



# The Fifth International Rice Blast Conference

## The Peabody Hotel, Little Rock, Arkansas, USA

### August 12 -14, 2010

#### KEYNOTE SPEAKERS:

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International Rice Research Institute (IRRI)  
the Philippines

**Jan Leach**  
Colorado State University, USA

**Ralph Dean**  
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**Barbara Valent**  
Kansas State University, USA

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USDA-ARS Dale Bumpers National Rice  
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**11-14 August 2010**  
**Little Rock, Arkansas USA**

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**2010 5<sup>th</sup> International Rice Blast Conference**  
**Wednesday, August 11, 2010**

1:00 – 6:00 p.m. Meeting registration – Peabody Hotel Lobby  
3:00 - 6:00 p.m. Set up posters in Arkansas Ball Room according to number

**Thursday, August 12, 2010**  
**PROGRAM**

7:30 – 8:30 a.m. Light breakfast, coffee, tea, juice outside the Conway Room  
8:30 – 9:00 a.m. Welcome and Introductory Remarks, the Conway Auditorium

**Plenary Presentations**  
(Chair: Jim Correll and Co-Chair: Yulin Jia)

9:00 – 9:30 a.m. Perspectives on Rice Blast, Research and the Broader Issues of Food Security and Evolving Production Practices  
**Robert S. Zeigler**

9:30 – 10:00 a.m. Next Generation Biology: Novel Insights into Pathogenicity of the Rice Blast Fungus, *Magnaporthe oryzae*  
Gowda, M., Oh, Y., Nunes, C., Sailsbery, J., Xue, M., Chen, F., Nelson, C., Brown, D.E., Meng, S., Mitchell, T., Hagedorn, C.H. and **Ralph A. Dean**

10:00 – 10:30 a.m. Wading Through Murky Paddies: Clarifying Broad Spectrum Resistance in Rice  
**Jan E. Leach**, Davidson, R.M., Snelling, J., Bruce, M., Liu, B., Zhu, X., Leung, H. and Vera Cruz, C.M.

10:30 – 10:55 a.m. Break (outside Conway Room)

10:55 – 11:30 a.m. Duck Walk – Honorary Duckmaster – Barbara Valent and Group Photo Meet in the hotel lobby water fountain area promptly at 11 a.m.

11:30 – 12 noon Biotrophic Invasion of Rice Cells by *Magnaporthe oryzae*: Where We Go from Here  
**Barbara Valent**

12:00 – 12:30 p.m. Plants and Pathogens Engage in Trench Warfare-Knowledge Learned from Natural Variation of the Rice Blast Resistance Gene *Pi-ta* and the Corresponding Avirulence Gene *AVR-Pital*  
**Yulin Jia**

12:30 – 2:00 p.m. Lunch on your own.

Thursday, continued

**I. Genomics and Functional Genomics of the Pathogen**

(Chair: Ane Sesma and Co-Chair: Naweed Naqvi)

- 2:00 – 2:15 p.m.  
I-O-70      Population Genomic Approaches to Understand *Magnaporthe*-Rice Interactions  
**Terauchi, R.**, Yoshida, K., Hiromasa, S., Okuyama, Y., Kanzaki, H., Tamiru M., Abe, A., Takano, Y., Chuma, I., Tosa, Y., Win, J. and Kamoun, S.
- 2:15 - 2:30 p.m.  
I-O-71      Novel Splicing Factors Control Infection-related Morphogenesis in *Magnaporthe oryzae*  
**Peng, Y.-L.**, Yang, J., Wang, W., Ding, S., Chen, X., Wang, R., Kong, L. and Xu, J.-R.
- 2:30 – 2:45 p.m.  
I-O-73      Alpha-1,3-glucan Functions as a Stealth Gear During Infection in *Magnaporthe oryzae*  
**Nishimura, M.** and Fujikawa, T.
- 2:45 – 3:00 p.m.  
I-O-74      A Novel NADPH-Dependent Genetic Switch Regulates Infection by the Rice Blast Fungus  
Fernandez, J., Quispe, C., Hartline, D., Wright, J. and **Wilson, R.A.**
- 3:00 – 3:15 p.m.  
I-CO-75      Functional Study of the *Hyr1* Gene, Potentially Involved in Detoxification of Plant-Defense-Generated Reactive Oxygen Species in the Rice Blast Pathogen  
**Huang, K.**, Czymmek, K., Caplan, J., Sweigard, J.A. and Donofrio, N.M. (Competing presentation)
- 3:15 – 3:30 p.m.  
I-O-76      Links Between Post-Transcriptional Regulatory Mechanisms and Pathogenicity in the Rice Blast Fungus  
**Sesma, A.**, Franceschetti, M., Bueno, E., Besi, M. and Galhano, R.
- 3:30 – 3:45 p.m.  
I-O-103      *Magnaporthe* Pathogenesis: Efflux, Effectors and Entry into the Host  
Patkar, R.N. and **Naqvi, N.I.**
- 3:45 – 4:15 p.m.      Break (upstairs outside the Arkansas Ball Room)
- 4:15 – 6:00 p.m.      Poster viewing in the Arkansas Ball Room
- 6:30 – 8:30 p.m.      Boscors Restaurant Reception (two blocks away from the Peabody Hotel)  
Dinner on your own

**2010 5<sup>th</sup> International Rice Blast Conference**  
**Friday, August 13, 2010**

**PROGRAM**

- 7:30 – 8:30 a.m. Light breakfast, coffee, tea, juice outside the Conway Room
- 8:30 – 8:45 a.m. Business Session in Conway Auditorium  
(organizing committees, session chairs and co-chairs)
- 8:45 – 9:00 a.m. Introductory Remarks, Field Day Plans and Meeting Logistics  
Remarks about PostDoc and Student Oral Competition – **Tom Mitchell**

**I. Genomics and Functional Genomics of the Pathogen (continued)**

- 9:00 – 9:15 a.m. Multiple Plant Surface Signals are Sensed by Different Mechanisms of the  
I-O-77 Rice Blast Fungus for Appressorium Formation  
**Xu, J. –R.**, Liu, W., Zhou, X., Li, G., Kong, L. and Wang, C.
- 9:15 – 9:30 a.m. Functional Characterization of *Magnaporthe oryzae*: Putative Effectors in  
I-O-80 the Infective Process of Rice  
**Burbano-Figueroa, O.**, Kim, S., Chen, S., Songkumarn, P., Wang, G.L.,  
and Mitchell, T.K.
- 9:30 - 9:45 a.m. The Biotrophic Interfacial Complex and Trafficking of *Magnaporthe*  
I-O-81 *oryzae* Effectors Inside Rice Cells  
**Khang, C.H.**, Berruyer, R., Giraldo, M.C., Kankanala, P., Park, S.-Y.,  
Czymmek, K., Kang, S. and Valent, B.
- 9:45 – 10:15 a.m. Break (outside Conway Room)
- 10:15 – 10:30 a.m. CFGP 2.0: An Integrated Web-based Environment for Comparative and  
I-CO-5 Evolutionary Genomics in Fungal Kingdom  
**Park, J.**, Choi, J., Cheong, K-C., Kim, D., Jung, K., Kim, S., Park, B.,  
Choi, D., Kang, S. and Lee, Y-H. (Competing presentation)

**II. Host Resistance, Signaling and Defense Responses**  
(Chair: Yinong Yang and Co-Chair: Guo-Liang Wang)

- 10:30 – 10:45 a.m. Functional and Evolutionary Characterization of Rice Blast Resistance  
II-O-102 Alleles at the *Pik* Locus  
Zhai, C., Yuan, B., Dong, Z., Zeng, X., Lin, F., Wang, L. and **Pan, Q.**
- 10:45 – 11:00 a.m. Characterization of Rice Lesion Mimic Mutants of 93-11 for a Better  
II-O-33 Understanding of General Host Defense Response to Both Rice Blast and  
Sheath Blight Diseases  
**Wang, X.**, Wu, D., Jia, Y. and Zhang, M.

Friday, continued

## II. Host Resistance, Signaling and Defense Responses (continued)

- 11:00 – 11:15 a.m. II-O-83 A Nep1-like Protein Toxin from *Magnaporthe oryzae* Interacts with a Conserved, Ubiquitin-like Rice Protein, and Elicits Plant Cell Death  
Liu, Z., Rohila, J. S., Liu, W., Wang, Q. and **Yang, Y.**
- 11:15 – 11:30 a.m. II-CO-84 New Insight for Two Major Rice Blast *R* Genes: *Pi-ta* and *Pi-km*  
**Costanzo, S.**, Yang, Y. and Jia, Y. (Competing presentation)
- 11:30 – 11:45 a.m. II-CO-85 Evolutionary Dynamics and Structure of the Rice Blast Resistance Locus *Pi-ta* in Wild, Cultivated and US Weedy Rice  
**Lee, S.**, Jia, Y., Costanzo, S., Olsen, K.M., Caicedo, A.L., Gealy, D.R. and Jia, M. (Competing presentation)
- 11:45 – 12:00 a.m. II-O-86 The Hop/Sti1-Hsp90 Chaperone Complex Facilitates the Maturation and Transport of a PAMP Receptor in Rice Innate Immunity  
**Chen, L.**, Hamada, S., Fujiwara, M., Zhu, T., Thao, N.P., Wong, H.L., Krishna, P., Ueda, T., Kaku, H., Shibuya, N., Kawasaki, T., and Shimamoto, K.
- 12:00 – 12:15 p.m. II-O-88 Durable Panicle Blast Resistance Gene *Pbl* Encodes an Atypical CC-NBS-LRR Protein and Generated by Acquiring a Promoter Through Local Genome Duplication  
**Hayashi, N.**, Inoue, H., Kato, T., Funao, T., Shirota, M., Shimizu, T., Hayano-Saito, Y. and Takatsuji, H.
- 12:15 – 12:30 p.m. II-O-100 Novel Functions of *AvrPiz-t* and APIP10 in the *Piz-t* Mediated Resistance to *Magnaporthe oryzae*  
Park, C.H., Chen, S., Zhou, B., Madhav, S., Yang, H., Li, W., Bellizzi, M., Jena, K.K. and **Wang, G.-L.**

## III. Molecular and Cellular Biology, Diversity, Evolution and Adaptation of the Pathogen

(Chair: Didier Tharreau and Co-Chair: Mark Farman)

- 12:30 – 12:45 p.m. III-O-91 Localization of *Magnaporthe oryzae* Secreted Proteins During Host Penetration and Growth in *Planta*  
Gong, X., Xu, J., Wang, B., Hurtado, O., Wu, C. and **Farman, M.**
- 12:45 – 2:00 p.m. Lunch on your own
- 2:00 – 2:15 p.m. III-O-92 Novel Mycovirus, *Magnaporthe oryzae chrysovirus* 1, Causing Hypovirulence to the Host Rice Blast Fungus  
Moriyama, H., Urayama, S., Kanemaki, A., Le M.-T., Fukuhara, T., Arie, T. and **Teraoka, T.**

Friday, continued

**III. Molecular and Cellular Biology, Diversity, Evolution and Adaptation of the Pathogen (continued)**

2:15 – 2:30 p.m.  
III-O-98 Genetic and Biological Evidence of Sexual Reproduction of *Magnaporthe oryzae* in a Rice Field of Yunnan Province of China  
Saleh, D., Fournier, E., Milazzo, J., Adreit, H., Ravigné, V., Bazin, E., Xu, P., Tao D., Li, C.Y., **Tharreau, D.**

2:30 – 2:45 p.m.  
III-CO-90 Characterization of the Cyclase-associated Protein *CAP1* in *Magnaporthe oryzae*  
**Zhou, X.-Y.**, Zhang, H.-F., Shaw, B., Li, G.-T. and Xu, J.-R. (Competing presentation)

**IV. Strategies for Managing Diseases Caused by *Magnaporthe species***  
(Chair: Barbara Valent and Co-Chair: Marta Cristina Filippi)

2:45 – 3:00 p.m.  
IV-O-105 Wheat Blast, a Potential Threat to Global Wheat Production  
**Urashima, A.**

3:00 – 3:15 p.m.  
IV-O-93 Marker Assisted Selection for the Improvement of Three-Line Hybrid Rice  
**Zhuang, J.-Y.**, Zhu Y.-J., Qi, F.-L. and Ying, J.-Z.

3:15 – 3:30 p.m.  
IV-O-94 Characterization of Novel Blast Resistant Genes for US Rice Breeding  
**Yan, W.G.**, Scheffler, B.E., Correll, J.C., Fjellstrom, R.G., Feng, C.D., Jackson, A. and McClung A.M.

3:30 – 3:45 p.m.  
IV-O-95 Cropping System to Limit Blast Disease in Upland Rice  
**Sester, M.**, Raveloson, H., Michelon, R., Dusserre, J. and Tharreau, D.

3:45 – 4:00 p.m.  
IV-O-96 Application of PCR for Diagnosis of Fungicide Resistance in the Rice Pathogen  
**Gomathinayagam, S.**, Rekha, M. and Sakthivelmurugan, S.

4:00 – 4:15 p.m.  
IV-O-101 Screening Rhizobacteria for Growth Promotion and Leaf Blast Suppression (*Magnaporthe oryzae*) on Aerobic Rice in Brazil  
**Filippi, M.C.C.**, Silva, G.B., Côrtes, M.V.C.B., Moraes, A.J.G. and Silva-Lobo, V.L.

4:15 – 4:30 p.m.  
IV-O-82 Mapping of the *PWT3* Locus of *Magnaporthe oryzae*, a Gene Involved in the Avirulence Reaction of the *Avena* Isolate on Wheat  
**Cumagun, C.J.R.** and Tosa, Y.

4:30 – 5:00 Break (upstairs outside the Arkansas Ball Room)

5:00 – 5:15p.m. Remarks about PostDoc and Student Poster Competitions – **Tom Mitchell**

5:15 – 7:00 Poster Viewing in the Arkansas Ball Room

7:00 – 7:30 Remove all posters  
Dinner on your own

## NOTES

**2010 5<sup>th</sup> International Rice Blast Conference**  
**Saturday, August 14, 2010**

**PROGRAM – Chair Fleet Lee**

7:00 – 7:30 a.m.	Load Buses at Peabody Hotel (Little Rock, AR)
7:30 a.m.	Depart Peabody Hotel
9:30 a.m.	Arrive University of Arkansas -Rice Research & Extension Center facilities. Unload for brief break.
10:00 a.m.	Depart for field tour to visit Rice Blast Nurseries.  <ol style="list-style-type: none"><li>1. Inclined plot blast nursery. Designed to evaluate differential flood management response in rice.</li><li>2. Standard hill plot field nursery located adjacent to the inclined plots. Designed to evaluate blast severity in individual experimental lines growing upland.</li></ol>
11:30 a.m.	End blast nursery tour and return for lunch.
12:00	Lunch. Arkansas Rice Farmers Conference Room.
12:30-1:00 p.m.	Facilities Overview Dr. Chris Deren; Dr. Dave Gealy
1:30 - 2:00 p.m.	Depart for Little Rock with one stop at blast infected grower field (time permitting).
5:00 p.m.	Arrive back at the Peabody Hotel.
5:45 p.m.	Depart for Clinton Library Walk (15 min.) or carpool from the front of the hotel
6:00 – 9:00 pm	Banquet and Awards ( <b>Chair: Barbara Valent and Co-Chair: Tom Mitchell</b> )
6:00 pm	Welcome
6:30 – 7:30 p.m.	Tour Library
7:30 – 9:00 p.m.	Dinner and awards Closing of the conference

**Sunday, August 15, 2010**

Hotel Checkout and Depart

## STATEMENT OF WELCOME

Rice blast disease is one of the most destructive diseases in the world and has been studied extensively worldwide. The International Rice Blast Conference (IRBC) is the premier conference on rice blast disease and typically is held every 3-5 years in a different country. Since the last meeting in Changsha China in 2007, availability of the genome sequences of rice and the blast fungus have resulted in significant advancement in understanding the molecular basis of host resistance, fungal virulence, and host-pathogen co-evolution.

We are thrilled to host the 2010 5<sup>th</sup> IRBC here in Little Rock, Arkansas where most of US rice is grown and rice blast has been one of the major constraints for stable rice production. The 5<sup>th</sup> IRBC will kick off with five keynote addresses to present an overview of perspective of rice blast disease, current understanding of genomics, functional genomics and host-pathogen interactions. The entire conference will take place in one technical session including 35 oral and 74 poster presentations. The meeting promises to present the most contemporary genomics-based research on rice blast, pathogen virulence, and host resistance along with novel disease management strategies. Over 175 participants will attend the conference, providing a unique opportunity to promote scientific collaboration. A field day to observe rice and rice blast disease in Arkansas will be held during the conference. An awards banquet where distinguished rice blast researchers will be recognized and winners of competitions of poster and oral presentations by students and postdoctoral research associates will be announced will be held in the William J. Clinton Presidential Library and the banquet will include a tour of the Library.

Finally, we would like to thank all members of the organizing committee and staff members of USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, Arkansas, the Department of Plant Pathology, University of Arkansas Experiment Station at Fayetteville, and the University of Arkansas Rice Research and Extension Center, Stuttgart, Arkansas for their tireless efforts in forging all of the contributions into a program.

Yulin Jia  
*2010 5<sup>th</sup> International Rice Blast Conference Program Chair*

James Correll  
*2010 5<sup>th</sup> International Rice Blast Conference Program co Chair*

## 2010 Lifetime Dedication to Rice Blast Research Award Recipients

### FRANCES LATTERELL

Dr. Frances Meehan Latterell was research plant pathologist at the US Army Biological Laboratories, Fort Detrick, Maryland, and plant pathologist, US Department of Agriculture, Agricultural Research Service in Frederick, Maryland. She conducted extensive research on cereal diseases, including rice blast. Her outstanding contributions to rice blast research spanned four decades. Her detailed analysis and evaluation of *Magnaporthe oryzae* pathogenicity and host specificity towards various rice cultivars defined blast differentials that are still in use today. She played a major role in the first global effort to standardize a set of “international differentials” for comparing *M. oryzae* pathotypes in populations from different rice-growing regions. She was the only female scientist to participate in a ground-breaking meeting on “The Rice Blast Disease” organized by IRRI in 1963. Her analysis contributed to understanding the stability of pathogenic races in *M. oryzae*. Dr. Latterell’s achievements in the area of plant pathology earned her the National Science Foundation Lifetime Achievement Award, the Distinguished Service Award of the Potomac Division of the American Phytopathological Society, and the Ruth Allen Award, a significant honor bestowed by the American Phytopathological Society. This award is presented in memorial. Dr. Latterell passed away after fighting cancer on the fifth of November in 2008.

### SALLY LEONG

Professor Leong, an emeritus faculty member of the University of Wisconsin, Madison, first began working on rice blast in 1986 at the request of the Rockefeller Foundation Rice Biotechnology Program when she was charged with the development of an RFLP map of the rice blast fungal genome. Since that time, she has received numerous awards recognizing this and subsequent work on the rice blast pathosystem, including the College of Agriculture and Life Sciences’ Pound Research Award and the Arthur S. Flemming Award for scientific achievement. Dr. Leong has pioneered numerous technologies such as Achilles’ cleavage and DNA polymorphism methodology for mapping and genotyping of the blast fungus and its rice host, as well as other grasses. This work has provided important understanding of the evolution of the genus *Magnaporthe* and its pathogenic specialization on grasses, as well as laying a foundation for the sequencing of the blast fungal genome. The discovery that many DNA polymorphisms are caused by transposable elements has led to the development of rapid fingerprinting and PCR-based diagnostic tools for identification of the blast fungus. The description of an avirulence gene, *AVRI-CO39*, its molecular evolution in grass-infecting strains of *M. oryzae*, and the discovery of a complementary disease resistance locus in rice represent major achievements that are likely to have broad practical application for plant protection in the future. Showing her commitment to the field, Dr. Leong also organized and hosted the 1st International Rice Blast Congress in Madison, Wisconsin, and co-edited the proceedings of that congress, which remain a landmark reference book for the disease after almost 20 years.

## TONI MARCHETTI

Dr. Marchetti conducted research in rice blast over 39 years, first as a Research Pathologist with the U.S Department of the Army, then with the U.S. Department of Agriculture. His research contributed significantly in the areas of pathogenic race identification and evolutionary development, discovery and inheritance of host resistance genes, epidemiology, and disease loss assessment. He was a member of the USDA-ARS rice varietal improvement team located at Beaumont, TX for 27 years where he was responsible for evaluating breeding lines and exotic germplasm for resistance to diseases of the Southern U.S., notably rice blast caused by *Magnaporthe oryzae* (*Pyricularia grisea*) and sheath blight caused by *Rhizoctonai solani*. Dr. Marchetti's main contributions to the field of rice blast research are in the areas of identifying host differentials useful for identifying blast races, inheritance of blast resistance based on both traditional and molecular research methods, and characterization of dilatory resistance in U.S. rices. He also collaborated with scientists at Purdue University in the first successful separation of blast fungus pathotypes by DNA fingerprinting. He has authored and coauthored some 150 scientific articles and presentations, and has presented papers and seminars on his blast research in the Philippines, Japan, Indonesia, France, and Egypt.

PLENARY SESSIONS

Thursday, August 12, 2010, 9:00-12:30 p.m.

ABSTRACTS

Perspectives on Rice Blast, Research and the Broader Issues of Food Security and Evolving  
Production Practices

**Zeigler, R.S.**

International Rice Research Institute, Los Baños, PHILIPPINES

Rice is, and will continue to be for the foreseeable future, the major staple cereal in developing countries. Around half the world's population eat rice every day and about 70% of the world's poor depend on rice as their major source of food energy. Anything that threatens global rice supplies can have major impact on the wellbeing of hundreds of millions of people. Local supply disruptions, such as those caused by a serious blast epidemic near flowering, can be equally devastating as most populations depend on locally sourced rice. Most rice production is concentrated in Asia but demand growth is particularly strong in Sub – Saharan Africa. The world will have to produce around 8 – 10 M tons more rice *each year* to meet future demand. Assuming only modest area increase, the required yield growth rate far exceeds that realized across Asia for the past decade.

Current rice production across most of Asia is both water – and labor – intensive. Thus it is under pressure as both are rapidly becoming scarcer. Production systems will likely rapidly evolve and adopt practices that use less water and less labor. These will include dry direct seeding and intermittent irrigation (to upland-like conditions) which will create conditions far more conducive to the development of blast outbreaks than current transplanted, continuously flooded systems. Stands of direct seeded rice, such as is common in Latin America, are typically much denser than transplanted rice and provide a microclimate more favorable for the development of blast epidemics. Farmers also tend to apply more nitrogen in the hopes of improving rice competition with weeds. Intermittent irrigation also creates conditions more suitable for blast to develop and results in more soil nitrate which has been shown to favor blast development.

Achieving needed yield growth rates under these conditions will require that future rice varieties have higher levels of broad spectrum blast resistance. While research continues on the biology of rice blast pathogen and has contributed to improved understanding of the nature of the disease, has this been translated into tools of use to breeders trying to develop more reliable blast resistance? Have farmers been empowered to better manage the problem than they could 20 years ago, when a major resurgence in research on the pathogen and the disease began? The answer to these questions is likely that after some initial successes, there has been little progress in the past decade. A major outcome of this conference should be a roadmap to developing tools and approaches that can be integrated by scientists and farmers to face what will most likely be a serious challenge from the blast pathogen ten to fifteen years from now.

Next Generation Biology: Novel Insights into Pathogenicity of the Rice Blast Fungus,  
*Magnaporthe oryzae*

Gowda, M.<sup>1,2</sup>, Oh, Y.<sup>1</sup>, Nunes, C.<sup>1</sup>, Sailsbery, J.<sup>1</sup>, Xue, M.<sup>1,3</sup>, Chen, F.<sup>4</sup>, Nelson, C.<sup>5</sup>, Brown, D.E.<sup>1</sup>, Meng, S.<sup>1</sup>, Mitchell, T.<sup>6</sup>, Hagedorn, C.H.<sup>5</sup> and **Dean, R.A.**<sup>1</sup>

<sup>1</sup>Fungal Genomics Laboratory, Center for Integrated Fungal Research, North Carolina State University, Raleigh, North Carolina, USA

<sup>2</sup>Plant Biology, Michigan State University, East Lansing Michigan, USA

<sup>3</sup>Department of Plant Pathology, China Agricultural University, Beijing, CHINA

<sup>4</sup>US DOE Joint Genome Institute, Walnut Creek, California, USA

<sup>5</sup>University of Utah School of Medicine & Huntsman Cancer Institute, Salt Lake City, Utah, USA

<sup>6</sup>Department of Plant Pathology, The Ohio State University, Columbus Ohio, USA

My research explores the biology of the rice blast, the causal agent of rice blast disease. Following the completion of the genome sequence of *Magnaporthe oryzae*, attention has focused on the transcriptional, including non-coding RNA, and post-transcriptional regulation of the infection process. Through analyses of small RNA, a novel class of small 5' methylguanosine capped and 3' polyadenylated RNAs (SCARs) were discovered that primarily map to transcription initiation and termination sites of protein coding genes. SCARs were positively correlated with gene expression, particularly for highly expressed genes including those encoding ribosomal proteins and genes involved in mycelial development. Further analyses of small RNA from infection cells and mycelia revealed evidence for siRNAs. SiRNAs mapping to repetitive elements were highly abundant in mycelia compared to infection cells. These data suggest small RNAs play an active role in regulating fungal growth and development. Whole genome microarray analysis of appressorium formation revealed a core set of differential genes, which included numerous genes involved in protein turnover and amino acid catabolism. Functional analyses showed that protein catabolism, including endo-proteases and key enzymes involved in shuttling carbon back into the Krebs cycle, is critical for successful host infection. Other research efforts in my laboratory are currently focused on examination of transcriptional networks using protein arrays and protein-protein interactions to define the circuitry regulating the infection process.

## Wading Through Murky Paddies: Clarifying Broad Spectrum Resistance in Rice

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Incorporation of quantitative trait loci (QTL) that control disease resistance into elite germplasm could help reduce crop losses, and may even confer broad-spectrum and durable resistance. Applied breeding programs, however, have not enthusiastically pursued QTL for crop improvement. A key reason for this lack of adoption is that reliable markers for accumulation of the QTL are not readily available because the genes that contribute to the quantitative trait are not known. Identifying these genes that function in QTL phenotypes has proven difficult partly because of the imprecision of QTL mapping and because the effects are small and can vary with environment. In rice (*Oryza sativa*), the availability of a high quality genome sequence and the ability to associate several types of phenotypic data to the physical map, is allowing new approaches to link complex phenotypes to genomic regions and even genes. As a proof of concept, we combined numerous sequence and expression datasets of the large multi-member rice germin protein family that is known to include genes involved with broad-spectrum disease resistance. By examining phylogenetic relationships and functional diversity of germins across diverse genera, including monocots and dicots, we predicted candidate germin gene lineages with possible relevance to disease resistance across taxa. The strategy to use bioinformatics to layer data types, detect candidates and connect them across plant species will become more powerful as an increasing number of crop genomes are sequenced and gene functions are determined.

Biotrophic Invasion of Rice Cells by *Magnaporthe oryzae*: Where We Go From Here

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While rice blast continues as a major constraint to rice production, wheat blast, caused by the wheat-adapted strains of *Magnaporthe oryzae*, now looms as a threat to global wheat production. Understanding the cellular and molecular biology of the early biotrophic invasion stages of blast disease remains critical. Recent progress has resulted from live cell imaging of fungus invading rice leaf sheath cells. *M. oryzae* sequentially invades living rice cells using specialized intracellular invasive hyphae (IH) that are enclosed in host-derived extracellular hyphal membrane. Blast IH specifically express numerous biotrophy-associated-secreted (Bas) proteins, including known effectors Avr-Pita1, Pwl1, and Pwl2. When secreted *in planta*, fluorescently-labelled effectors and many BAS proteins accumulate in a highly-localized pathogen-induced structure, the biotrophic interfacial complex (BIC). Fluorescent Pwl2 and diverse Bas proteins are translocated into the cytoplasm of invaded rice cells, and they subsequently move into uninvaded neighbor cells. During biotrophic invasion, the fungus appears to manipulate host plasmodesmata for its own cell-to-cell movement, and for sending effectors into neighboring cells, presumably to prepare these host cells before invasion. This talk will focus on our current understanding of biotrophic invasion in the context of the major challenges for future research.

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- Khang, C.H., Berruyer, R., Giraldo, M.C., Kankanala, P., Park, S-Y, Czymmek, K., Kang, S. and Valent, B. 2010. Translocation of *Magnaporthe oryzae* effectors into rice cells and their subsequent cell-to-cell movement. *Plant Cell* 22: 1388-1403.

Plants and Pathogens Engage in Trench Warfare-Knowledge Learned from Natural Variation of the Rice Blast Resistance Gene *Pi-ta* and the Corresponding Avirulence Gene *AVR-Pita1*

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Plants and pathogens have existed together and coevolved for millions of years. As a result of this relationship, sophisticated, multifaceted recognition and resistance mechanisms have evolved in plants in order to prevent or reduce infection by pathogens. This selection process may drive the evolution of pathogen genes to counteract plant defenses and promote more disease. Rice blast disease is one of the most destructive plant diseases that threatens stable rice production in the U.S. and worldwide. For several decades, the *Pi-ta* gene in rice has been deployed which confers resistance to races of the blast fungus containing the corresponding avirulence gene *AVR-Pita1*. How *Pi-ta* has evolved to cope with *AVR-Pita1* has been the subject of our investigation at the USDA-ARS Dale Bumpers National Rice Research Center.

