach.

Selected BB resistance genes and their potential for Japonica rice improvement

In our previous studies we found that BB races/isolates in Korea are dynamic in nature (Jeung et al. 2006). The near-isogenic lines (NIL) possessing different BB resistance genes expressed variable BB resistance against 16 virulent BB isolates collected from the field. Most of the major resistant genes such as *Xa1, Xa2, Xa3 and Xa4* present in Japonica cultivars are highly susceptible to the new BB isolates. However, the recessive resistance gene *xa5* and the dominant gene *Xa7* expressed low level of susceptibility to the Korean BB races compared to high susceptibility of *Xa1* gene. In contrast, the *Xa21* gene showed susceptibility to specific isolates belonging to K1 and K3 races. The R gene, *xa13* expressing BB resistance in South and Southeast Asia showed complete susceptibility to the Korean races (Jeung et al. 2006). The R genes with different combinations either in two or three-gene status expressed increased level of resistance to Korean races. Quantitative complementation was prominent in the R-gene combinations, *Xa4+Xa21* and *Xa4+xa5+Xa21* as the R genes interacted with each other independently and additively. Moreover, inoculation experiment on NILs with the recently evolved field isolate, K3a showed high susceptibility to IRBB4 (*Xa4*), IRBB7 (*Xa7*) and moderate resistance to IRBB21 (*Xa21*), whereas IRBB50 (*Xa4+xa5*), IRBB54 (*xa5+Xa21*), and IRBB57 (*Xa4+xa5+Xa21*) expressed strong resistance. With these findings we deduced that the R-gene pyramid of *Xa4*, *xa5*, and *Xa21* would be the most prospective genotype for improving Japonica cultivars.

Advantages of using pyramided *Xa4*, *xa5* and *Xa21* genes for Japonica rice

There are several advantages on using *Xa4*, *xa5* and *Xa21* genes for improvement of Japonica rice cultivars. The R-gene *Xa4* is derived from the traditional cultivar TKM 6 and has been widely used for BB resistance in many countries until its breakdown in

the 1970s. The gene *Xa4* is reported to be linked with the cloned gene *Xa26* which produces receptor-like Kinase protein (Sun et al. 2004). The recessive R-gene *xa5* derived from an Aus Boro line, DZ192 expressed resistance to the Korean BB races and the gene has been cloned that encodes a transcription factor gamma subunit (TFIIA) (Iyer and McCouch 2004). The R-gene *Xa21* derived from the wild species (*O. longistaminata*) is highly effective against BB races of South and Southeast Asia and also expressed resistance to the most virulent isolates in Korea. The *Xa21* gene is developmentally regulated and expressed effective resistance at adult stages. The *Xa21* gene has been cloned and encodes receptor-like kinase type protein (Song et al, 1995). With our understanding on gene functions of *Xa4*, *xa5* and *Xa21*, it is predicted that the R-gene combinations of *Xa4+xa5+Xa21* would exhibit broad-spectrum durable resistance against newly evolved *Xoo* strains in temperate rice environment.

Strategies for pyramiding BB resistance genes

We used dual selection strategies for the incorporation of *Xa4+xa5+Xa21* into the Japonica cultivar, Mangeumbyeo. In this approach the Mangeumbyeo cultivar has been used as female parent and IRBB57 carrying three R genes is used as the pollen parent. We used conventional breeding to produce F_1, BC_1, BC_2, BC_3 and BC_4 progenies and simultaneously carried out selfing for the development and identification of three R-gene homozygous plants. Molecular marker-assisted selection (MAS) has been successfully applied starting from BC_1F_1 generation progenies using cloned gene sequences as primers for *xa5* and *Xa21* and tight linked markers for the *Xa4* for gene detection along with BB resistant phenotype characterization of the progenies expressing resistance to K3a race of *Xoo*. 160 progenies with *Xa4+xa5+Xa21* genes in homozygous state and expressing strong resistance to the most virulent strain of K3a race (HB01009) are selected in advanced backcross population of BC_3F_3 and BC_2F_4 generations (Fig. 1).

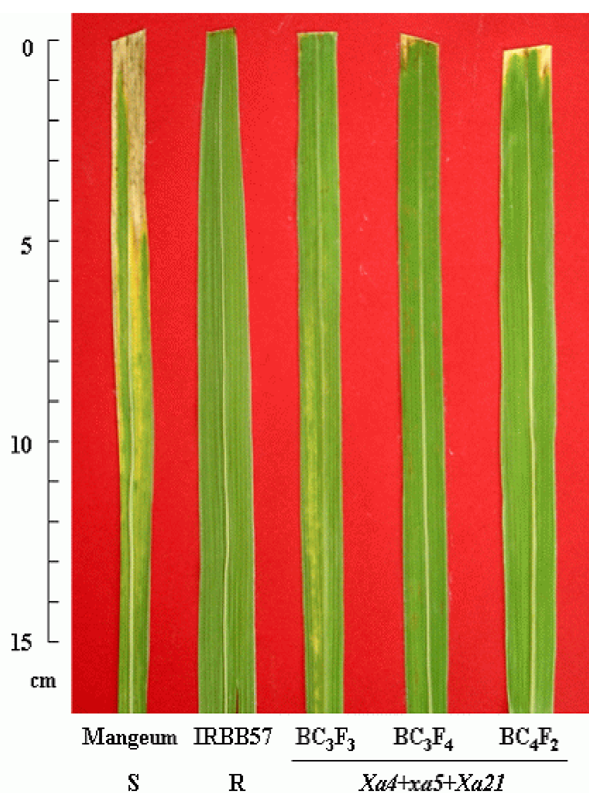


Fig. 1: Reactions of the susceptible (Mangeum) and resistant (IRBB57) parents, and advanced BC progenies to the K3a race of *Xoo* in Korea. S= susceptible, R= resistance

Background and foreground selection of fixed pyramid lines with BB resistance

We analyzed 14 BC_3F_3 plants possessing BB resistance genes and the parents for foreground selection using R-gene specific markers. All plants are confirmed with the presence of homozygous alleles of *Xa4, xa5 and Xa21*. These BC_3F_3 BB resistant plants are analyzed for background selection using AFLP approach. Three hundred and fourteen (314) informative AFLPs were generated by using 10 primer sets. The percent conversion of BC_3F_3 progenies to recurrent parent genome with integration of the R genes for BB resistance ranged from 57 to 90 suggesting the resistance progenies attaining maximum homozygosity for the recurrent parent genome. We are continuing to analyze the BC_3F_3 progenies with SSR markers for background selection and selecting desirable plant types with strong resistance to K3a race of *Xoo*.

Perspectives

Development of broad spectrum durable BB resistant rice cultivars is one of the major goals of rice breeding in temperate as well as tropical irrigated rice ecosystems. Japonica rice cultivars in Korea and Japan mainly possess the genes, *Xa1*, *Xa3* and *Xa4* for BB resistance. However, in recent years these resistance genes are becoming susceptible to new races of BB and there is breakdown of resistance in the high yielding Japonica varieties. It is imperative to look for novel R-genes or gene combinations for improvement of Japonica rice resistant to BB races. Advances in

rice genomics, availability of makers for candidate resistance genes, molecular understanding on BB pathogen virulence mechanism and MAS for pyramiding resistance genes have provided ample opportunities for the development of BB resistance in Japonica rice. Single gene resistance is often race-specific for a particular geographical location and expression of R genes depends on different growth stages of the cultivars. However, combination of three R genes, *Xa4+xa5+Xa21* proves to be an ideal gene pyramiding system for improving BB resistance in Japonica cultivars that has narrow genetic base.

References

He Q, Li D, Zhu Y, Tan M, Zhang D, Lin X (2006) Fine mapping of Xa2. a bacterial blight resistance gene in rice. Molecular breeding 17: 1-6

Iyer AS, McCouch SR (2004) The rice bacterial blight resistance gene xa5 encodes a novel form of disease resistance. Mol Plant-Microbe Interact 17: 1348-1354

Jeung JU, Heu SG, Shin MS, Vera Cruz CM, Jena KK (2006) Dynamics of *Xanthomonas oryzae* pv. *oryzae* population in Korea and their relationship to Korean bacterial blight resistance genes. Phytopathology 96: 867-875

Khush GS (2001) Green revolution: The way forward. Nature Rev Genet 2: 815-822

Lee KS, Khush GS (2000) Genetic analysis of resistance to bacterial blight, *Xanthomonas oryzae* pv. *oryzae* in rice. Rice Genet Newslett 17: 72

Liu DO, Ronald PC, Bogdanove AJ (2006) *Xanthomonas oryzae* pathovars: model pathogens of a model crop. Mol Plant Pathol 7: 303-324

Mew TW, Alvamz AM, Leach JE, Swings J (1993) Focus on bacterial blight of rice. Plant Disease 77: 5-12

Noh TH, Lee DK, Kang MH, Shin MS, Na SY (2003) Identification of a new race of *Xanthomonas oryzae* pv. *oryzae* (Xoo) in Korea. (Abstr.) Phytopathology 93 (suppl.): S66

Ou SH (1985) Rice Diseases. Kew, Surrye: Commonwealth Agricultural Bureau

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P (1995) A receptor kinase–like protein encoded by the rice disease resistance gene, Xa21. Science 270: 1804 - 1806

Sun X, Cao Y, Yang Z, Xu C, Li X, Wang S, Zhang Q (2004) Xa26, a gene conferring resistance to *Xanthomonas oryzae* pv. *oryzae* in rice, encodes an LRR receptor kinase-like protein. Plant J. 37: 517-527

Three novel bacterial blight resistance genes identified, mapped and transfer to cultivated rice *O. sativa* L.

Kuldeep Singh¶, Yogesh Vikal, Ritu Mahajan, Kuljit K Cheema, Dharminder Bhatia, Rajiv Sharma, Jagjeet S Lore and TS Bharaj

Dept. Plant Breeding, Genetics and Biotechnology, Punjab Agricultural University, Ludhian 141004, India

¶Corresponding author (kuldeep35@yahoo.com)

Abstract

Bacterial Blight (BB) of rice caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*) is one of the major constraints in sustaining rice production in South East Asia as yield losses in severely infected fields may be as high as 50%. The strategy of using major genes, individually or in combination, appears to be the most effective approach for management of BB. Currently more than two dozen genes conferring resistance to BB have been identified but not all of these genes are effective in all regions against all the pathotypes. The challenge therefore is to continue to expand the pool of effective and potentially durable genes. Wild species continue to be an important reservoir of the resistance genes including bacterial blight. More than 300 accessions of 14 *Oryza* species were screened against seven prevalent *Xoo* pathotypes over a period of 3–4 years and 67 accessions were identified that were resistant or moderately resistant to all pathotypes. These comprised 13 accessions of *O. glaberrima*, 5 of *O. barthii*, 10 of *O. rufipogon*, 4 of *O. longistaminata*, 22 of *O. nivara*, 6 of *O. officinalis*, 2 of *O. rhizomatis* and 5 of *O. minuta*. One accession each from *O. nivara* (IRGC81825), *O. glaberrima* (IRGC 102600B) and *O. barthii* (IRGC100119) were used for studying inheritance, mapping and transferring these genes into cultivated rice *O. sativa*. Resistance in *O. nivara* was found to be governed by a single dominant gene whereas the resistance in *O. glaberrima* and O. *barthii* was found to be governed by recessive genes. Inheritance and mapping of resistance in *O. nivara* acc. IRGC81825 was studied by using F_2, BC_2F_2 and BC_3F_1 populations of the cross involving *O. sativa* cv PR114 and the *O. nivara* acc. 81825. Bulked segregant analysis (BSA) of F_2 population using 191 polymorphic SSR markers identified ~35 cM chromosomal region on 4L, bracketed by RM317 and RM562, to be associated with the BB resistance. Screening of additional 74 BC_3F_1 and 175 BC_2F_2 plants and their analysis using more than 30 polymorphic SSR markers in the region bracketed by RM317 and RM562 led to mapping BB resistance gene in between the markers RM17499 and RM17502 which is ~176 kb region. In accessions of *O. glaberrima* and *O. barthii*, the inheritance and mapping was carried by crossing these with *O. sativa* cv Pusa 44. Due to recessive nature of BB resistance in *O. glaberrima* and *O. barthii* and complete male sterility in the F_1, BC_1F_1 was generated by backcrossing the F_1 with *O. sativa* cv Pusa44. Of more than 100 BC_1F_1 plants in each of the two backcross populations, only eight and four plants respectively in crosses with *O. glaberrima* and *O. barthii* showed partial fertility and set BC_1F_2 seed upon selfing. The BC_1F_2 populations of all the twelve plants were planted and inoculated with most virulent *Xoo* strain. Of all the twelve progenies, two progenies from cross with *O. glaberrima* and one progeny in cross with *O. barthii* showed segregation, all others were susceptible. Two BC_1F_2 progenies statistically fit well to 1:3 segregations and one population showed distortion in favors of susceptible plants. The two BC_1F_2 populations, one each with *O. glaberrima* and *O. barthii* were used for mapping of BB resistance genes with SSR markers using Bulk Segregant Analysis (BSA). A total of 170 and 191 SSR markers spanning all the 12 linkage groups were analyzed for polymorphism between *O. glaberrima* vs Pusa44 and *O. barthii* vs Pusa44 respectively. Polymorphism level of