*Pi-ta* is located near the centromere of a rice chromosome, a region that is relatively stable. *Pi-ta* encodes a predicted cytoplasmic protein with nucleotide binding sites and leucine rich repeats (NBS-LRR) that resembles most cloned plant resistance gene products. Our survey of rice germplasm revealed that there is only one resistant *Pi-ta* allele, however, it produces 12 proteins each capable of preventing infection of up to 10 races of the blast fungus. Moreover, the fungus is known to have its own Houdini-like talents to attack the host plant. Not only is the *AVR-Pita1* gene located near the telomere, an unstable region of blast fungus, but also the *AVR-Pita1* protein has been shown to be frequently altered in field blast populations. Taken together, these findings suggest that *Pi-ta* engages in trench warfare with *AVR-Pita1* with both organisms maintaining an array of strategies to cause/prevent disease.

Trench warfare hypothesis of *Pi-ta/AVR-Pita1* needs to be further tested. However, it could have a significant impact on crop protection since gene-for-gene interactions have been observed in plant responses to numerous destructive bacterial, viral, fungal pathogens and insects.

## ABSTRACTS - ORAL PRESENTATIONS

- I. Genomics and Functional Genomics of the Pathogen
- II. Host Resistance, Signaling and Defense Responses
- III. Molecular and Cellular Biology, Diversity, Evolution and Adaptation of the Pathogen
- IV. Strategies for Managing Diseases Caused by *Magnaporthe species*

O## = Oral Presentation

CO## = PostDoc and Student Competition, Oral Presentation

Presentations in competition:

I-CO-5

I-CO-75

II-CO-84

II-CO-85

III-CO-90

Population Genomics Approaches to Understand *Magnaporthe*-Rice Interactions

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To subvert host defense, *M. oryzae* is thought to secrete a battery of effector molecules, a subset of which have avirulence (AVR) activity, i.e. they are recognized by host resistance (R) proteins resulting in rapid and effective activation of innate immunity. With the objective of isolating novel avirulence (AVR) genes from *M. oryzae* by association studies, we carried out a large-scale DNA polymorphism study of secreted protein genes predicted from the genome sequences of isolates 70-15 and Ina168. Remarkably, a total of 1.68-Mb regions, comprising 316 candidate effector genes, were present in Ina168 but absent in the assembled sequence of isolate 70-15. Association analyses of these 316 genes revealed three novel AVR genes, *AVR-Pia*, *AVR-Pii* and *AVR-Pik/km/kp*, whose products are recognized inside rice cells possessing the cognate R-genes. *AVR-Pia* and *AVR-Pii* have evolved by gene gain/loss processes, whereas *AVR-Pik/km/kp* has evolved by nucleotide substitutions as well as gene gain/loss (Yoshida et al. 2009). We are beginning the process to identify host target molecules and to elucidate trafficking mechanisms of the AVRs. Association study was also applied to rice to identify candidate *Pia* R-genes. A large-scale rice mutant screen combined with protoplast complementation assay revealed that a *Pia* R-gene requires two ORFs coding for NBS-LRR type proteins. The whole genome DNA polymorphisms study coined as “population genomics” will be a powerful approach to understand *Magnaporthe*-rice interactions and pathogen-host interactions in general.

## Reference:

Yoshida, K., Saitoh, H., Fujisawa, S., Kanzaki, H., Matsumura, H., Yoshida, K., Tosa, Y., Chuma, I., Takano, Y., Win, J., Kamoun, S. and Terauchi, R. 2009. Rice blast association genetics reveals three novel avirulence genes, *AVR-Pia*, *AVR-Pii* and *AVR-Pik/km/kp*, whose products are recognized inside rice cells. *Plant Cell* 21:1573.

Novel Splicing Factors Control Infection-related Morphogenesis in *Magnaporthe oryzae*

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Recently, the authors identified *COM1* and *MgCON2* as two pathogenicity-related genes in *Magnaporthe oryzae*. *COM1* encodes a novel nuclear protein and is essential for normal conidium morphology and full virulence. *MgCON2* also encodes a novel nuclear protein and is required for conidiogenesis, vegetative hyphal growth and pathogenicity.

In order to investigate molecular functions, the *Com1*-interacting proteins were pulled down and subjected to MS analysis. From 4 independent pull-down experiments, 49 putative nuclear localized proteins were identified, of which 25 proteins were homologous to components of the RNA spliceosome. To further verify the interaction between these proteins and *Com1*, 7 of them were selected for yeast-two-hybrid assays. The assay confirmed that 3 Sm snRNP proteins were directly associated with *Com1*, and that the C-terminal region of *Com1* was essential for the interactions. These results indicate that *Com1* is a novel component of the RNA spliceosome. The pull-down technology was also used to isolate *MgCon2*-interacting proteins, and 46 putative nuclear proteins were identified in 3 independent experiments. For 27 of them, their orthologs function as pre-mRNA splicing factors in yeast. Yeast-two-hybrid assays confirmed that at least 3 core components of the RNA spliceosome, *MgCwc2*, *MgCwf4*, and *MgPrp17*, were directly associated with *MgCon2*. Since the orthologs of these 3 proteins are components of the activated spliceosome complex C in yeast and human cells, it is supposed that *MgCon2* is a novel component of the same complex. To determine the function of *MgCon2* in mRNA processing, the transcriptomes of the wild type and the  $\Delta mgcon2$  null mutant were generated. In comparison with the wild type, introns in transcripts of over 420 genes were not fully spliced in the  $\Delta mgcon2$  null mutant, including *CON7*, *ICL1*, *MoHOX4*, and *MoATG9*, which are known to play important roles in pathogenicity and conidiogenesis. In addition, the authors also identified a novel virulence gene, *MoPPF3*, whose deletion resulted in less penetration. Intron of its transcripts was also not completely spliced in the  $\Delta mgcon2$  null mutant. These data indicate that *MgCon2* controls the efficiency of intron-splicing and that decreased splicing efficiency of *CON7*, *ICL1*, *MoHOX4*, *MoATG9* and *MoPPF3* is a key mechanism responsible for the defects of the  $\Delta mgcon2$  null mutant.

Above all, the present studies provide strong evidences that RNA splicing factors play important roles in infection-related morphogenesis in *M. oryzae*.

Alpha-1,3-glucan Functions as a Stealth Gear During Infection in *Magnaporthe oryzae*

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Oligosaccharides derived from cell wall of fungal pathogens evoke primary immune responses in host mammalian and plant cells. Upon activation of the primary immune systems, plants secrete various enzymes that hydrolyze fungal cell walls, e.g. chitinase and beta-glucanases, as a defense mechanism.

To understand fungal strategies circumventing the host plant immune responses, we investigated localization of major cell wall polysaccharides of the rice blast fungus *Magnaporthe grisea* during rice infection using fluorescent labels. Our cytological studies showed that major polysaccharides at accessible surface of infectious hyphae were alpha-1,3-glucan and chitosan; beta-1,3-glucan and chitin became detectable after enzymatic digestion of alpha-1,3-glucan. Immunoelectron microscopic observation of infectious hyphae revealed that alpha-1,3-glucan was localized more outwardly in the cell wall compared to beta-1,3-glucan. The accumulated alpha-1,3-glucan interfered with the digestion of chitin in the cell wall by chitinase. Taken together, alpha-1,3-glucan physically and functionally masks other cell wall polysaccharides in the infectious hyphae. Since genes encoding alpha-1,3-glucanase are not found in the rice genome, alpha-1,3-glucan is suggested to protect the fungal cell wall from digestive enzymes produced by plant cells during infection. In support of this, a deletion mutant in the alpha-1,3-glucan synthase was non-pathogenic to rice plants. Moreover, a cutin monomer 1,16-hexadecanediol activated Mps1 MAP kinase that led to accumulation of alpha-1,3-glucan on the fungal cell wall.

Our results demonstrate that *M. grisea* masks surface of the cell wall with alpha-1,3-glucan in response to a plant cue to circumvent the innate immunity attack of the host rice.

Reference:

Fujikawa, T., Kuga, Y., Yano, S., Yoshimi, A., Tachiki, T., Abe, K., and Nishimura, M. 2009. Dynamics of cell wall components of *Magnaporthe grisea* during infectious structure development. *Molecular Microbiology* 73: 553-570.

## A Novel NADPH-Dependent Genetic Switch Regulates Infection by the Rice Blast Fungus

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To cause rice blast disease, *Magnaporthe oryzae* has distinct morphogenetic stages that allow it to breach the surface of the host leaf and invade the plant tissue. How the fungus monitors the transition from the nutrient-free surface to the nutrient-rich interior of the leaf, and what controls the genetic reprogramming necessary to produce infectious hyphae, is not understood. Shedding light on this process, recent work (Wilson, et al. submitted) has shown that trehalose-6-phosphate synthase (Tps1) monitors the nutritional status of the cell and regulates fungal virulence in *M. oryzae* via a novel NADP(H)-dependent genetic switch. Tps1 is required for production of NADPH from glucose-6-phosphate in the oxidative pentose phosphate pathway (Wilson et al. 2007), but also directly binds to NADPH and is essential for fungal pathogenicity<sup>1</sup>. The activity of Tps1 is regulated by a transcriptional co-repressor complex, comprising three Nmr proteins predicted to bind NADP (Lamb et al. 2003), and controls the expression of a set of virulence-associated genes, including the known virulence factors *ALB1* and *MPG1* and at least 10 genes encoding NADPH-requiring enzymes. Therefore, the initiation of rice blast disease requires a novel regulatory mechanism involving an NADPH sensor protein (Tps1), an NADP-dependent transcriptional co-repressor complex and, uniquely, the non-consuming inter-conversion of NADPH and NADP acting as signal transducer.

NADPH is the dominant electron donor for anabolic reactions such as reductive biosynthesis, the generation of reactive oxygen species (ROS), and the protection of cells from oxidative damage, but a role for NADPH in cellular signalling has not been previously described. Here, using high-throughput gene deletion techniques to target processes involved in NADP(H) metabolism, we explore the dynamics of the NADPH-dependent genetic switch to understand how NADPH production and depletion is balanced in the cell, how crucial NADPH-requiring processes are regulated by the availability of NADPH, and how these processes impact the ability of the fungus to cause disease.

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Functional Study of the *Hyr1* Gene, Potentially Involved in Detoxification of Plant-Defense-Generated Reactive Oxygen Species in the Rice Blast Pathogen

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During plant-pathogen interactions, plants may mount several types of defense responses to either block the pathogen completely or ameliorate the amount of disease. Such responses include release of reactive oxygen species (ROS), as well as formation of cell wall appositions (CWA). A successful pathogen will likely have its own ROS detoxification mechanisms to cope with this inhospitable environment. We are studying one such candidate mechanism in the rice blast fungus, *Magnaporthe oryzae*, governed by a gene called *Hyr1*. This gene (MGG\_07460) encodes a protein containing a glutathione peroxidase (GSHPx) domain, and its homologue in yeast was reported to be a glutathione-dependent phospholipid peroxidase (PhGpx) that specifically detoxifies phospholipid peroxides (Delaunay et al., 2002). We have successfully generated a deletion mutant ( $\Delta$ *hyr1*) and characterized it in *M. oryzae*. The  $\Delta$ *hyr1* mutants showed increasing levels of growth inhibition when grown in increasing amounts of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). We also observed that the knockout mutants are less able to break down ROS *in planta*, including ROS found around CWA, and they cause significantly smaller lesion sizes on both barley and rice. Gene expression studies using quantitative realtime RT-PCR suggest that this gene regulates expression of genes involved with ROS cleavage. Thus far, we conclude that *HYR1* is important for allowing the fungus to tolerate H<sub>2</sub>O<sub>2</sub> both *in vitro* and *in planta*, and this may be directly related to pathogenicity.

Reference:

Delaunay, A., D. Pflieger, et al. (2002). "A thiol peroxidase is an H<sub>2</sub>O<sub>2</sub> receptor and redox-transducer in gene activation." *Cell* 111(4): 471-81.

## Links Between Post-Transcriptional Regulatory Mechanisms and Pathogenicity in the Rice Blast Fungus

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We are investigating the involvement of post-transcriptional mechanisms that regulate *Magnaporthe oryzae* pathogenesis. RNA-binding proteins play a major role during all steps of RNA processing. They associate with non-coding RNAs (ncRNAs) and messenger RNAs (mRNAs) to form RNA-protein complexes (ribonucleoprotein complexes or RNPs). RNPs are highly dynamic complexes and proteins may dissociate from the RNP particle and others incorporate later to develop distinct RNPs during RNA life cycle (Anderson and Kedersha, 2009).

The *M. oryzae* T-DNA mutant M35 was identified as being defective in plant colonization. M35 has undergone insertional inactivation of a gene encoding a fungal-specific RNA-binding protein (RBP35). The *M. oryzae* RBP35 contains two protein modules, a RRM domain and six RGG boxes. A His-tagged version of RBP35 binds specifically poly(G)<sub>30</sub> RNA homopolymers and not ssDNA or dsDNA. RBP35-cherry protein fusion constructs show a steady-state nuclear localisation. Fluorescence recovery after photobleaching (FRAP) experiments revealed that RBP35 forms different protein complexes in the nucleus of appressoria and conidia, suggesting that RBP35 play a distinct role during appressorium development. A transcriptomics and proteomics comparison between the  $\Delta rbp35$  mutant and the wild-type strain showed that several enzymes required for flavonoid and melanin synthesis were up- and down-regulated in the  $\Delta rbp35$  mutant. Interestingly, a protein variant of RBP35 lacking the arginine aminoacids within the six RGG boxes accumulates in cytoplasmic granules located near the fungal cell wall, possibly due to the involvement of RBP35 with mRNAs related to melanin synthesis. Other evidence of the link between post-transcriptional regulation and pathogenicity derives from the requirement of the karyopherin EXP5 for *M. oryzae* full virulence (Tucker et al., 2010), suggesting that ncRNAs transported by this nucleocytoplasmic receptor may have an important role during fungal plant infection.

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*Magnaporthe* Pathogenesis: Efflux, Effectors and Entry into the Host

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ATP-binding cassette (ABC) transporters are able to couple hydrolysis of ATP to the transport of a variety of substrates either into or out of the cells. Our previous observations (Sun et al. 2006) suggest that the reduced viability of the *abc3Δ* appressoria could be due to the excessive accumulation of a cytotoxic metabolite(s), presumably the efflux target of *Abc3* transporter in *Magnaporthe*. Here, we designed a novel yeast-based assay to ascertain and guide the purification of *Abc3*-Transporter Substrate (ATS) from the *abc3Δ* appressoria. ATS was identified by LC-APCI-MS as a Digoxin-like steroidal glycoside and showed cytotoxic activity against several yeasts and fungi. Further co-immunoprecipitation and gene-deletion studies suggest that ATS targets a Translation Elongation Factor EF1- $\alpha$  (Tef2) in fission yeast. Exogenously applied ATS specifically increased the sensitivity of WT *Magnaporthe* to Na<sup>+</sup> and Ca<sup>+2</sup> ions, likely through perturbed ion homeostasis during appressorial development. However, when applied in excess to *Magnaporthe*, ATS significantly blocked appressorial function of breaching the host surface, and induced Hypersensitive Response in rice leaf tissue. We propose that ATS plays a key role in appressorial function and is likely a novel effector during establishment of rice blast disease.

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Sun C.B., Suresh A., Deng Y.Z. and Naqvi N.I. 2006. A multidrug resistance transporter in *Magnaporthe* is required for host penetration and for survival during oxidative stress. *Plant Cell*, 18: 3686-3705.

## Multiple Plant Surface Signals are Sensed by Different Mechanisms the Rice Blast Fungus for Appressorium Formation

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Surface recognition and penetration is one of the most critical plant infection processes in foliar pathogens. In *Magnaporthe oryzae*, the *Pmk1* MAP kinase regulates appressorium formation and plant penetration. Its orthologs also are known to be required for various plant infection processes in other phytopathogenic fungi. Although a number of upstream components of this important pathway have been characterized, the upstream sensor genes have not been identified. *Pmk1* is orthologous to yeast *Kss1*, which plays a key role in regulating filamentous growth. In yeast, *Msb2* (a signaling mucin) and *Sho1* are involved in *Kss1*-mediated filamentous growth pathway. Because the conserved nature of the *Pmk1* and *Kss1* MAPK cascades and reduces expression of the *MSB2* ortholog in the *pmk1* and *mst12* mutants, in this study we identified and characterized the *MoSHO1* and *MoMSB2* genes. While the *Moshol* mutant was only reduced slightly, the *Momsb2* mutant was significantly reduced in appressorium formation and virulence. The *Moshol Momsb2* double mutant rarely formed appressoria on artificial surfaces, had a reduced *Pmk1* phosphorylation level, and was nonresponsive to exogenous cAMP and cutin monomers. However, it still formed melanized appressoria on plant surfaces and caused a few small lesions on rice leaves. On artificial hydrophilic surfaces, leaf surface waxes and two long chain primary alcohols, but not paraffin waxes and two alkanes, stimulated appressorium formation in the *Momsb2 Moshol* double mutant but more efficiently in the *Momsb2* single mutant. Furthermore, melanized appressoria were formed on hydrophilic surfaces by transformants of the *Momsb2* mutant expressing a dominant active *MST7* allele. These results indicate that *MoMsb2* and *MoSho1* may have overlapping functions in the activation of the *Pmk1* cascade. While *MoMsb2* plays a critical role in the recognition of surface hydrophobicity and cutin monomers, primary alcohols, a major component of epicuticular leaf waxes in grasses, are likely recognized by *MoSho1* and other unknown sensor genes as chemical signals at the plant surface to trigger appressorium formation in *M. oryzae*.

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Functional Characterization of *Magnaporthe oryzae*: Putative Effectors in the Infective Process of Rice

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Using a protoplast transient expression system, genes *MGG00194* and *MGG03356* were identified as putative effectors of host defense. We worked to determine *in planta* secretion patterns and functionally characterize each by over expression and mutational analysis. Each gene was fused to GFP and controlled by its native promoter or the constitutive ribosomal promoter and transformed into line 70-15. Successful colonization of the host by this strain was obtained only using barley. *MGG\_00194::GFP* showed an accumulation in the blast interfacial complex (BIC) and the hyphal tips as they moved across cell walls during colonization of neighboring cells. *MGG\_03356::GFP* did not show an evident fluorescent localization pattern.

Transformants obtained from strains KJ201, PO6-6, CHNOS and Guy11 were used for rice infection. KJ201 *MGG\_03356::GFP* under the constitutive promoter showed a significant difference in lesion area values in a whole-plant pathogenicity assay on *Pi-2* resistant plants. This over expression strain caused lesions in *Pi-2* plants similar to those produced by wild type KJ201 on the susceptible Nipponbare plants. Breaking of *Pi-2* resistance was observed in all 3 independent mutants and 3 biological replications. KJ201 was not able to cause lesions or provoke small necrotic spots in *Pi-2*. The *MGG\_00194::GFP* mutants also showed a significant increased value on lesion area in *Pi-2*-plants (bigger specks and lesions), but not to the extent of *MGG\_03356::GFP*. No lesions were obtained by either constitutive of wild type such as resistant *Pi-9* plants.

Cytological evaluations were obtained using sheath assay. KJ201 and *MGG\_03356::GFP* constitutive mutants were able to infect Nipponbare plants as determined by healthy globulous invasive hyphae growing profusely before moving to the next cell. KJ201 in *Pi-2* plants showed an intense autofluorescence reaction surrounding appressorium, neighboring cells, and infected host cells accompanied by cytoplasmic granulation across the host cells with little or no fungal growth. However for the *MGG\_03356::GFP* constitutive mutant on *Pi-2*, granulation was limited to immediately under the appressorium. Invasive hyphae showed high growth across the cells without a well defined necrosis in the initially infected cell. Later stages in infection (60 hpi) showed extensive vertical growth and proliferation. *MGG\_00194::GFP* under the constitutive promoter did not show any visible difference in growth compared with the wild type. No growth or autofluorescent surrounding appressorium were visible. Reduced growth accompanied by an intense vesiculation was observed in *MGG\_03356::GFP*. Both proteins fused to GFP and under the native promoter did not show a distinguishable signal from autofluorescent during sheath assay. New Guy11 and KJ201 native promoter GFP fusion mutants using red fluorescent protein (RFP) now are available and will be used for the sheath assay. No knockout mutants using 70-15 and KJ201 were obtained. A new knockout cassette is being developed for transformation of KJ201 and CHNOS protoplasts.

The Biotrophic Interfacial Complex and Trafficking of *Magnaporthe oryzae* Effectors Inside Rice Cells

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*Magnaporthe oryzae* causes the globally devastating blast disease on rice (*Oryza sativa*) and other crops. As with other biotrophic pathogens, *M. oryzae* delivers effectors into host cells to facilitate disease development, but some also trigger the host resistance gene-mediated hypersensitive response. However, only a few blast effectors have been identified and nothing is known about how effectors enter host cells. Understanding the mechanism of effector delivery into host cells will provide new targets for controlling blast disease and new strategies for identifying new effectors that involve the same delivery mechanism.

To investigate secretion and translocation of blast effectors into rice cells, we used live-cell imaging of fungal transformants that expressed fluorescently tagged effectors (*AVR-Pita1*, *PWL1*, and *PWL2*) during invasion of rice cells. We identified a highly localized structure, the biotrophic interfacial complex (BIC), which accumulates effectors secreted by invasive hyphae (IH). Effectors were first secreted into BICs at the tips of the initially filamentous hyphae in each newly entered rice cell. These tip BICs were left behind beside the first-differentiated bulbous IH cells as the fungus continued to colonize the host cell. Fluorescence recovery after photobleaching experiments showed that the fluorescently tagged *PWL2* continued to accumulate in BICs after IH were growing elsewhere. Two BIC-targeted proteins, *PWL2* and *BAS1* (for biotrophy-associated secreted protein 1), but not a non-BIC-targeted protein *BAS4*, were translocated into the rice cytoplasm. Preliminary data indicates that both the effector promoter and signal peptide play a role in effector secretion into BICs and subsequent translocation to the rice cytoplasm. Our working hypothesis is that BICs mediate effector delivery into host cells. Interestingly, the translocated *PWL2* and *BAS1* moved into uninvaded neighbor cells, presumably preparing host cells before invasion. A robust *in planta* translocation assay is now available to facilitate studies of translocation mechanisms and identification of a blast effector translocation motif.

Reference:

Khang, C.H., Berruyer, R., Giraldo, M.C., Kankanala, P., Park, S-Y, Czymmek, K., Kang, S. and Valent, B. 2010. Translocation of *Magnaporthe oryzae* effectors into rice cells and their subsequent cell-to-cell movement. *Plant Cell* 22: 1388-1403.

## CFGP 2.0: An Integrated Web-based Environment for Comparative and Evolutionary Genomics in Fungal Kingdom

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Since the whole genome of *Saccharomyces cerevisiae* was sequenced in 1996, more than 250 fungal genomes were sequenced and more genomes will be sequenced with the aid of next generation sequencing technologies. To support various comparative and evolutionary analyses based on these sequences, Comparative Fungal Genomics Platform 2.0 (CFGP; <http://cfgp.snu.ac.kr/>) has been developed. The CFGP 2.0 now archives 254 fungal genomes from 127 species in a standardized form and provides 26 bioinformatics tools including BLAST, ClustalW, and BLASTMatrix which draws graphical distribution of homologous genes along with taxonomy. CFGP 2.0 also archives 126 non-fungal genomes covering from bacteria to human as well as SwissProt/UniProt and MSIP sequences to support comparative analyses across kingdoms. SNU Genome Browser (SNUGB; <http://genomebrowser.snu.ac.kr/>) was fully implemented inside the CFGP 2.0 for quick access of genomic contexts.

Favorite, a personalized virtual space, serves the 26 bioinformatics tools based on the Data-driven User Interface (DUI). The DUI provides a simple way for diverse analyses instead of endless copy and pastes: i) select a Favorite, ii) choose the sequences, iii) click the 'add to Cart' button, and iv) execute bioinformatics tools. This process will increase efficiency and decrease errors during analysis.

Based on the data warehouse of the CFGP 2.0, several databases in various areas have been constructed: i) *Agrobacterium tumefaciens*-mediated transformation database (ATMT; <http://atmt.snu.ac.kr/>), ii) T-DNA Analysis Platform (TAP; <http://tdna.snu.ac.kr/>), iii) Fungal Transcription Factor Database (FTFD; <http://ftfd.snu.ac.kr/>), iv) Fungal Cytochrome P450 Database (<http://p450.riceblast.snu.ac.kr/>), v) Phytophthora Database (PD; <http://www.phytophthoradb.org/>), vi) Fungal Secretome Database (FSD; <http://fsd.snu.ac.kr/>), and vii) Fungal EST Database (<http://fedb.snu.ac.kr/>). With these interconnected databases, the CFGP 2.0 would be a web-based platform for further analyses toward systems biology in the fungal kingdom.

Functional and Evolutionary Characterization of Rice Blast Resistance Alleles at the *Pik* LocusZhai, C., Yuan, B., Dong, Z., Zeng, X., Lin, F., Wang, L. and **Pan, Q.**

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The *Pik* locus, located on rice chromosome 11, which consists of at least five alleles, i.e., *Pik*, *Pik-s*, *Pik-p*, *Pik-m*, and *Pik-h*, is one of the most important resistance gene resources for improving rice blast resistance worldwide. Resistance spectra of these alleles were characterized with 8 populations (464 isolates) of *Magnaporthe oryzae* collected from various regions in China. The results showed that all alleles expressed higher resistance in the south part of China except for *Pik-s*. Dynamics of resistance of these alleles was characterized with 11 populations (725 isolates) of *Magnaporthe oryzae* collected from Guangdong province, China. The results showed that the alleles again expressed higher and stable resistance in the province except for *Pik-s* and *Pik*. For molecular characterization of the alleles, the genetic and physical maps of these alleles, respectively, were constructed using genomic position-ready markers. Then, the alleles were isolated, respectively, through an approach called map-based cloning, *in silico*. The function of each allele was dissected using both forward and reverse genetic approaches. The evolutionary relationships among alleles were determined by the allele-specific functional nucleotide polymorphisms (FNPs) using a large range of rice germplasms. The detailed results will be presented in the meeting.

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Characterization of Rice Lesion Mimic Mutants of 93-11 for a Better Understanding of General Host Defense Response to Both Rice Blast and Sheath Blight Diseases

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Rice lesion mimic mutants (LMM) exhibit necrotic lesions resembling programmed cell death (PCD). PCD is one of the significant hallmarks of disease resistance genes mediated defense responses. LMM can be used to study the mechanisms of plant disease resistance.

In the present study, a total of 133 rice lesion-mimic mutants of rice restorer line 93-11, induced by Co<sup>60</sup>-irradiations, were identified for further characterization. The lesions of the mutants were first observed at the five- to six-leaf stage without any treatment in the field, and all of the lesions can transfer to next generation. Among 133 mutants five distinct phenotypes of LMM were observed. Genetic analysis indicated that each of these five phenotypes was conditioned by a single recessive gene, respectively. Sequencing analysis of the cloned lesion mimic genes between 93-11 and mutants indicated that those five phenotypes were controlled by novel genes. Bacterial blight isolate IV and blast isolates ZF1 and ZG1 were used to perform standard pathogenicity assay. Results of pathogenicity tests demonstrated that these mutants exhibited significantly enhanced resistance to both rice blast and bacterial blight diseases. These findings suggest that the lesion mimic mutation in rice may be involved in general disease resistance.

Progress on mutant characterization for enhanced disease resistance and genetic mapping and cloning will be presented.

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A Nep1-like Protein Toxin from *Magnaporthe oryzae* Interacts with a Conserved, Ubiquitin-like Rice Protein and Elicits Plant Cell Death

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The *Magnaporthe oryzae* genome encodes over a thousand of secreted proteins, which are likely critical for the fungal pathogenesis in rice plants. Among them is a small family of 25-34 kD secreted proteins that share sequence homology with the necrosis and ethylene-inducing peptide1 (Nep1)-like proteins (NLPs). NLPs are conserved in a diverse array of microorganisms including bacteria, fungi and oomycetes and were previously shown to elicit necrotic cell death in dicotyledonous plants. In this study, we have characterized a family of four *M. oryzae* NLPs (*MoNLP1-4*), demonstrated their ability to elicit necrotic cell death in both dicot and monocot plants, and identified a conserved, ubiquitin-like rice protein that interacts with *MoNLP1*.

Among the four NLPs from *M. oryzae*, *MoNLP1* and *MoNLP2* contain two conserved cysteine residues and share a high sequence identity (51%) to Nep1 from *Fusarium oxysporum*. By contrast, both *MoNLP3* and *MoNLP4* contain four conserved cysteine residues. The *Agrobacterium*-mediated transient expression demonstrated that *MoNLP1* and *MoNLP2* were capable of inducing necrotic cell death in tobacco leaves at 30-48 hours post-inoculation whereas *MoNLP3* induced cell death within 60-72 hours. However, the transient expression of *MoNLP4* in tobacco leaves did not elicit cell death even after three to seven days. When introduced into rice protoplasts, the *MoNLP1*, *MoNLP2* and *MoNLP3* genes caused significant cell death, as evidenced by reduction in the GUS activity. In addition, *MoNLP1* purified from *E. coli* was able to rapidly induce necrotic cell death on tobacco, rice, maize and sorghum leaves within 18 hours. Application of purified *MoNLP1* protein into rice seedlings via the root also resulted in the activation of pathogenesis-related protein (PR) genes such as *PR1* and *PR10*.