55.2% and 51.3% was observed in cross between *O. glaberrima* and Pusa44 and *O. barthii* and Pusa44 respectively when SSR markers were resolved on 2.5% agarose gel. BSA indicated presence of *O. glaberrima* resistance gene on chromosome 5 and that of *O. barthii* on chromosome 6. Analysis of population with SSR markers in the region defined by BSA was carried out for both the populations. The BB resistance gene in *O. glaberrima* mapped on chromosome 5 flanked by markers RM548 and RM593 at a distance of 1.7 and 1.1cM respectively. Similarly the BB resistance gene in *O. barthii* mapped on terminal region of chromosome 6 at a distance of 9.3cM proximal to RM 588. Based on disease reaction of these accessions to seven *Xoo* pathotypes, chromosomal location and markers linked to the genes, all the three are novel and we propose to designate the genes from *O. nivara*, *O. glaberrima* and *O. barthii* as *Xa*30(t), *xa*31(t) and *xa*32(t) respectively.

Inheritance of resistance to bacterial blight in 21 cultivars of rice (*Oryza sativa* L.)

K.S. Lee, S. Rasabandith, E.R. Anggeles and G.S. Khush
International Rice Research Institute (IRRI), DAPO Box 7777, Metro Manila, Philippines

ABSTRACT
Genetic analysis for resistance to bacterial blight (*Xanthomonas oryzae* pv. *oryzae)* of 21 rice (*Oryza sativa* L.) cultivars was carried out. These cultivars were divided into two groups based on their reaction to Philippine races of bacterial blight. Cultivars of group I were resistant to race 1 and those of group 2 were susceptible to race 1 but resistant to race 2. All the cultivars were crossed with TN 1. Which is susceptible to all the Philippine races of *X. oryzae* pv. *Oryzae*. F1 and F2 populations of hybrids of group I cultivars were evaluated using race 1 and F1 and F2 populations of hybrids of group II cultivars were evaluated using race 2. All the cultivars showed monogenic inheritance of resistance. Allelic relationships of the genes were investigated by crossing these cultivars with different testers having single genes for resistance. Three cultivars have *Xa4*. another three have *xa5*, one has *xa8*, two have *Xa3*, eight have *Xa10*, and one has *Xa4* as well as *Xa10*. Three cultivars have new, as yet undescribed genes. Neb Bha Bong To has a new recessive gene for moderate resistance to race 1, 2 and 3 and resistance to race 5. This gene is designated *xa26(t)*. Arai Raj has a dominant gene for resistance to race 2 which segregates independently of *Xa10*. This gene is designated as *Xa27 (t)*. Lota Sail has a recessive gene for resistance to race two which segregates independently of *Xa10*. This gene is designated as *xa28 (t)*.

Keywords: durable resistance, genetic engineering, independent segregation, isogenic lines, pyramided lines.

Bacterial Blight resistance and semidwarfing genes transferred to improve plant architecture and productivity of two traditional basmati varieties

Dharminder Bhatia, Yogesh Vikal, G.S.Mangat, Ashok Patnaik and Kuldeep Singh*
* Corresponding author (E-mail. kuldeep35@yahoo.com)

Traditional Basmati varieties grown in Northern India are low yielding, tall, and lodge even under low nitrogen fertilizer dose. In addition to lodging, all the traditional Basmati varieties are susceptible to bacterial blight, thus making it a high risk crop for farmers. These unimproved features make basmati varieties very low yielding as compared to non-aromatic rice varieties and hence less remunerative despite the crop being sold at premium price due to its export potential. Although semi-dwarf basmati varieties have been developed that are high yielding but these are photoperiod insensitive and do not develop the aroma equivalent to traditional Basmati varieties. For improving the productivity and sustainability of basmati rice, we have introgressed semi dwarfing gene *sd*-1, two bacterial blight resistant genes *xa*13 and *Xa*21 into Basmati370 and Basmati386 from donor parent PR106-P2 (IET17948) without disturbing the typical basmati traits of aroma , amylose content and photoperiod sensitivity using marker assisted selection. In BC_1F_1, out of 474 plants of cross Bas370/IET17948//Bas370 and 304 plants of cross Bas386/IET17948//Bas386, 30 and 35 plants were found to be heterozygous for *Xa*21 and homozygous for major aroma locus respectively. In BC_1F_2, the selected 65 plants were planted as plant to row with 92 plants for each progeny at CRRI, Cuttack. In BC_1F_3, a total of 54 dwarf BC_1F_2 plants from the cross Bas370/IET17948//Bas370 and 42 plants from the cross Bas386/IET17948//Bas386 were selected for further analysis and backcrossing. For molecular analysis of these progenies, DNA was isolated from leaf samples collected from 15 plants of each progeny in bulk. Based on molecular analysis, five progenies were selected for individual plant analysis. The selected plants were again backcrossed to respective recurrent parents to obtain BC_2F_1 seeds. The selected BC_2F_1 plants were again planted at CRRI, Cuttack.A total of 2498 BC_2F_2 plants were planted at PAU, Ludhiana. These plants were artificially inoculated with most virulent strain of *Xoo*. Out of these, 1198 plants were selected which were resistant to *Xoo*, dwarf and photoperiod sensitive. Further selection among these plants was based on *Xa*21 marker analysis, grain type and aroma. A total of 152 selected plants were planted at CRRI, Cuttack. Based on molecular analysis of 152 plants for all the markers, three plant from the cross Bas370/PR106-P2//2*Bas370 were found to be homozygous for all the markers. Thirteen plants from the cross 370/PR106-P2//2*Bas370, only four plants were selected. Similarly out of 340 plants of the cross Bas386/PR106-P2//2*Bas386 and seven plants from the cross Bas386/PR106- P2//2*Bas386 were found to be having only *Xa*21 gene. Out of these, three plants of Basmati 370 and four plants of Basmati 386 were heterozygous for amylose with all other genes in the homozygous condition.

Twenty two plants of Basmati 370 and three plants of Basmati 386 were having *Xa*21 in homozygous and *xa*13 in heterozygous condition, while three plants of Basmati 370 were having *Xa*21 in heterozygous and *xa*13 in homozygous condition. One plant of Basmati 370 had only *xa*13 gene with all other genes in the homozygous condition. These plants are bacterial blight resistant, dwarf with all the typical basmati characteristics. These plants are put in multilocation yield trial at Punjab Agricultural University, Ludhiana this season.

Resistant classification of Korean rice varieties to Bacterial Blight

Ki-Young Kim[1]*, Mun-Sik Shin[2], Seo-Ho Shin[1], ji-Ung Jeung[3], Tae-Hwan Noh[1],
Bo-Kyeong Kim[1], Jae-Kwon Ko[1]
[1]*Honam Agricultural Research Institute, NICS., Iksan 570-080, Korea*
[2]*Yeongnam Agricultural Research Institute, NICS., Milyang 627-803, Korea*
[3]*National Institute of Crop Science, RDA, Suwon 441-857, Korea*
* Corresponding Author Email: kkyoung@rda.go.kr

The spreading of bacterial blight disease occurrence is increasing in all areas of Korea because of emergence of new BB races, K_{3a} and breakdown of resistant varieties having *Xa1* or *Xa3* gene (Noh *et al.*, 2003). This study was carried out to classify the resistant response of rice varieties developed in Korea and identify resistant gene. Two hundred thirty seven varieties developed from 1972 to 2006 in Korea were tested their reaction to four Korean races, HB01013 (K_1), HB01014 (K_2), HB01015 (K_3), HB01009 (K_{3a}). At maximum tillering stage, four plants per race were inoculated by clipping method with bacterial suspension of 10^9cells/ml. The lesion length was measured at 21 days after inoculation. The reaction of resistance was classified into two categories; Resistance(< 5cm), susceptible (>5.1cm). Two hundred thirty seven varieties were classified into five groups by the reaction to Korean races. Eleven Tongil-type varieties and one hundred fifteen japonica varieties were susceptible to four races; K_1, K_2, K_3, and K_{3a}. Twelve Tongil-type varieties and thirty two Japonica varieties were resistant to one race; K_1. Nine Tongil-type varieties and one Japonica variety were resistant to two races; K_1 and K_2. Three Tongil-type varieties and forty Japonica varieties were resistant to three races; K_1 , K_2, and K_3 . Thirteen Tongil-type varieties and one Japonica varieties were resistant to four races; K_1 , K_2, K_3, and K_{3a}. SNP marker, 16PFXa (Shin *et al.*, 2006) was used to identify the *Xa1* gene. STS markers, 9643.T4 and 10571.T14, were used for conforming *Xa3* gene and STS marker, 10571.T14 (unpublished) was also used for identifying *Xa4* gene. Varieties resistant to only K_1 race were conformed as having *Xa1* gene on the basis of SNP marker, 16PFXa and those resistant to three races (K_1 , K_2, and K_3) were conformed as having *Xa3* gene on the basis of STS markers, 9643.T4 and 10571. T14. Ten Tongil-type (Samgangbyeo, Taebagbyeo, Yongmunbyeo, Hangangchalbyeo, Namcheonbyeo, Singang -byeo, Baegyangbyeo, Baegunchalbyeo, Milyang42, and

Namyeongbyeo) resistant to four races(K_1 , K_2, K_3, and K_{3a}) were showed as having *Xa4* gene by using STS marker, 10571. T14.

Key words : Rice, varieties, bacterial blight, resistant gene, molecular marker

Reference

Noh, T. H., D. K. Lee, M. H. Kang, M. S. Shin, H. K. Shin, and S. Y. Na. 2003.Identification of new race of *Xanthomonas oryzae*. pv. *oryzae*(Xoo) in Korea. Phytopathology 93. s66.

Shin, W. C., S. H. Baek, S. S. Seo, H. G. Kang, C. K. Kim, M. S. Shin, G. S.Lee, J. H. Hahn, and H. S. Kim. 2006. Improvement of selection efficiency forbacterial blight resistance using SNP marker in rice. Korean J. of Plant Biotechnology 33(4): 309-313.

Confirmation of *Xa* genes introgressed into elite Basmati derived lines

M.Y.V. Reveche, A. Das, E.F.L. Mercado, M.G.C. Carrillo, I. P. Oña, S.N. Begum, V. Lopena, G.S. Capilit, P. Virk and C.M. Vera Cruz

Plant Breeding, Genetics and Biotechnology Division, IRRI, DAPO Box 7777, Metro Manila, Philippines

m.reveche@cgiar.org

It is essential in any rice breeding program to develop high yielding varieties and breeding lines with resistance to biotic and abiotic stresses in combination with desirable agronomic traits such as grain quality and aroma. Bacterial blight (BB) is a major disease in rice and introgression of effective BB resistance genes (*Xa* genes) singly or in combination into susceptible varieties is the most economical approach to manage the disease. Basmati rice is an aromatic rice favored for its good eating and cooking qualities especially in South and Southeast Asia, but is susceptible to BB. To improve its resistance, *Xa* genes were pyramided into basmati rice by crossing a basmati-derived line IR 71730-51-2 with IRBB60 (a pyramid line containing *Xa4, xa5, xa13* and *Xa21* genes). Elite breeding lines were selected for aroma, BB resistance, and grain yield and other desired agronomic traits, including grain quality related physico-chemical traits. To select for BB resistance, phenotyping against selected diagnostic strains of *Xoo* was done until F_8. Marker-assisted selection or confirmation was practiced at F_2, F_5, F_7 until F_9 generations. Fifteen promising lines with genes for aroma, BB resistance, along with good agronomic performance and other grain quality traits were identified. These elite materials were evaluated in large replicated yield trials at IRRI. The resistance of these elite lines to BB was assessed based on their reaction to 12 strains which represent 10 Philippine races. Four lines were resistant to all strains, five lines to 8-11 strains and six-lines were resistant to 5-7 strains. The fragrance gene was also detected in 7 of these lines using a PCR based marker. To confirm the BB resistance genes in each line, we used a gel-free dot blot

assay developed in our lab using gene specific primers and SNP-based probes for *xa5*, *xa13* and *Xa21*. Initial results show that *Xa21* is present in 7 lines. Screening for *xa5* and *xa13* is in progress. All 15 lines carry *Xa4*, which was confirmed by an STS-linked marker for the gene. These lines would serve as useful genetic resource in breeding for resistance to bacterial blight of aromatic rice.

Development of bacterial blight and blast resistant indica rice cultivars by gene pyramiding using marker assisted breeding

Manoj S. Phalak[1]*, Girish T. Nikhade[1], D. S. Mukadam[2], G. V. Umalkar[1], L.P. Aurangabadkar[1]
1. Molecular Breeding Division, Ankur Agricultural Research Laboratory, Nagpur (India)
2. Dr. B. A. M. University, Aurangabad. (India)
* Corresponding author: msphalak@ankurseeds.com

Rice bacterial blight (BB) and blast are the most wide spread and devastating diseases of rice. These diseases are the major constraints for rice farmers and can cause yield loss as high as 50% in some areas of Asia. The severity and significance of damage caused by both diseases have necessitated the development of strategies to control and manage them to reduce crop loss and to avert an epidemic. In the present scenario the exploitation of host resistance appears to be the only reliable method of disease management. Fortunately advantages of MAS can be effectively used to compliment conventional breeding for the development of inbuilt resistance in rice cultivars.
The present study aims at development of bacterial blight and blast resistant, agronomically superior and diversified rice genotypes. Fifteen diverse rice genotypes having Pi-1 and Pi-2(t) genes from blast resistance breeding program and 13 genotypes having xa-5, xa-13, Xa-21 genes from bacterial blight resistance breeding program were selected as a possible parentage for the further breeding program. These lines were evaluated for quantitative resistance parameter by screening blast resistant lines for BB resistance and vice versa.
Blast resistant genotype AOSB-3, AOSB-15 and BB resistant genotypes AOSBB-7 and AOSBB-10 were selected as parents based on the quantitative resistance offered by these genotypes in absence of the major disease resistance genes. In the present study first a cross with in the blast resistant parents (AOSB-3 X AOSB-15) was taken to produce a hybrid AOSBH-1 which is homozygous for Pi-1 and Pi-2(t). Similarly BB resistant parents (AOSBB-7 X AOSBB-10) were crossed to produce hybrid AOSBBH-1 which is homozygous for xa-5, xa-13 and Xa-21.
Single cross hybrids AOSBH-1 and AOSBBH-1 were crossed to produce a four-way genetically diverse hybrid having all the five genes (Pi-1, Pi-2(t), xa-5, xa-13 and Xa-21) in heterozygous condition. The selfed progeny of four way cross hybrid was subjected to MAS to recover genotypes having all the five genes in homozygous condition.
Total 15652 F_2 seedlings of four way hybrid were analyzed with subtractive MAS.

Marker pTA 248 identified 4008 plants homozygous for Xa-21 gene. These 4008 plants were analyzed by marker RG 136 for xa-13 gene and identified 1049 plants homozygous for xa-13. Marker RG 556 identified 238 plants out of 1049 plants homozygous for xa-5 gene. Marker RG 64 identified 68 plants from 238 plants homozygous for Pi-2(t) gene. Marker RM 144 identified 16 plants out of 68 plants homozygous for Pi-1 gene. Thus the sequential analysis of 15652 plants for five different genes produced 16 plants homozygous for all the five genes. Further selection and stabilization for morphological and economical characters yielded 14 diversified, bacterial blight and blast resistant, agronomically superior rice genotypes which can be used for large scale commercial cultivation or as parents for hybrid rice production or standard donor for disease resistance genes.

Transgenic rice expressing a harpin-encoding gene (*hrf1*) exhibits nonspecific resistance to *Magnaporthe grisea*

Min Shao, Wanchun Huang, Lei Zhang, Wenqi Li, Shanshan Xiao, Jinsheng Wang

Key Laboratory of Monitoring and Management of Plant Diseases and Pests, Ministry of Agriculture of China, Department of Plant Pathology, Nanjing Agricultural University, Nanjing, 210095, China.

Abbreviations footnote: *hrf1*: a gene encoding a harpin protein derived from *Xanthomonas oryzae* pv. *oryzae*.

Abstract: Rice blast caused by the fungus *Magnaporthe grisea* is one of the most destructive diseases on rice. Management of this disease poses a tremendous challenge because the pathogen population changes rapidly (Zhu et al., 2000; Talbot, 2003). Exogenous applications of harpins, protein elicitors isolated from plant pathogenic bacteria, induce systemic resistance in plants to pathogens through activating the host defensive response (Wei et al. 1992; He et al. 1993; Strobel et al. 1996; Lee et al. 2001, Peng et al. 2004). Here we show that expression of a harpin-encoding gene (*hrf1*), derived from *Xanthomonas oryzae* pv. *oryzae* (Wen et al. 2003), confers nonspecific resistance in rice to *M. grisea*. Transgenic plants and their T1-T5 progenies are highly resistant to major *M. grisea* races in rice-growing areas along the Yangtze River, China. In an incompatible interaction induced by *hrf1* expression, formation of melanized appressoria, which is essential for foliar infection, was inhibited on transgenic plants. This is, to our knowledge, the first report that expression of a harpin-encoding gene transformed into a monocot confers durable nonspecific resistance and suggests that harpins may offer new opportunities for generating broad-spectrum disease resistance in other crops. We performed comparative transcriptome analysis on R109 and trangenic plant NJH12 (a line of resistance to rice blast) by microarray. Results show that there are 192 genes enhanced expression in NJH12. These results suggest that expressing a

harpin-encoding gene in rice can triggers a lot of genes expression and enhanced disease resistance.

Key words: *hrf1*gene, *Magnaporthe grisea*, nonspecific resistance, microarray

Introduction

Rice blast causes 10-30% yield losses in rice, posing a constant threat to the supply of a staple food for nearly half of the world's population. Control of *M. grisea* relies on the utilization of resistant cultivars and application of fungicides, but neither is particularly effective. Host resistance in rice to *M. grisea* functions via a classical gene-for-gene interaction where a single dominant resistant gene corresponds to a dominant avirulence gene in the pathogen (Hammond-Kosack and Jones, 1997). Because of apparent instability in the genome of *M. grisea*, new pathogenic races evolve rapidly and thus host resistance typically lasts only for a few years (Zhu et al., 2000; Talbot, 2003). Few fungicides are available for effective control of rice blast as rapid mutation in the pathogen leads to emergence of fungicide-resistant variants (Takagaki et al. 2004) and high dose applications pose risks to both humans and the environment. Generation of cultivars that possess non-specific resistance to *M. grisea* will provide an economically effective and environmentally sound approach to rice blast control. One promising approach to achieving non-specific resistance to *M. grisea* is to incorporate genes that elicit general defense responses into rice (Dangl and Jones, 2001; Stuiver & Custers, 2001). Harpins induce systemic acquired resistance (SAR) in plants to pathogens through activating defense pathways mediated by salicylic acid (SA) (He et al. 1993, Dong et al., 1999) and jasmonic acid (JA)) or ethylene (ET) (Kariola et al. 2003), as well as inducing a range of pathogenesis-related (PR) genes (Lee et al. 2001). Here, we report that transformation of harpin-encoding (*hrp*) genes into rice has the potential to enhance general resistance to pathogens.

Methods

Hrf1 transformation and expression in rice

The full-length *hrf1* gene was excised from pET30(a) with restriction enzymes *Xba*I and *BamH*I and inserted into the corresponding sites in pBI121 between the CaMV35S promoter and the gusA gene (Sambrook et al. 1989). The resulting pBMH9 (or pBI-*hrf1*) (Fig. 1) was sequenced to verify the correct orientation of genes and transferred into *A. tumefaciens* EHA105 using a freeze-thaw method (Huang et al. 2001). Rice (3-wk old seed calli of cultivar R109) was transformed with pBMH9 by soaking the calli with *A. tumefaciens* suspensions and co-cultivated for 2 days in N_6 medium containing 2,4-D (1 mg/L) and acetosyringone (200 mg/L). After being thoroughly washed with water, calli were plated on N_6 medium with 2,4-D (1 mg/L), carbenicillin (250 mg/L) and kanamycin (50 mg/L) for selection for at least one month. Resistant calli were transferred to MS medium containing carbenicillin and kanamycin, and plantlets were then transferred to ½ MS medium without hormones to induce root formation (Huang et al. 2001).

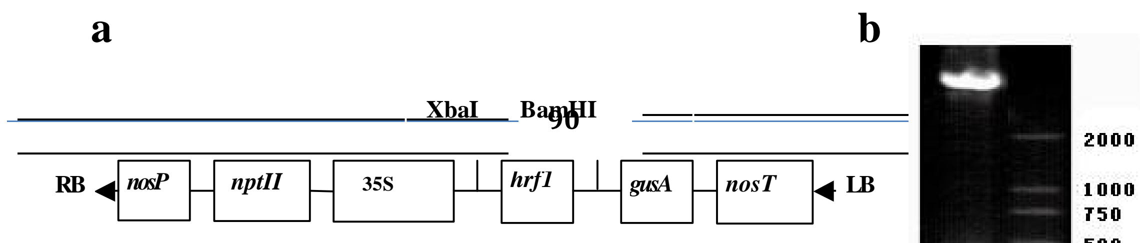


Fig. 1 The *hrf1*-transformation unit pBMH9 constructed in the vector pBI121 and enzyme digestion of plant expression plasmid pBMH9. **a**, Schematic representation of transformation plasmids pBMH9. *nosP* — promoter of gene encoding nopaline synthetase at 5'termination region of T-DNA from *Agrobacterium tumefaceins*; *nptII* — report gene from Kan^r eubacteria; CaMV35S — cauliflower mosaic virus 35S promoter; *hrf1* — harpin protein-encoding gene from *Xoo*; *gusA* — *E. coli* β-glucuronidase gene (selection marker gene); *nosT* — 3'termination region from *Agrobacterium* nopaline synthetase gene. **b**, Digestion of plant expression plasmid pBMH9: 1 — pBMH9 (*Xba*I-*BamH*I), 2 — Marker (DL-2000).