To better understand the mode of action and potential host cellular target(s) of microbial NLP toxins, *MoNLP1* was used as a bait to screen for interacting rice proteins by the yeast two-hybrid assay. Independent screenings and repeated confirmation tests led to the identification of OsNPI1 (*Oryza sativa* NLP Interactor1), a ubiquitin-like protein which is highly conserved in eukaryotes. Quantitative RT-PCR revealed that OsNPI1 is constitutively expressed in rice plants during the infection of *M. oryzae* and in response to purified *MoNLP1* treatment. Interestingly, knockout of the NPI1 orthologue in *Arabidopsis* T-DNA mutants led to retarded growth and lethal phenotype. Functional characterization of OsNPI1 at the protein level and analysis of suppression and overexpression transgenic rice lines should shed light on the role of OsNPI1 as a potential *MoNLP1* target which mediates necrotic cell death and disease development.

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Ottmann, C., Luberacki, B., Küfner, I., Koch, W., Brunner, F., Weyand, M., Mattinen, L., Pirhonen, M., Anderluh, G., Seitz, H. U., Nürnberger, T., and Oecking, C. 2009. A common toxin fold mediates microbial attack and plant defense. Proc. Natl. Acad. Sci. USA 106: 10359-10364.

New Insight for Two Major Rice Blast *R* Genes: *Pi-ta* and *Pi-km*Costanzo, S.<sup>1</sup>, Yang, Y.<sup>2</sup> and Jia, Y.<sup>1</sup><sup>1</sup>USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, Arkansas, USA<sup>2</sup>Plant Pathology Dept., Pennsylvania State University, University Park, Pennsylvania, USA

In rice breeding programs across the world, the introgression of major resistance (*R*) genes remains the most cost-effective method to control blast epidemics caused by the fungal pathogen *Magnaporthe oryzae*. During the last two years, we have examined two loci, on chromosome 12 and 11, which harbor previously characterized *R* genes *Pi-ta* and *Pi-km*, respectively.

At the *Pi-ta* locus, we studied the gene expression, and identified new alternative splicing events. A total of 12 transcript variants were predicted with open reading frames encoding 11 distinct putative products between 315 and 1033 amino acids. Among them, five preserved complete nucleotide binding sites-leucine rich repeats (NBS-LRR) domains and two couple the original NBS-LRR domain of the *Pi-ta* protein with a C-terminal thioredoxin domain. These post-transcriptional modifications of *Pi-ta* produce a series of transcript isoforms that could have a significant role in *R* gene regulation and/or may increase protein diversity. Next, we explored the sequence variation for this locus. Proximal to the *Pi-ta* promoter region we discovered a 3364 bp fragment encoding a predicted transposase consistently associated with the resistance phenotype. Additionally, a previously un-characterized NBS-LRR gene located 10 kb (3-prime) from the *Pi-ta* locus was cloned and transformed in a rice cv. Kitake. Preliminary inoculation tests suggest this gene may contribute to enhanced blast resistance.

At the *Pi-km* locus, we initially explored the existing sequence diversity for alleles of the two genes responsible for resistance specificity of this gene. The analysis of 15 rice cultivars revealed that the majority of nucleotide polymorphisms were only associated with the *Pi-km1* gene. Interestingly, the correspondent amino acid variation was localized within the predicted coiled-coil domain of the putative *Pi-km1* protein. In contrast, the sequence of *Pi-km2* alleles was highly conserved even within distantly related cultivars. Furthermore, disease reactions of the selected cultivars to five *M. oryzae* races, as well as their determined *Pi-km1* allele, showed a good correlation with the known *Pi-k* genes (*-k/-kh/-km/-ks/-kp*) historically reported for these cultivars. Based on these findings, specific primer sets have been designed to successfully discriminate among the various *Pi-km* alleles. The new information obtained from our studies has contributed to a better understanding of the gene-for-gene complex mechanisms regulating this plant-pathogen interaction as well as simplifying resistance breeding efforts by introducing more selective tools for marker assisted selection.

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Evolutionary Dynamics and Structure of the Rice Blast Resistance Locus *Pi-ta* in Wild, Cultivated and US Weedy Rice

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The *Pi-ta* gene in rice has been used to control the rice blast pathogen *Magnaporthe oryza* in rice growing areas worldwide for decades. To understand the evolutionary process and natural selection of *Pi-ta* during rice domestication, we first examined sequences of the genomic region of *Pi-ta* in geographically diverse rice accessions of AA genome *Oryza* species: *O. sativa*, *O. rufipogon*, *O. nivara*, *O. meridionalis*, *O. barthii*, *O. glaberrima*, and *O. glumaepatula*. A single amino acid change at position 918 of the *Pi-ta* protein known to determine resistance specificity was consistently found in *O. sativa* and *O. rufipogon*. A 3364 bp fragment encoding a predicted transposon was also consistently found in the proximity of the *Pi-ta* promoter region associated with the resistance phenotype. In *O. rufipogon*, patterns potentially consistent with recent directional selection were found in the *Pi-ta* region, while no significant deviation from neutral evolution was found in *O. sativa* groups. Results of sequence variation in flanking regions around *Pi-ta* in *O. sativa* suggest that the size of the resistance *Pi-ta* introgressed block was at least 5.4 Mb in all elite resistant cultivars, but not in the cultivars without *Pi-ta*, suggesting that the *Pi-ta* region has evolved under extensive selection pressure during crop breeding.

In a complementary study, in order to understand the evolutionary dynamics of the *Pi-ta* locus in US weedy rice, we analyzed nucleotide sequences and SSR markers at/around the *Pi-ta* genomic region in US weedy rice samples. The level of genetic diversity in the US weedy rice population was low compared with that in cultivated and wild rice groups. The same resistant *Pi-ta* in cultivated rice was found in the majority of US weedy rice. Phylogenetic relationships of *Pi-ta* in weedy red rice suggest that they are genetically closer to both Asian and US cultivated rice than to *O. rufipogon*. Evidence for direct gene flow of *Pi-ta* between US cultivars and US weedy rice was not found, but possible evidence of natural hybridization at the *Pi-ta* locus was observed between three weedy rice accessions (1025-01, 1214-02, and TX4) and three US cultivars (Edith, Carolina Gold, and Blue Rose). In addition, the *Pi-ta* genomic region (8 Mb) in the weedy rice accession 2002-2-pot 21 was identical to that of Lemont, Cypress, Delitus, Rexoro, CL121, and CL161, presenting the evidence of hybridization events occurring at the *pi-ta* locus between cultivated and weedy rice. Details and significance of these new findings will be presented.

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The Hop/Sti1-Hsp90 Chaperone Complex Facilitates the Maturation and Transport of a PAMP Receptor in Rice Innate Immunity

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Recognition of pathogen-associated molecular patterns (PAMP) by pattern recognition receptors (PRRs) represents a critical first step of innate defense in plants and animals. However, maturation and transport of PRRs is not well understood. We find that the rice chitin receptor OsCERK1 interacts with Hsp90 and its co-chaperone Hop/Sti1 in the endoplasmic reticulum (ER). Hop/Sti1 and Hsp90 are required for efficient transport of OsCERK1 from the ER to the plasma membrane (PM) via a pathway dependent on Sar1, a small GTPase which regulates ER-to-Golgi trafficking. Further, Hop/Sti1 and Hsp90 are present at the PM in a complex (designated the 'defensome') with OsRac1, a plant specific Rho-type GTPase. Finally, Hop/Sti1 was required for chitin-triggered immunity and resistance to rice blast fungus (*Magnaporthe oryzae*). Our results suggest that the Hop/Sti1-Hsp90 chaperone complex plays an important and likely conserved role in the maturation and transport of PRRs and may function to link PRRs and Rac/Rop GTPases.

Durable Panicle Blast Resistance Gene *Pb1* Encodes an Atypical CC-NBS-LRR Protein and Generated by Acquiring a Promoter Through Local Genome Duplication

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Rice blast, caused by *Magnaporthe oryzae*, is one of the most widespread and destructive diseases of rice. Breeders have used disease resistance (R) genes that mediate fungal race-specific ‘gene-for-gene’ resistance to manage rice blast, but the resistance is prone to breakdown due to the high pathogenic variability of blast fungus. Panicle blast, occurring after heading stage, causes decreased yield and lowered quality of brown rice. *Pb1* (Panicle blast 1) is a panicle blast resistance gene, which was identified as a major quantitative gene in rice cultivars derived from an *indica*-type rice cultivar, Modan (Fujii et al., 2007). The blast resistance by *Pb1* is more effective during adult stages (adult resistance), in particular for panicle blast, than during young stages, and has not experienced breakdown for more than 30 years in Japan (durable resistance).

We isolated the *Pb1* gene by map-based cloning to investigate the mechanisms underlying its characteristic traits of resistance. An F<sub>2</sub> segregating population, which derived from a cross between panicle blast resistant St. No.1 due to *Pb1* and susceptible Norin 8, was screened for recombinant lines. Then, F<sub>4</sub> homozygous lines derived from this population were tested in an experimental paddy field at the Aichi Agricultural Research Center, Mountainous Region Agricultural Research Institute, where high pressure of blast disease and its progress are well controlled. High-resolution genetic mapping and sequencing of a St. No.1 BAC clone overlapping with neighboring markers narrowed down the *Pb1* region to 25.8kb genetic interval in a region where 60-kb sequence was tandemly repeated (repeat 1 and 2). Six open reading frames were predicted in the *Pb1* region (repeat 2), but gene expression was detected for only P15, P16 and P18. Complementation tests showed that genomic sequence encompassing P15, but not P18, conferred panicle blast resistance to rice; therefore, we concluded that P15 is *Pb1*. *Pb1* encoded a coiled-coil–nucleotide-binding site–leucine-rich repeat (CC–NBS–LRR) protein of 1,296 amino acids. The protein sequence of *Pb1* differed from those previously reported for R-proteins, particularly in the NBS domain, in that P-loop was absent and some other motifs were degenerated. In *Pb1*+ cultivars, *Pb1* transcript level was low in young seedling but increased during development, which accounts for adult/panicle resistance. *Pb1'*, a paralog of *Pb1* in repeat 1, was almost identical with *Pb1* (one amino-acid difference). Expression levels of *Pb1* was 150-300 times higher than those of *Pb1'*. Promoter:GUS analysis indicated that a sequence that had been placed upstream of *Pb1* coding sequence due to local genome duplication drove a *Pb1*-characteristic expression pattern. These results indicate that the genome duplication played a crucial role in the generation of *Pb1* by transcriptionally activating an otherwise inactive ‘sleeping’ resistance gene.

Novel Functions of *AvrPiz-t* and APIP10 in the *Piz-t* Mediated Resistance to *Magnaporthe oryzae*

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To understand the molecular basis underlying the broad-spectrum resistance to *Magnaporthe oryzae*, we cloned three blast resistance (R) genes, i.e., *Pi2*, *Pi9* and *Piz-t*, which confer high levels of resistance to diverse blast isolates. Sequence analysis in both cultivated and wild rice species revealed that the R locus contains a complex cluster of NBS-LRR genes that span about 100 kb region on chromosome 6. We have recently constructed a fine physical map of three new R genes, i.e., *Pi40(t)*, *Pi-TY(t)* and *Pi-Jeff(t)*, that are located at the same locus. To understand the avirulence (*Avr*) gene function, we cloned the *AvrPiz-t* gene in *M. oryzae* that encodes a novel secreted protein. Yeast two-hybrid screening identified 12 *AvrPiz-t* interacting proteins (APIP1-12) in a rice cDNA library when using *AvrPiz-t* as the bait. Among them, four *APIP* genes encode novel proteins that are involved in the ubiquitination pathway, indicating a possible role of the ubiquitination-mediated pathway in the *AvrPiz-t* and *Piz-t* interaction. *In vitro* E3 ligase activity assays showed that *AvrPiz-t* interferes with APIP2, 6 and 10's E3 ligase activity and all three APIPs can ubiquitinate the effector protein. Interestingly, silencing of APIP10 leads to strong hypersensitive response (HR)-like cell death in the *Piz-t* background but weak HR cell death in the non-*Piz-t* background, suggesting that APIP10 plays a negative role in the *Piz-t* mediated resistance to rice blast.

Localization of *Magnaporthe oryzae* Secreted Proteins During Host Penetration and Growth *in planta*

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Plant pathogenic fungi secrete many proteins with diverse roles in plant-microbe interactions. However, information on the temporal and spatial patterns of protein secretion during fungal infection of plants is extremely limited, so in most cases it is not clear which proteins are expressed/secreted at different infection stages. Likewise, it is not known if secretion occurs at specific locations within invasive hyphae, or if the secreted proteins are targeted to different regions of the pathogen-host interface. To address these gaps in understanding, we are performing high throughput localization of *Magnaporthe oryzae* secreted proteins during penetration of, and growth inside, rice leaf sheaths.

An analysis of *M. oryzae* predicted genes using stringent criteria identified 939 genes that are predicted to code for secreted proteins. Fusions to green and/or red fluorescent protein (GFP/RFP) are generated using the Gateway system and the resulting constructs are introduced into *M. oryzae* via Agrobacterium-mediated transformation. Transformants are inoculated onto rice leaf sheaths and live cell imaging with epifluorescence and confocal microscopy is used to detect protein localization during penetration and invasive growth. To date, we have studied the localization patterns of over 120 proteins and another 400 are at various stages of the pipeline. We have found that RFP is much better than GFP for detecting proteins in *Magnaporthe* and we have shown that fusions generated using the Gateway system yield localization patterns that are equivalent to those obtained with direct fusions. At least 15 distinct localization patterns have been identified and examples are presented. One interesting class of secreted proteins under study consists of cell wall degrading enzymes (CWDEs). Most are abundant in conidia and several show interesting patterns of developmental regulation during germination, appressorium formation and penetration. Many CWDEs were abundantly expressed in appressoria on leaf sheaths but were not present in appressoria formed on artificial surfaces. None of the CWDEs studied were detected in biotrophic hyphae growing within rice cells but some were still present in the overlying appressorium, even as late as 48 hours after inoculation, when the fungus had already proliferated extensively within the host. Though they were repressed during biotrophic growth, most were re-activated and expressed very highly at the onset of lesion formation, approximately 96 to 120 h after inoculation. Our preliminary data support the predicted roles for CWDEs during penetration and lesion formation. In addition, however, they show the importance of plant-derived signals in CWDE expression and, further, suggest that appressoria continue to serve a role in pathogenesis long after penetration has already occurred.

Novel Mycovirus, *Magnaporthe oryzae chrysovirus* 1, Causing Hypovirulence to the Host Rice Blast Fungus

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Several novel mycoviruses were found in isolates of *Magnaporthe oryzae* distributed in the Mekong Delta. One of them detected in the isolate S-0412-II 1a had 4 double-stranded RNAs, dsRNA 1 (3,554 bp), dsRNA 2 (3,250 bp), dsRNA 3 (3,074 bp), and dsRNA 4 (3,043 bp), in an isometric particle (about 35nm in diameter). The dsRNA1 encoded a gene product highly homologous to viral RNA-dependent RNA polymerase derived from the *Chrysoviridae* family. The novel dsRNA mycovirus was named *Magnaporthe oryzae chrysovirus* 1 (MoCV1). By curing with cycloheximide and single spore isolation, virus-free cured strains were obtained from the original isolate S-0412-II 1a. In comparison with the MoCV1-infected original isolate, the cured strains showed more rapid mycelial growth and more abundant sporulation *in vitro*, and also had more virulence to increase the lesion numbers and to enlarge the lesion sizes on infected leaves. These results revealed that this MoCV1 caused hypovirulence to the host fungus. When cultured the MoCV1-infected strains in liquid media, the MoCV1 was released to the culture filtrate with the incubation time. When co-cultured with the filtrate, the virus-free strains were easily infected with the MoCV1. Additionally the filtrate had preventive effects on rice blast infection when sprayed on leaves just before inoculation. These results strongly suggested that the MoCV1 had potential as a biocontrol agent for rice blast disease.

Genetic and Biological Evidence of Sexual Reproduction of *Magnaporthe oryzae* in a Rice Field of Yunnan Province of China

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As shown by recent examples of human fungal pathogens, sexual reproduction is sometimes difficult to detect *in natura*, even for well-studied organisms. The genetic structure observed for *M. oryzae* populations from rice is consistent with clonal reproduction in the nearly whole distribution area of this plant pathogen. Moreover, sexual reproduction of *M. oryzae* has never been detected directly *in natura*. Production of the sexual stage requires the presence of strains of opposite mating types (Mat1.1 and Mat1.2) and of at least one female-fertile strain, that is, one strain able to produce perithecia. However, several arguments are in favor of sexual reproduction in limited regions of the putative centre of origin of the disease, namely in the Himalayan foothills. First, the genotypic structure of some populations from this region suggests recombination. Second, both mating types and female fertile isolates are found in some of these populations. Finally, the sexual reproduction can be obtained between chosen strains in controlled conditions in the laboratory.

Here we present genetic and biological evidences that one *M. oryzae* population isolated from rice in Yunnan Province of China may reproduce sexually in the field. In this population, we have found high levels of genotypic diversity and low linkage disequilibrium. The two mating types required for sexual reproduction are in balanced frequencies, and female-fertile isolates are found in high proportion (ca 90 %). This population structure was observed for samples from the same field obtained during two consecutive years. Viable progeny from crosses between different isolates of this population were obtained and showed Mendelian segregation for molecular markers and mating type. Computer simulations of population evolution show that clonal reproduction alone cannot explain the observed levels of genetic diversity and linkage disequilibrium.

Although one exception to clonal reproduction of a *M. oryzae* rice population has been hypothesized in North India, sampling, rather than sexual reproduction, may explain these formerly observed results. Hence, our study represents the first clear demonstration of sexual reproduction of the rice blast pathogen in the field. Extended survey with population genetics and biological tools may reveal other sexually reproducing population of the rice blast fungus.

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Characterization of the Cyclase-associated Protein *CAP1* in *Magnaporthe oryzae*Zhou, X.-Y.<sup>1</sup>, Zhang, H.-F.<sup>1</sup>, Shaw, B.<sup>2</sup>, Li, G.-T.<sup>1</sup> and Xu, J.-R.<sup>1</sup><sup>1</sup>Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, USA<sup>2</sup>Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, USA

In many plant pathogenic fungi, surface recognition and penetration are critical processes in their infection cycles. Previous studies have shown that the cyclic AMP (cAMP) signaling pathway is important for surface recognition and pathogenesis in *Magnaporthe oryzae*. Intracellular cAMP is synthesized by adenylate cyclase. Deletion of the adenylate cyclase gene *MAC1* affected aerial hyphal growth, conidiation, and appressorium formation. The cAMP-PKA pathway also plays a critical role in various developmental and infection processes in a number of fungal pathogens.

To better understand this important signal transduction pathway, we used the affinity purification approach to identify proteins that associate with the *Mac1* adenylate cyclase *in vivo*. One of the *Mac1*-interacting proteins is the adenylate cyclase-associated protein (named *Cap1*). The interaction between *Mac1* and *Cap1* was further confirmed by yeast two-hybrid and co-IP assays. *Cap1* has all typical structural features of CAP proteins, including an N-terminal cyclase-binding domain (CB), two proline-rich domains (P1, P2), and a C-terminal actin-monomer binding domain (AB). Deletion of the *CAP1* gene reduced the growth rate and conidiation on oatmeal medium but these defects can be partially rescued when grown on complete medium, indicating growth defects of the  $\Delta cap1$  mutant were nutritionally dependent. The  $\Delta cap1$  mutant formed melanized appressoria but often produced long, wide and branching germ tubes on hydrophobic surfaces. The intracellular cAMP level and transcripts of *MAC1* were reduced significantly in the  $\Delta cap1$  mutant. Exogenous cAMP partially suppressed its defects in germ tube growth and branching and stimulated appressorium formation on hydrophobic surface. The  $\Delta cap1$  mutant rarely caused typical blast lesions on rice leaves and it was defective in invasive hyphae growth.

*Cap1*-eGFP fusion proteins localized to the tips of hyphal and germ tubes, which is similar to the localization pattern of actin. In mature appressoria, *Cap1*-eGFP localized to small circular structures on the bottom. Surprisingly, the AB domain was dispensable for its actin-like subcellular localization because *Cap1* <sup>$\Delta AB$</sup> -eGFP had the same localization pattern with *Cap1*-eGFP. Further deletion analysis with the P1 and P2 poly-proline regions showed that P2 was responsible for its actin-like localization pattern, indicating that binding with profilins or actin by SH3 domain at P2 region may be essential for the subcellular localization of *Cap1*. Nevertheless, the *CAP1* <sup>$\Delta AB$</sup>  allele partially suppressed the defects of the  $\Delta cap1$  mutant in vegetative growth, germ tube growth, and virulence. Therefore, the actin-binding domain is important for the function of *Cap1*. Interestingly, exogenous cAMP induced the formation of melanized conidium compartments in transformant expressing *CAP1* <sup>$\Delta AB$</sup>  on hydrophilic surfaces. *GAS2*, an appressorium-specific gene, was expressed in these melanized conidium compartments, suggesting that they were appressorium-like structures. Exogenous cAMP appeared to stimulate appressorium formation without conidium germination in *CAP1* <sup>$\Delta AB$</sup>  transformants. Together, these data showed that *CAP1* is important for proper regulation of adenylate cyclase and appressorium morphogenesis.

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## Wheat Blast: a Potential Threat to Global Wheat Production

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Brazil produces 5 million tons of wheat per year but it is just half of the national need. The Brazilian policy of wheat expansion to new regions has faced a major obstacle to national self-sufficiency due to blast disease. Despite the release of wheat cultivars adapted to warmer regions, the impact of wheat blast on yield loss has been so severe that it threatens the plan of policy makers. Wheat blast caused by *Magnaporthe grisea* (*Pyricularia grisea*) has been a major disease of wheat in Brazil since its first outbreak in 1985 and has already spread to other South American countries. The impact blast disease has caused to wheat production in Bolivia and Paraguay, and its spread to Argentina and Uruguay, are examples of the risk wheat blast imposes to other wheat producing countries around the globe. The seriousness of the problem led CIMMYT and Embrapa to organize a meeting in May 2010 with scientists from eleven countries to discuss the global threat to wheat production. The reasons for CIMMYT's concern are the fact that in countries where the disease is already established, general strategy of resistant varieties and chemical control has not been effective and the yield loss observed fifteen years later was similar to the early 1990's despite constant release of new varieties (Urashima et al., 2009). Although some interesting aspects have already been elucidated, like the difference between the causal organism of wheat and rice blast (Valent, 1990) and the existence of two distinct populations of wheat blast disease based on DNA fingerprinting and sexual characterization (Urashima et al., 2005), there are still many aspects in the epidemiology of the disease to be investigated. The objective of this talk is to let rice blast colleagues know the seriousness of the wheat blast disease in South America and the potential threat to global wheat production on other continents. Some data on current status of the wheat blast disease will be presented.

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## Marker Assisted Selection for the Improvement of Three-Line Hybrid Rice

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Marker-assisted selection (MAS) for major genes has been extensively applied in the rice breeding programs in China. Facilitated by advances in the gene mapping and cloning, the MAS practice has been extended from a single gene to multiple genes and from a single trait to multiple traits. Two studies of MAS for multiple traits are reported here.

*Pi25* is a blast resistance gene identified from Gumei 2, a semi-dwarf *indica* rice line showing durable blast resistance. Three rice lines carrying *Pi25* were crossed to new restorer lines developed by the China National Rice Research Institute. A total of 394 recombinant inbred lines (RILs) were developed and screened with DNA markers tightly linked to *Pi25*. Lines carrying the resistant homozygote were genotyped with DNA markers tightly linked to genes *Rf3* and *Rf4* for fertility restoration. Twenty candidate restorer lines and 27 candidate maintainer lines were selected. Blast resistance and fertility restoration of the 47 lines were tested with phenotypic evaluation. Results suggest that the step-by-step approach used in this study is feasible for marker-assisted selection of multiple traits.

*Indica* rice Teqing is a high-yielding inbred variety and a restorer line of the three-line hybrid rice, and IRBB51 is an *indica* rice line carrying bacterial leaf blight (BB) resistance genes *Xa4* and *xa13* in the genetic background of the restorer line IR24. Genotype of Teqing and IRBB51 at five gene loci were determined through literature reading and gene based marker detection. While IRBB51 carries resistance alleles at the *Pib*, *Xa4* and *xa13* loci and high-grain-quality allele at the *wx* locus, Teqing carries resistant alleles at the *Pib*, *Pita* and *Xa4* loci and unfavorable allele at the *wx* locus. New restorer line Zhonghui 161 which carries beneficial alleles at all the five target loci was developed from the Teqing/IRBB51 RIL population. New hybrid rice Zhongyou 161 bred by crossing Zhonghui 161 to cytoplasmic male sterile line Zhong 9A was released commercially in 2009. It is suggested that adding beneficial genes which are carried by either parental line of a rice population to those carried by both parental lines might be a quick way of gene pyramiding in the breeding of commercial rice varieties.

## Characterization of Novel Blast Resistant Genes for US Rice Breeding

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Blast resistance genes, such as *Pi-ta* conveying resistance to 8 common US races of the pathogen (*Magnaporthe oryzae*), have been used for over 20 years in the US rice (*Oryza sativa*) industry. However, *Pi-ta* is susceptible to two known US races, IE-1K that has caused blast outbreaks in Arkansas, and IB33 that was identified under greenhouse conditions, but overcomes all known resistance genes in the US. Thus, novel resistant genes are needed for rice production security. Screening the USDA rice world collection has identified resistant germplasm including lines 4484 (PI 615022) and Shufeng (Shu) 121 (PI 615015) introduced from China.

Rondo and Shu 121-1655, derived from 4484 and Shu 121, respectively, are cultivars having improved agronomic traits using mutation breeding. They were found to be resistant to all 10 US common races of blast, including IE-1K and IB33. Preliminary analysis, with molecular markers linked to *Pi-ta*, *Pi-d*, *Pi-i*, *Pi-k*, and *Pi-z*, indicated that none of these resistant genes were present in the two cultivars. Further testing using the *Pibdom* marker demonstrated that both Rondo and Shu 121-1655 possess *Pi-b* which does not convey resistance to races IB33 and IB54. We developed a mapping population derived from Francis (female) and Shu 121-1655 (male) and evaluated 300 F2:3 families for resistance to IB33 and IB54 by scoring homozygous resistance (RR), heterozygous resistance (Rr) or homozygous susceptibility (rr) for each family. Disease segregation data fit a 1:2:1 pattern for both races, indicating a single dominant gene responsible for resistance to IB33 and IB54, respectively. Meanwhile, we identified 108 genome-wide polymorphic SSR markers between Francis and Shu 121-1655. Analysis of the F2 population with these polymorphic markers and with response to IB33 indicated that the resistance is not linked to any of the previously mapped US blast resistance genes. Resistance to IB54 was mapped between the markers RM7364 at 9.56 Mb and RM3912 at 10.83 Mb on rice chromosome 9. This region harbors three resistance genes *Pi 5-1*, *Pi 5-2* and *Pi 5-3* in the same cluster. Further effort is being applied to map the resistance gene for IB-33 and fine-map the resistance gene for IB54. While the blast resistant germplasm, Rondo and Shu 121-1655, have been used in several US breeding programs, the development of markers linked to these novel resistance genes will assist breeders in selecting cultivars with broad spectrum resistance to blast disease.

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## Cropping System to Limit Blast Disease in Upland Rice

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Cropping system is an essential aspect to take into account to manage blast disease (caused by the fungus *Magnaporthe oryzae*). In addition to the selection of resistant cultivars, studies report opportunities to limit blast incidence by managing mineral amendment (N, Si, P, etc.), cultivar mixtures or other cropping system adaptations. In Madagascar, rice is the staple crop and food. Farmers traditionally grow irrigated or rainfed lowland rice wherever possible. In the mid-1980s, CIRAD and FOFIFA launched a research program for the highlands to extend upland rice growing areas in high elevation areas of the tropics. This program was consolidated with research on cropping practices that ensure the sustainability of upland rice based cropping systems in this poor and fragile environment. New varieties were obtained, adapted for rainfed cropping up to 1800 m altitude (Dzido *et al.*, 2004). However, farmers had to face attacks of blast disease. Due to the small genetic basis of these varieties, the fungus quickly overcame resistant or tolerant lines selected by breeders.

Observations of blast epidemics in different regions made us consider the soil as a key factor for rice susceptibility. In a first experiment, we measured the potential of our very susceptible varieties to tolerate blast when cropped in different soil conditions. During two years, we transported volcanic soil from a very fertile area close to the experimentation site and where blast pressure is much lower. We observed a decrease of blast symptoms on rice cropped on that soil compared to the soil of the experimental site, both on leaves and panicles. That decrease was bound to a significant yield increase, demonstrating the importance of plant nutrition on blast incidence. Our aim was then to develop cropping systems that could influence blast incidence through an improvement of soil functions.

Direct-seeded, mulch-based (DSMB) cropping systems were first used in Madagascar to limit erosion in upland areas. During 4 years, we compared blast epidemics between a traditional cropping system with ploughing each year and a DSMB cropping system, on a mid-susceptible variety specific of the highlands conditions. Two fertilisation levels were also tested. A significant difference between the two systems was observed, both at leaf and panicle stage.

The fact that DSMB cropping system reduced the effect of N-fertilisation made us consider N as the determinant factor of the interaction between cropping system and blast incidence. The determinants of this interaction must then be explained to enable new and durable cropping systems to be developed to manage blast epidemics, in addition to cultivar improvement. This is the objectives of the starting project GARP (ANR-Systema) which is conducted in Bolivia, Brazil, France, and Madagascar. Its aim is to quantify the interactions between cropping system, N-nutrition and blast resistance in upland rice. Hypotheses and preliminary results of the project will be presented.

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## Application of PCR for Diagnosis of Fungicide Resistance in the Rice Pathogen

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A procedure was developed for detecting point mutations in the beta-tubulin gene of benomyl resistant laboratory mutant of *Bipolaris oryzae* using the Polymerase Chain Reaction (PCR) in combination with Allele Specific Oligonucleotide (ASO) analysis. PCR was used to amplify a specific 11,919 –bp DNA sequence of the beta-tubulin gene in DNA extracts. The amplified DNA sequence was then probed with 18- mer end labelled oligonucleotides specific for the sensitive phenotype or for the benomyl-resistant phenotypes. The point mutations, converting codon 198 from glutamic acid in the sensitive strain to lysine or alanine, respectively, in high resistant and very high resistant strains were detected by ASO analysis. A point mutation, converting codon 200 for phenylalanine in sensitive strain to tyrosine, in moderately resistant strains was detected by ASO analysis. ASO analysis was a useful tool for detecting and characterizing benomyl-resistant strains *B. oryzae* and other pathogenic fungi in field and under laboratory conditions.

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Screening Rhizobacteria for Growth Promotion and Leaf Blast Suppression (*Magnaporthe oryzae*) on Aerobic Rice in Brazil

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Rice blast caused by *M. oryzae* [(T.T. Hebert) M.E.] (*Pyricularia grisea* (Cooke) Sacc.) has the potential to cause 100% grain yield loss, depending upon the degree of cultivar resistance, planting time and climatic conditions. Rhizobacteria utilize specific mechanisms to colonize roots of host plants, and their use has been widely increasing in agriculture, because it offers an alternative disease control method and growth promotion (Choudhary et al., 2007). The objective of this investigation was to identify rhizobacteria showing potential for plant growth stimulation and resistance induction under greenhouse conditions. The rhizosphere bacteria from rice plants were collected in soils of the Amazon basin in the municipalities of Paragominas and Dom Eliseu, PA. Out of 148 isolates eighteen were selected based on the capacity to promote plant growth and to reduce leaf blast severity when drenched before challenge inoculation with *M. oryzae*. To confirm the potential to reduce disease severity, a third experiment was conducted using three replications and three application methods (drenching the soil, 15 and 2 days, and spraying two days before challenge inoculation) of the two isolates, R-46 and R-55, under controlled conditions in the greenhouse. Also, the enzymatic tests were conducted to quantify the presence of proteins related to pathogenesis “PRP’s” during the induction process of resistance by rhizobacteria. Out of 148 isolates evaluated in massal screening for promotion growth, 12.7% stimulated Length of aerial part (LAP), 52% length of root (LR), total biomass (TB) and total root (TR) resulting in a gain of 10.07 cm (22%) on LAP, 11.83 cm (60.02%) on LR, 31.37 g (47.45%) to TB and 1.5g (45.83%) on TR. Two best rhizobacteria isolates (Rizo-46, and Rizo-55) when inoculated to prior challenged inoculation reduced to 90% leaf blast severity when compared with non-inoculated control. The capacity to suppress leaf blast by isolates Rizo-46 and Rizo-55B varied according to the mode of rhizobacteria application. The enzyme activity of peroxidase (POX) was found to be greatly increased, followed by  $\beta$ -1,3-glucanase (PR2), and chitinase (PR3). The same results were also obtained in greenhouse tests with reference to leaf blast severity. These results conclusively showed the increased enzymatic activity when Rizo-46 was inoculated by drenching the soil 15 days before challenge inoculation and Rizo-55B was sprayed two days prior to challenge inoculation.

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Mapping of the *PWT3* Locus of *Magnaporthe oryzae*, a Gene Involved in the Avirulence Reaction of the *Avena* Isolate on Wheat

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Cultivar specificity of *Magnaporthe oryzae* is known to be very unstable. During the past 50 years, resistance genes introduced into rice cultivars have been quickly overcome by new races of *M. oryzae*. On the other hand, plant species specificity appears to be stable. In the mechanisms of plant species specificity, we may find a clue to the establishment of a breeding system for durable resistance. *Pwt3* (pathogenicity to wheat) is a locus conditioning the specificity of *Avena/Triticum* isolates of *M. oryzae* on wheat identified among F<sub>1</sub> progeny derived from a cross between an *Avena* isolate Br58 and a *Triticum* isolate Br48. The avirulence allele, *PWT3*, is involved in the avirulence reaction of the *Avena* isolate on wheat.

Among 59 Simple Sequence Repeat (SSR) markers from an existing genetic map of *M. oryzae*, only seven (12%) showed polymorphism between Br48 and an F<sub>1</sub> strain 73Q2 carrying *PWT3*. Using a BC<sub>1</sub>F<sub>1</sub> population, *PWT3* locus was mapped on chromosome 6 between two flanking markers MGM134 and MGM 402 with genetic distances of 20 cM and 25 cM, respectively. The SSR marker most closely linked to *PWT3* is MGM130 with a distance of 5 cM. To finely map the *PWT3* locus, a BC<sub>2</sub>F<sub>1</sub> progeny (h12-16) was backcrossed with Br48 producing 118 random progeny of BC<sub>3</sub>F<sub>1</sub> population. *Triticum aestivum* 'Norin 4' was used for the determination of genotypes at the *PWT3* locus by infection assay of the 25 recombinants between the two SSR flanking markers. Nine cultures were identified as *PWT3* carriers and 16 cultures as *pwt3* carriers. These results provided a starting point for cloning and characterization of *PWT3*. An F<sub>1</sub> culture with *PWT3* was backcrossed with the *Triticum* isolate four times resulting in the establishment of near isogenic lines of the *Triticum* isolate carrying *PWT3*. Thirty one hexaploid, 34 tetraploid wheat cultivars and 26 barley cultivars were inoculated with Br58, Br48 and a near isogenic line of Br48 (h31-2-7) to determine their reaction to *PWT3*. A majority of the cultivars responded to *PWT3*. When representative cultivars of hexaploid and tetraploid wheat and barley were inoculated with 31 BC<sub>4</sub>F<sub>1</sub> tetrad progeny (16 *PWT3* carriers and 15 *pwt3* carriers), all *PWT3* carriers were avirulent on all representative cultivars whereas all *pwt3* carriers were virulent, suggesting that they recognize *PWT3*. *PWT3* was also recognized by the same cultivars when inoculated with 48 random progeny of the same cross. A resistance gene *Rwt3* corresponding to *PWT3* was identified in wheat and barley cultivars. These results suggest that *PWT3* is ubiquitously involved in the avirulence of the *Avena* isolate on wheat and barley because its corresponding resistance gene is ubiquitously distributed in wheat and barley.

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## ABSTRACTS - POSTER PRESENTATIONS

- I. Genomics and Functional Genomics of the Pathogen
- II. Host Resistance, Signaling and Defense Responses
- III. Molecular and Cellular Biology, Diversity, Evolution and Adaptation of the Pathogen
- IV. Strategies for Managing Diseases Caused by *Magnaporthe species*

P## = Poster Presentation

CP## = PostDoc and Student Competition, Poster Presentation

Presentations in competition:

I-CP-2  
I-CP-3  
I-CP-4  
I-CP-11  
I-CP-12  
I-CP-23  
I-CP-24  
I-CP-79  
II-CP-30  
III-CP-78  
IV-CP-51  
IV-CP-57

## Insertional Mutagenesis to Identify Novel Determinants of Pathogenicity in Rice Blast Disease

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Rice blast is caused by the filamentous fungus *Magnaporthe oryzae* and is the most destructive disease of cultivated rice. It was the first plant pathogenic fungus with its genome sequence published that opened up the opportunities to discern possible attributes that confer pathogenicity on the fungus. The availability of the genome sequence has presented fresh challenges in terms of converting sequence data into meaningful biological information. Functional genomics studies involving the generation of large mutant collections and comprehensive screening has the potential to identify novel pathogenicity or virulence determinants. In this study, we have utilized random insertional mutagenesis to study the infection mechanism of *M. oryzae* and this method has previously been exploited to identify novel determinants of pathogenicity in rice blast disease such as *PDE1*, *PDE2*, *IGD1*, *MET1* and *GDE1* [1]. More recently *Agrobacterium tumefaciens* mediated transformation (ATMT) has been developed as a large-scale gene tagging method in *M. Oryzae* and other filamentous fungus due to high efficiency of transformation, random insertion [2] and subsequent identification of the tagged genes e.g. *MoRIC8* [3]. We have recently generated 10,000 *M. Oryzae* ATMT mutants and developed a high-throughput screening system. To date we have screened more than 7,000 mutants and confirmed 6 potential mutants either reduced or lacking in pathogenicity for gene functional analysis. It is hoped that the use of this high-throughput genetic screen will allow Identification of novel genes to develop a greater insight into the processes required for appressorium morphogenesis and more integrated understanding of appressorium mediated plant infection. A detailed understanding of the molecular basis of plant infection by *M. oryzae* will benefit the development of new strategies to control the disease and understanding the molecular basis of plant-fungus interactions.

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Genome-Wide Expression Profiling of Transcription Factor Genes in *Magnaporthe oryzae*

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*Magnaporthe oryzae* is a filamentous ascomycete that causes rice blast disease and is an important model organism for investigating fungal pathogenicity due to its genetic tractability and availability of genome information. Although much effort has been focused on understanding the molecular nature of pathogenicity, little is known about transcriptional regulation of pathogenicity-related genes at the genome level in this fungus. As a first step, we initially identified all putative transcription factors (TFs) using bioinformatic tools in the genome and achieved them in the database, Fungal Transcription Factor Database (FTFD, <http://ftfd.snu.ac.kr>). Here, we present expression profiling of 224 *M. oryzae* TF genes under 32 conditions including developmental stages and several stress conditions using quantitative real time-PCR. We were able to cluster into seven groups according to expression patterns. Principal component analysis revealed that expression patterns under developmental stages, carbon starvation, and CuSO<sub>4</sub>-treated conditions had strong associations with Cluster 1 and 2. To verify our expression profiles at functional levels, we generated knock-out mutants of two TF genes (MGG\_09869.6 and MGG\_08463.6) and characterized their phenotypes. Functional analysis revealed that expression patterns under specific conditions could suggest functional roles of the TFs. Our comprehensive analysis of expression profiling of TF genes would provide a new paradigm to decipher molecular mechanisms of gene expression and pathogenicity in the rice blast fungus.

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Laboratory Information Management System for Genetic Research of *Magnaporthe oryzae*

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A Laboratory Information Management System (LIMS) is laboratory computer software for the management of all laboratory information. LIMS supports information gathering in the lab network, decision making in the lab process, solutions of bottlenecks in the workflow, calculation, documentation and review, using an online database. Particularly, web-based LIMS implementations require no special client-side installation. However, LIMS offers users the opportunity to see analytical results in real time from any web browser and from any operation system.

*Magnaporthe oryzae* is an important model organism for investigating fungal developments and pathogenicity owing to its genetic tractability and availability of genomic sequence information. Although much effort has been made to establish the standard experimental protocols, there is no globally accepted standardization especially for quantitative phenotype assays. Therefore, we designed LIMS to provide not only an integrated management system for *M. oryzae* genetic research, but also a standardized process that has a guideline of phenotype assays and data acquisition formats.

Our goal of LIMS for *M. oryzae* is to develop the platform to manage all experimental data from genetic and phenotypic analyses of the genes. Initially we planned to make a standard guideline for phenotype characterization such as mycelial growth on various media, conidiation, conidial germination, appressorium formation, and pathogenicity. As a first step, we collected over 100 published research papers of *M. oryzae* that describe mutant phenotypes through gene knock-out experiments. All experimental protocols and data acquisition methods were compared and analyzed for specific phenotypes using a spreadsheet program. Then, we developed a web-based work process management system. All work processes are divided by time-dependent and -independent manners. Time-independent processes contain information generated in course of making gene knock-out mutants including primer design, vector construction/confirmation, and genotype identification. On the other hand, time-dependent processes manage steps of phenotypic assays for fungal development and pathogenicity in a standardized format. All data generated by each step can be stored in preformed database files and they can be interconnected and managed by our LIMS database. Standardized experimental data in genetic and phenotypic analyses will provide new paradigm to understand molecular mechanisms of fungal development and pathogenicity at genome level in *M. oryzae*.

Development of Phenotype Based Screening (PBS) System for Support of Bidirectional Genetic Approach in *Magnaporthe oryzae*

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Genetic analysis in *Magnaporthe oryzae* was enlivened with the release of genome sequences and application of several gene manipulation tools. *Agrobacterium tumefaciens*-mediated transformation (ATMT) and targeted gene deletion strategies are most frequently used for forward and reverse genetics, respectively. The efficiency of gene replacement relies on the frequency of homologous recombination (HR) events. However, many of filamentous fungi including *M. oryzae* exhibited low frequency of HR due to the dominance of non-homologous end joining (NHEJ). Efforts had been made to increase HR frequency through the inactivation of NHEJ-associated components in *M. oryzae*, but the HR frequency turns out to be loci-dependent and unstable.

To improve the efficiency of functional genetic study in *M. oryzae*, we developed an add-on system, designated as Phenotype Based Screening (PBS), to supplement the targeted gene deletion strategy. This system utilizes ectopic transformants, which can be easily generated in a transformation process for targeted gene replacement. Ectopic integration can disrupt a gene in a random manner, providing a pool of disruption mutants. We screened many ectopic transformants for various phenotypic defects in mycelial growth, pigmentation, colony morphology, and sporulation. Single ectopic integration was detected by the Southern blot analysis, and location of ectopic integration was verified by inverse-PCR and sequencing. To prove the efficacy of this system, two ectopic mutants were selected and characterized in detail. We confirmed that two loci, MGG\_00839 (homolog of *Saccharomyces cerevisiae* *VPS74*) and MGG\_14904 (homolog of *S. cerevisiae* *HIS5*) were disrupted with an ectopic integration in each mutant. Phenotypic defects were proven to be correlated with the disruption of each corresponding gene. Taken together, the combined bidirectional genetic approach -PBS system and targeted gene deletion- would be a novel platform to elucidate the molecular functions of both known and novel genes, which can be facilitated in many species.

*COM1* Encodes a Novel Component of Spliceosome and is Required for Conidium Morphology and Virulence in *Magnaporthe oryzae*

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The spliceosome is a dynamic ribonucleoprotein complex. Although major components of the spliceosome have been characterized, many dynamic components remain to be functionally investigated. In a previous study, the authors identified *Com1* as a novel protein required for normal conidium morphology and full virulence in *Magnaporthe oryzae*. In this study, the authors further characterized functional domains and isolated proteins that interacted with *Com1*.

Sequence alignment and pBLAST search suggested that there were 7 characteristic structure regions in *Com1*. Deletion and complementation assays revealed that two conserved C-terminal regions and one variable KP-rich region were essential for the functions. Deletion of NLS sequences indicated that *Com1* was a nuclear localized protein and contained PPVKRPRE and PLAKKFK as two NLS required for the nuclear localization and full functions of *Com1*. Microarray analysis revealed that a total of 17 and 59 genes were up- and down-regulated over 2-fold in the  $\Delta com1$  null mutant. In particular, expression of several genes that are likely involved in glycogen and lipid metabolism were affected by deletion of *COM1*. The *Com1*-3Flag tag was made and used to isolate *Com1*-interacting proteins. Forty-nine putative nuclear proteins were obtained from 4 independent pull-down experiments. Interestingly, a majority of these nuclear proteins was shown to be similar to the components of spliceosome identified in human and yeast cells. To verify which protein directly interacted with *Com1*, yeast-two-hybrid assays were conducted. Results showed that *Com1* interacted with at least 3 putative Sm snRNP proteins, including *MoSmD1*, *MoSmD2*, and *MoSmE*, and that the C-terminal region of *Com1* was required for the interactions.

In summary, the authors identified *Com1* as a novel component of RNA spliceosome that is required for conidium morphology and plant infection in *M. oryzae*.

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Identification of the PacC Signaling Pathway Genes in *Magnaporthe oryzae*

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Although extensive studies were carried out on pH signaling pathways in *Saccharomyces cerevisiae*, *Candida albicans* and *Aspergillus nidulans*, few reports deal with pH signaling pathways in *Magnaporthe oryzae*. From an ATMT library of *M. oryzae*, the authors obtained 9 co-segregated mutants that resembled in sensitivity to alkaline pH and showed similar defects in mycelial growth and sporulation. Isolation and analysis of the flanking sequences revealed that 5 of the mutants were disrupted in the same gene, *MGG\_10150*, whose product was highly similar to the transcription factor PacC, the remaining 4 were disrupted in genes whose coding proteins were homologous to PalB, PalC, PalF and PalH in *A. nidulans*, respectively. These *M. oryzae* genes were therefore named as *MoPacC*, *MoPalB*, *MoPalC*, *MoPalF* and *MoPalH*, respectively. Single gene deletion mutants of these 5 genes were generated, and were all sensitive to alkaline pH and defective similarly with respect to mycelial growth, sporulation and virulence, indicating that they were key components of PacC-pH signaling pathway genes. The authors also made single gene deletion mutants of *MoPalI*, *MoPalA* and *MoVps32*, however, no visible defects were observed in these mutants, demonstrating that these 3 genes were not important in the pH signaling pathway.

Microarray analysis revealed that 330 genes and 365 genes were down- or up-regulated in *ΔmopacC* mutant over 2-fold compared with those in the wild-type strain. Among them, 190 down- and 156 up-regulated genes have 5'-GCCAAG-3' *cis*-element in their promoter regions, respectively, indicating that *MoPacC* may function either as a transcription activator or transcription repressor. To clarify how *MoPacC* is switched between activator and repressor, the author investigated changes in *MoPacC* and found that *MoPacC* existed as a full-length protein mainly located in cytoplasm under acidic pH and that the protein was processed into a truncated form mainly located in nuclei under alkaline pH. The yeast transcription assay showed that the processed but not the full length form functions as a transcription activator. The EMSA assay proved that both the full-length and the processed forms of *MoPacC* could bind to the *cis*-element 5'-GCCAAG-3'. These results indicated that *MoPacC* is expressed in different forms in response to environmental pH and may play distinct roles in regulating downstream gene expression.

To identify roles played by downstream genes, *MoPrg1* (PacC repressed gene) was chosen for analysis in detail. *MoPrg1* has the *cis*-element in its promoter 950 bp upstream of the translation site. qRT-PCR confirmed that *MoPrg1* was evidently activated in *ΔmopacC*. Mutation of the *cis*-element in the *MoPrg1* promoter activated expression of *MoPrg1* in both *Δmoprg1* and the wild type and resulted in reduced colony growth. However, defects in sporulation and virulence were not observed in the transformants. These results indicated that *MoPrg1* functions as a repressor of mycelial growth and that there may be distinct branches downstream of *MoPacC* to regulate hyphal growth, sporulation and virulence.

Identification of '-ATATAT-' as a Novel *cis*-element Bond by Both bHLH and JAZ Proteins

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Identification of key *cis*-elements in promoter and transcription factors binding to the elements is important to elucidate the regulatory mechanisms of gene expression. In this study, the author cloned *OsPinA*, a gene encoding Borman-Birk type protease inhibitor whose expression was inducible by infection of *Magnaporthe oryzae* and treatments with SA, MeJA and mechanical wounding.

In order to identify key *cis*-elements that regulate the gene expression, the 1,480 bp DNA fragment upstream the translational start site was fused with the reporter gene *GusA*, and its series of deletions were generated and transformed into rice. By assaying the GUS enzyme activity in the transgenic rice, it was found that deletion of the fragment between -913 and -864 bp (50 bp) resulted in significant reduction in GUS activity, indicating that a positive *cis*-element existed in the region. By using the 50 bp as bait, the authors carried out yeast one-hybrid screening and obtained two cDNAs that encoded a putative bHLH protein and a JAZ protein, respectively. Re-transformation of yeast confirmed that both the JAZ and the bHLH protein bond the 50 bp DNA fragment. Further, OsJAZ-GST and OsbHLH-GST fusion proteins were expressed and purified from *E. coli*. EMSA assays showed that either the JAZ-GST or the bHLH-GST protein specifically bonded the 50 bp DNA fragment. Deletion and base-substitution analysis revealed that both OsJAZ-GST and OsbHLH-GST bonded '-ATATAT-' in the fragment. In addition, the OsJAZ-GFP and OsbHLH-GFP fusion proteins were located in nuclei of onion epidemic cells. Yeast assays showed that OsbHLH but not OsJAZ has transcription activation activity, inferring an interaction between the two proteins. By fusing the OsJAZ with Gal4 BD domain and OsbHLH with Gal4 AD domain, the authors conducted yeast two-hybridization assays and confirmed interaction between the two proteins. Constructs of the OsJAZ fused with YFP<sup>N</sup> and OsbHLH with YFP<sup>C</sup> were also made, YFP fluorescence was observed in nuclei of *N. benthamiana* leaf epidermis co-infiltrated with *A. tumefaciens* harboring the two constructs, confirming that OsbHLH and OsJAZ interact *in planta*.

In this study, the authors identified '-ATATAT-' as a novel *cis*-element in plant gene promoters and demonstrated that OsJAZ and OsbHLH were able to bind the *cis*-element.

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Systematic Characterization of SCP Domain Proteins in *Magnaporthe oryzae*Zhang, Y.-P.<sup>1,2</sup>, Zhou, X.-Y.<sup>2</sup> and Xu, J.-R.<sup>2</sup><sup>1</sup>Graduate School of Central South University, Changsha, Hunan, CHINA<sup>2</sup>Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, USA

SCP domain (SMART SM00198) proteins or Cysteine-Rich secretory Proteins (CRISPs, Pfam 00188) are conserved in a variety of organisms ranging from bacteria, fungi, plants, and animals. However, their exact biological functions are not clear. There are two and three hypothetical proteins with unknown functions in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, respectively. The *M. oryzae* genome contains six predicted genes, MGG\_03085, MGG\_03755, MGG\_05100, MGG\_06772, MGG\_07807, and MGG\_13936 that encode proteins with the SCP or CRISP domain. They are named *SDP1* to *SDP6* (for SCP Domain-Contain Proteins) genes in this study. Phylogenetic analysis suggests that SDP genes are more abundant in phytopathogenic fungi and have species-specific family expansion.

To determine the functions of these SDP genes, we first assayed their expression levels by qRT-PCR with RNA samples isolated from vegetative hyphae, conidia, appressoria (24 h), and infected rice leaves (5 dpi). While *SDP1* and *SDP2* had the highest expression level in infected leaves, transcripts of *SDP3* were more abundant in conidia. Deletion of any of these SDP genes had no effects on the growth rate. Only the *sdp3* and *sdp6* mutants appeared to reduce half conidiation and produce less aerial hyphae than the wild type. While *SDP4*, *SDP5*, and *SDP6* were dispensable for plant infection, the *sdp1*, *sdp2*, and *sdp3* mutants were reduced 60%, 66%, and 36% in virulence compared with the wild type. The *sdp1 sdp2* and *sdp2 sdp3* double mutants were more significantly reduced in virulence than the single mutants in infection assays with rice or barley seedlings. These results indicate that the *SDP1*, *SDP2*, and *SDP3* genes may be novel virulence factors in *M. oryzae*. The *SDP1*-, *SDP2*-, and *SDP3*-eGFP fusion constructs have been generated for determining their expression and localization during invasive growth. We also have generated constructs for the *SDP1*-*SDP6* genes for Agro-infiltration assays with *Nicotiana benthamiana*. For the *SDP1* and *SDP2* genes, Agro-infiltration constructs without the signal peptide sequences also have been generated. In addition, GST-fusion proteins were purified for the *SDP1* and *SDP2* genes. Data from infiltration assays with the Agrobacteria and GST-fusion proteins will be presented. Overall, the systematic characterization of these well-conserved SCP genes will be helpful to determine their functions in *M. oryzae* and other plant pathogens.

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## Functional Characterization of MoCRZ1 Regulated Genes Identified by ChIP-chip Analysis

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We performed a comprehensive ChIP-chip analysis of the MoCRZ1 binding sites. MoCRZ1 (*M. oryzae* Calcineurin Responsive Zinc Finger) acts as a downstream regulator in Ca<sup>2+</sup>-dependent signaling for gene expression and pathogenicity. MoCRZ1::eGFP localizes to the nucleus following calcium treatment. The *mocrz1* knockout mutant shows impaired growth in the presence of Ca<sup>2+</sup> and in the presence of SDS. To understand the regulatory circuit of calcineurin signal transduction via MoCRZ1, we identified its binding sites *in vivo* by chromatin immunoprecipitation and DNA tiling microarrays (ChIP-chip). To facilitate this study, we developed a non-coding region array of promoter and all non-coding sequences of the *M. oryzae* genome in collaboration with NimbleGen. Of particular note, genes involved in vesicle mediated secretion necessary for establishing host associations were also found. MoCRZ1 itself was a target, suggesting a previously unreported autoregulation control point. The data also implicated a previously unreported feedback regulation mechanism of calcineurin activity as well as functions in development and pathogenicity manifest through multiple layers of regulation. Candidate MoCRZ1 regulated genes were selected for functional characterization using knock out mutagenesis approach.

Genome Sequencing, Alignment, and Comparison Among 70-15 and Two *Magnaporthe oryzae* Field Isolates

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Rice (*Oryza sativa*) production has been challenged worldwide by increased new virulent *M. oryzae* isolates. In past decades, the lack of genetic diversity study of rice blast presented a challenge for rice crop protection. In an effort to develop effective methods to control rice diseases, it is essential to examine and compare different *M. oryzae* isolates at the whole genome scale. Also, the continuously decreasing price for sequencing and the rapid development of computational tools for whole genomic analysis make it possible to do such analysis within a reasonable budget and time. In this study, genome sequences of two rice blast field isolates from China, 19311 and 81278, were sequenced using Illumina sequencing, and the short reads were assembled by Velvet and Edena assemblers. Contigs from these two isolates were aligned against *M. oryzae* 70-15 genome sequence version 6 released by the Broad Institute. 85% of the field isolate assembly contigs successfully aligned to 70-15 with over 70% identical matching. For contigs which could not be aligned, GeneMark, an ab initio gene finder, was employed to predict genes. The genes were analyzed for protein motifs and homology to genes from other organism.

Understanding the Molecular Mechanism of Instability of the Avirulence Gene *AVR-Pita1* in Field Isolates of *Magnaporthe oryzae*

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The avirulence gene *AVR-Pita1* in *Magnaporthe oryzae* triggers a resistance response in rice plants that contain the resistance gene *Pi-ta*. Understanding the evolution of the *AVR-Pita1* gene in field isolates should benefit the deployment of *Pi-ta* for the control of rice blast disease. A total of 187 field isolates of *M. oryzae* collected from the U.S., China, Colombia, Egypt, India, and the Philippines over a 40 year period were used for this analysis.

We first determined the pathogenicity of isolates on rice cultivars with and without *Pi-ta*. It was found that 151 isolates were avirulent toward *Pi-ta* containing rice cultivars while 36 were virulent. Using *AVR-Pita1* specific primers, *AVR-Pita1* was amplified by PCR, and the resulting PCR products were sequenced from the DNA of all 151 avirulent isolates as well as 4 virulent isolates collected from China. Alignment of sequence assemblies revealed 38 highly similar *AVR-Pita1* haplotypes. It was found that most DNA sequence variation occurs in the exon regions, and the majority of this variation resulted in amino acid substitutions. A total of 27 highly variable haplotypes excluding *AVR-Pita1* was predicted based on the 38 different DNA sequence assemblies. For the 4 virulent isolates from which the *AVR-Pita1* allele was amplified, two additional nucleotides were found in the first exon of *AVR-Pita1* of these virulent isolates. The insertion of these two nucleotides resulted in a frame-shift that produced a predicted truncated *AVR-Pita1* metalloprotease; in the 32 virulent isolates where *AVR-Pita1* was undetectable by PCR, the 5' portion of the *AVR-Pita1* allele was predicted to be deleted from the genomes of 29 isolates while the entire *AVR-Pita1* was predicted to be deleted in the remaining 3 virulent isolates using Southern blot analysis. These findings suggest that frame-shift, partial and complete deletions of *AVR-Pita1* are three mechanisms that the fungus uses to overcome *Pi-ta* mediated resistance. To determine the function of *AVR-Pita1*, field isolates from Arkansas were transformed with one to many copies of *AVR-Pita1* from isolate O-137 by homologous based recombination. The presence of *AVR-Pita1* in virulent isolates was confirmed by PCR using *AVR-Pita1* specific primers and the results were verified by DNA sequencing and Southern blot analysis using the *AVR-Pita1* coding region as the probe. Results of pathogenicity assays demonstrated that isolates transformed with *AVR-Pita1* were restored to avirulence on *Pi-ta*-containing rice cultivars.

In summary, we demonstrated the molecular diversity and instability of *AVR-Pita1* among field isolates of *M. oryzae*. The impact of these findings on the understanding of the molecular mechanisms of disease resistance in rice will be discussed.

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Inside a Rice Cell: Uncovering the Secretion Mechanism during Biotrophic Invasion by the Blast Fungus *Magnaporthe oryzae*

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Rice blast caused by the fungus *Magnaporthe oryzae* remains a threat to global food security. This pathogen infects other important cereal crops such as wheat, barley and millets, as well as turf grasses. In addition to economic importance, rice blast is a model pathosystem for difficult- to-study biotrophic fungi and fungal-plant interactions. Our research focuses on studying proteins, called effectors, fungi secrete inside living cells to block plant defenses and control host cell processes. To date mechanisms for secretion and delivery of effectors inside host cells during disease establishment remain unknown.