PCR analysis was performed using *hrf1* specific primers (Zhu et al. 2000; Wen et al. 2003) and CaMV35S promoter primers through amplifying a 420bp band of *hrf1* and 800bp band of 35s promoter (Table 1). Expression of *hrf1* in transgenic lines was examined by using protein extracts of transgenic plant leaves and the concentration of proteins was estimated with the BioRad reagent. Western analysis was carried out with anti-HRF1 antibodies (Dual-spiral) and total proteins were separated by 12% SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were incubated with the primary antibody (anti-HRF1 antiserum) and then with goat anti-rabbit alkaline phosphate-conjugated IgG as a secondary antibody. Protein bands were visualized using a western blotting detection kit (Amersham) following the manufacturer's protocol.

RT-PCR evaluation of defense genes

Total RNA was prepared from well-expanded 6-wk-old leaves using a Tripure kit (Roche) following the manufacturer's instructions, and treated with RNase-free DNase (Promega). The *EF1a* gene was used as a standard and amplified with specific primers, resulting in a 495-bp product (Table 1).

Table 1 Characteristics of *hrf1*, defense, and signal transduction-related genes determined in this study

Gene	**GenBank accession no.**	**Primers**	**Size (bp) PCR or RT-PCR product**

hrf1	AY139030	5'-ATGAACTCTTTGAACACACAATTCG-3' 5'-CTATTACTGCATTGATGCGCTTCC-3'	420
35S promoter	AF485783	5'-GCCTTTTCAATTTCAGAAAGAATGC-3' 5'-CGTGTTCTCTCCAAATGAAATGAAC-3'	800
OsPR1a	AJ278436	5' AATTAATGGCGAGTTCGTC 3' 5' CAGCTTTATTTATTTATTCATCGG 3'	683
OsPR1b	AF306651	5'-AGGTAGCCAAGCTGGCCATT-3' 5'-TATGGACCGTGGACCTGTTT-3'	700
Chia4a	AB096140	5'-TGTCTGTAGAGAGCGTGGTCACCGA-3' 5'-ATCACCTGGTGCACGTTGTTCATCC-3'	473
PAL	X16099	5'-GAGGCCATCCACCAAGCTGCTCAACA-3' 5'-GGAACACCTTGTTGCACTCCTCGCC-3'	1524
CHS	AB058397	5'-CGGCAGGACATCGTCGTCGTC-3' 5'-GTCGAGGATGAAGAGCACGCAGGC-3'	700
GLU	AB070742	5'-TGAAGTCGTTCAGCTATATGCCTCG-3' 5'-GCTCACATGGTTAATCAGGTTCTGG-3'	727
NPR1	NM191394	5'-GAGGTGGTGAGCCTGAATC-3' 5'-TAATAGCTGGCTCTCTCCTCATA-3'	700
COI1	AY168645	5'-CGCGCCTTCGGGAGCGGTTC-3' 5'-GCACAAGCCGAAAGTCGTATAGATTTTTG-3'	1022
EF1a	AF181492	5'-AGACCACCAAGTACTACTGCAC-3' 5'-CCACCAATCTTGTACACATCC-3'	495

Plant materials and growth conditions

Rooting transgenic plants (T0) were transferred to soil and placed in a growth chamber (12 h photoperiod, 28°C, 30000lx light strength) and a slow release fertilizer was applied. Seeds collected from T0 plants (i.e., T1) were grown in the field to maturity in Pujiang, Sichuan. T2 and T4 progenies of NJH12 and untrangenic R109 plants were planted in Sanya, Hainan Province for generation advancement in the winter. T3 and T5 progenies were grown in Pujiang, Sichuan and Anhou, Hunan during the regular rice-growing season.

Specificity of NJH12 resistance to *M. grisea* races

The specificity of the transgenic plant resistance to *M. grisea* races was determined in a controlled environment at Jiangsu Academy of Agricultural Sciences (JAAS), Nanjing, China. *M. grisea* conidia were produced on a rice stalk powder agar medium (25 g rice stalk powder, 40 g cornmeal and 18 g agar per liter, pH7.0), grown under fluorescent light and then in dark to induce conidiation, and harvested with DI H_2O washing. Three-wk old NJH12 (T2) and R109 plants were inoculated with *M. grisea*

by spraying a fungal spore suspension (2×10^5 conidia/ml with 500µg/ml Tween 20), maintained in the dark for 24 h and transferred to a greenhouse with humidity (RH > 95%) and temperature (26-28°C) being manipulated to optimize germination and appressorium formation of *M. grisea*. Six isolates (ZB_{13}, ZC_3, ZD_1, ZE_3, ZF_1, and ZG_1), each representing the most virulent isolate in six major *M. grisea* races in China, were selected to test *M. grisea* resistance of transgenic lines. Disease severity was recorded 7-10 d after the inoculation (see below). The experiment was repeated twice with 180 transgenic plants being examined each time.

Field evaluation of *M. grisea* resistance in transgenic plants and their progenies

Resistance of NJH12 progenies to *M. grisea* was examined in the field in Anhou, Hunan (T3 and T5) and Pujiang, Sichuan (T1, T3, and T5). Surrounded by mountains, both sites experience prolonged foggy mornings and high diurnal temperature amplitudes, leading to local environmental conditions that highly favor *M. grisea* growth and infection. The composition of *M. grisea* was very diverse since rice cultivars and presumably their associate pathogens across China have been introduced to these two sites over the last two decades.

For T3 and T5 progenies, NJH12 plots alternated with R109 ones (50 plants in each plot of 5 rows), with 3 replicate plots in field. To ensure the density of *M. grisea* inoculum, experimental plots were encircled by *M. grisea* infested disease-inducing plants (Shanyou 63), leading to a ratio of inducing to test plants at 1:10. Disease inducing plants were seeded 2-wk prior to the test plants, inoculated with *M. grisea*-infested rice residues 2-wk after seed germination, and transplanted into the field plots together with test plants. All 50 plants in each plot were examined for leaf and panicle blast using a very rigorous criterion: as long as one leaf or panicle has blast, the whole plant is taken as susceptible and the disease severity is recorded using the most damaged leaf or panicle. Each panicle was visually examined by experienced personnel to estimate the percentage of branches that were necrotic due to infection by *M. gresea* and given a rating from 0 to 9 with no visible lesion in grade 0 and extensive death in grade 9.

Microscopic examination of growth and infection of *M. grisea* pathogens on R109 and NJH12 plants

Freshly cut rice leaves (5 cm long) from 2~3 wk-old R109 and NJH12 (T3) plants (one leaf only from each plant) were taped at their tips to glass slides and placed face up on a layer of moistened paper towels in a clear culture box (Liu and Dean, 1997). Multiple 5µl droplets of conidia (2×10^5 conidia/ml containing 500µg/ml Tween 20) were placed on the leaf surface. Conidia, germ tubes, and appressoria were stained in lactophenol-cotton blue solution (a mixture of 5 ml lactic acid, 5 ml phenol, 15 ml H_2O, and 10 mg cotton blue) for 3 min, and examined with a light compound microscope. Appressorium formation was checked periodically from 18 to 48 h. At

each time period, fifteen inoculation sites (5 on each leaf) were examined microscopically for both transgenic and untransformed plants.

Rice microarray analysis

To identify the genes whose expressions are dramatically induced in transgenic rice plants which express *hrf1* gene and resistant to rice blast , a microarray containing 51,279 trascripts unique rice ESTs spotted in duplicate on a single array, representing the entire rice genome (Affymetrix,GeneChip RICE Genome Array), was used to perform a broad monitoring of transcriptome changes in R109 and NJH12. Microarray hybridization was performed by Gene Tech Biotechnology Co.Ltd. Data analysis was performed with Plant Expression Database (PLEXdb) [Shen et al., 2006], GCOS1.4 and GeneSpring7.3 [Dudoit et al., 2003].

RT-PCR analysis

To further test the reliability of the microarray results, a series of oliogonucleotide primers were designed for determining whether the up-regulation genes expressed higher levels in NJH12 than in R109. RNA extracted from R109 and NJH12 at six weeks after growth. PCR reactions were performed containing gene-specific primers as following: *Os-4*,Up 5'-CAGCGGTAACTCCACGAA-3', Down 5'-CAAGAACAACATCGAACACAGC-3; *Os-6*,Up 5'-CTGCCAAAGTGGGATGAT-3', Down 5'-TGCTCCTCGATGCTCATT-3; *Os-10*, Up 5'-CTGCGAAGGAATACGACA -3',Down5'-CTGGTGGACTGTCATAAG CA-3; *Os-24*, Up 5'-ATGTGGGGGTGCTTGTATGG-3', Down 5'-CGCAACAACACTGA AGACGA -3; *Os-26*,Up 5′-GTTGAATGCATGCCTGG -3′, Down 5′-CCGACAGAAGAAGAT GC-3′; *Os-27*, Up 5′-GCACTGGGCTGTTGTTG -3′, Down 5′-ATAAGTTGCGGCTGCTG -3′.

Results and discuss

We transformed rice (*Oryzae sativa* subsp. *Japonica*) with the harpin$_{Xoo}$ (*hrf1)* gene under the 35S promoter derived from *Xanthomonas oryzae* pv. *oryzae* (Wen et al. 2003). Rice cultivar, R109, susceptible to rice blast, was transformed with *hrf1*-containing pBMH9 by *Agrobacterium*-mediated transformation (Huang et al., 2001) (Fig. 1). PCR and Southern blot analysis confirmed that the T-DNA was integrated into the rice genome. Plant morphology and yield were unaffected in transgenic plants. The active harpin protein was isolated from leaves of transgenic plants and a single band, identical in size to its original bacterial protein, was detected using Western blot analysis, indicating that the transferred *hrf1* gene was normally expressed in transgenic lines (Fig. 2a). Protein levels increased in leaves during the growing season, reaching a maximum by flowering (data not shown). To determine whether *hrf1* expression induces host defense responses similar to exogenous applications (Dong et al. 1999; Strobel et al. 1996), we examined the expression of

eight defense-related genes in 4 transgenic resistant T1 lines using reverse transcription (RT)-PCR. The genes tested are involved in plant basal defense pathways mediated by SA and JA (Dangl and Jones, 2001), which includes six for defense (*CHS, GLU*, *PR1a*, *PR1b*, *Chia4a* and *PAL)* (Durrant and Dong, 2004) and two for signal transduction (*COI1* and *NPR1*) (Spoel et al., 2003) (Table 1). Expression of *OsPR1a*, *OsPR1b, Chia4a, PAL* and *NPR1* was constitutively enhanced in four T1 transgenic lines, but not in untransformed parental R109 plants (Fig. 2b) (see below).