To cause blast disease, *M. oryzae* crosses rice cell walls, grows as thin filamentous primary hyphae, and then differentiates into bulbous invasive hyphae (IH) that colonize living rice cells while enclosed in plant-derived extra-invasive-hyphal membrane. Primary and bulbous IH secrete effector proteins to block plant defenses and control rice cell processes. Fluorescently-labeled effectors accumulate in a novel structure, the biotrophic-interfacial complex (BIC), found first at the primary hyphal tip and then beside the first bulbous IH cells after pseudohyphal differentiation. We previously characterized secretion patterns for additional biotrophy-associated-secreted (BAS) proteins: BAS1 preferentially accumulates in BICs like known effectors; BAS2 and BAS3 accumulate in BICs and at the points where IH cross rice cell walls; and BAS4 localizes to the extracellular matrix, uniformly accumulating around subsequently-formed IH cells. We now report localization studies with *M. oryzae* orthologs of conserved secretion machinery components to investigate secretion mechanisms for effectors showing preferential BIC accumulation and for non-BIC proteins such as BAS4. Especially bright Yup1:GFP fluorescence adjacent to BICs suggests that this t-SNARE endosomal protein is involved in effector secretion. These studies will also provide insight on secretion by *M. oryzae* pseudohyphal IH cells that mediate biotrophic invasion.

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Efflux of a Steroidal Glycoside ATS is Required for Appressorial Function in *Magnaporthe oryzae*

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ATP-binding cassette (ABC) transporters are able to couple hydrolysis of ATP to the transport of a variety of substrates either into or out of the cells. Our previous observation (Sun et al. 2006) suggests that reduced viability of the *abc3Δ Magnaporthe* infection structures (appressoria) could be due to the excessive accumulation of a cytotoxic metabolite(s), presumably the efflux target of Abc3p. We designed a novel yeast-based assay to ascertain and guide the purification of Abc3-Transporter Substrate (ATS) from the *abc3Δ* appressoria. ATS was identified by LC-APCI-MS as a Digoxin-like steroidal glycoside that showed cytotoxic activity against yeasts. Further co-immunoprecipitation and gene-deletion studies suggest that ATS targets a Translation Elongation Factor EF1- $\alpha$  (Tef2) in fission yeast. Exogenously applied ATS specifically increased the sensitivity of WT *Magnaporthe* to Na<sup>+</sup> and Ca<sup>+2</sup> ions, probably through perturbed ion homeostasis during appressorial development. However, when applied in excess to *Magnaporthe*, ATS significantly blocked appressorial function of breaching the host surface, and induced Hypersensitive Response in rice leaf tissue. We propose that ATS plays a key role(s) in ion homeostasis during appressorial function in *Magnaporthe*.

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Characterizing the Role of Two GATA-family Transcription Factors in Pathogenicity by the Rice Blast Fungus *Magnaporthe oryzae*

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The filamentous fungus, *Magnaporthe oryzae*, responsible for rice blast disease, destroys about 10-30% of the world's rice crop annually. Infection begins when the specialized infection structure, the appressorium, develops on a hydrophobic surface (i.e. the leaf surface) and generates enormous internal turgor pressure through the accumulation of glycerol. This turgor acts on a penetration peg emerging at the base of the cell, causing it to breach the leaf surface allowing infection. Understanding how this process of infection is regulated is key to developing durable control strategies against rice blast disease.

Tps1 is a central regulator of the infection process and regulates the transition from appressorium development to infectious hyphal growth. Its activity is mediated via the Nmr transcriptional co-repressor complex (NRC), which regulates the activity of GATA-family transcription factors. In eukaryotic organisms, GATA transcription factors are well-studied gene activators involved in important cellular and metabolic processes. They have high affinity binding for a motif of the consensus T/A (GATA) A/G sequence. We hypothesize that in *M. oryzae*, some GATA transcription factors might control pathogenicity-associated gene expression downstream of Tps1 and be essential for disease progression. However, which GATA factors are required for pathogenicity is not known. Previous yeast-two hybrid studies demonstrate that physical interactions occur between the NRC and at least two GATA transcription factors, Asd4 and Pas1. We therefore sought to characterize the role of these two GATA transcription factors in infection by *M. oryzae*.

Gene functional analysis of *PAS1* and *ASD4* was achieved by high throughput targeted gene replacement of the respective coding sequences, using the split marker strategy, which replaces the gene of interest by a selectable marker conferring sulphonyl urea resistance. Subsequent phenotypic analysis demonstrated morphological differences of the resulting  $\Delta asd4$  and  $\Delta pas1$  mutant strains compared to the wild type Guy 11 strain. Compared to Guy11,  $\Delta asd4$  mutant strains were significantly reduced in sporulation and radial growth, were severely retarded in appressorium formation and were non-pathogenic. In contrast,  $\Delta pas1$  mutant strains demonstrated increased radial growth and sporulation, compared to the wild type and were fully pathogenic. Intriguingly,  $\Delta pas1$  mutant strains alone formed appressoria on hydrophilic, non-inductive surfaces.

Therefore, we report using yeast two-hybrid analysis and split marker gene deletion strategies to identify two new genes that regulate appressorium development in *M. oryzae*: *ASD4*, a positive regulator that is essential for appressorium morphogenesis; and *PAS1*, a negative regulator that prevents the development of appressoria on non-inductive surfaces. This work provides new insights into the genetic regulatory network that enables *M. oryzae* to cause disease.

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Inhibition of Histone Deacetylase Causes Reduction of Appressorium Formation in the Rice Blast Fungus *Magnaporthe oryzae*

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Post-translational modifications (PTMs) are important for cellular functions. The regulation of histone acetyltransferases (HATs) and histone deacetylases (HDACs) is one of important PTMs for the epigenetic control, protein activity and protein stability. The regulation of acetylation of the N-terminal histone tails of core histone affects gene expression. Two class I HDAC genes and two class II HDAC genes have been identified in the *Magnaporthe oryzae* genome. Treatment with Rpd3/Hda1 family (classical) HDAC inhibitor inhibited the appressorium differentiation of *M. oryzae*. Treatment with trichostatin A, a classical HDAC inhibitor, also decreased pathogenesis. Furthermore, analyses of HDAC mutants indicated that MoHda1 and MoHos2 were required for vegetative growth and conidiation, and MoHos2 was required for appressorium formation. Disruption *MoRPD3* was unsuccessful, as the case with *Aspergillus nidulans RpdA*. These data indicated that HDACs have important roles in the asexual differentiation of *M. oryzae*.

## Involvement of Chitinase and Peroxidase in Basal Resistance of Rice Against Blast

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Plants naturally express variable levels of resistance against different groups of their pathogens. This kind of primary defense response is known as basal, general, partial, polygenic or multigenic resistance that is controlled by several genes. Basal resistance partially protects plants against challenge infection by pathogens and decreases the progress and destructive effects of disease. It is obtained by cooperation of multiple molecular and cellular defense responses and involvement of various signaling pathways.

In this study, the molecular and cellular changes of a partially resistant (CO39) and a susceptible Samba mahsuri (BPT 5204) rice line after infection with the hemibiotrophic blast fungus *Pyricularia oryzae* were compared. The expression of defense related genes such as chitinase (*RC24*) and peroxidase (*POCI*) in infected rice cultivars was investigated using semiquantitative reverse transcription-polymerase chain reaction (RT-PCR). This method revealed considerably elevated levels of expression for both genes in the partially resistant cultivar compared to the susceptible cultivar. Cytological observations of infected rice seedling samples revealed that lower level of disease symptoms in the partially resistant cultivar is associated with decreased plant colonization by the pathogen. One of the most prominent facets of basal plant defense responses is the formation of physical barriers at sites of attempted fungal penetration. These structures are produced around the sites of potential pathogen ingress to prevent pathogen progress in plant tissues. We investigated formation of lignin, as one of the most important structural barriers affecting plant resistance, using thioglycolic acid assay. A correlation was found between lignification and higher level of resistance in CO39 compared to BPT 5204 line. These findings suggest the involvement of chitinase, peroxidase, and lignin formation in defense responses of rice against *P. oryzae* as the most destructive fungal pathogen of rice worldwide.

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Characterization of a *Magnaporthe oryzae* MAS-3 Homolog and its Role in Pathogenicity

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*Magnaporthe oryzae* secretes a diverse range of proteins, of which some are likely virulence factors involved in the disease process. In order to identify more fungal genes that play a major role in this pathosystem, we undertook a global gene expression experiment using microarrays. Among the differentially expressed genes, we identified one with similarity to a MAS3 (*Magnaporthe* appressoria specific) virulence factor gene. This gene was induced at 72 hours post-inoculation of rice and barley, and during *in vitro* stress conditions of temperature up shift, oxidative, and nutrient limitations. Database searches with the sequence of this gene revealed the presence of a CAS (capsule-associated) transmembrane domain from *Cryptococcus neoformans*, a human pathogen. This gene also presented protein homology to two other previously described *M. oryzae* genes, termed *GAS1* and *GAS2*, which were involved in pathogenicity by decreasing the ability of the fungus to invade the plant. Targeted replacement of the MAS3-similar gene was performed in three *M. oryzae* strains and defects in appressorium formation and decreased pathogenicity in rice and barley were observed. Phenotypic characterization of the MAS3 mutants will be presented.

*AVR-Pia* of *Magnaporthe oryzae* is Expressed During Appressorium Formation and in Invasive Hyphae

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*AVR-Pia* is one of the *Magnaporthe oryzae* AVR genes and was cloned from strain Ina168 by the mutant-aided cloning strategy using the host specificity mutant Ina168m95-1 which gained virulence toward *Pia* (Miki *et al.*, 2009). *AVR-Pia* is 255 bp in length and encodes 85 aa with 19-aa-secretion signal peptide sequence, but its function is still unknown. Through the cloning of *AVR-Pia*, we found that *AVR-Pia* driven by constitutive *A. nidulans TrpC* promoter could not complement the avirulence in the mutant strain, but that by the *AVR-Pia* native promoter could. Another transformant of Ina168 which had two *AVR-Pia* genes driven by each of two promoters showed the avirulence. These results suggested that the timing of the *AVR-Pia* expression was important to activate the *Pia*-mediated plant immunity. Therefore we analyzed it using quantitative realtime PCR (qRT-PCR) in this study.

Ina168 possesses three copies of *AVR-Pia* with the native promoter. A spore suspension of Ina168 was inoculated onto either compatible or incompatible rice. Inoculated leaves were harvested in a time-dependent manner (0, 12, 18, 24, 30, 36 and 48 hours post inoculation) and total RNA was immediately extracted from them. The *AVR-Pia* expression was then analyzed by qRT-PCR and evaluated as the relative expression of *AVR-Pia* by compared with the constitutively expressed gene, *actin*. On both inoculation assays, the expression was first detected at 24 hours post inoculation. After that, on compatible rice, the expression was increased until 60 hours post inoculation, whereas it was reduced after 30 hours post inoculation on incompatible rice. These results indicated that the transcription of *AVR-Pia* is induced at 24 hours post inoculation, which corresponds to appressorium formation, and continuously expressed in invasive hyphae in compatible rice, suggesting the function of *AVR-Pia* as invasive effectors in compatible rice.

Reference:

Miki *et al.*, *Mol. Plant Pathol.* (2009) 10, 361-374

Protein Interaction Analysis of *AVR-Pia* Derived from *Magnaporthe oryzae* by Yeast Two Hybrid Assay

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The avirulence gene *AVR-Pia*, which induces homologous recombination (HR) of rice cultivars with the resistance gene *Pia* was isolated from *Magnaporthe oryzae* strain Ina168 (Miki *et al.*, 2009). *AVR-Pia* is 255-bp encoding 85 amino acids, including N-terminal 19 amino acids which are predicted to be signal peptides. Two resistance gene analogs *RGA4* and *RGA5* which have a nucleotide binding site and a leucine rich repeat (NBS-LRR) are located on *Oryza sativa* *Pia* locus and required for the function as *Pia*. Interaction of proteins encoded by these genes was investigated in this study.

The interaction between *AVR-Pia*, *RGA4* and/or *RGA5* was analyzed by using Matchmaker Gold Two Hybrid system (Clontech) which utilizes *HIS3*, *ADE2*, *AURI-C* and *MEL1* as reporter genes. Bait and Prey plasmids were constructed with In-Fusion Advantage PCR Cloning Kit (Clontech). Full length *AVR-Pia* (*AVR-Pia F*), *AVR-Pia* without signal peptide (*AVR-Pia-sp*), *RGA4* and *RGA5* were expressed independently in the yeast cells. The interaction between those proteins was investigated by reporter gene expression after mating of these yeast cells. All reporter genes were expressed in the mated cells between two *AVR-Pia-sp* as Prey and Bait. On the other hand, the interaction in other combination mating was not observed although the expression of proteins in the yeast cells was confirmed by the Western blotting. Therefore there is a possibility that *AVR-Pia* has the interaction between itself and dimer or oligomer of *AVR-Pia* participates in the plant cells during the infection. *AVR-Pia ΔC12*, truncated version of *AVR-Pia*, was constructed by insertion of a stop codon and C terminal 12 amino acids are deleted, which caused the loss of function of *AVR-Pia* in inoculation assay. The interaction between *AVR-Pia ΔC12* and itself was found to be relatively weak, showing slow growth on *HIS3* and *ADE2* selective media and no growth on the media with Aureobasidin A. These results suggest that the formation of *AVR-Pia* homo multimers is related to the function as *AVR-Pia* protein.

Reference:

Miki S., *et al.* (2009) *Mol. Plant Pathol.* 10, 361–374

## DNA Homologous Recombinational Repair Participates in the Growth and Pathogenicity of the Rice Blast Fungus

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One of the important issues in rice blast control is the emergence of a new pathogenic race in the field. Mutations of avirulence (AVR) genes are the direct cause of the appearance of a new race. To date, several avirulence (AVR) genes have been isolated from *M. oryzae*, and gene deletions or transposon insertions have been revealed as the mechanism of this mutation (Miki et al. 2009). In addition, chromosomal length polymorphisms are often observed in field isolates of the pathogen. The common feature among the above phenomena is the participation of DNA recombination. Therefore, DNA recombination is expected as an important factor participating in the variability of *M. oryzae*. In eukaryotes, two major systems for DNA recombination, homologous recombination (HR) and non-homologous end-joining (NHEJ) are known. In order to clarify the role of DNA recombination in pathogenesis, genes involved in DNA recombinational repair have been analyzed in this study.

*Rhm51*, *Rhm52* and *Rhm54* are orthologs for *S. cerevisiae* *RAD51*, *RAD52* and *RAD54*, respectively, that participate in HR pathway. *Rhm51* and *Rhm54* were deleted from strains Ina168 and Ina86-137 using pDEST system (Abe et al. 2006). The deletion mutants showed multiple defects such as retarded growth, elevated sensitivity to mutagen, reduced conidiation and reduced pathogenicity due to the defects in the appressoria development. On the other hand, *Khm70*, *Khm80* and *Mglig4*, which are orthologs of *Ku70*, *Ku80* and *LIG4* in the NHEJ pathway, are not required for virulence. *Rhm50*, the ortholog of *RAD50*, which participates in both pathways, was also disrupted. The deletion mutant showed severe defects in growth, appressoria development and pathogenicity.

In order to clarify the mechanism of defects observed in HR mutants, cytological analyses were performed using *rhm51* deletion mutants. DAPI nuclear staining of the developing appressoria revealed the intact nuclei in the germinated conidia 24 hours post inoculation (hpi) indicating the inhibition of the autophagy in  $\Delta$ *rhm51* mutants. Simultaneous staining of nuclei and septa of vegetative hyphae using propidium iodide and calcofluor white respectively, revealed the formation of mitotic nuclei in hyphal tips followed by septation in wild-type strains, which could not be observed in the  $\Delta$ *rhm51* mutants. Neutral comet assay revealed unrepaired double-strand breaks (DSBs) in vegetative hyphae of *rhm51* mutant. These results indicate that *M. oryzae* suffers DSBs during its life cycle and uses HR as the main repair pathway. The accumulation of unrepaired DSBs triggers the G<sub>2</sub> checkpoint and consequently retards vegetative growth and inhibits appressorium maturation and autophagy of germinated conidia in  $\Delta$ *rhm51* mutants.

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Analysis of Small RNA Libraries in *Magnaporthe oryzae*, and the Role of Different sRNA Biosynthetic Genes on Pathogenicity

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Endogenous small RNAs (sRNAs), including small interfering RNAs (siRNAs) and microRNAs (miRNAs) are critical components of gene regulation in many organisms. Understanding the molecular mechanism of biogenesis of sRNAs and their effect on the pathogenicity of an organism is important for gaining insight into host-pathogen interactions; namely, how pathogenicity mechanisms are regulated. We were interested in examining sRNA species and biosynthetic genes in *Magnaporthe oryzae*, and to what degree these elements regulated fungal stress responses as well as pathogenicity. To achieve these goals, we characterized small RNAs under different physiological stress conditions, which have not yet been examined, using the next generation sequencing method, SBS. The resulting libraries had datasets composed of more than 83000 distinct signatures mapping to coding sequences, retrotransposons, inverted repeats, tandem repeats and other repeats etc of the genome. The 24-nt class of sRNAs was predominant, likely reflecting a high degree of heterochromatic siRNAs. We also made targeted deletions of sRNA biosynthetic genes resulting in mutants having phenotypes different from wild type. The results obtained from deep sequencing studies and phenotypic analyses of several mutants are discussed in detail.

## Identification of Cell Wall-degrading Enzyme Genes in Fungi

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Cell wall-degrading enzymes (CWDEs) play significant roles throughout the fungal life including acquisition of nutrients and decomposition of plant cell walls. Even though a large number of fungal genomes are available, there is no systematic platform for dissecting CWDEs. To understand fungal CWDEs in kingdom level, we developed a new web-based pipeline (<http://phygh.riceblast.snu.ac.kr/>) to identify and classify CWDEs from fungal genomes. Six CWDE families were defined using 19 InterPro terms in the 91 characterized CWDEs from *Aspergillus* spp. Among the 20 genomes which contained more than 20 CWDEs, 14 were plant pathogens including *Fusarium* spp., *Stagonospora nodorum*, and *M. oryzae*. In contrast, 15 genomes out of 22 human pathogens including *Cryptococcus neoformans*, *Histoplasma* spp., and *Coccidioides* spp. contained only 2 CWDEs on average, which is natural in the way that composition of the animal cell wall is different from that of plant. Most CWDEs of six families were predicted as secretory proteins by the pipeline of the Fungal Secretome Database (FSD; <http://fsd.snu.ac.kr/>) reflecting that their target substrates could be met in extracellular space. In *M. oryzae*, comparisons with fungal ESTs managed by the Fungal EST Database showed that CWDEs were expressed at several stages of development: i) MGG\_01147.6 (alpha-N-arabinofuranosidase A) was found at infectious stage, ii) MGG\_09095.6 and MGG\_14726.6 (alpha-L-arabinofuranosidases) were expressed at appressorium stage, and iii) MGG\_02245.6 (xylanase), MGG\_05879.6 and MGG\_15479.6 (putative rhamnogalacturonan acetylsterase), and MGG\_01389.6 (alpha-N-arabinofuranosidase) were expressed at conidiation stage. Furthermore, their encoded proteins were predicted to have signal peptides except MGG\_01389.6, implying that these CWDEs could contribute to recognition and penetration of host plant surface. Taken together, the PHYGH will be a fundamental resource for understanding fungal CWDEs with the aid of EST, microarray, and mutant libraries.

Peroxisomal Protein Importing is Required for Fungal Pathogenicity in *Magnaporthe oryzae*

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Peroxisomes are single-membrane-bound organelles that compartmentalize many cellular activities in eukaryotes. Previous studies indicate that peroxisomal functions are important for plant infection in many phytopathogenic fungi. However, detailed relationships between fungal pathogenicity and peroxisomal functions still remain unclear. Here we report the importance of peroxisomal protein importing through Peroxisomal Targeting Signal 2 (PTS2) in fungal pathogenicity. One pathogenicity-defective mutant from *Agrobacterium tumefaciens*-mediated transformation library was identified that T-DNA was inserted in PTS2 receptor gene of *Magnaporthe oryzae*, *MoPEX7*. Gene disruption of *MoPEX7* abolished the peroxisomal localization of a thiolase (MoTHL1) containing PTS2, supporting its role in peroxisomal protein importing machinery.  $\Delta$ *Mopex7* showed significant reduction in mycelial growth on media containing valerate or butyrate as a sole carbon source.  $\Delta$ *Mopex7* produced less numbers of conidiophores and conidia, but conidial germination was normal. Conidia of  $\Delta$ *Mopex7* were able to develop appressoria, but they failed to cause disease in plant cells except for wound inoculation. Appressoria formed by  $\Delta$ *Mopex7* showed a defect in turgor generation due to delay in lipid degradation and increased cell wall porosity during maturation. Taken together, our results suggest that MoPEX7-mediated peroxisomal matrix protein importing system is required for pathogenicity of *M. oryzae*.

Preparation and Analysis of *AVR-Pia* Product from *Magnaporthe oryzae*

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*AVR-Pia*, the AVR gene in *M. oryzae* that triggers HR in rice cultivars with the *Pia* R gene, was selected for the present *in vitro* biochemical and structural study. *Pia* was found originally in the Japanese rice cultivar Aichi-asahi, which is one of the Japanese blast differential cultivars, and has been introduced subsequently into many Japanese cultivars, including multilines. R-gene analogs RGA4 and RGA5, were found in *O. sativa* *Pia* locus and both were necessary for *Pia* function. Our interest here is to clarify how the product of *AVR-Pia* gene is recognized by the product of the R-gene analogs, in molecular level.

In order to obtain protein samples, we tried to prepare recombinant proteins of these genes. We tried refolding method to obtain *AVR-Pia* protein from inclusion bodies in *E.coli*. The *AVR-Pia* protein was successfully purified by using size exclusion chromatography and purified protein was evaluated using the circular dichroism. The *AVR-Pia* protein showed that most of the protein forms random coil region, suggesting this protein could be an intrinsically disordered protein and structure formation is promoted on binding with its counterparts. Crystallization for the X-ray study has been tested with sparse matrix method with sitting drop procedure after concentrating the protein up to 10 mg/mL. On the other hand, from the DNA sequences of RGA4 and RGA5, it is proposed that there is an ATP binding domain followed by a leucine rich region (LRR). Leucine rich region is expected to form a leucine rich repeat that is famous for its capability to recognize a wide range of different molecules including proteins, nucleic acids, and lipopolysaccharides, thus can be important for *AVR-Pia* recognition. We designed several constructs from RGA4 that cover the ATP domain, LRR and the whole protein region for the *E.coli* expression system. The detail of the properties of these proteins will be discussed.

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Understanding the Function of *AvrPiz-t* in Pathogenesis and Host Defense Interference in Rice

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Plant diseases are one of the major limitations for stable crop production in the US and other countries. Frequent applications of chemicals for disease control not only significantly reduce farmers' profits but also damage the environment. Elucidation of the molecular basis of the host and pathogen interaction is a key to design novel strategies for breeding new resistant cultivars. Our laboratory cloned three broad-spectrum blast resistance genes in rice (*Pi2*, *Pi9* and *Piz-t*) and also cloned the *AvrPiz-t* gene from *Magnaporthe oryzae*, which is the avirulence gene of *Piz-t*. When *AvrPiz-t* was expressed in the leaves of *Nicotiana benthamiana*, it significantly suppressed programmed cell death (PCD) that was triggered by the mouse BAX protein in *Agrobacterium tumefaciens*-mediated transient assays, suggesting that *AvrPiz-t* may contribute to virulence fitness in the fungus by suppressing the basal defense signaling in plants. To search for the host targets of *AvrPiz-t*, we recently performed yeast-two-hybrid (Y2H) screens using *AvrPiz-t* as the bait and twelve putative interacting proteins called *AvrPiz-t* interacting proteins (APIPs) were identified. Among them, three are E3 ligases that are involved in ubiquitination-mediated protein degradation pathway. All three APIPs have E3 ligase activity and can ubiquitinate *AvrPiz-t* in vitro. In return, *AvrPiz-t* interferes with APIP's E3 ligase activity. These data support a growing body of evidence that pathogen effectors may target the ubiquitination-mediated protein degradation system in the host to interfere with the defense response.

*MoRAP1* Is Required for Appressorium Development and Pathogenicity in *Magnaporthe oryzae*Wang, Y.<sup>1,2</sup>, Liu, W.<sup>2</sup> and Xu, J.-R.<sup>2</sup><sup>1</sup>College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, CHINA<sup>2</sup>Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, USA

In various eukaryotic organisms, intracellular cyclic adenosine monophosphate (cAMP) and the cAMP-dependent protein kinase PKA play important roles in cell growth and differentiation. While cAMP can stimulate cell growth in many cell types, it inhibits growth in others, which may be related to specific mechanisms involved in the inhibition or activation of MAP kinases by cAMP. In mammalian cells, RAP1 is a member of Ras family and one downstream target of cAMP signaling regulates MAP kinase activities in a cell-specific manner. In *Magnaporthe oryzae*, a hemitrophic ascomycetous pathogen that causes great yield losses on rice throughout the world, the cAMP signaling and *Pmk1* MAP kinase pathways are known to regulate the recognition of hydrophobic surfaces and appressorium formation, respectively. However, it is not clear about the interaction between these pathways and how the surface recognition signal is relayed to the activation of *Pmk1*. In this study, we identified and characterized the *RAP1* ortholog in *M. oryzae* (*MoRAP1*).

RAP1 orthologs are well conserved in filamentous fungi. *MoRAP1* shares its highest homology with the predicted gene NCU02167 in *Neurospora crassa*. The ligation-PCR approach was used to delete the *MoRAP1* gene in *M. oryzae*. The *Morap1* mutant had no defects in vegetative growth and colony morphology. However, it was reduced in the efficiency of appressorium penetration in penetration assays with onion epidermal cells. It also was reduced in virulence in infection assays with rice seedlings. On artificial hydrophobic surfaces, the *Morap1* mutant was delayed in germination and appressorium formation. Although they were melanized, a majority of the appressoria formed by the *Morap1* mutant were longer but narrower than the wild-type appressoria. On hydrophilic surfaces, the *Morap1* mutant still responded to exogenous cAMP and formed appressoria. However, the rate of induced appressorium formation in the mutant was much lower than that of the wild type. In addition, over 80% of *Morap1* appressoria induced by cAMP were morphologically abnormal. These results indicate that *MoRAP1* is not important for surface recognition and cAMP signaling. However, it plays a critical role in normal appressorium morphogenesis and virulence. Further characterization of the *MoRAP1* gene and its role in the activation of *Pmk1* or *Mps1* MAP kinases are in progress.

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Systematic Characterization of Chitin Synthase Genes in the Rice Blast Fungus *Magnaporthe oryzae*

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Chitin and beta-glucan are two major components of fungal cell wall and well-known fungicide targets. Unlike the glucan synthase genes, filamentous fungi normally have multiple chitin synthase genes. Some of them may play regulatory roles in fungal development and pathogenesis. In the rice blast fungus *Magnaporthe oryzae*, there are eight chitin synthase genes named *MoCHS1* to *MoCHS8*. To determine their roles in *M. oryzae*, we assayed the expression profiles and generated targeted deletion mutants of these *MoCHS* genes. None of the *MoCHS* genes was required for appressorium formation and colony surface hydrophobicity. *MoCHS3*, *MoCHS5*, and *MoCHS7* are important for virulence. Deletion of the *MoCHS1*, *MoCHS4*, or *MoCHS5* gene had no obvious defects in growth, conidiation, appressorium formation, and virulence, indicating that these three genes are dispensable or may have overlapping functions with other chitin synthase genes. The  $\Delta Mochs2$  mutant was slightly reduced in vegetative growth but was as virulent as the wild type. In addition to the reduced growth rate, the  $\Delta Mochs8$  mutant was reduced in appressorium formation on artificial surfaces but it was normal in invasive growth on rice leaf sheath penetration assays and virulence on rice seedlings. Therefore, the *MoCHS2* and *MoCHS8* genes are not essential for plant infection. The  $\Delta Mochs3$  mutant was significantly reduced in conidiation and it produced conidia with abnormal morphology. Most of the  $\Delta Mochs3$  conidia had no or one septum and rounded spore tips. Because it had no obvious changes in growth and colony morphology, *MoCHS3* appeared to play a specific role in conidiogenesis. Localization of MoCHS3-eGFP to the tip of developing conidia was observed. On artificial surfaces, only a small percentage of  $\Delta Mochs3$  conidia could germinate and form appressoria. In rice and barley infection assays, the  $\Delta Mochs3$  mutant was significantly reduced in virulence although appressoria formed by the mutant still penetrated and produced infectious hyphae in rice leaf epidermal cells. The  $\Delta Mochs5$  and  $\Delta Mochs7$  mutants also were significantly reduced in virulence but they produced normal pyriform conidia. The  $\Delta Mochs5$  mutant was significantly reduced in hyphal growth and conidiation. Most appressoria formed by the  $\Delta Mochs5$  mutant failed to develop penetration pegs. The  $\Delta Mochs7$  mutant was defective in appressorium formation on artificial surfaces but formed melanized appressoria on the rice sheath. These data indicate that the *MoCHS* genes play diverse roles in growth, conidiogenesis, and pathogenesis in *M. oryzae*. Double mutants of selected MoCHS genes based on their expression profiles and phylogenetic analysis have been generated and will be presented.