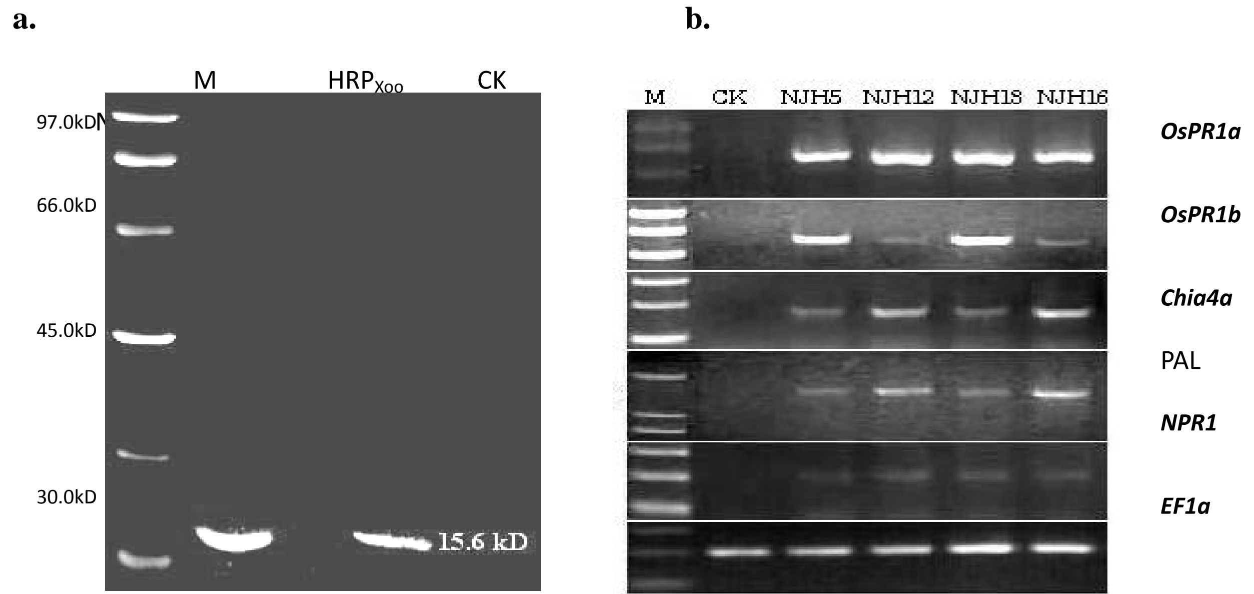


Fig. 2 Harpin and defense response expression in transgenic rice. **a**. Expression of *hrf1* gene in transgenic rice plants confirmed by Western blot analysis. NJH12: *hrf1* transgenic line; CK: the untransformed control R109; HRP_{Xoo} is a *hrf1* expression protein in plasmid pET30(a). **b**. Expression of defense-related genes in *hrf1*-expressing rice (lines NJH5, NJH12, NJH12 and NJH16) and their untransformed R109 (CK). Expression of defense-related genes was enhanced in lines NJH5, NJH12, NJH12 and NJH16 that were resistant to *M. grisea* but not in R109 plants.

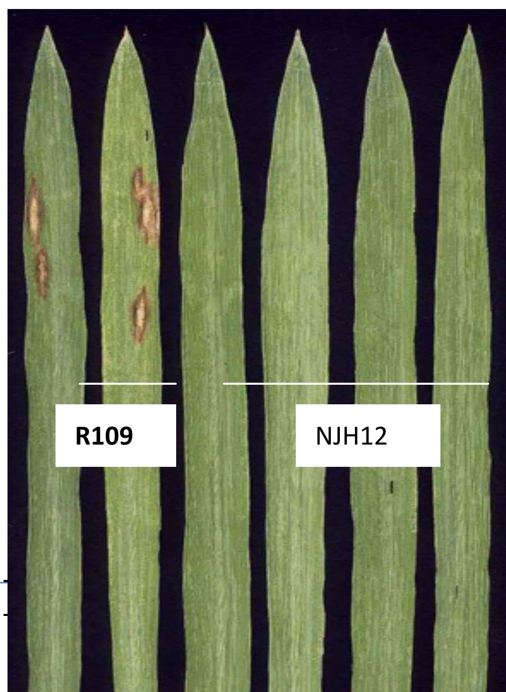
To determine whether *hrf1* expression leads to resistance to *M. grisea*, we planted un-transformed R109, and all 29 transformed T1 lines in a disease evaluation nursery at the National Center for Resistant Crop Identification in Pujiang County, Sichuan Province. In addition to the diverse population of local isolates, dominant by isolates of ZB race (NCRTRB, 1980; Jin and Chai, 1990), a mixture of *M. grisea* races was also introduced at the seedling stage through introducing *M. grisea*-infested disease-inducing plants (see Method). All R109 plants exhibited severe leaf and panicle blast (Disease severity, DS = 9), nine of the 29 transgenic lines, including NJH5, NJH12, NJH12 and NJH16, showed resistance to *M. grisea* (Fig. 3a). No leaf or panicle blast was observed on 35 out of 50 plants in the NJH12 line.

To examine whether *hrf1* expression induces general resistance to different *M. grisea* races, seedlings of NJH12 (T2) and R109 were separately inoculated with six *M. grisea* isolates (ZB_{13}, ZC_3, ZD_1, ZE_3, ZF_1 and ZG_1) in China under growth chamber conditions. Each of ZB_{13}, ZC_3, ZD_1, and ZG_1 represents the most virulent isolate of four major *M. grisea* races in rice-growing regions along the Yangtze River, China whereas ZE_3 and ZF_1 were representatives from the two dominant races (ZE and ZF) in northern China (NCRTRB, 1980; Jin and Chai, 1990). While R109 plants were highly susceptible to all six isolates (leaf blast, DS = 9.0), NJH12 plants were highly resistant to ZC_3, ZD_1, and ZG_1 with no disease lesions being observed (Fig. 3b). NJH12 plants were also resistant to ZB_{13} (DS =2.7) but susceptible to ZE_3 and ZF_1 (DS = 9.0). These results directly illustrate that the *hrf1* expression in rice confers non-specific resistance to all major *M. grisea* races in the Yangtze River region.

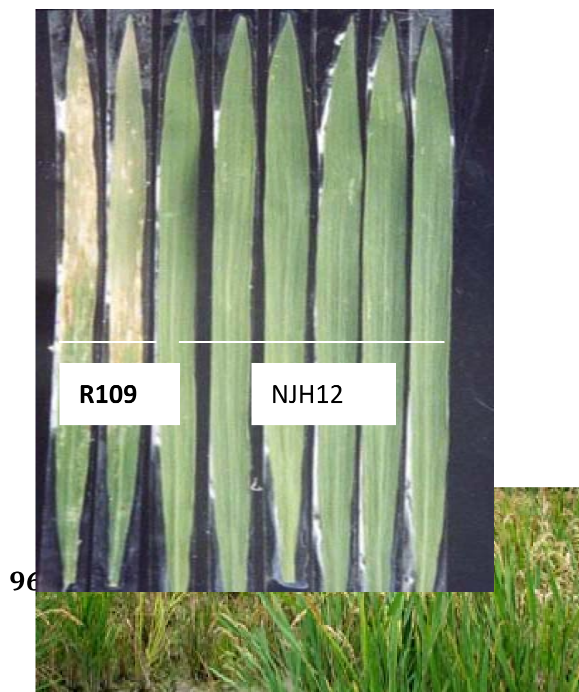
NJH12 progenies (T3 and T5) and untransformed parental R109 plants were further evaluated for their resistance to *M. grisea* in the field at the National Center for Rice Blast Resistance Identification in Anhou County, Hunan Province, and again in Pujiang, Sichuan in 2003 and 2004. *M. grisea* isolates of ZB and ZC races dominated the natural population in Anhou, although isolates of ZG and other races exist at both sites (Jin and Chai, 1990). Leaf blast was very severe in Anhou in 2003 (DS = 9.0), leading to the death of most R109 plants (> 95%) before flowering. Remarkably, T3 progenies of NJH12 line grew well and matured normally in spite of high disease pressure (Fig. 3c). Disease incidence in general was less

In 2004, leaf blast was severe on plants of both R109 (DS = 8.0) and NJH12 (DS = 7.0) in Anhou. However, while R109 plants exhibited extensive panicle blast (DS = 9.0), T5 progenies of NJH12 line remained healthy and matured normally with little evidence of panicle blast. At Pujiang, leaf and panicle blast was very severe on R109 plants (DS = 9), however, NJH12 progenies were resistant to *M. grisea* and no panicle blast was observed. Consistently lower disease severity on panicles than on leaves suggests that *hrf1*-induced resistance increases as the plants mature, likely related to the level of the harpin protein accumulation.

a. T1 Plants



b. T2 Plants



R109 NJH12

b

R109 NJH12

Fig. 3 Enhanced resistance of transgenic rice expressing of the *hrf1* gene. **a,** NJH12 (T1) plants were resistant to leaf and panicle blast but R109 plants were highly susceptible in a disease evaluation nursery at Pujiang, Sichuan. **b,** Non-specific

resistance in T2 progenies to *M. grisea.* Seedlings of NJH12 line were resistant to isolates ZB_{13}, ZC_3, ZD_1 and ZG_1 with no lesions being visible on their leaves, but extensive rice blast was severe on untransformed R109 plants. **c,** Enhanced resistance in transgenic plants to *M. grisea* in field. T3 progenies of NJH12 line were highly resistant to *M. grisea* in 2003 evaluation nurseries and matured normally (Anhou, Hunan), but all R109 plants died before flowering (most of them during the seedling stage). Disease incidence in general was less severe in Pujiang, but was not statistically evaluated because a flood killed all plants.

Taken together, our results document consistent resistance in the NJH12 progenies to diverse *M. grisea* races across large geographical distances. Enhanced specific resistance to *M. grisea* has recently been reported in transgenic rice transformed with genes encoding antifungal compounds of both plant (Krishnamurthy et al. 2001) and microbial origins (Coca et al. 2004). However, non-specific resistance remains elusive and no durable resistance in the field has so far been achieved in transgenic rice. To our knowledge, results presented here are the first report of transgenic rice possessing non-specific resistance to *M. grisea* that is effective in several highly disease-conducive environments.

M. grisea has evolved a highly specialized structure known as appressorium that is essential for foliar infection (Chumley &Valent, 1990, Dean 1997). We examined *M. grisea* conidial germination and subsequent development on the transgenic and the untransformed control plants. Leaves of NJH12 (T3) and R109 plants were inoculated with the conidia of ZC_3 race and monitored under a microscope for a period of 48 h. Marked differences in conidial development were consistently observed between the NJH12 and their control R109. On R109 leaves, germ tube tips became enlarged by 24 h and heavily melanized appressoria were visible by 36 h (Fig. 4). In contrast, conidia germinated abnormally on the NJH12 leaves: multiple germ tubes formed by 30 h, no appressoria were visible by 36 h, and the conidiospores collapsed thereafter (Fig. 4). Inhibition of appressorium formation on NJH12 leaves appeared to occur

prior to pathogen penetration, suggesting that host plant defenses are activated prior to infection.

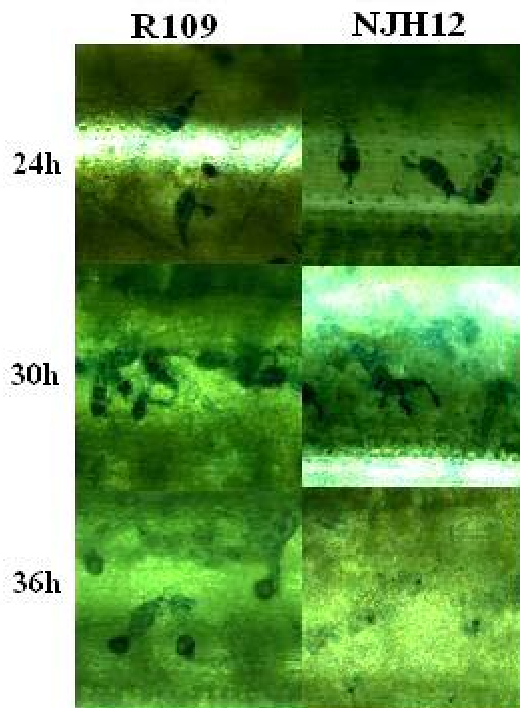


Fig. 4 Microscopic evaluation of germination and development of *M. grisea* (isolate ZC_3) on leaves of NJH12 (T3) and R109 plants. Conidia of *M. grisea* isolate ZC_3 were inoculated on leaves of 2 wk-old rice seedlings. Photographs were taken 24, 30 and 36h after the inoculation. Magnification = × 200.

The high level of resistance in NJH12 plants to leaf and/or panicle blast and inhibition of appressorium formation indicate that the *hrf1*-expression may induce multiple resistance mechanisms. Enhanced expression of the defense-related genes, which encode basic or acidic PR proteins, may be essential for the *hrf1*-induced resistance to *M. grisea* in transgenic plants (Fig. 2b). The NPR1 protein modulates defense pathways mediated by SA and JA (Spoel et al., 2003) and both induce SAR in rice against infection from blast fungus (Lee et al. 2001; Yang et al. 2004). Also, rice genes *OsPR1a* and *OsPR1b* are highly responsive to the rice blast pathogen (Agrawal et al. 2001) and constitutive expression of chitinase genes enhances blast resistance in rice (Nishizawa et al. 1999). However, whether and how enhanced expression of these genes is related to the observed inhibition of appressorium formation remains to be investigated. Because harpin_{Xoo} itself did not impact *in vitro* growth of *M. grisea* (data not shown), inhibition of appressorium formation suggests that some unidentified compounds produced by the transgenic plants might interfere with development in this fungus (Rodrigues et al., 2003).

In order to gain further understanding of the molecular mechanisms of *hrf1* transgenic rice plants resistant to rice blast, we subjected the RNA from R109 and

NJH12 and microarray analysis to identify the genes that are specifically involved in NJH12 resistant to rice blast. These results show that 306 genes were significantly modulated in NJH12 by the microarray analysis (Fig.5). In particular, 192 genes were up-regulated in NJH12. 62% of differential expressed genes were functional unknown (unclassified). 38% functional known genes could be observed in following functional classes: binding, catalytic activity, chaperone activity, defense immunity protein activity, signal transducer activity, transport activity, cell communication, cell growth and-or maintenance, death, development and cell (Fig.6a). These data provide insight into the role of *hrf1* gene expression in rice plants. We choose 6 of up-regulated genes to detect the difference expression between NJH12 and R109. Our RT-PCR analysis revealed that the express of *Os-4*, *Os-6*, *Os-10*, *Os-24*, *Os-26* and *Os-27* was higher in NJH12 than in R109 (Fig.6b). Whether all up-regulated and down-regulated genes are related to the blast resistant remain to research in future.

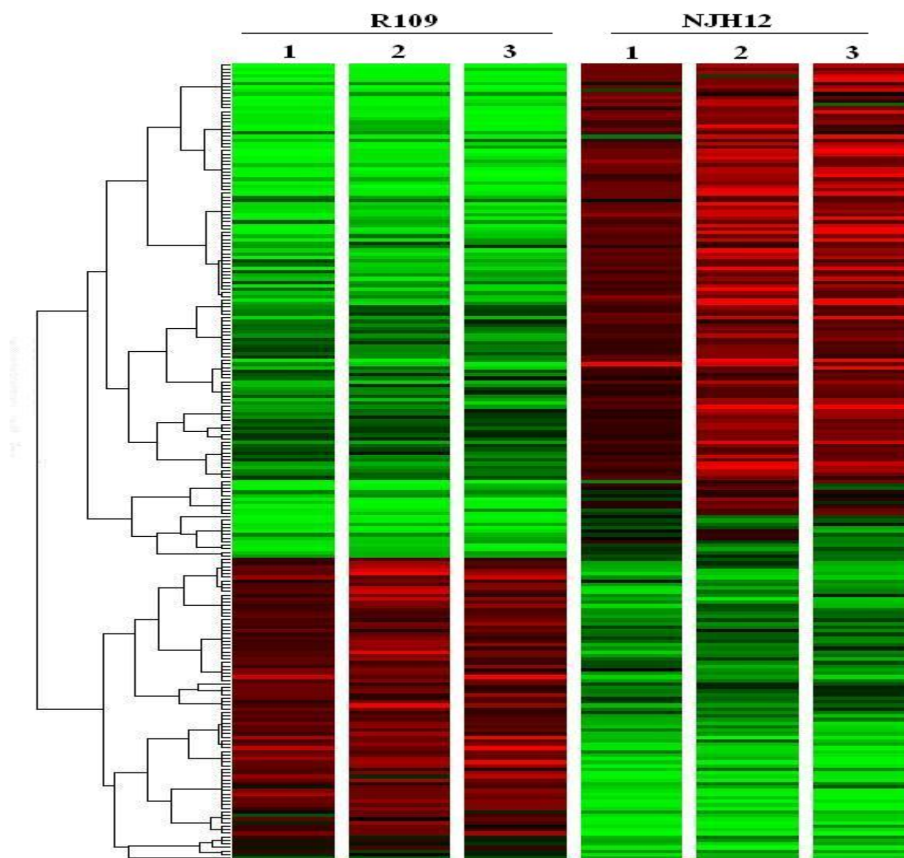


Fig. 5 Clustering of the isolated up-regulated and down-regulated transcripts from the microarray analysis. 1,2,3 columns represent three repeats. Red and green reflect transcriptional activation and repression respectively.

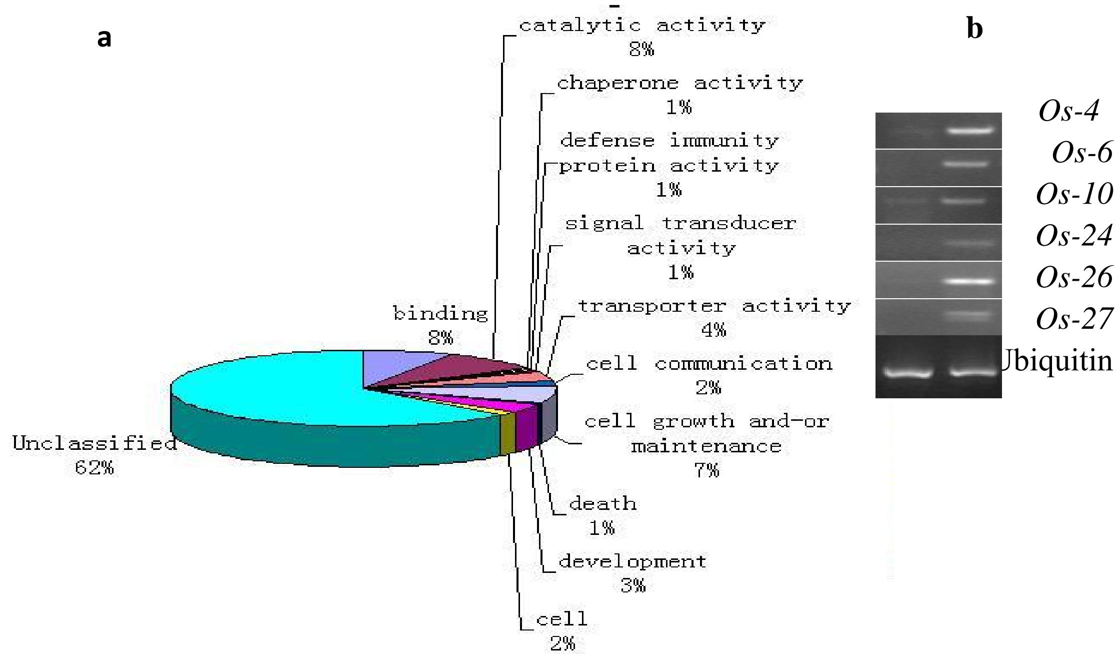


Fig. 6 Distribution of functional categories within the group of genes with an either higher and lower expression in NJH12 (a) and RT-PCR analysis of up-regulated genes (b).Determination by RT-PCR of the rice RNA of R109 and that express *Os-4*, *Os-6*, *Os-10*, *Os-24*, *Os-26* and *Os-27*.Ubiquitin was amplified as a loading control.

In conclusion, our results illustrate that *hrf1*-expression confers non-specific resistance in rice to *M. grisea*, likely through SAR and activating general defense responses that subsequently interfere with the growth and development of the pathogen. Over the last decade, numerous *hrp* genes have been identified in several gram-negative phytopathogenic bacteria (Lee et al. 2001; Noel et al. 2002). It remains to be tested whether the expression of these genes will confer durable resistance to *M. grisea* and other pathogens of economic significance in rice or other crops, but the success of NJH12 transgenic rice reveals a clear path for the direction of further experimentation.

Reference

1. Agrawal, G.K., Rakwal, R., Jwa, N.S. & Agrawal, V.P. Signalling molecules and blast pathogen attack activates rice *OsPR1a* and *OsPR1b* genes: A model illustrating components participating during defence/stress response. *Plant Physiology and Biochemistry* **39**,1095-1103 (2001)
2. Chumley, F.G. & Valent, B. Genetic-analysis of melanin-deficient, nonpathogenic mutants of *Magnaporthe grisea*. *Molecular Plant-Microbe Interactions* **3**, 135-143 (1990).
3. Coca, M., Bortolotti, C., Rufat, M., Penas, G., Eritja, R., Tharreau, D., del Pozo, A.M., Messeguer, J. & San Segundo, B. Transgenic rice plants expressing the antifungal AFP protein from *Aspergillus giganteus* show enhanced resistance to the rice blast fungus *Magnaporthe grisea. Plant Molecular Biology* **54**, 245-259 (2004).

4. Dangl, J.L. & Jones, J.D.G. Plant pathogens and integrated defence responses to infection. NATURE **411**, 826-833 (2001).
5. Dean, R.A. Signal pathways and appressorium morphogenesis. *Annual Review of Phytopathology* **35**, 211-234 (1997).
6. Dong, H.S., Delaney, T.P., Bauer, D.W., Beer, S.V. Harpin induces disease resistance in *Arabidopsis* through the systemic acquired resistance pathway mediated by salicylic acid and the *NIM1* gene. *Plant Journal* **20**, 207-15 (1999)
7. Dudoit S., Gentleman R.C., Quackenbush J. (2003) Open source software for the analysis of microarray data. *Biotechniques (Suppl),* 45-51.
8. Durrant, W.E., Dong, X. Systemic acquired resistance. *Annual Review of Phytopathology* **42**, 185-209 (2004)
9. HammondKosack, K.E. & Jones, JD.G. Plant disease resistance genes. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**, 575-607 (1997).
10. He, S.Y., Huang, H.C. & Collmer, A. *Pseudomonas syringae* pv. *syringae* harpinPss: A protein that is secreted by the hrp pathway and elicits the hypersensitive response in plants. *Cell* **73**, 1255–1266. (1993).
11. Huang, J.Q., Wei, Z.M., An, H.L. & Zhu, Y.X. *Agrobacterium tumefaciens*-mediated transformation of rice with the spider insecticidal gene conferring resistance to leaf folder and striped stem borer, *Cell Research* **11**, 149-15 (2001).
12. Jin, M.Z. & Chai, R.Y. Advances in studies on physiological races of *Magnaporthe grisea* in China. *Plant Protection* **3**, 37-41 (1990).
13. Kariola, T., Palomaki, T.A., Brader, G. & Palva, E.T. *Erwinia carotovora* subsp *carotovora* and *Erwinia*-derived elicitors HrpN and PehA trigger distinct but interacting defense responses and cell death in Arabidopsis. *Molecular Plant-Microbe Interactions* **16**, 179-187 (2003)
14. Krishnamurthy, K., Balconi, C., Sherwood, J.E. & Giroux, M.J. Wheat puroindolines enhance fungal disease resistance in transgenic rice. *Molecular Plant-Microbe Interactions* **14**, 1255-1260 (2001).
15. Lee, J., Klessig, D.F. & Nurnberger, T. A harpin binding site in tobacco plasma membranes mediates activation of the pathogenesis-related gene *HIN1* independent of extracellular calcium but dependent on mitogen-activated protein kinase activity. *Plant Cell* **13**, 1079-1093 (2001).
16. Liu, S.H. & Dean, R.A. G protein αsubunit genes control growth, development, and pathogenicity of *Magnaporthe grisea*. *Molecular Plant-Microbe Interactions* **10**, 1075-1086 (1997).
17. NCRTRB (National Coordinating Research Team on Rice Blast). Evaluation of rice varietal resistance to blast (*Pyricularia grisea*). *Scientia Agricultura Sinica* **4**, 44-52 (1980).
18. Nishizawa, Y., Nishio, Z., Nakazono, K., Soma, M., Nakajima, E., Ugaki, M., Hibi, T. Enhanced resistance to blast (*Magnaporthe grisea*) in transgenic *Japonica* rice by constitutive expression of rice chitinase. *Theoretical and Applied Genetics* **99**, 383-390 (1999).
19. Noel, L., Thieme, F., Nennstiel, D. & Bonas, U. Two novel type III-secreted proteins of *Xanthomonas campestris* pv. *vesicatoria* are encoded within the hrp pathogenicity island. *Journal of Bacteriology* **184**, 1340-1348 (2002).