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## Application of Next Generation Sequencing to Study Rice Blast Disease

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In Brazil rice blast fungus caused by *Magnaporthe oryzae* is a major constraint in production and causes losses of up to 100% of the yield depending on cultivar susceptibility, environmental conditions and crop management system. The molecular basis of the defense response to rice blast remains poorly characterized. A thorough understanding of the molecular response mechanisms against rice blast may provide new methods in devising strategies to control rice blast disease. The identification of host genes involved in the early defense responses is one of the most critical steps leading to the elucidation of resistance mechanism in plants. The recent advent of tools enabling the transcriptional profiling of infected plant tissues using next-generation sequencing methods provides an unprecedented depth of analysis permitting application of powerful statistical techniques for discovery of functional relationships among treatments.

We used the Illumina/Solexa technology to study gene expression of rice after infection with *M. oryzae*. This ultra high-throughput sequencing technology produced a digital expression profile with millions of short reads for each treatment. mRNA populations extracted from treatments were subjected to the Genome Analyzer with a total of 16,995,679 signatures being generated reflecting the depth at which the treatments have been sampled. Then individual signatures counting were performed to assign the quantitative variation between treatments to assign the gene expression level. A total of 711,284 distinct signatures were generated, showing a higher number than previously reported. Signatures covered 35% of the total rice genome. Taking advantage of the information content in the 21-bp tag, it was identified signatures from *M. oryzae* messages among the total signatures isolated from blast-infected rice leaves, representing 10% of the analyzed transcript in the blast-infected rice leaves. This opens a possibility to directly study the gene expression of two organisms at the foci of interaction. The signatures that could not be assigned to known transcripts are a rich source of information about the part of the transcriptome that is not yet characterized. Comparison among treatments was performed and then 200 more expressed genes were selected and twenty were used to run qRT-PCR. The comparison of the infection treatments obtained with digital expression profiles (quantification of transcripts) to those obtained with RNA extracted leaves, highlighted the same expressed rate when comparing distinct treatments. Among a subset of 20 these genes, 70% were validated by qRT-PCR. The results presented here are a preliminary analysis of these data. Currently, there are ongoing projects to study and perform detailed functional analysis of the selected candidate genes. Illumina technology promises to be a valuable addition to the repertoire of methodologies for functional genomics.

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A Novel Pathogenicity Gene Is Required in the Rice Blast Fungus to Suppress the Basal Defenses of the Host

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For successful colonization and further reproduction in host plants, pathogens need to overcome the innate defenses of the plant. We demonstrate that a novel vacuolar protein gene, *DESI*, in *Magnaporthe oryzae* regulates counter-defenses against host basal responses. The *DESI* gene was identified by screening for pathogenicity-defective mutants in a T-DNA insertional mutant library. This gene encodes a serine-rich protein that has unknown biochemical properties, and its homologs are strictly conserved in filamentous *Ascomycetes*. Targeted gene deletion of *DESI* had no apparent effect on developmental morphogenesis, including vegetative growth, conidial germination, appressorium formation, and appressorium-mediated penetration. The *Δdes1* mutant, however, was hypersensitive to exogenous oxidative stress, and the activity of extracellular enzymes including peroxidases and laccase were severely decreased in the mutant. In the interaction with a susceptible rice cultivar, the *Δdes1* mutant displayed a significant reduction in infectious hyphal extension, which caused a reduction in pathogenicity. Rice cells inoculated with the *Δdes1* mutant exhibited strong defense responses accompanied by brown granules in primary infected cells, the accumulation of reactive oxygen species (ROS), the generation of autofluorescent materials, and pathogenesis related gene induction in neighboring tissues. Notably, the suppression of ROS generation by treatment with diphenyleneiodonium (DPI), an inhibitor of NADPH oxidases, resulted in a significant reduction in the defense responses in rice tissues challenged with the *Δdes1* mutant. Furthermore, the *Δdes1* mutant recovered its infectious growth in DPI-treated plant tissues. These results suggest that *DESI* functions as a novel pathogenicity gene that regulates the activity of fungal proteins, compromising ROS-mediated plant defense.

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GEMO: a Project on Evolutionary Genomics of *Magnaporthe oryzae*

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Developing integrated control methods against pests of cultivated plants can significantly contribute to increasing food production while reducing inputs threatening the environment. The durability of a control method can be improved by a better knowledge of the pathogen's genetic determinants that are responsible for this adaptation. We were granted by the French National Research Agency for a project that aims at sequencing the genomes of several strains of the phytopathogenic model species *Magnaporthe oryzae*, exploiting these complete sequences to characterize the repertoire of genes involved in pathogenicity and host specificity, and studying their evolution. We will sequence 7 strains of the species *M. oryzae* representing different genetic groups pathogenic of different species of Poaceae and one strain of the sister species *M. grisea*. ESTs produced during the infection by two strains pathogenic of rice and wheat on their respective host will also be sequenced. Different available annotation pipelines will permit to list and do comparative analyses of different gene families known or speculated to be involved in pathogenicity. Transcriptomic data of the two strains with different host specificities will be compared to identify key genes in specialization to the host. Genome fluidity will be characterized by synteny analyses and by the identification and localization of repeated elements. The impact of these rearrangements on pathogenicity genes and host specificity genes will be tested. Molecular signatures of positive or purifying selection in coding and regulatory sequences will be searched for by different methods. The whole set of data will be integrated in a database that will be designed to be publicly accessible.

*MoSFL1*, Transcription Factor Involves Pathogenicity and Heat Sensitivity in *Magnaporthe oryzae*

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Rice blast caused by the ascomycete *Magnaporthe oryzae* is one of the most devastating diseases that affects rice production worldwide. Like many other foliar pathogens, *M. oryzae* produces appressoria for plant penetration. Appressorium formation is regulated by the Mst11-Mst7-Pmk1 MAP kinase cascade. *MST12*, one transcription factor regulated by *Pmk1*, is important for penetration and invasive growth but dispensable for appressorium formation. Transcription factors other than *MST12* must be responsible for regulating appressorium formation by the *Pmk1* pathway. In this study, we functionally characterized the *MoSFL1* gene that encodes a putative downstream transcription factor of *Pmk1*.

*MoSfl1* is orthologous to yeast *Sfl1*. It was first identified as a protein that was phosphorylated by *Pmk1* but not by *Mps1* or *CpkA* in phosphorylation assays with a *M. oryzae* protein chip. Like other *Sfl1* orthologs, the *MoSfl1* protein has the HSF-like domain. When expressed in yeast, *MoSFL1* functionally complemented the flocculation defects of the *sfl1* mutant. The interaction between *Pmk1* and *MoSfl1* was further confirmed by co-immunoprecipitation assays. *MoSfl1* also has one MAP kinase-docking site and three putative MAP kinase phosphorylation sites. The *Mosfl1* deletion mutant had normal growth rate but was slightly reduced in conidiation. It also was normal in conidium formation, appressorium formation, which is different from the *cpkA* or *pmk1* mutant. However, in both infection assays with rice or barley leaves, the *Mosfl1* mutant was significantly reduced in virulence. Consistent with this observation, the *Mosfl1* mutant was defective in invasive growth in penetration assays with rice leaf sheath epidermal cells. When cultured at 30°C, the *Mosfl1* mutant was more sensitive to the elevated temperature. Similar defects were observed in the *pmk1* mutant. When assayed by qRT-PCR, the transcription levels of the *HSP30* and *HSP98* genes were reduced 10- and 3-fold, respectively, in the *Mosfl1* mutant, indicating that the *MoSfl1* transcription factor may regulate responses to heat shock and other stresses in *M. oryzae*. Further characterizations of the *Mosfl1* mutant and structural elements of the *MoSFL1* genes are in progress and will be presented.

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Li, Y., Su, C., Mao, X., Cao, F., and Chen, J. 2007. Roles of *Candida albicans* Sfl1 in Hyphal Development. *Eukaryotic Cell* 6:2112–2121

Ubiquitin Mediated Protein Turnover is Essential for Fungal Development and Pathogenicity in  
*Magnaporthe oryzae*

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Rice blast is the most important disease of rice worldwide, and is caused by the filamentous ascomycete fungus, *Magnaporthe oryzae*. Ubiquitin-mediated protein degradation is highly conserved and is known to regulate a number of biological processes including cellular differentiation and pathogenesis in fungi. Gene expression analysis during infection structure development and nitrogen starvation of *M. oryzae* revealed that a number of ubiquitination associated genes, including a polyubiquitin encoding gene, *MGG\_01282*, were regulated developmentally as well as in response to nitrogen starvation. Inhibition of ubiquitin-proteasome dependent proteolysis using the 26S proteasome inhibitor, bortezomib, significantly delayed spore germination and appressorium formation of *M. oryzae*. In addition to a significant reduction in protein, ubiquitination as determined by immunoblot assays, targeted gene deletion of *MGG\_01282* resulting in pleiotropic effects on *M. oryzae* including abnormal spore morphology, reduced growth and sporulation, reduced germination and appressorium formation and the inability to cause disease. Our study suggests that ubiquitin-mediated proteolysis, which is known to be highly selective, plays an important role in nutrient assimilation, morphogenesis and pathogenicity of *M. oryzae*.

## A 14-3-3 Protein Negatively Regulates Cell Death and Resistance

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The 14-3-3 protein family is found in all eukaryotes and is implicated in diverse biological functions including cell cycle regulation and signal transduction. In rice, the 14-3-3 gene family has at least eight members, named *GF14a-h*. One member, *GF14e*, colocalizes with disease resistance QTL in multiple mapping populations. *GF14e* expression, as measured by reverse transcriptase PCR (RT-PCR) in susceptible and resistant rice lines, did not show differences correlated with host-pathogen interactions. Silencing of *GF14e* by RNAi in a stable transgenic rice line resulted in a lesion mimic phenotype that appears at approximately 25 days after sowing. Other 14-3-3 family members (*GF14b* and *GF14c*) showed downregulation in the silenced lines, though to a lesser degree than *GF14e*. The silenced lines exhibit enhanced resistance (reduced bacterial numbers and shorter lesions) to the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae*; and the enhanced resistance is observed prior to the appearance of lesion mimic lesions. Silenced plants with lesion mimic phenotypes also showed enhanced resistance to the sheath blight pathogen *Rhizoctonia solani*. Current experiments are focused on understanding the role of *GF14e* in cell death by exploring potential interactions between the *GF14e* protein and plant defense response proteins.

Expression Profiling of Common and Specific Defense Responses of Rice to *Magnaporthe oryzae* Infection Using Deep Sequencing Technologies

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Rice blast caused by *Magnaporthe oryzae* is a serious disease in rice production. Wild type Nipponbare and transgenic rice plants (carrying the *Pi9* blast resistance gene) were challenged with the rice blast strain KJ201 to identify the early, mid and late host responses to *M. oryzae* infection at the transcriptional level. Deep transcriptome analysis was performed using Illumina's massively parallel signature sequencing (MPSS) and sequencing-by-synthesis (SBS) technologies. Thirteen MPSS and eight SBS libraries were constructed from compatible and incompatible interactions to identify putative genes involved in host susceptibility and resistance mechanisms. About 1 to 1.5 million and 2 to 12 million signatures were obtained from each library using MPSS and SBS technologies, respectively. More than 80% of the signatures matched the Nipponbare genomic sequence. About 70% of the signatures identified overlapped in the MPSS and SBS libraries. About 30% more transcriptome data was generated using SBS compared to MPSS. Functional classification of the genes expressed in these libraries was carried out using KEGG (<http://www.genome.jp/kegg/>). RT-PCR was performed to validate putative early, mid and late responsive host susceptibility genes. Transcription factors in rice and secreted proteins from *M. oryzae* expressed in the compatible and incompatible interactions were also analyzed. The comprehensive transcriptome data obtained in this study have built the foundation for in-depth elucidation of the molecular basis of defense responses and the development of cultivars with durable blast resistance.

Mapping of MAP Kinase Interactome in *Oryzae sativa* Reveal Diverse Putative Signaling Pathways

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Mitogen-activated protein kinase (MAPK) cascade is a complex and fundamental signaling module in eukaryotic cells. They function downstream of sensors/receptors and regulate cellular responses to various external and endogenous stimuli. To date the identity of regulatory components that link sensors/receptors to target genes and other cellular response in case of *Oryzae sativa* is not well studied. However, various studies have proved several molecules, including receptors, kinases and transcription factors, as suitable candidates for common players that are involved in crosstalk between various stress signaling pathways. Hence, it is crucial to identify the regulatory component of Map Kinase cascade and their interacting partners to better understand the signaling pathway mediated by these Map kinases upon reception of various stress.

Here, using stringent, medium-scale yeast two hybrid system, we have generated a protein-protein interaction map of Mitogen Activated Protein Kinase (MAPK) cascade genes of *Oryzae sativa* by indentifying 60 different novel interactors. For testing protein-protein interactions we used high density rice cDNA library as bait and others MAPKKs and MAPKs as prey. Our analyses revealed *OsMPK1* as a major gene in this interactome which interacts with 50 different novel stress related proteins. Further, some of these interactions were confirmed in planta by bimolecular fluorescence complementation and in vitro by pull down analysis. Most of the interacting proteins belong to the functional categories of various biotic and abiotic stresses like drought, salinity, metal, light, defense and hormone related signaling pathway. Additionally, various protein phosphatases were also identified which are known to repress the particular signaling by dephosphorylating the Mapk Cascade genes. Subcellular localization analysis of Map Kinase proteins and their interactors showed same subcellular localization in most of the cases, prerequisite to being of biological relevance. In addition, resistance or susceptible phenotypes obtained from pathogenicity test of each interacting MKKs mutants support the hypothesis of potential roles of MPK signaling cascade in defense response. We hypothesize that signaling cascades control transcription through a set of common response pathways and subsets of highly specific targets activated by combinations of RLKs, MKKs and MPKs. Hence, we anticipate that our MAPK interactome allows the exploration of the nature of protein-protein interaction in rice and provides a basis for future functional characterization of these interactions.

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## Role of Rice Endogenous Peptide Elicitors in Defense Signaling and Disease Resistance

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Plant disease resistance is generally initiated by host perception of pathogen-associated molecular patterns (PAMP) or effectors and mediated by salicylic acid (SA), jasmonic acid (JA), ethylene (ET) and/or abscisic acid (ABA) signaling pathways. Although extensive efforts have been made to study PAMPs, effectors and hormone-mediated host defense pathways, little is known about the role of endogenous plant peptide elicitors in activation and amplification of defense signaling and disease resistance response. Recently, a small family of *Arabidopsis* peptide elicitors (AtPeps) was shown to regulate defense gene activation and basal resistance. In this study, we have identified a similar family of endogenous peptide elicitors from rice and attempt to elucidate their role in activation of defense signaling and rice disease resistance.

The rice genome contains seven peptide elicitor precursor genes (*OsPROPEP1-7*), encoding putative mature peptides with lengths ranging from 25 to 42 amino acids. Based on publicly available rice microarray data and our quantitative RT-PCR analysis, *OsPROPEP1*, *4*, and *7* were found to be induced significantly by *Magnaporthe oryzae* infection, wounding, JA and/or ET treatments. A 25-amino acid peptide, *OsPep7* (putative mature product of *OsPROPEP7*), was synthesized and is being tested for its activation of defense genes and rice blast resistance. Transgenic rice lines defective in JA, ET and ABA pathways are being used to determine which defense pathway(s) might mediate the endogenous peptide signaling. In addition, we have generated more than twenty transgenic rice lines with overexpression of *OsPROPEP7* via the *Agrobacterium*-mediated rice transformation. Preliminary observations indicate that overexpression of *OsPROPEP7* may lead to reduced plant growth and yellowish leaves. Further evaluation of transgenic plants is being conducted for altered defense gene expression and basal resistance against *M. oryzae* and other rice pathogens. Besides the PAMP- and effector-triggered immunity, genetic manipulation of endogenous plant peptide elicitors (one type of damage-associated molecular pattern) may provide an alternative approach for broad-spectrum disease resistance.

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## Effects of Combination Usage of Two Resistance Genes on Rice Blast Lesion Expansion

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For the effective use of various resistance genes characterized by quantitative reduction in blast development, we examined the efficacy of combinations of two genes between three blast resistance genes, *Pi34*, *Pi35(t)*, and *pi21*. We used near isogenic lines (NILs) with similar genetic background of the rice cultivar Koshihikari except for the blast resistance, and measured the leaf blast suppression by resistance genes. We compared the ability to suppress blast disease of a NIL with a single resistance gene and a NIL with two resistance genes. Penetration of blast fungus into rice epidermal cells was observed 40h after inoculation. Significant difference of penetration frequency was not observed between all rice lines used. However, growth rates of infection hyphae at 40h after inoculation varied among rice lines used. In a NIL with a single resistance gene, infection hyphae grew slower than in Koshihikari. In a NIL with both *Pi34* and *Pi35(t)*, growth rate of infection hyphae was a little bit lower than in a NIL with *Pi34* or in a NIL with *Pi35(t)*. We also measured the lesion length 20 days after spot inoculation. *Pi34* and *Pi35(t)* significantly decreased the lesion length while *pi21* did not. Lesion length in a NIL with both *Pi34* and *Pi35(t)* was shorter than that in a NIL with *Pi34* or in a NIL with *Pi35(t)*. Combination usage of *Pi34* and *Pi35(t)* suggested to have more suppressive effect on leaf blast expansion than single use of these two genes.

The Evolutionary Assay of the *Pi2/9* Locus Against the LRR Polymorphism in 28-zhan and Six Other Cultivars

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Most plant disease resistance (R) genes encode intracellular proteins with nucleotide-binding site and leucine-rich repeat (NBS-LRR), and related putative amino-terminal signaling domains. They are termed NBS-LRR proteins. The LRRs of a wide variety of proteins from many organisms serve as protein interaction platforms, and as regulatory modules of protein activation. Genetically, the LRR region rather than the NBS domain determines specificity to pathogen elicitors. It is the most variable region in closely related NBS-LRR proteins and is under selection to diverge and can lead to plant cell death in the form of the familiar hypersensitive response (HR).

28-zhan is an *indica* variety which was widely used as a blast resistant donor for local rice breeding in South China. Most of the varieties derived from this donor appeared broad spectrum and had stable resistance to rice blast. A new member, temperately denominated as *Pie(t)* of blast resistant genes family at *Pi2/9* locus, was identified in 28-zhan and finely tagged at the region of the reference clone AP005659. Since at least 7 rice blast R genes (*Pi2*, *Pi9*, *Pie(t)*, *Pigum2(t)*, *Pigum4(t)*, *Piz*, and *Piz-t*) were identified in the target region, it is necessary to analyze the evolutionary relation of the target gene and other related R genes in the special region.

In order to survey the evolutionary relation of the target genes at *Pi2/9* locus in the seven representative cultivars including 28-zhan (*Pie(t)*), C101A51 (*Pi2*), Toride 1 (*Piz-t*), 75-1-127 (*Pi9*), Fukunishiki (*Piz*), Gumei 2 (*Pigm2(t)*) and Gumei 4 (*Pigm4(t)*), two marker primers, based on the *Pi2* and *Pi9* LRR region sequences, were used to amplify the related LRR region sequences of the seven cultivars. The evolutionary relations of these seven LRR genes were analyzed by ClustalW software. The results indicated that these genes were classified into four distinct subgroups: *Pie(t)/Piz-t* (I), *Pi2*(II), *Piz/Pi9*(III), *Pigm2(t)/Pigm4(t)* (IV). Race specific resistances of the seven R genes were also tested. Genotyping and phenotyping data provides new insight into the evolution of the race specificity resistance to rice blast.

Cloning and Sequence Analysis of the *Pi-ta* allele in Wild Rice Species in Yunnan Province of China

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Rice blast is one of the most serious diseases in rice production. This study was designed to explore new resistant resources from wild relatives of rice, specifically Jinghong erect type of *O. rufipogon* with the strongest blast resistance for modern rice breeding. In the present study, resistance sources to rice blast in *O. rufipogon* from different areas within Yunnan were evaluated with 8 strains of rice blast fungi.

The *Pi-ta* gene in rice is one of the efficient and broad-spectrum major blast resistance genes deployed worldwide. Three pairs of DNA primers were synthesized based on DNA sequences of the *Pi-ta* gene (AF207842 and AK066558) to amplify the target gene fragments by polymerase chain reaction (PCR). Two fragments at 4,672 bp and 3,961 bp were amplified by PCR respectively from Jinghong erect type with purple-stalk and green-stalk. The fragment 4,672 bp including the entire open reading frame (ORF), intron and 331 bp downstream after termination codon has been cloned from Jinghong erect type with purple-stalk. Sequence analysis suggested that the fragment shares 99.86% homology with the corresponding regions of the reported rice cultivar Yashiro-mochi. Compared with the *Pi-ta* gene from Yashiro-mochi, there are 4 nucleotide differences in the coding region and 6 in intron of the *Pi-ta* gene, which lead to three amino acid changes. But the functional amino acid Alanine at 918 of *Pi-ta* is the same between purple-stalk wild rice and Yashiro-mochi suggesting the *Pi-ta* gene from Jinghong erect type with purple-stalk is a resistance *Pi-ta* allele. However, the fragment 3,961 bp was found to carry a portion of the *Pi-ta* gene from green-stalk without the functional amino acid suggesting that there is a new *Pi-ta* allele in green stalk. Research progress in determining the function of this allele will be presented.

## A Monogenic Differential Set for Blast Resistance and Fine-mapping of Some Novel Resistance Genes in Rice

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Rice blast, caused by *Magnaporthe oryzae* (*M. oryzae*), is one among the most devastating diseases of rice worldwide. Knowledge of the pathotype composition and variation of the pathogen in rice fields is essential for rational development and deployment of rice resistant cultivars. A number of near isogenic lines (NILs) and monogenic lines (MLs) have been developed in the world so far. We evaluated 24 LTH (Lijiangxintuanheigu)-MLs and 6 LTH-NILs for their resistance to 585 isolates collected from different rice cropping regions of China, and screened 12 MLs/NILs as monogenic differentials by clustering and principal factor analysis of the 30 MLs/NILs combining with their resistance-spectrum analysis, *i.e.*, IRBLks-S, IRBLi-F5, IRBLta-K1, IRBLta-CT2, IRBLkp-K60, IRBL5-M, IRBL12-M, F-80-1(*Pik*), F-128-1(*Pita-2*), IRBLb-B, IRBL1-CL and IRBLz-Fu. A multiple regression model showed these 12 lines had very good representation for the 30 MLs/NILs. This differential set could differentiate 375 *indica*-derived, 210 *japonica*-derived, and total 585 tested isolates into 225, 112 and 301 pathotypes, respectively, and showed higher differentiating ability to both *indica*- and *japonica*-derived blast isolates than did other sets of differentials once used in China. We suggest that these selected differentials could be applied as a common differential set for *M. oryzae* pathotype assay in China.

In addition, we pay much attention to exploitation of rice germplasms and genes with broad- spectrum or durable resistance to rice blast. By now, we have identified and finely mapped several blast resistance genes, including two genes *Piym1(t)* and *Piym2(t)* from one *japonica* landrace, three genes *Pi93-1(t)*, *Pi93-2(t)* and *Pihm(t)* from two *indica* cultivars. Among them, the genes *Piym1(t)*, *Pi93-1(t)* and *Pihm(t)* were deduced to be novel blast resistance genes, which were anchored within 50 – 150 kb regions on chromosomes 4, 11 and 2, respectively. Cloning of these finely mapped genes is underway.

Molecular and Cellular Characterization of a Koshihikari NIL, Introducing the Partial Resistance Gene *Pi34*

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The partial resistance gene *Pi34* is proposed to confer durable resistance to blast in rice. To investigate mechanisms of the partial resistance genetically, transcriptome analysis between Koshihikari and *Pi34*-NIL was conducted with SuperSAGE method (Matsumura et al. 2003), resulting in libraries containing 218,443 tags. Significant tags were selected with statistical method and their expression profiles were investigated. Two tags, located in the mapped region of *Pi34* (Zenbayashi et al. in this conference), OMG-01 and OMG-02, presented distinct expression depending on the presence of *Pi34*. Expression pattern of OMG-01 was constitutive, whereas OMG-02 was infection inducible. We speculated that OMG-02 was the primary candidate of *Pi34* because of its sequence and expression.

To analyze the partial resistance microscopically, we observed penetration of *Magnaporthe oryzae* to rice leaf blades with whole cell clearing method (Koga and Kobayashi 1980). *Pi34*-NIL and Chubu32, which is a donor cultivar of *Pi34*, did not inhibit penetration of *M. oryzae*. We also investigated penetration and infection in rice by intact leaf sheath inoculation method (Koga et al. 2004). The epidermal cells of *Pi34*-NIL did not prevent *M. oryzae* from penetrating but infecting to adjacent rice cells. Cell death and accumulation of active peroxide in epidermal cells of *Pi34*-NIL under appressoria showed similarity to that of *Pib*-NIL. Consequently, *Pi34* presented common cytological phenotypes with *Pib*, which classified into the true resistance gene, except for penetration.

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Kito, H., Zenbayashi, K., Koizumi, S., and Nakajima, T. 2009. Control effects of the field resistance gene *Pi34* in infection with rice blast disease. Ann. Rept. Prot. North Japan. 60:25-29.

Fine Mapping of the Partial Resistance Gene to Blast, *Pi34*, and Analysis of its Gene-for-Gene Relationship in Rice

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Partial resistance to blast is characterized by the reduction of the extent of pathogen reproduction in compatible combination. To date, a few partial resistance genes have been precisely mapped or isolated, e.g. *pi21*, *Pi39* and *Pb1*. The *japonica* rice cultivar Chubu32 has a high level of partial resistance to blast, which is mainly controlled by a dominant gene *Pi34*, located on chromosome 11. To identify and isolate *Pi34*, we conducted high-resolution genetic and physical mapping. Chubu32 was crossed with blast susceptible 98G-98-5. From 4,012 segregating individuals, 213 recombinants in the *Pi34* region were screened by using PCR-based markers. Resistance was also tested in the field and greenhouse. We constructed a bacterial artificial chromosome library of Chubu32 and selected the clone containing *Pi34*. The size of *Pi34* region in Chubu32 was estimated 67kb by RFLP map, and four expressed genes were identified in this region by RT-PCR. This result was integrated with the data of super-SAGE analysis (presented by Kito et al in this conference), and we selected the two candidate genes OMG-01 and OMG-02.

Koizumi and Fuji reported that Chubu32 is severely infected with particular rice blast isolates and suggested that the partial resistance in Chubu32 is isolate-specific. On the basis of this result, we hypothesized that the interaction between *Pi34* and a corresponding avirulence gene follows the gene-for-gene model. We used the Chinese isolate Y93-245c-2 and Japanese isolate IBOS8-1-1 which shows strong aggressiveness against Chubu32. Both isolates are virulent to Chubu32 (*Pi34+*) and Koshihikari (*Pi34-*). Chubu32 holds a high level of partial resistance to Y93-245c-2 but low level to IBOS8-1-1, whereas Koshihikari has low level of resistance to both these isolates. At first, the 69 blast isolates from the F<sub>1</sub> progeny produced by the cross between Y93-245c-2 and IBOS8-1-1 were tested for aggressiveness on Chubu32 and Koshihikari. The progeny segregated at a 1:1 ratio for strong to weak aggressiveness on Chubu32. Secondly, we inoculated Y93-245c-2 with the 32 F<sub>3</sub> lines selected from the mapping population of *Pi34*. The lines which possess *Pi34* are more highly resistant to Y93-245c-2 than the line lacking *Pi34*. From these results, Y93-245c-2 has one gene encoding avirulence to *Pi34* (*AVRPi34*), and IBOS8-1-1 is extremely aggressive on Chubu32 because of the absence of *AVRPi34*.

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Towards Map-Based Cloning of the Broad-Spectrum Resistance Gene *Pi40(t)* to Rice Blast

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Utilization of resistance gene is the most effective and economical method of controlling the worldwide devastating blast disease of rice. Previous genetic mapping analysis showed that the broad-spectrum blast resistance gene *Pi40(t)* is located on the short arm of chromosome 6. We have validated the mapping results with the F2 and F3 populations from a cross between IR65482-4-136-2-2(*Pi40(t)*) x CO39. Using the newly identified linked markers and reference genome, a physical map of the *Pi40(t)* locus was established, which was located within a 91 Kb region. To determine the gene organization in the *Pi40(t)* region and to identify the candidate genes, a 10X genome coverage bacterial artificial chromosome (BAC) library was constructed. Polymerase chain reaction (PCR) screening and several rounds of colony hybridizations were employed to identify the candidate BAC clones from the pools of BAC libraries. Using co-segregating STS marker NBS4-*Pi2*, we identified 4 BACs spanning the *Pi40(t)* locus, and we have sequenced a positive contig C10. It is showed that this locus appears to be allelic to the previously established *Pi2/Pi9* locus. The identified linked markers are useful for molecular breeding of blast resistance, and the candidate genes are useful in cloning the blast resistance gene *Pi40(t)*.

*PIC5* Regulates Appressorium Differentiation and Plant Infection in the Rice Blast Fungus  
*Magnaporthe Oryzae*

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In the rice blast fungus *Magnaporthe oryzae*, the *PMK1* mitogen-activated protein (MAP) kinase gene is known to regulate appressorium formation and invasive growth. Its orthologs in many other fungi also play critical roles in fungal development and pathogenicity. However, the targets of this important MAP kinase and its interacting genes are clear. (Did he mean 'are not clear'? Previous studies have shown that other factors may be required to stabilize the interaction of *Pmk1* with its upstream MAP kinase kinase *Ste7*.)