20. Peng, J.L., Bao, Z.L., Ren, H.Y., Wang, J.S., Dong, H.S. Expression of harpin$_{Xoo}$ in transgenic tobacco induces pathogen defense in the absence of hypersensitive cell death. *Phytopathology* **94**, 1048-1055 (2004).
21. Rodrigues, F.A., Benhamou, N., Datnoff, L.E., Jones, J.B., Belanger, R.R. Ultrastructural and cytochemical aspects of silicon-mediated rice blast resistance. Phytopathology **93**, 535-546 (2003)
22. Sambrook, J., Fritsch, E.F., & Maniatis, T. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press (1989).
23. Shen L.S., Xin J.Q., Caldo, R. A. Nettleton D., Brendel V., Wise R., Dickerson J.A. PLEXdb –Plant And Pathogen Expression Database And Tools For Comparative And Functional Genomics Analysis, Plant & Animal Genomes XIV Conference, Town & Country Convention Center, San Diego, CA，P947(2006).
24. Spoel SH, Koornneef A, Claessens SMC, Korzelius JP, Van Pelt JA, Mueller MJ, Buchala AJ, Metraux JP, Brown R, Kazan K, Van Loon LC, Dong XN, Pieterse CMJ. NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. *Plant Cell* **15**, 760-770 (2003).
25. Stuiver, M.H. & Custers, J.H.H.V. Engineering disease resistance in plants. *Nature* **411**, 865-868 (2001).
26. Takagaki M, Kaku K, Watanabe S, Kawai K, Shimizu T, Sawada H, Kumakura K, Nagayama K. Mechanism of resistance to carpropamid in *Magnaporthe grisea*. *Pest Management Science* **60**, 921-926 (2004).
27. Talbot, N.J. On the trail of a cereal killer: Exploring the biology of *Magnaporthe grisea*. *Annual Review of Microbiology* **57**, 177-202 (2003).
28. Wei, Z.M., Laby, R.J., Zumoff, C.H., Bauer, D.W., He, S.Y., Collmer, A. & Beer, S.V. Harpin, elicitor of the hyper- sensitive response produced by the plant pathogen *Erwinia amylovora. Science* **257**, 85–88 (1992).
29. Wen, W.G., Shao, M., Chen, G.Y. & Wang, J.S. Defense response in plants induced by harpinXoo, a elicitor of hypersensitive response from Xanthomonas oryzae pv. oryzae, Journal of Agricultural Biotechnology 11, 192-197 (2003).
30. Yang, Y.N., Qi, M. & Mei, C.S. Endogenous salicylic acid protects rice plants from oxidative damage caused by aging as well as biotic and abiotic stress. *Plant Journal* **40**, 909-919 (2004).
31. Zhu, W.G., Magbanua, M.M. & White, F.F. Identification of two novel *hrp*-associated genes in the *hrp* gene cluster of *Xanthomonas oryzae* pv. *oryzae*. *Journal of Bacteriology* **182**:1844-185 (2000).
32. Zhu, Y.Y., Chen, H.R., Fan, J.H., Wang, Y.Y., Li, Y., Chen, J.B., Fan, J.X., Yang, S.S., Hu, L.P., Leung, H., Mew, T.W., Teng, P.S., Wang, Z.H. & Mundt, C.C. Genetic diversity and disease control in rice. *Nature*. **406**, 718–722 (2000).

Acknowledgements: The project was supported by grants from the National Key Basic Research Program of China (2003CB114204), and the National Special Program for Research and Industrialization of Transgenic Plants (JY03-B-12).We thank G.Y. Chen, X.B. Wang and H.S. Zhang for contributing to the design and execution of the field experiment. Thanks are also

extended to F.H. Xiao and Y. Liu at Anhou, D.H. Lu and G. Yao at Pujiang, and T.C. Gao at Qianshan for evaluating the *M. grisea* resistance, Y.J. Zhou for providing the *M. grisea* conidia and access to the *M. grisea* evaluation facility at JAAS, and J.Y. Zhuang at China National Rice Research Institute for Si determination.

Correspondence and requests for materials should be addressed to Min Shao (e-mail: minshao@njau.edu.cn).

Topic 6. Chemical and biological control strategies against Xoo

Structural proteomics of *Xanthomonas oryzae* pv. *oryzae* (Xoo) for anti-bacterial drug development against bacterial blight (BB)

Jeong-Gu Kim[1], Sungguan Cho[1], Junho Jung[2], Jaewook Jung[2], Myoungki Hong[2], Seunghwan Kim[2], Taeyoung Kim[2] and Lin-Woo Kang[2,¶]

[1]Microbial Genetics Division, National Institute of Agricultural Biotechnology, 225 Seodun dong, Suwon 441-707, South Korea; [2]Department of Advanced Technology Fusion, Konkuk University, Hwayang-dong, Gwangjin-gu, Seoul 143-701, South Korea.
[¶]Address correspondence to: Lin-Woo Kang, Department of Advanced Technology Fusion, Konkuk University, Seoul 143-701, South Korea; Tel. 82-2-450-4090; Fax. 82-2-444-6707; E-mail: lkang@konkuk.ac.kr

Abstract
Bacterial blight (BB) is the most destructive bacterial disease of rice, which is caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). In 2006 BB caused a hundred of million dollars worth damage only for South Korea. Although several anti-bacterial drugs against *Xoo* are already in use, most of them are barely effective to treat BB. Here, we try to develop the anti-bacterial drug against *Xoo* by rational drug design based on the atomic resolution three dimensional structures of target proteins in *Xoo*, which are closely related with pathogenicity to rice. About a hundred of genes in *Xoo* are selected and are being cloned into several expression vectors, which contains bacterial expression vectors and a novel *Xoo* expression vector developed by our own lab. Up to now expression of *Xoo* genes in *E.coli* is not so successful and the better systematic approach to secure native materials of target proteins is essential. One of the best ways to obtain the native conformation target proteins is to purify the proteins directly from the source organism. We are in the middle of developing a novel *Xoo* expression vector derived from the previous broad host range shuttle vectors of *E.coli* and *Xoo* such as pML122, pHM1, and pUFR027. The purified proteins or protein complexes will be tried to crystallize and the crystal structure of target protein will provide important information to develop anti-bacterial drug development against BB.

Study on the efficacy of rice cultivar mixtures for rice bacterial blight management

Guanghai Ji[1], Lan-Fang We[1],Yue-qiu He[1] , Shi-Guang Zhang[1], Yongchen Li[2]
1.The Key Laboratory for Plant Pathology of Yunnan Province, Yunnan Agricultural University, Kunming 650201, China;
2.Institute of Agricultural science research of Honghe prefecture , Menzhi 662000, China;
Tel:086-0871-5227904 E-mail:jghai001@yahoo.com.cn

Bacterial blight of rice (*Oryza sativa* L.), caused by *Xanthomonas oryzae* pv. *oryzae*, is a serious disease of global importance.Cultivar mixtures have been shownto impact a spectrum of plant diseases,and their commercial use is increasing.Rice cultivar mixtures recently have been used to control rice blast in Yunnan Province China, with great success. However, Information on rice cultivar mixtures is largely lacking for the bacterial blight disease of rice in China. Thus, we conducted field experiments to determine the effects of different rice cultivar mixtures and inter-planting mode on BB control, grain yield and epidemic progression. Four *indica*(Hybrid) rice of Diantun 502(A),Yunhui290(B), Hongyou No.6(C), and II You725(D), two glutinous cultivars of Huangkenuo(E),Bendinuo(F) were selected based on the genetic background, resistance and agronomic traits. Plot experiments of 16 mixed-planting combinations of A/E(4:1), B/E(4:1), C/E(4:1),D/E(4:1),A/F(4:1) , D/F(4:1),A/E(6:1),A/E(8:1), D/E(6:1), D/E(8:1), E/A(4:1),E/A(6:1),E/A(8:1), E/D(4:1), E/D(6:1),E/D(8:1) were conducted in farmer's fields at three locations of the Mengzhi, Kaiyuan, Yuanmou county of Yunnan province in southern west, China, during the wet seasons from 2004 to 2006.and the six rice cultivars pure stands as the control in each experiment. The results demonstrated that rice BB management was more effective in combinations of resistant *indica* rice cultivars inter-planting with glutinous susceptible cultivars than mixtures of two susceptible rice lines, compared with their pure stand. when the treatments were inoculated with Xoo pathogen in two years, Disease progressed steadily throughout the season in both years at Mengzhi county in two years, For the highly susceptible glutinous varieties, the disease incidence and index in mixed planting was significantly less than the mean of its component pure stands. The BB control efficiencies of glutinous varieties in different mixture combinations reached to 43%-62%, the BB control efficiencies of indica(Hybrid) cultivars varied from 15-57% ;The distance of disease progressed in mixed cultivation plots reduced by about one-thirds as compared with its pure stand; Grain yield of *indica* and glutinous cultivars in mixed cultivation plots averaged 3%-42.6% higher than that in pure stand. The results also showed that the indica(Hybrid) rice cultivars and glutinous varieties should be planted at a ratio of 1 landraces :4 or 6 indica rows in mixed inter-planting conditions, which could be effective in disease control and increase grain yield.

Miscellous

Development of DNA microarray of *Xanthomonas oryzae* pv. *oryzae* and its validity in gene expression profile analysis

Xinjian Zhang, Shiqiang Gao, Maosen Wu and Chenyang He*

*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China. *E-mail: cyhe@caas.net.cn*

To develop DNA microarrays as a comprehensive tool for gene expression profiling analysis in *Xanthomonas oryzae* pv. *oryzae* (Xoo), a collection of 371 predicted protein-coding genes consisting of pathogenicity-related genes, species-specific genes, regulator genes and conserved genes in plant pathogenic bacteria was arrayed in high density on glass slides to establish the PCR fragment-based microarrays, based on the complete genome sequence of Xoo KACC10331. Primer pairs were designed to amplify internal gene-specific DNA fragments (400–600bp) from Xoo JXOI. To ascertain the quality of microarrays and to validate gene expression analysis in Xoo, hybridizations of microarrays with Cy3- and Cy5-labeled probes based on RNA samples obtained from bacterial cells co-incubated for 15 min with the detached leaves of rice were performed. The fabrication and application of microarrays has been optimized. Fifth-six genes were identified from the gene expression profiling revealed by DNA microarray analysis, which showed significant changes in gene expression resulting from the bacterial interactions with rice leaf tissues. Among the differentially-expressed genes, 50 genes were up-regulated, whereas 6 genes were down-regulated. Thus, the fabricated microarrays and optimized technologies have been successfully employed in gene expression profiling analysis of Xoo.

Keywords: *Xanthomonas oryzae* pv. *oryzae*; DNA microarrays; gene expression profile; differentially-expressed genes; functional genomics

Design of genome-directed primers (GDPs) for selective labeling of transcripts of *Xanthomonas oryzae* pv. *oryzae* and its application for bacterial expression profiling during early infection revealed by DNA microarray analysis

Xinjian Zhang, Jingsheng Xu, Shiqiang Gao, Maosen Wu and Chenyang He*

*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China. *E-mail: cyhe@caas.net.cn*

DNA expression microarrays require the substantial amounts of bacterial RNA to generate the probes for hybridization. A computer-based algorithm for prediction of the minimal number of primers to specifically anneal to all genes in a given bacterial genome was used in this study. The algorithm predicts that 74 oligonucleotides should prime all genes in the *Xanthomonas oryzae* pv. *oryzae* (Xoo) genome. The usefulness of the genome-directed primers (GDPs) was tested for *in planta* gene expression

profiling of Xoo during early bacterial infection using DNA microarrays. GDPs were used to generate fluorescent-labeled probes and to hybridize to an array of 371 bacterial genes. This approach could be useful for accurate genome-wide expression analysis, especially for *in vivo* gene expression profiling.