In this study, we constructed the yeast two-hybrid libraries and used the *Pmk1* bait to screen for *Pmk1*-interacting genes. A number of *Pmk1*-interacting clones (named PIC) were identified and confirmed by re-transformation into yeast. Two of them, *PIC1* (MGG\_11168) and *PIC5* (MGG\_08600), were selected for further characterization. *PIC1* encodes an 832-amino acid protein with no known functional domains but a nuclear localization signal and a MAP kinase phosphorylation site. *PIC5* encodes a 271-amino acid protein which contains one transmembrane domain and two unknown functional CTNS motifs. It is homologous to a mannose-P-dolichol utilization defect 1 protein (*Mpu1*) in *Gibberella moniliformis*. The interactions of *Pmk1* with *Pic1* and *Pic5* were further confirmed by co-immunoprecipitation assays. Targeted gene deletion of *PIC1* had no apparent effects on vegetative growth and pathogenicity but resulted in a significant reduction in conidiation. The  $\Delta$ *pic1* mutant formed branching germ tubes and multi-appressoria on onion epidermal cells. This defect was not observed on artificial hydrophobic surfaces. Like the  $\Delta$ *pic1* mutant, deletion of *PIC5* had no apparent effects on vegetative growth but led to a reduction in conidiation. The  $\Delta$ *pic5* mutant was defective in appressorium differentiation on glass cover slips. It was significantly reduced in appressorial penetration and virulence on rice seedlings. Quantitative RT-PCR analysis revealed that the *PIC1* gene was expressed in conidium, appressorium, and infected rice leaves. In transformants expressing the *PIC5*-eGFP construct, GFP signals mainly localized to the cytoplasm in all the tissues examined. These data indicate that *PIC5* is involved appressorium differentiation and pathogenicity in *M. oryzae*. However, it is not essential for appressorium formation. Other PIC genes or downstream targets of *Pmk1* must exist and play regulatory roles in appressorium differentiation.

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Localization of Secreted Proteins of the Rice Blast Fungus during Early Invasive Growth *in Planta*

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The rice blast fungus, *Magnaporthe oryzae*, is a devastating fungal pathogen that threatens stable rice production worldwide. The fungus develops specialized intracellular invasive hyphae (IH) in living rice cells and interacts with host cells across a plant-derived extrainvasive-hyphal membrane (EIHM). Although secreted proteins from the fungus play important roles in manipulating the host responses during the interaction, current information on secreted proteins is limited. Previous microarray analysis in our laboratory identified fungal genes that were highly expressed during the early biotrophic stage of rice blast disease. We used the SIGNALP program to identify secreted protein candidates showing 10-fold and higher levels of up-regulation compared to expression during the vegetative growth stage. We labeled the proteins with either C-terminal GFP or RFP by a high throughput Gateway cloning system and localized the fusion proteins *in planta* using the rice sheath assay. We identified 46 fluorescently labeled proteins that were specifically expressed by biotrophic invasive hyphae and that localized in the Biotrophic Interfacial Complex (BIC) similar to the known blast effector proteins AVR-*Pita*, *PWL1* and *PWL2*. In addition, 12 of the BIC-localized proteins were translocated across the EIHM into the rice cytoplasm, and 9 of these translocated proteins moved into adjoining uninvaded cells, possibly preparing these host cells for subsequent fungal invasion. These results will accelerate cataloging candidate blast effector proteins and elucidating the secretion mechanism of effectors during fungal-plant interactions.

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Comparative Molecular Analysis of *Pyricularia oryzae* Populations Causing Rice Blast in Iran and Uruguay

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Blast disease, caused by hemibiotrophic fungus *Pyricularia oryzae* Sacc. (teleomorph: *Magnaporthe oryzae* (Hebert) Barr.), previously known as *Magnaporthe grisea*, is the most serious disease of rice and causes high yield losses in most rice growing regions every year. Almost all commercially grown rice cultivars are susceptible to this disease, and prolonged periods of humid weather will provide favorable conditions for progress of the disease. Blast is an endemic disease in all Iranian and Uruguayan rice fields, with yield losses up to 90 %. Knowledge of different rice blast pathogen populations and factors affecting genetic structure of the isolates in some of main rice growing countries, such as Iran and Uruguay, is still scarce. However, understanding disease epidemiology and plant-pathogen interaction, and finally sustainable disease management is highly dependent on knowledge of the diversity and genetic structure of the pathogen.

The genetic variability of 55 *P. oryzae* isolates from Iran and 32 isolates from Uruguay was analysed using amplified fragment length polymorphism (AFLP). Cluster analysis using different methods based on the AFLP data from 679 monomorphic and polymorphic bands generated with eight primer combinations, was performed. The clustering of the isolates revealed 5 separate AFLP groups among a total of 87 isolates, which typically showed more than 78% fingerprint similarity. Within each AFLP group, three or more haplotypes were detected with a genetic similarity of 100%. Overall genetic similarity was greater than 82% between Iranian and Uruguayan populations. Little evidence for gene flow between the two populations was observed. Analysis of molecular variance (AMOVA) revealed that rice varietal type and geographic region were the dominant factors affecting genetic structure of *P. oryzae* populations.

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Wandering of *AVR-Pita* on Chromosomes during the Course of Evolution of *Pyricularia* Species

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*AVR-Pita* is an avirulence gene of *Pyricularia oryzae* corresponding to the *Pita* resistance gene of rice (Orbach et al. 2000; Khang et al. 2008). We have examined processes of evolution of the *AVR-Pita* family with a special focus on its chromosomal locations. Here, we show that *AVR-Pita* has frequently changed its chromosomal locations, or has “wandered” on chromosomes, during the course of speciation and parasitic specialization of *Pyricularia* spp. (Hirata et al. 2007).

We employed 99 isolates belonging to nine species in the genus *Pyricularia*. *AVR-Pita* homologs were detected not only in the rice-specific subgroup but also in non-rice subgroups of *P. oryzae* and other cryptic species. Most of homologs carried by non-rice subgroups maintained the function as an inducer of *Pita*-mediated resistance. To identify chromosomes carrying these homologs, we separated chromosomes of the isolates on contour-clamped homogeneous electric field (CHEF) gels and assigned chromosome numbers to each band using genetic mapping of chromosome-specific markers and CHEF-Southern analysis with those markers. Chromosomes carrying the homologs were different among species of *Pyricularia* and host-specific subgroups of *P. oryzae*, and were extraordinarily variable among isolates in the rice-specific subgroup. Telomeric repeats were detected at variable positions on either side of some *AVR-Pita* homologs. Comparative analyses of structures of flanking regions suggested that insertion of a retrotransposon, Inago1, into flanks of an ancestral *AVR-Pita* homolog was a key event associated with its mobility. We propose that one of mechanisms of the *AVR-Pita* wandering may be its frequent loss from the genome and recovery from populations of the same or different species. The outstanding wandering in the rice-specific subgroup is considered to be attributable to extensive interactions with the *Pita* resistance gene in rice.

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Genotypic and Phenotypic Diversity of *Pyricularia oryzae* in the Contemporary Rice Blast Pathogen Population in Arkansas

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Rice blast, caused by *Pyricularia oryzae* (teleomorph: *Magnaporthe oryzae*), is one of the most economically important diseases on rice worldwide, including Arkansas. Pathogen population analysis could provide a better understanding of the genetic diversity of the pathogen, help in choosing appropriate isolates for screening for disease resistance, and aid in the development of strategies to breed for durable resistance to rice blast disease. Earlier studies of the pathogen focused on pathogenic/virulence variability, while more recent studies have utilized genetic and molecular markers to characterize population diversity (Correll, 2009). More recently, SSR markers are being employed to characterize pathogen diversity and have the advantages of being highly polymorphic, multi-allelic, highly reproducible, and easily detected by PCR (Tharreau, 2009).

Previous analysis of *P. oryzae* in the U.S. identified 8 MGR586 DNA fingerprint groups (designed as A-H), but only 4 groups (group A, B, C and D) have been identified since 1991 (Correll, 2009). These 4 MGR lineages also correspond to 4 distinct vegetative compatibility groups, or VCGs. The objective of this study was to examine the genotypic and phenotypic diversity of *P. oryzae* in the contemporary population in Arkansas. The weather during the 2009 rice season was particularly conducive for disease development and over 500 isolates were recovered from symptomatic rice cultivars in Arkansas and are being examined for their genotypic and phenotypic diversity. The isolates were evaluated for vegetative compatibility, MGR586 DNA fingerprint diversity, and virulence on a set of 40 commercial cultivars or advanced breeding lines. Examination of isolates from 2009 indicated that 3 VCGs were recovered (US-01, US-02, and US-04) and that one VCG (US-01) predominated (>50%). Using SSR markers and virulence tests, the genotypic and phenotypic diversity within and between lineages of *P. oryzae* from Arkansas will be evaluated.

In addition, the stability of the *AVR-Pita* pathogen avirulence gene will be evaluated *in-vitro*. When certain mutations occur in *AVR-Pita*, isolates are able to overcome resistance conveyed by the *Pi-ta* resistance gene (Zhou, 2007). However, considerable variation in the *AVR-Pita* gene has been observed among field isolates of *M. oryzae* that are not able to overcome *Pi-ta* and therefore appears to remain functional. Thus, efforts are underway to assess mitotic stability of *AVR-Pita* under controlled conditions.

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Mitogen-Activated Protein Kinases are Required for Cell-to-Cell Movement of Invasive Hyphae in *Magnaporthe oryzae*

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Rice blast disease is caused by the filamentous ascomycete fungus *Magnaporthe oryzae* and is one of the most severe diseases of cultivated rice worldwide. The pathogen forms appressoria for plant penetration. In the penetrated cell, it produces invasive hyphae that could invade neighboring cells. In *M. oryzae*, the *Pmk1* MAP kinase (MAPK) regulates appressorium formation. The *Mps1* MAPK is not required for appressorium formation but is important for hyphal growth and conidiation. Both of them are essential for appressorium penetration and invasive growth. Their orthologs also are known to regulate various plant infection processes in other phytopathogenic fungi.

In this study, we expressed the *hopAII* gene in *M. oryzae* to determine its effects on the biotrophic invasive growth. *HopAII* is a well-conserved effector protein in plant and animal bacterial pathogens. It inactivates MAPKs to suppress PAMP-induced immunity in plants. When expressed under the control of the *MIR1* promoter, *HopAII* had no effects on appressorium formation but inhibited the colonization of the neighboring plant cells by invasive hyphae formed in penetrated cells. No typical blast lesions were formed by the resulting transformants on inoculated rice seedlings. Similar results were obtained when the *BAS1* or *BAS4* promoters were used to express *HopAII*. While *MIR1* is highly induced during plant infection, the *BAS1* and *BAS4* genes are specifically expressed in the biotrophic growth stage. We also used the promoter of *GAS1*, a gene highly expressed at late stages of appressorium formation, to express *HopAII*. The resulting transformants still formed melanized appressoria but were defective in plant infection. Constitutive expression of *HopAII* with the *TrpC* promoter in *M. oryzae* resulted in multiple defects, including reduced vegetative growth and appressorium formation and loss of pathogenicity. When assayed with an anti-TpEY specific antibody, the phosphorylation levels of both *Pmk1* and *Mps1* were reduced in the transformants expressing *HopAII*. These results indicated that the *Pmk1* and *Mps1* MAP kinases are required for the spreading of invasive hyphae in infected plant tissues. Based on the phenotypes of transformants expressing *HopAII* under the control of different promoters, *HopAII* appeared to be more effective in blocking the *Mps1* than *Pmk1* MAP kinase signaling. *Mps1* may play a more critical role than *Pmk1* in regulating the biotrophic invasive growth in plant tissues.

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Functional Analysis of Carbohydrate Binding Module (CBM)-Containing Effectors in  
*Magnaporthe oryzae*

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Rice blast disease, caused by the fungus *Magnaporthe oryzae*, is one of the most serious diseases of rice. To elucidate the molecular basis of interactions between rice and *M. oryzae* upon infection, we employed a multifaceted approach that combined gene expression profiling, high throughput gene cloning, and rapid protoplast transient expression assay for large-scale identification of *M. oryzae* effector proteins. Eight effector proteins were found to induce cell death in rice protoplasts and in *Nicotiana benthamiana*. Among them, three effectors which are CBM1, CBM3 and MGG18 contain a carbohydrate binding module (CBM). Structure-function analysis of three CBM-containing genes indicated that carbohydrate binding modules are required for full function of cell death induction. We show that MGG18 effector protein induced cell death is light dependent, and is suppressed by Bcl xL, an apoptotic inhibitor, and is suppressed by LaCl<sub>3</sub>, an inhibitor of calcium channel. In addition, enhanced expression of pathogenesis-related (*PR*) genes are observed, when *MGG18* is transiently expressed in *N. benthamiana*. A yeast-two-hybrid screen identified a rice DnaJ protein that moderately interacts with MGG18 effector protein in yeast. Characterization of these fungal effector proteins and their targets in rice cells will provide new insights into the molecular basis of rice and *M. oryzae* interactions.

Studies on Interactions between Rice and *Magnaporthe grisea* in Jilin Province, P. R. China

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Jilin Province is an important Japonica-type rice producing area in Northeast China, and rice blast is a serious disease in local agriculture. Combining molecular biotechnology and conventional agricultural methods, we focus on resistance disease breeding, molecular marker assisted selection, and scientifically distributing cultivars according to the information of interactions between cultivars and blast fungi.

The DNA markers linked to resistance genes are powerful tools to detect the presence of genes and are used to select breeding material through marker assisted selection. 10 pairs of dominant primers of resistance genes were used to amplify target gene fragments in 40 varieties, 3-10 target genes were amplified from different varieties respectively. The broad spectrum resistance gene *Pi9* was detected in 18 varieties.

Seedlings of the 40 varieties were inoculated with 60 isolates of *Pyricularia grisea*. Among all isolates, at the most 24 strains infected the varieties containing the *Pi9* gene, and 8 strains infected the varieties containing the *Pi5* gene. Selected 4 varieties containing different resistance genes and arranged by 5:5 lines ratio mixing, the incidence of rice blast disease decreased 50%, against single variety planted in same field.

In addition, based on the avirulent genes information of *Pyricularia grisea* and resistance genes information of varieties, we are improving varieties resistance by combining the traditional breeding and molecular biotechnology with approaches such as gene pyramiding and molecular marker selection and progress will be presented.

Identification of the Blast Resistance Gene *Pit* in Rice Cultivars using Functional Markers

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DNA markers that allow for identification of resistance genes in rice germplasm have a great advantage in resistance breeding because they can assess the existence of the genes without laborious inoculation tests. Functional markers, which are designed from functional polymorphisms within the sequence of genes, are unaffected by nonfunctional allelic variation and make it possible to identify an individual gene. We previously showed that the resistance function of the rice blast resistance gene *Pit* in a resistant cultivar, K59, was mainly acquired by up-regulated promoter activity through the insertion of a long terminal repeat (LTR) retrotransposon upstream of *Pit*. Here, we developed PCR-based DNA markers derived from the LTR-retrotransposon sequence and used these markers to screen worldwide accessions of rice germplasm. We identified five cultivars with the LTR-retrotransposon insertion out of 68 rice accessions. The sequence and expression pattern of *Pit* in the five cultivars were the same as those in K59 and all showed *Pit*-mediated blast resistance. The results suggest that the functional *Pit* allele, identified using the markers, was derived from a common progenitor. Additionally, comparison of the *Pit* coding sequences between K59 and susceptible cultivars revealed that one nucleotide polymorphism, causing an amino acid substitution, offered another target for a functional marker. These results indicate that our DNA markers should enhance prediction of *Pit* function and be applicable to a range of rice varieties/landraces cultivated in various regions worldwide, including *Oryza sativa* var. *japonica*, *O. sativa* var. *indica*, and *O. sativa* tropical *japonica* cultivar.

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Breeding of Restorer Lines with Dual Resistance to Blast and Bacterial Blight in Rice  
Through Marker-Assisted Selection

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Rice blast and bacterial blight (BB) are two of the most destructive diseases leading to severe yield loss in rice production throughout the world. Genetic improvement is one of the most effective strategies to prevent rice from rice blast and bacterial blight diseases.

Several advanced generation breeding populations with different resistant sources were subjected to screening for plants homozygous for resistance genes through marker-assisted selection (MAS), and eventually, five lines with *Pi25* (a new blast resistance gene) and five lines carrying various combinations of genes *Xa21*, *xa13* and *xa5* (BB resistance genes) were selected from one F<sub>6</sub> population consisting of 76 plants and three other F<sub>6</sub> populations consisting of 203 plants, respectively. A total of 25 crosses were made by using the two sets of lines either carrying *Pi25* or carrying a combination of genes *Xa21*, *xa13* and *xa5* as the parents.

Polymorphism detection between the parents using available DNA markers revealed that only the cross, DH146/TM487, was suitable for MAS analysis and then was subjected to pyramid multiple blast and BB resistance genes into one breeding line through MAS. The F<sub>2</sub> population of the cross DH146/TM487 was inoculated with *M. grisea* isolate 05-20-1 to eliminate those susceptible plants, followed by MAS analysis using markers tightly linked to various resistance genes. Lines homozygous for *Pi25* and BB resistance genes *Xa21*, *xa13* and *xa5* were screened by markers RM3330 and A7, pTA248, RM433 and RM153, respectively.

Lines carrying various combinations of resistance genes were obtained, and inoculation with five *Xoo* isolates demonstrated that the selected lines reached the same resistance level as TM487 and IRBB60 (check). Finally, after selection for agronomic traits and restoration ability of the pyramided lines, we acquired a good restorer line, H1, which can be used directly in hybrid rice breeding. The combination Zhong 9A/H1 was entered into the national trial of south China and ranked first place among all tested varieties. This study provides a paradigmatic example that MAS is a practical, feasible tool in effectively pyramiding multiple resistance genes.

Allele Mining of a Collection of *Indica* Rice Genotypes for Blast Resistance Gene *pi2(t)* and Bacterial Blight Resistance Gene *Xa 13*

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Worldwide, rice blast and bacterial blight are two of the most widespread and destructive diseases of rice. Host-plant resistance is the most economical and logical approach to reducing yield losses. As pathogens can rapidly evolve to overcome resistance genes, development of resistant cultivars utilizing a single resistance source is difficult. Breeding for durable and broad spectrum resistance by pyramiding multiple R genes is the most effective strategy. Hence identification of additional genetic resources having multiple stress resistance genes is imperative. Microsatellites are PCR markers that provide an attractive high-throughput and labor-saving means to tag resistance genes, particularly for breeding programs that screen thousands of lines.

Here we present the resistance spectra of blast resistant gene *pi2(t)* and bacterial blight resistant gene *Xa 13* in a collection of 76 *indica* rice genotypes of India, analyzed with suitable molecular markers. The varieties, 'Ptb3', 'Ptb10', 'Ptb14', 'Ptb17', 'Ptb18', 'Sagara', and the landrace 'Mavilan' are potential genetic resources for both blast resistance and bacterial blight resistance. Blast resistance genes are also observed in the varieties 'Ptb8', 'Suraksha', and in two more landraces, namely 'Kayama', and 'Malayudumban'. The blight resistance gene *Xa 13* was also detected in 46 more genotypes. Out of these genotypes, 'Ptb17', 'Ptb18' and 'Sagara' phenotypically express moderate resistance to blast, but none of the 76 genotypes phenotypically express blight resistance. Varieties 'Kairaly', 'Aswathy', 'Kanchana', 'Remya', 'Kanakom', 'Remamika', 'Ptb1', 'Ptb22', 'Ptb25', 'Ptb26', and 'Ptb28', which phenotypically express moderate resistance to blast, seem to be lacking the blast resistance gene *pi2(t)*.

Presence of resistance genes of both bacterial blight and blast diseases in phenotypically non-expressible genotypes indicates the potential of these genotypes for bacterial blight resistance and blast resistance to be exploited through hybridization and recombination and for gene tagging. The phenotypic non-expression may be due to the presence of suppressing genes or retro-transposons having a gene-silencing role. Further, a suitable genetic background is important for the efficient expression of a gene, and more truly when the loci are complex.

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Identification of Rice Blast Resistant Gene *Pi-z(t)* in NSGC Using DNA Markers and Pathogenicity Assays

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Rice blast disease, caused by the fungus *Magnaporthe oryzae* (formerly *Magnaporthe grisea*) is a major fungal disease threatening rice production worldwide. Genetic resistance in rice to *M. oryzae* typically belongs to a classic gene-for-gene system where a resistance (*R*) gene is effective in preventing infections by races of *M. oryzae* containing the corresponding avirulence (*AVR*) gene. Presently the use of *R* genes is the most economical and environmentally benign method to manage blast disease. Thus far, more than 80 race-specific *R* genes to *M. oryzae* have been identified and some of them have been molecularly cloned and tagged for marker-assisted selection (MAS).

Among the tagged blast *R* genes, the *Pi-z(t)* gene initially identified by Kiyosawa has been effectively introgressed into numerous rice cultivars around the globe to prevent infection by a wide range of races of the rice blast pathogen. In the U.S., *Pi-z(t)* was first identified in the medium grain cultivar 'Zenith'. This gene has been shown to confer resistance to five U.S. races of blast, IH-1, IG-1, IC-17, IE-1 and IE-1k, and susceptibility to two races, IB49 and IB33, respectively.

The objective of this present study was to characterize *Pi-z(t)* in 131 rice germplasm accessions selected from the NSGC using DNA markers and pathogenicity assays. Four simple sequence repeat (SSR) markers (RM527, AP4791, AP5659-1, AP5659-5) closely linked to *Pi-z(t)* were used to predict *Pi-z(t)* in rice germplasm and the results were verified using pathogenicity assays with an avirulent strain (IE1k) and two virulent races, IB33 and IB49. The presence of the *Pi-z(t)* gene in 98 germplasm accessions out of 131 was verified using both SSR markers and pathogenicity assays. The remaining 33 germplasm accessions containing unexpected SSR alleles were found to be different in their responses to blast races, IB33, IE1k and IB49, suggesting the presence of additional *Pi-z(t)* independent *R* gene(s). These characterized germplasms should be useful for genetic studies and marker-assisted breeding for improving blast resistance worldwide.

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Identification and QTL Mapping of Blast Resistance in Wild *Oryza* SpeciesEizenga, G.C.<sup>1</sup>, Prasad, B.<sup>2</sup>, Jackson, A.K.<sup>1</sup> and Jia, M.H.<sup>1</sup><sup>1</sup>USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, Arkansas, USA<sup>2</sup>Rice Research and Extension Center, University of Arkansas, Stuttgart, Arkansas, USA

Leaf blast disease of rice (*Oryza sativa* L.) caused by *Magnaporthe oryzae* B. Couch is one of the most devastating rice fungal diseases worldwide. Wild relatives of rice (*Oryza* spp.) may contain novel genes for biotic and abiotic stress resistance lost during domestication. A collection of 67 wild *Oryza* spp. accessions was evaluated for reaction to eight U.S. races of *M. oryzae* and four accessions showed resistance to all races (Eizenga et al., 2009). Subsequently, we developed an advanced backcross mapping population with one *O. nivara* accession, as the wild donor parent. Our objective was to identify the QTLs associated with blast in this mapping population and compare the location of these putative QTLs to known blast (*Pi*-) genes.

Bengal (PI 561735) is a popular U.S. medium grain rice variety which is susceptible to blast races IB-1 and IB-49. Bengal was chosen as the recurrent parent and *O. nivara* (IRGC104705) from Maharashtra, India as the donor parent. The advanced backcross method was used to develop the mapping population because this method has proven to be effective for simultaneously identifying QTLs and improving germplasm, especially when the donor parent is not adapted. The resulting population, composed of 253 BC<sub>2</sub>F<sub>2</sub> families, was evaluated for reaction to blast disease in the greenhouse using races IB-1 and IB-49 (Eizenga et al., 2010). The BC<sub>2</sub>F<sub>1</sub> founder lines were genotyped with 133 SSR markers distributed throughout the 12 rice chromosomes. Products of the PCR reactions were visualized on an ABI Prism 3730 and “allele calling” completed with GeneMapper 3.7. The linkage map was created using JoinMap 4.0 and QTLs identified using multiple interval mapping as performed by QGene 4.3.6. Graphical Genotype (GGT) was used to determine the percentage of the genome heterozygous for Bengal/*O. nivara*. Preliminary results of the inoculation suggest approx. 10 BC<sub>2</sub>F<sub>2</sub> families are resistant to both blast races, IB-1 and IB-49. QTL mapping results will be presented. The location of the putative blast QTLs identified will be compared to that of known blast genes and the marker–trait associations previously identified in the collection (Eizenga et al., 2009).

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## Screening and Association Mapping of Rice Blast Disease Resistance Using a Diverse Collection of Rice Germplasm.

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Rice blast, caused by the fungus *Magnaporthe oryzae* B. Couch, is a very serious disease in rice (*Oryza sativa* L.) worldwide. Incorporation of new blast resistance genes into breeding lines is an important objective of many rice breeding programs. A diverse collection of 409 *O. sativa* accessions described as the “Rice Diversity Panel” was purified by single plant selection for two generations and genotyped with SSR markers and 44,000 SNP markers. Based on SSR markers, the five rice subpopulation groups represented in this panel are *aus* (59 accessions), *indica* (90), *temperate japonica* (108), *tropical japonica* (104), and *aromatic/ GroupV* (15), as well as, admixture groups representing the *Indica* (11 accessions) and *Japonica* (22) varietal groups. The objectives of this study were to 1) rate the Diversity Panel for reaction to blast disease, 2) identify new germplasm as a source of blast resistance using SSR/SNP markers associated with *Pi*-genes, and 3) discover novel “marker-blast trait” associations.

The diversity panel was scored on a “0” (no disease) to “9” (dead) scale for reaction to *M. oryzae*, after field inoculation with a mixture of the U.S. blast races, IB-49, IC-17 and IE-1K. Accessions in the *indica* subpopulation were the most resistant (mean=3.0) to these races while *temperate japonica* was the most susceptible (mean=7.0). The *Pi-b* resistance gene on chromosome 2 confers resistance to races IC-17 and IE-1K. Only 16 accessions, mostly *indica*, carried the *Pi-b* allele and rated  $\leq 3.0$ . Similarly for *Pi-ta* on chromosome 12, only 30 accessions, mostly *indica*, carried the *Pi-ta* resistance allele and 23 of the 30 accessions rated  $\leq 3.0$ . The *Pi-ta* gene confers resistance to races IB-49 and IC-17. A Genome Wide Association (GWA) scan using genotypes from the 44,000 SNP array identified marker-blast trait associations in the complete diversity panel and only within four of the five subpopulation groups (*aus*, *indica*, *tropical* and *temperate japonica*). Physical positions of these associations were compared with the position of approx. 12 cloned blast resistance genes (Liu et al. 2010) located on chromosomes 1, 2, 4, 6, 8, 9, 11 and 12 using the TIGR/MSU v6 sequence annotation. There was strong signal around the *Pi-ta* gene in the GWA scan and SNPs in the neighboring regions of some of the other cloned genes. Different sets of *Pi*-genes were identified across the different subpopulations illustrating the genetic heterogeneity of blast resistance. Further investigation of these marker-blast trait associations should identify new SNP markers linked to the blast resistance and possibly new germplasm sources of resistance genes. Both the new SNP markers and novel candidate *Pi*-genes could be used to incorporate blast resistance into new rice cultivars.

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Breeding for Resistance to Blast Disease in the University of Arkansas Semidwarf Rice Breeding Project

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During the last 3 years, over 53% of rice production area in Arkansas has been sown to rice cultivars rated as susceptible or highly susceptible to blast disease. This results in pressure on pesticides and agronomic management practices to provide disease control, and increases the probability of a major epidemic. Stakman and Harrar stressed the importance of resistant cultivars due to the increase in costs of production associated with alternative control methods. Our breeding project has as a major objective to develop blast disease resistant cultivars. We use field and laboratory methods to identify resistant germplasm and breeding lines.

Screening of F<sub>2</sub> populations and yield trial entries is done under field conditions at the Pine Tree Experiment Station at Colt, Arkansas. Our approach includes the use of susceptible spreader rows, high nitrogen fertilization and upland growing conditions. Leaf and neck blast evaluations are made at approximately 50 and 100 days after planting. F<sub>3</sub> to F<sub>5</sub> plants and backcrosses are screened for individual blast genes (*Pi-ta*, *Pi-b* and *Pi-z*, routinely) using Molecular Aided Selection (MAS). A bulk seed extraction method has been developed to greatly increase efficiency of MAS. Over the last three years, phenotypic blast screening has been conducted on an average of 94 Advanced Yield Trial entries, 335 Preliminary Yield Trial entries, and 724 F<sub>2</sub> populations per year. In addition, 258 Working Collection entries are being screened in 2010. In the past 3 years, an average of 3450 F<sub>3</sub> and 2300 F<sub>4</sub> and F<sub>5</sub> individual rows have been screened and selected for individual blast genes using MAS. Our project has released Cybonnet and AR1124 long grain cultivar and germplasm line, respectively, with strong blast resistance. We are in the process of incorporating the *Pi-ta* resistance gene into a high yielding, high quality, but blast susceptible, medium grain rice breeding line.

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Development of Marker-Free Transgenic Rice Carrying the Broad-Spectrum Blast Resistance Gene *Pi9* via Co-transformation of Double Binary Vectors

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Rice blast, caused by the fungus *Magnaporthe oryzae*, is one of the most devastating diseases in rice production. Utilization of rice blast resistance genes is the most effective and economical strategy for control of the blast disease. *Pi9* is a broad-spectrum blast resistance gene cloned in rice and has been transformed into rice cultivars for development of blast-resistant transgenic rice. In this study, we used a new approach to produce marker-free *Pi9* transgenic rice. The cloned *Pi9* gene was transformed into rice cultivars Zhejing 22, Zhehui H414, Nipponbare, Kongyu 131 and Yuetai B *via* co-transformation of two *Agrobacterium* binary vectors. One of the binary vectors (pLJ42) carried a 13.5-kb *Pi9* genomic sequence in the T-DNA region. The T-DNA of another binary vector (pSK51) contained the hygromycin resistance gene (HPT) as the transformation selection marker and green fluorescent protein (GFP) gene as the visual marker to facilitate screening of marker-free transgenic progeny. A total of 487 T<sub>0</sub> transgenic rice plants were generated and T<sub>1</sub> seeds of 321 transgenic lines were obtained. Genomic DNA of 91 Zhejing 22 T<sub>0</sub> plants and of 35 Zhehui H414 T<sub>0</sub> plants was PCR analyzed using primers specific to *Pi9*, T-DNA borders, HPT and GFP. A total of 26 T<sub>0</sub> plants carried both pLJ42 T-DNA (*Pi9*) and pSK51 T-DNA (HPT/GFP). T<sub>1</sub> plants of transgenic lines carrying the *Pi9* T-DNA were inoculated with the Philippine rice isolate PO6-6 and 20 blast isolates collected in Zhejiang Province, China. Co-segregation of blast resistance phenotype and the *Pi9* transgene was observed in Zhejing22 T<sub>1</sub> and Zhehui H414 T<sub>1</sub> populations based on blast inoculation results. Through PCR analysis of pLJ42 T-DNA (*Pi9*) and pSK51 T-DNA (HPT/GFP) of T<sub>1</sub> plants, marker-free transgenic progeny plants carrying the *Pi9* blast resistance gene were obtained from 2 independently transformed Zhejing 22 transgenic lines.

Reference:

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

Mapping Quantitative Trait Loci for Resistance to Rice Blast Using Physiological Races of the Fungus

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It has been known for sometime that genetic resistance to blast caused by *Magnaporthe oryzae* is controlled by major resistance (*R*) and minor resistance genes. Minor resistance genes, each providing a different extent of resistance, have been identified on different rice chromosomes as quantitative trait loci (QTLs). The qualitative data converted from category scale have been successfully used to map and clone major blast *R* genes worldwide.

In this study, we mapped blast resistant QTLs using composite interval mapping with the six races IB1, IB45, IB49, IB54, IC17, and ID1 of *M. oryzae* using a recombinant inbred line (RIL) population derived from a cross of the moderately- susceptible *japonica* cultivar Lemont with the moderately- resistant *indica* cultivar Jasmine 85. Disease reactions of 227 RILs were evaluated using a category scale of ratings from 0, representing the most resistant, to 5, representing the most susceptible. The category data we collected were used for mapping resistant QTLs. A total of eight QTLs responsive to different degrees of phenotypical variation ranging from 5.2 to 26.5% were identified on chromosomes 3, 8, 9, 11, and 12.

This study demonstrates the usefulness of studying blast resistant QTLs using physiological races by composite interval mapping under greenhouse conditions.

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Identification of QTLs for Partial Resistance to Blast in European Rice Varieties and Their Use in Breeding for Durable Resistance

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Blast is the only pathogen of rice treated with pesticides in Europe, where most of the rice growing takes place in fragile areas like river deltas and damps, with special environmental protection. The use of resistant rice varieties to avoid fungicide applications is a cost effective method to control the disease, but it is hampered by the difficulty of breeding for durable resistance. The GENBLAST project aimed at identifying the important genes for durable blast resistance and creating resistant varieties adapted for European growing conditions, through the combined efforts of different teams and methodologies: characterization of European blast populations, evaluation of resistance to blast in the field and in controlled conditions, study of expression patterns of genes involved in defense mechanisms, characterization of allelic variability, and QTL analysis.

For QTL detection, two rice populations have been used, derived from crosses between cultivars considered as potential donors of resistance and elite Spanish varieties: Jsendra x Gigante Vercelli (JSxGV) and Senia x Kalao (SxKl). Both were genotyped with SSR markers evenly distributed along the rice genome, with higher density in chromosomal regions where specific resistance genes and metaQTLs have been previously found. For evaluation of partial resistance, F2 plants were inoculated with European representative blast isolates, and all the corresponding F3 lines were evaluated in the field in three growing areas. Several QTLs of interest have been detected in both populations, and used for marker assisted selection of resistant F4 lines of the JSxGV background.

Improvement of International Blast Differential Varieties with *Japonica*-type Genetic Background

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Differential varieties (DVs) for blast disease are essential tools for research and breeding programs for blast resistance in rice. Monogenic lines (MLs) for blast resistance were developed as the first set of international standard DVs using standard blast isolates from the Philippines for selection under the IRRI-Japan Collaborative Research Project (Tsunematsu et al. 2000, Telebanco-Yanoria et al. 2008). As of now, these MLs with genetic background of a *japonica*-type variety Lijiangxintuanheigu (LTH) were used in more than 30 countries as DVs. However, many of the MLs showed differences with LTH in several agronomic traits. This may be due to introgression of large chromosomal segments from donor varieties, because the MLs are derived from only one to three backcrossings. Recently, 27 near-isogenic lines (NILs) for blast resistance with the genetic background of an *indica*-type variety CO 39 suitable for the tropical environment were developed by six backcrossings under the same Project (Telebanco-Yanoria et al. 2010). However, development of NILs with a *japonica*-type background is still required. In our study, NILs for single blast resistant genes were developed by six backcrossings with the genetic background of LTH to improve the MLs of *japonica*-type DVs.

Twenty NILs with 11 blast resistance genes—*Pib*, *Piz-5*, *Pi9*, *Pi3*, *Pia*, *Pik-s*, *Pik*, *Pik-h*, *Pi7(t)*, *Pita*, and *Pita-2*—derived from 19 donor varieties in a genetic background of LTH were developed by recurrent backcrossing. The resistant plants were selected in each backcross generation using a specific avirulent blast isolate to the targeted resistance genes. The NILs showed identical or similar reaction pattern with that of the corresponding MLs against 20 standard isolates from the Philippines, suggesting the target resistance genes were successfully introduced. The introgression of targeted resistance genes was further confirmed by SSR markers that were located within the region where the resistance gene was previously mapped. Genome survey using SSR markers showed that LTH NILs had more similar genetic composition with the recurrent parent LTH than MLs. Morphological characters of each NIL were almost identical to those of LTH. These NILs with genetic background of *japonica*-type variety are useful materials for blast resistance studies and breeding programs.

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Effect of Rice Blast Resistance Genes to Variation of *Pyricularia grisea* Population Over Space and Time in the Mekong Delta of Vietnam

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In the Mekong Delta of Vietnam, one of the Asian countries with the most intensive rice cropping practices, the released and promising rice varieties have become susceptible to blast caused by the fungus *Pyricularia grisea* Sacc. Understanding the variation and population structure play important roles in blast management, gene deployment and breeding for blast resistance. About 500 blast isolates have been identified over 4 years in 10-13 provinces in the Mekong Delta. Genetic diversity and virulence analyses were done by genotyping and phenotyping based on *Pot2* primers and the 31 LTH monogenic lines. Races were classified based on a new differential system of LTH monogenic lines. The breakdown of durable resistance genes at *Pik* and *Piz* loci were also analyzed.

The results showed that resistance genes *Pik* and *Piz* loci were durable. The influence of blast resistance genes on population structure were identified by frequency of occurrence of race groups, significant genetic differentiation of fungus population, as well as population diversity. Eighty eight races were classified into 19 groups. There was race-specificity in each province. The *P. grisea* population between and within provinces, seasons and monogenic lines had significant genetic differentiation. Over time three resistance genes *Piz-5* (IRBLz5-CA and IRBLz5-CA (R)), *Piz-t* (IRBLzt-T) and *Pik-m* (IRBLkm-Ts) started displaying compatibility to the *P. grisea* population. The other two resistance genes *Pik-p* (IRBLkp-K60) and *Piz* (IRBLz-Fu) are still highly effective. Gene pyramiding should be considered to enhance resistance and maintain the resistance longevity.

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Reaction of Rice Lines with *Pi*-genes Against *Magnaporthe grisea* Under Rainfed Situations of Karnataka, India

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Blast disease caused by *Magnaporthe grisea* (*Pyricularia grisea*) is a major threat to rice production under upland situations. Host resistance is often broken down by shift in the virulence pattern of this pathogen. To contain the genetic plasticity of blast pathogen resistant, *Pi*-genes may be introgressed or pyramided into elite background. In this context rice lines possessing *Pi*-genes in Co-39 background were field evaluated against blast during kharif 2008-10 under rainfed situations at Agricultural Research Station (Paddy) Mugad, Karnataka. The entries were sown in a uniform blast nursery and screened by adopting 0-9 scale. HR-12 was used as the susceptible check. Observations on disease severity as well as lesion types were recorded soon after the first appearance of the disease and then 15 days later.

The near isogenic lines Co 101 LAC, Co 101 A51 and Co 105 TTP having the *Pi-1*, *Pi-2* and *Pi-4b* gene respectively, exhibited high level resistance with leaf blast scores of 1-2 and B lesion type. The disease severity was not enhanced further. The NILs having a *Pi*-gene, either *Pi-3* or *Pi-4a*, were highly susceptible to blast (score 7). The recombinant parent Co-39 with *Pi-a* gene was highly susceptible to blast (score 8). Resistance status of RILs having *Pi-5* was very poor with high blast incidence (score 8 with lesion type E (D) or E(C)). However *Pi-7* gene (RIL-29) offered good resistance level (score 2) with lesion type C. Pyramiding of *Pi*-gene offers a good opportunity to manage blast disease effectively. Performance of two gene combinations (*Pi-1+Pi-2* or *Pi-2+Pi-4*) was superior to a three gene combination (*Pi-1 + Pi-2 + Pi-4*) except in the *Pi-1 + Pi-4* combination (score 5). Performance of *Pi-1 + Pi-4* combination in field was not good against IB-41 race, Blast reaction on International differentials indicated the prevalence of IB-41 race at the test site which may be attributed to a masking effect of *Pi-4* gene on *Pi-1* gene. Thus indiscriminate combinations of major genes may be ineffective against some populations.

The gene combination of *Pi-1 + Pi-2* offered high resistance throughout the crop stage with 'A' lesion type and disease severity of 1. Pyramiding of *Pi-1* and *Pi-2* genes appears promising to obtain long lasting resistance against blast where such virulence pattern persists.

## Rice Blast Disease in Colombia

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Rice is an important food source worldwide including Latin America and The Caribbean (LAC). Rice in this region is grown under flooded or irrigation conditions corresponding to variable environmental conditions of savannas and upland, respectively. Such variability makes CIAT's Rice breeding program, in collaboration with local entities, a key starting point for the development of rice materials that can be adapted easily across the LAC region. In fact, this breeding program is an important source of germplasm for several countries.

The Santa Rosa experiment station in the Eastern plains of Colombia is an internationally known blast hot spot (Correa-Victoria and Zeigler, 1993). The pathogenic variability at Santa Rosa is very high due to the diversity in gene pools present in the host. The source of such variability is because this location is used by several breeding programs, including CIAT, for field evaluation against blast disease. In Colombia, fungal strains have shown the capacity to break down resistance in rice varieties 1 to 3 years after deployment by farmers, except for Oryzica Llanos 5 and Fedearroz 50 that remain as resistant after more than one decade of their release (Correa et al, 2002). On the other hand, varieties with no source of resistance, such as Fanny, are completely destroyed by the disease in the first 3 weeks after germination.

Fingerprinting studies have shown that Colombian blast population is composed mainly by 6 genetic families but a direct relationship with the avirulence structure is not very clear. The use of varieties with more than one resistance gene or unknown sources of resistance can somehow limit these types of studies. For this reason the use of differential varieties hosting a single resistance gene is very helpful in establishing pathotype composition of the fungal populations that can be used as a foundation in research toward avirulence genes identification.

We recently introduced into the blast evaluation program at CIAT a new differential system, developed by an IRRI-Japan collaboration, composed of monogenic lines for 24 resistance genes. This new set of differentials is being used for blast characterization in greenhouse and field experiments. Preliminary results show that most of the resistance genes can be defeated by blast fungus in Santa Rosa. Interestingly, few genes seem to resist the pathogen attack of the natural field population. Responses to blast infection obtained from the new set of differentials evaluated in Santa Rosa are also being compared with those obtained from old differentials and other breeding materials with gene pyramids.

These studies will guide inclusion or avoidance of specific resistance genes in breeding programs to manage disease through the use of resistant cultivars and also identify strains harboring specific avirulence genes.

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## Development of Strategies to Manage Rice Blast Disease in the US

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Rice blast disease has been a serious threat to stable rice production in the southern US. Blast disease has been causing yield losses for decades. Severity of blast epidemics has always been influenced by a combination of the following three factors: 1) rice cultivars deployed with different combination of major and minor resistance (*R*) genes, 2) weather conditions during rice growing stages, particularly high relative humidity before and during heading, and 3) race composition of field pathogen populations. In the US successful deployment of rice degree days, known as the “DD-50” program, erratic irrigation (shallow flood, intermittent flooding, furrow irrigation, pivot irrigation attempts) and improved varieties carrying both major and minor *R* genes have greatly contributed to the effectiveness of blast management.

Since the fourth International Rice Blast Conference held in Changsha, the map positions, resistance spectra, DNA sequence variation, and evolution of the six most commonly used blast *R* genes, *Pi-ta*, *Pi-b*, *Pi-kh/s(m)*, *Pi42(t)*, *Pi43(t)*, *Pi-z(t)* and eight blast resistant QTLs in the US have been investigated. Progress will be presented by Costanzo, Lee, Dai, Roy-Chowdhury, Wang, Liu, Zhou, Reddyvari-Channarayappa, and Zhai. Additional accomplishments are listed below:

1. Through a combination of DNA sequencing, genetic crosses and pathogenicity assays a large linkage block at the *Pi-ta* region was identified. Genotyping of selected rice germplasm and six mapping populations revealed that such a linkage block is a result of the combination of selection of blast resistance, recombination suppression, and segregation distortions;
2. A resistant spectrum of the *Pi-ta* gene in the US was determined. The *Pi-ta* gene was found to confer resistance to 10 races of the US blast fungus; and
3. A total of 182 mono and digenic rice lines with different combinations of *Pi-ta*, *Pi-kh* and *Pi-ks* and agronomic traits have been created to facilitate the genetic studies of epistatic interactions of yield, yield components, and blast resistance.

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Dissecting the Relationship Between Leaf and Panicle Blast Resistance in Rice by Disease Phenotyping and Gene Mapping Using a BC<sub>4</sub>F<sub>3</sub> Population

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Rice blast can be generally categorized as leaf blast and panicle blast according to the infected parts in the rice plant. The relationship between leaf and panicle blast resistance in rice is still unclear. To understand the correlation between leaf and panicle blast resistance in rice, an advanced BC population (BC<sub>4</sub>F<sub>3</sub>) derived from a durable blast-resistant variety and a susceptible variety (recurrent parent) was developed. Interval planting was conducted to evaluate the leaf and panicle blast resistance of BC<sub>4</sub>F<sub>3</sub> lines at the same time in the blast nursery in Guangdong, China and gene mapping was also performed. The disease evaluation indicated that the correlation between leaf blast resistance and panicle blast resistance in BC<sub>4</sub>F<sub>3</sub> was significant, but the correlation coefficient was not high ( $r = 0.440$ ). Three regions associated with leaf blast resistance were mapped on chromosome 2, 6 and 9, while three regions associated with panicle blast resistance were mapped on chromosome 3, 6 and 9, respectively. The region associated with leaf blast resistance on chromosome 9 co-localized with the region associated with panicle blast resistance on chromosome 9. The regions associated with leaf blast resistance and with panicle blast resistance on chromosome 6 were linked. However, the regions associated with leaf blast resistance on chromosome 2 and with panicle blast resistance on chromosome 3 were different. The mapping results could well explain the close relationship and the lower correlation coefficient between leaf blast resistance and panicle blast resistance in disease phenotypic analysis. Our results suggest that rice leaf blast resistance and panicle blast resistance have different mechanisms.

## Breeding Strategy for Rice Blast Disease Resistance in the Highland Production Environments of Africa

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The highland environments in Africa effect the countries of Burundi, Rwanda, Madagascar, Eastern Democratic Republic of Congo and the zone around Mont Kilimanjaro in Tanzania where rice (*Oryza sativa* L.) is a key staple and cash crop grown on over one million hectares in mixed irrigated/rainfed inland valleys. Rice areas in those valleys are located 1000 to 2100 masl (meters above sea level) with the average day and night time air temperatures ranging from 18 to 20°C and 15 to 10°C, respectively, with humidity constantly above 80%. Rice germplasm collections have limited diversity for rice improvement that can be successfully exploited in these agro-ecosystems because there is severe cold stress throughout the crop growth and development phases coupled with the prevalence and often epidemics of blast disease caused by *Magnaporthe oryzae* B. Couch. Research efforts aimed at managing rice blast disease also need to simultaneously consider the cold stress and the associated sheath rot (*Sarocladium oryza* and *Pseudomonas fuscovaginae*) disease complex which together cause significant grain yield and quality losses.

To identify sources of new germplasm in order to develop improved cultivars, a large scale screening of diverse rice germplasm was conducted in Rwanda in 2009 and 2010. The screening was conducted at 11 locations using the augmented design with six popular cultivars as controls (Gakire, Intsinzi, Intsindagirabigega, IR64, Zong geng and Yunkeng). At each location 350 accessions were evaluated including several *temperate* and *tropical japonica* cultivars reported to be cold tolerant. Accessions were also evaluated for days to heading, days to maturity, plant height and yield components (1000 grain weight, number of unfilled and filled grains per panicle, productive tillers/m<sup>2</sup> and grain yield/hectare). The results of the screening suggest accessions originating from West Africa and distributed by the Africa Rice Center in Benin may be good parents to incorporate improved cold tolerance and resistance to rice blast and sheath rot disease into the cultivars adapted to these environments. Seventy-two percent of the selected accessions were derived from the *O. glaberrima* accession, CG 14 (IRGC96717) and exhibited multiple resistances to blast and sheath rot diseases and cold tolerance, as well as, early maturity and the preferred long and bold grain type.

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## Rice Blast Disease in Texas

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Rice is an important agricultural commodity in Texas, with an economic impact of more than \$1 billion annually. Rice blast, caused by *Magnaporthe oryzae*, is one of the most devastating diseases in rice. Texas Rice Belt provides a warm, humid climate favorable for the infection and reproduction of *M. oryzae*. Current agronomic practices including dense stands and excessive use of nitrogen fertilizer increase the likeliness of the epidemic of the disease. Blast poses a constant threat to Texas rice production. The objectives of this study are to update the occurrence of rice blast and the research on control of the disease in Texas.

Rice blast has historically been a major disease in Texas. Prior to 1950's, blast did not cause substantial yield and quality loss of rice. However, with the increased usage of nitrogen fertilizer following World War II, blast became a major disease in rice. Increase in severity of the disease also coincided with the change in virulence of *M. oryzae* over time. The dominant races of *M. oryzae* in Texas have changed to IC-17, IB-19 and IE-1 from IG-1 during the 1960's through 1970's. Continued efforts in improving varietal disease resistance using major resistance genes including *Pi-b*, *Pi-kh(m)/s* and *Pi-ta*, and employing proper agronomic measures such as land and water management have contributed to the successful control of this disease. Currently, blast is not a widespread problem in Texas; severe blast has not been observed for many years. However, blast is highly adaptive to the host and potential race shifts could cause severe loss to the crop.

The development of blast-resistant varieties and lines adapted to Texas and other southern states has been a major focus of the plant pathology program in collaboration with rice geneticists and breeders at Beaumont, TX for many decades. An upland blast disease nursery has been established at the Beaumont Center since 1964. Approximately 200 varieties and lines are screened for resistance to blast each year. DNA marker-assisted selection has been used for incorporating major and minor blast resistance genes. As a result, many blast-resistant varieties and lines such as Madison (*Pi-ta*), Saber (*Pi-b*), and Jasmine 85 (resistant QTLs) have been released. In addition, a field research trial has been established at the Beaumont Center to allow the conduction of fungicide efficacy evaluation for control of blast under the Texas environment.

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Method for Evaluating Rice Wild Relatives (*Oryza* spp.) Reaction to Blast DiseaseEizenga, G.C.<sup>1</sup>, **Grunden, Q.P-H.**<sup>1</sup> and Lee, F.N.<sup>2</sup><sup>1</sup>USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, Arkansas, USA<sup>2</sup>Rice Research and Extension Center, University of Arkansas, Stuttgart, Arkansas, USA

Rice wild relatives (*Oryza* spp.) are an important source of novel pest resistance genes, as well as, tolerance to abiotic stress and yield-enhancing traits. Resistance to rice leaf blast caused by *Magnaporthe oryzae* B. Couch has been reported in *Oryza* spp. accessions. Most of the *Oryza* spp. accessions have low seed set, poor germination, and shatter seed, thus it is advisable to grow the *Oryza* spp. accessions in the greenhouse. In order to determine the reaction of these accessions to U.S. races of *M. oryzae*, it is necessary to adapt the procedures used for evaluating reaction to blast disease for use with the *Oryza* spp. accessions and their progenies. Our objective is to describe the procedure used to screen the *Oryza* spp. for reaction to *M. oryzae*.

To ensure the *M. oryzae* isolate of the specified blast race being used for inoculation was virulent, the culture was started from a stock culture of the isolate stored at -2°C by placing the dried culture on an oatmeal agar medium or an agar medium containing alfalfa pellets and rice hulls. After the initial culture grew, it was sub-cultured to obtain the number of spores needed for inoculation and if necessary, subcultures were started that could be stored for future use.

Seeds of the *Oryza* spp. accessions or progenies to be inoculated were dehulled, surface sterilized, and placed on an orchid medium in a lighted incubator set at 27°C with 12 hr light/12 hr dark for about 10 days. After the seedlings were approximately 12 cm tall, 4-6 plants were transferred to 6.5 cm square pots containing a soil mixture (1 part soil: 2 parts RediEarth). When the seedlings were at the 3 to 4 leaf stage, they were drought stressed in preparation for inoculation. Drought stress was induced by dumping the excess water from the flooded pots/trays and allowing the soil to dry down until no moisture was evident (~24-48 hours at room temperature). The *M. oryzae* spores were placed in a xanthene gum mixture (0.1 g xanthene gum per 400 ml water), counted using a Hemacytometer, and diluted to 100,000 to 200,000 spores per ml. The spore solution was applied to the leaves of the drought stressed seedlings with an airbrush, and the seedlings subsequently were placed in a tight plastic bag for 24 hr at room temperature. After the seedlings were removed from the plastic bag, they were watered with a 20-20-20 (N-P-K) liquid fertilizer solution and rated approximately one week later for blast symptoms on a 0 to 9 scale (0=no disease to 9=dead). Initially, a collection of *Oryza* spp. was screened using a slight variation of this method (Eizenga et al., 2009). Currently, this method is being used to evaluate mapping populations for reaction to blast disease (Eizenga et al., 2010).

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Characterization of *Magnaporthe grisea* (*Pyricularia grisea*) from Oat in Brazil**Urashima, A.S.** and Silva, C.P.

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Blast caused by *Magnaporthe grisea* (*Pyricularia grisea*) is a disease that occurs in many important gramineous plants in Brazil such as rice, wheat, triticale and barley. In 2005, the presence of this disease was reported on black oat (*Avena strigosa*) at different locations of Paraná state. Due to little information of *M. grisea* that is infecting this host, the present work aimed to characterize it at molecular, sexual and pathogenic level.

For molecular assay the RFLP/Southern blot technique was employed where total DNA was digested with *EcoR*I and hybridized with probe *Pot2*. Sexual compatibility between *M. grisea* from oat and rice, wheat, triticale and barley was also examined. Pathogenic assays included host range and cross-inoculation between the pathogens from oat and rice, wheat, triticale and barley.

The present data showed that *M. grisea* from black oat formed a homogenous and genetic distinct group of its own different from other hosts. Isolate 15720 was the only exception because it was similar to wheat isolate. There was no sexual compatibility between *M. grisea* from oat and rice, wheat, triticale or barley. In pathogenic terms, isolates of *M. grisea* from oat infected triticale, wheat, rye, millet, barley, sorghum and rice; cross-inoculation was positive with wheat, triticale, and barley.

Effect of Period of Wetness and Chemical Control on Wheat Seedlings with Different Reaction  
to *Magnaporthe grisea*

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Although wheat blast has been confined to Brazil, Bolivia and Paraguay, it has been a major disease of wheat since its first outbreak. The Brazilian policy to wheat expansion to other states of the Country has been jeopardized because this disease has already been reported recently. Therefore, it is spread to all wheat producing areas of the Country where severe yield loss is frequent. The warmer and wetter winter of the 2009 crop season favored blast epidemics on all wheat producing regions and complete yield loss occurred in many municipalities of the traditional states of Parana and Sao Paulo despite four applications of fungicides. In order to better understand the epidemiology of this intriguing disease we have initiated a study to examine if different periods of wetness and chemical control vary according to resistance of wheat varieties to *Magnaporthe grisea*. Five periods of wetness and two varieties, with and without chemical treatment, were employed in a split plot design with four replications. Preliminary data have shown that 14h was the minimal period of wetness to disease expression regardless of varietal resistance and the efficacy of chemical treatment decreased according to the increase of wetness period.

## Genotyping and Monitoring of Resistance to Blast Disease in Hybrid Rice from Sichuan Province

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Since the popularization of hybrid rice in China, the loss caused by *Magnaporthe oryzae* has been aggravated due to the breakdown of single genotypes of resistance in the varieties that have been widespread on an unprecedented large scale. The number of hybrid rice varieties has increased since the late 1990s in Sichuan. The genotyping of the resistance to blast disease in 134 commercial rice varieties, of which the majority were hybrid rice, were postulated by inoculating 21 differentiated isolates of *M. oryzae* of different compatible spectra to 24 lines of which each had a single, different known resistance gene. Only 2 out of the tested hybrid rice, Lu You No. 1 and Hong You 44, showed the same compatible spectra to the 21 differentiating isolates. The cluster analysis of the compatible spectra of the tested varieties classified them into 6 groups when the similarity index computed by DPS software was at 0.80. The genotypes of 2 hybrid rice, D Xiang 287 and Q You NO.2, could be clearly postulated. The former probably contained  $Pi-k^m$ ,  $Pi-z^t$  and  $Pi-7$  and the latter was considered to contain  $Pi-k$ ,  $Pi-I$ ,  $Pi-t$  and  $Pi-19$ . Unknown resistant genes were found to be present in other tested varieties. The field resistance of 24 NILs, 126 commercial varieties, largely hybrid rice, as well as Lijiang Xintuan Heigu (LTH) was tested at blast disease nurseries located at Pujiang, Xuyong, Ya'an or Jiangyou in Sichuan Province. The NILs of  $Pi-k^m$ ,  $Pi-k^p$ ,  $Pi-k^h$ ,  $Pi-z^5$  and  $Pi-9$  were resistant or less susceptible to blast disease than other lines. D You 202 was moderately resistant to leaf and panicle blast disease at Xuyong, Pujiang and Ya'an. D You 287 was found to be resistant or moderately resistant to leaf blast at Xuyong and Ya'an and resistant to panicle disease at Xuyong and Pujiang, whereas Q You 2 was susceptible to leaf and panicle blast at all locations, suggesting the accordance to result to the NILs of the same known genes. The differences in rate of panicle blast were significant between cultivars and nurseries, and the interaction between cultivars and nurseries was also remarkable. 98.41% of the 126 tested varieties were susceptible to panicle blast at Xuyong and Pujiang, while that rate was 63.43% at Ya'an.

## Test of Some Hybrid Combinations to Rice Blast

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Rice blast disease caused by *Magnaporthe oryzae* is one of the most devastating rice diseases worldwide. Blast resistant cultivars are recognized as the most efficacious and economical way to control this disease. Genetic resistance to rice blast is generally governed by a few major genes, often in combination with partial resistance genes. Multiple blast resistant cultivars have been released and utilized in the southern US. However, the blast pathogen is highly adaptable and new blast races have quickly evolved as resistant cultivars were utilized in rice production. For example races IB-1, IB-49, IC-17, and IG-1 are the most common in the southern US. The cultivar Katy, containing the *Pi-ta* gene, was released as resistant to these common races. However, a new virulent race IE-1k emerged to attack cultivars possessing *Pi-ta*. Recently, breeders have endeavored to combine blast resistance genes and introduce new sources of resistance for the deployment of new cultivars that have broader spectrum resistance in the southern US.

Hybrid breeding is accepted as a new way to improve yield and blast resistance for rice production in the southern US. Thirty hybrid rice combinations and selected parents were evaluated for reaction to a bulk spore suspension of seven common blast races including IE-1, IB-1, IH-1, IG-1, IB-49, IC-17 and IE-1k in greenhouse tests conducted at the University of Arkansas Rice Research and Extension Center (RREC), near Stuttgart, Arkansas in the Spring of 2010. Seedlings were inoculated at the four-leaf stage by spraying with  $16 \times 10^4$  to  $22 \times 10^4$  spores mL<sup>-1</sup>. The inoculated plants were held in the chambers with over 90% humidity at about 25° C for 18-24 hours, then moved to the greenhouse at 28° to 35° C. Disease reactions were determined 7 days after inoculation when typical blast lesions appeared on the leaves of susceptible checks. The Arkansas RREC Blast Leaf Rating Scale System (0 to 9 scale) was used to determine disease reactions. Nine rice combinations and 7 parents were found to be resistant to the mixture of the seven blast races. The resistant rice combinations and parents will be evaluated for subsequent hybrid rice development and progress will be presented

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