Keywords: *Xanthomonas oryzae* pv. *oryzae*; GDPs; early bacterial infection; gene expression profiling; differentially-expressed genes

Quantitative *Forecasting Method* for *Xanthomonas oryzae* pv. *oryzae*

Tae Hwan Noh[1], Jong Chul Park[1], Du Ku Lee[1], Hyeong Kwon Shim[1], Mi Hyung Kang[2], Byoung Moo Lee[3] and Jae Duk Kim[1]

[1]Honam Agricultural Research Institute, NICS, RDA, Korea
[2]Namwon Horticultural Experiment Station, JARTS, Korea
[3]Nation Institue of Agricultural Biotechnology, RDA, Korea

Recently, the disease incidence of bacterial leaf blight of rice has been increase centering around southern region of Korea. In general, the bacterial density in irrigation water was use to forecast the disease incidence even if it shows unstable results caused by several bacterial strain. So we developed new forecasting method for BLB caused by *Xanthomonas oryzae* pv. *oryzae* (*XOO*) using by Real-Time PCR. We used the highly characterized and specific two primer such as XOO-taqF and XOO-taqR. XOO-taq FAM probe primer pairs from *hpaA* gene. These primers showed distinct detection rseults that was not response to *Burkholderia glumae, Acidovorax avenae subsp. avenae, Pantoea herbicola, Pseudomonas syringae pv. syringae,* including rice seed related bacteria and other 12 isolates from paddy field water. The method is effective in using a various templates such as *Xoo* DNA extraction, pure colonies or liquid culture sources, and contaminating water. In addition, this assay can quantitate the population density of *Xoo* in paddy field. This method showed higher efficiency than bacteriophage method for disease occurrence forecasting about one month earlier before diseased diffusion.

N-terminal mutants of *Xanthomonas oryzae* harpins that abolish the activity to elicit hypersensitive reaction retain to induce systemic acquired resistance on tobacco

Xiaoyu Wang [a], Jiahuan Zhang [a], Yan Zhang [a], Guiying Zhang [a], Jinsheng Wang [a,*]

[a] The key Laboratory of Monitoring and Management of Plant Diseases and Insects of Chinese Ministry of Agriculture;Department of Plant Pathology, Nanjing Agriculture University, Nanjing 210095, China.
*, Corresponding author

Abstract

Harpins are encoded by many gram negative phytopathogenic bacterial *hrp* genes and induce hypersensitive reaction (HR) and associated defense response on some nonhost plants. HR characterized by the rapid collapse of tissue accompanies localized hypersensitive cell death [HCD] that resembling animal programmed cell death [PCD]. The intact purified harpins, when infiltrate into the plant leaves, display visible HR, while spraying on leaves only microscopic-HR is observed. A set of N-terminal and C-terminal mutants were previously created from hpa1 homologues of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *X. o.* pv. *oryzicola* (*Xooc*) and displayed that only N-terminal portions are essential for elicitation of HR. The mutants, hpa1xoo-N and hpa1xooc-N, deleted 12 highly hydrophilic amino acids(H_2N-QGISEKQLDQLL-COOH) that are partially overlapped with the N-terminal α-helical region, and $Harpin_{xoo}$(L51P) and $Harpin_{xooc}$(L53P) are two single missense mutants that are CTT-CCT base pair substitution caused Leu. to Pro. at appreciated sites. These mutants all abolished the activity to elicit HR when infiltrate into tobacco leaves but save the activity to induce systemic acquired resistance (SAR) in treated tobacco. The treated leaves displayed the microscopic cell death (micro-HR) after stained with trypan blue accompanied with enhanced transcript accumulation level of several HR and SAR marker genes such as *NPR1*, *HSR515* and *PR2*. To the end we suggest that HR is not a prerequisite for SAR in HarpinX-tobacco system.

Cloning and characterization of a *hrf1* homolog from rice (*Oryza sativa* L.)

Min Shao, Ling Guo, Wanchun Huang, Lin Li, Jinsheng Wang

Key Laboratory of Monitoring and Management of Plant Diseases and Pests, Ministry of Agriculture of China, Department of Plant Pathology, Nanjing Agricultural University, Nanjing, 210095, China.

Abstract: Over the last decade, numerous *hrp* genes have been identified in several gram-negative phytopathogenic bacteria. In this study, we found *hrf1* homolog, named *hpfr1*, from *O. sativa* L. cv R109, when we researched expression *hrf1* gene in rice R109. With this information in hand, we then proceeded with the isolation of homolog from 9311 rice cultivar, and named *hpfr2*. In comparison with *hrf1* gene, *hpfr1* showed high-level homology of 92% identity, and *hpfr2* was 48% identity. The presence of *hpfr* in R109 and 9311 rice cultivar was examined by Southern hybridization with *hrf1* as a probe. Western blot analysis showed that hpfr1 protein was not detected in protein extraction of rice plants. *hpfr1* gene was cloned into pET30, overexpressed protein in *E.coli* BL21 (DE3) strain by isopropyl-β-D-thiogalactopyranoside (IPTG) induction. The product of overexpression *hpfr1* is about 14.5kD. 14.5kD hpfr1 protein has been purified by nondenaturing electrophoresis. Our research results indicated that hpfr1 protein have the common characteristics of harpin, such as heat stable, rich-Gly and induce hypersensitive response (HR) and resistance. This is the first report of HR-eliciting encoding gene identified from plant. During a long coevolution of pathogens and their hosts, horizontal gene transfer may be present between pathogens and hosts.

Key words: rice, *hpfr1* gene, hypersensitive response, induce resistance
Correspondence and requests for materials should be addressed to Min Shao (e-mail: minshao@njau.edu.cn).

Generation and Characterization of an Anti-idiotypic Antibody of Harpin (subscript Xoo), a Proteineous Elicitor of *Xanthomonas oryzae* pv. *oryzae*

D.F.Chen, G.L.Qian, Q.Sun, C.Y.Gu, B.S.Hu and F.Q.Liu*
Lab of Immunology & Quarantine, Department of plant pathology, College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China

Harpin is a group of proteins produced by gram-negative plant pathogenic bacteria which triggers hypersensitive response in nonhost plants. Protein harpin(subscript Xoo) is encoded by the hpa1 gene in the hrp cluster of *Xanthomonas oryzae* pv. *Oryzae*. A monoclonal anti-idiotypic antibody (Ab2) was elicited by using a $F(ab')_2$ fragment generated by digesting IgG, purified polyclonal antibody(Ab1) against Protein harpin(subscript Xoo), with immobilized pepsin. After several selections by means of competition ELISA, 25 Ab2 clones were chosen to be fully characterized. These Ab2 clones competed with protein harpin(subscript Xoo) for binding to Ab1 indicating its anti-idiotypic character and the internal image property. The relevance of antigen mimicry was further demonstrated by eliciting a third generation antibody(Ab3), which was shown to not only recognize Ab2 but also react with Protein harpin(subscript Xoo). Taken together, these results demonstrate functional and biochemical mimicry of Ab2 for harpin(subscript Xoo) and suggest that

anti-idiotypic antibody can substitute harpin to study localization of its receptor in tobacco.

hrpF and *hpa1* Genes of *Xanthomonas oryzae* pv. *oryzicola* Determine the Development of the Leaf Streak Symptom, but Have No Effects on Infection Route in Rice

Zou LF, Wang YP, Zhou D, Li YR, and Chen GY (gyouchen@njau.edu.cn)

Department of Plant Pathology, Nanjing Agricultural University, Nanjing China 210095

The *hrp* cluster of *Xanthomonas oryzae* pv. *oryzicola* (*Xooc*) endows the pathogen with the ability to elicit hypersensitive response (HR) in non-host tobcco and pathogenicity in rice and encodes components of type III secretion system (T3SS), through which T3SS effector proteins are secreted and delivered via HrpF translocon into plant cells. Hpa1 is a T3SS effector eliciting HR in tobacco. However, what type-III effectors determine the formation of bacterial leaf streak (BLS) symptom and the infection route through stomata in rice are still unclear. We hear knocked out the *hrpF* and *hpa1* genes individually and simultaneously. The *hrpF* mutant failed to produce bacterial leaf streak symptoms when it was sprayed on rice leaves. Observations by scan electro-microscopy (SEM) revealed that the double *hrpF* and *hpa1* mutant lost the ability to assemble near, to penetrate the stomata, and to infect the rice callus, suggesting that the *hrpF* and hpa1 genes have no effects on bacterial infection route through stomata, but on disease symptom development or/and on parasitism in rice.

Genome-wide microarray analysis of transgenic cotton expressing harpinxoo from *Xanthomonas oryzae* pv. *oryzae*

Weiguo Miao, Ming Li, Yu Wang, Congfeng Song, Jinsheng Wang

Key Laboratory of Monitoring and Management of Plant Diseases and Insects, Ministry of Agriculture, Nanjing Agric Univ, Weigang I, Nanjing 210095, P.R.China.

Harpins are a group of glycine-rich, heat-stable and protease K-sensitive proteins that are able to elicit disease and insect resistance in plants and, can also promote plant growth besides inducing many other plant-reaction phenotypes, such as enhance crop yield and quality. In *Xanthomonas oryzae* pv. *oryzae*, the harpin protein was given the name $harpin_{Xoo}$ and the corresponding gene designated *hpa1* (GenBank

accession no. AY205561). The crude protein preparation of harpin$_{Xoo}$ can induce wide and systemic disease resistance when sprayed onto plants leaves. In this study, the *hpa1* was introduced into the cotton(*Gossypium hirsutum* L.) using the pollen-tube pathway transformation technique and the transgenic cotton plants exhibit an enhanced resistance to *Verticillium* wilt disease. To understand the mode of action of Harpin at the molecular level, DNA microarray analysis of gene expression profiling in transgenic cotton leaves or roots were conducted. About 530 genes in leaves and 121 genes in roots were either up-regulated or down-regulated in transgenic cotton compared with non transgenic cotton. These differentially expressed gene involved 162 pathways. Functional annotations of the 530 most differentially expressed genes from transgenic cotton leaves were conducted.

Cloning and Expression of hpa1 homology sequence from *Triticum aestivum* L.

Congfeng Song, Peipei Li, Na Li, Xiaolan Peng, Jinsheng Wang

Key Laboratory of Monitoring and Management of Plant Diseases and Insects, Ministry of Agriculture, Nanjing Agric Univ, Weigang I, Nanjing 210095, P.R.China.

By using RT-PCR and nest-PCR, a *hpa1* homology sequence named TA50-10 was cloned from *Triticum aestivum* cutivar Xiaoyan 54. Sequcence analysis showed it is 371bp long and has 59% identity with *hpa1* of *Xanthomonas oryzae* pv. *oryzae* .The G+C content is 68.22% which is slightly higer than that of *hpa1* (60.48%). The deduced product is a tyrosine kinase like protein. The construct pET30-TA50-10 was expressed in *Escherichia coli* BL21 strain. The expressed protein induced by Isopropyl β- D -1-thiogalactopyranoside (IPTG) has the size of about 12Kda. It is thermostable after water boiled for 10min. When sprayed onto the tobacco leaves, both the cruded or heat treated protein solution at the concentration of 15μg/ml of expressed TA50-10 protein can induce strong resistance of tobacco against TMV. The resistance can last as long as 10 days with no obvious loss of the resistance inducing activity. However, the resistance is locally rather than systemically induced.

Acknowledgements: The research was supported by grants from National natural sciences foundation (30671355); the National Key Basic Research Program of China (2003CB114204).