International Rice Blast Conference

2013  Jeju, Korea

Translation from Genomics to Disease Management

Auditorium, Ramada Plaza Jeju Hotel, Jejudo, South Korea

August 20-24, 2013
Official Language: English

http://www.irbc2013.com
STATEMENT OF WELCOME

On behalf of the 6th International Rice Blast Conference (IRBC) organizing committee, we would like to thank you all for joining the 6th IRBC in a beautiful island of South Korea, Jeju. In particular, we are grateful to the distinguished guests from all over the world.

Rice blast disease remains the most destructive disease of cultivated rice worldwide. Considering that rice is the staple food for more than half of human population, the disease is a significant threat to food security for many nations. It is therefore imperative to devise the novel and stable control strategies for the disease, which requires understanding of the pathogen, rice blast fungus and its interaction with rice or other host plants. IRBC has been the forum to foster collaboration among scientists around the world. Not surprisingly, much progress has been made again in a wide range of research topics on the biology, genomics, host-pathogen interactions, resistance, and disease management since the last Rice Blast Conference in Little Rock, Arkansas, USA.

Here, we have many distinguished scientists from home and abroad. We are convinced that this meeting will serve as an exciting venue to share the recent advancement in scientific researches and to provide opportunities for collaboration. Such sharing and promotion of collaboration among participants would enable “Translation of Genomics into Disease Management” as the main theme of this conference.

We would like to thank you again for being here with us, and wish all the participants meaningful and pleasant stay in the UNESCO Heritage and the New 7 Wonders of Nature!

Organizing Committees
6th International Rice Blast Conference
Jeju, South Korea
International Rice Blast Conference

2013 Jeju, Korea

Lifetime Achievement Award for Rice Blast Research

Bharat Chattoo
Eunjong Lee
Hei Leung
THE LIFETIME ACHIEVEMENT AWARD FOR RICE BLAST RESEARCH

Bharat Chattoo

Prof. Chattoo pioneered the molecular genetic and proteomic analyses of the rice-blast fungus towards understanding the natural variability in the blast populations. Prof. Chattoo’s group discovered and characterized in detail several new transposable genetic elements in the rice blast fungus *Magnaporthe oryzae*; one of these, a SINE element, was reported for the first time in a lower eukaryote. He also analyzed the organization of repeated DNA sequences, leading to a better understanding of genomic flux in this fungus and a new PCR-based DNA fingerprinting method, now used internationally. His group contributed to the development of near-isogenic lines for mapping of dominant blast resistance genes, in collaboration with IRRI and the Rockefeller Foundation. His lab also pioneered the development of RAPD and SCAR techniques for molecular mapping and fingerprinting of such blast resistance genes. More importantly, since 1987 he has been an exemplary mentor and supervisor and has trained a number of graduate students and postdoctoral fellows in Rice Blast research.
THE LIFETIME ACHIEVEMENT AWARD FOR RICE BLAST RESEARCH

Eunjong Lee

As a plant pathologist, Dr. Eunjong Lee has made significant contributions to understanding pathology of the rice-blast fungus, and to development of control strategies against the rice blast disease by establishing a strong foundation for rice blast research in Korea. His contribution includes introduction of differential varieties for race identification, breeding of resistant rice varieties, establishment of disease control system, and systematization of resistant screening. In addition, he has devoted himself to managing and coordinating agricultural research in both academic and industrial sectors. He also helped build and improve research capacity in Rural Development Administration (RDA) in Korea as its administrator. His contribution to basic research and professional and administrational services to research communities were vital to greatly advancing the development of rice blast research in Korea.
THE LIFETIME ACHIEVEMENT AWARD FOR RICE BLAST RESEARCH

Hei Leung

Dr. Leung, a Principal Scientist at IRRI, is one of the pioneering plant pathologists in molecular analysis of the rice blast fungus Magnaporthe oryzae. In the last 30 years, he has made significant contributions to many areas of rice-pathogen interactions, including a genetic analysis of pathogenicity in the rice blast pathogen, the application of pathogen population biology to disease control, and the dissection of qualitative and quantitative disease resistance in rice. In addition, he has helped build research capacity in a number of research and breeding institutions in developing countries to enable them to develop better disease resistance varieties through the application of new knowledge in host-pathogen interactions and plant genomics. Through collaborative research and training, he has helped the National Research Systems in the developing countries to become proficient in the application of molecular marker technology for rice resistance breeding.
## COMMITTEE MEMBERS

**International Organizing Committee**

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
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<tbody>
<tr>
<td>Barbara Valent</td>
<td>Kansas State University, USA</td>
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<td>Guoliang Wang</td>
<td>Ohio State University, USA</td>
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<td>Yinong Yang</td>
<td>Pennsylvania State University, USA</td>
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<td>Ralph Dean</td>
<td>North Carolina State University, USA</td>
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<td>Jin-Rong Xu</td>
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<td>Mark Farman</td>
<td>University of Kentucky, USA</td>
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<td>Nicholas J. Talbot</td>
<td>University of Exeter, UK</td>
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<td>Ane Sesma</td>
<td>John Innes Centre, UK</td>
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<td>Yong-Hwan Lee</td>
<td>Seoul National University, South Korea</td>
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<td>Didier Tharreau</td>
<td>CIRAD-CA, France</td>
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<td>Robert Zeigler</td>
<td>International Rice Research Institute (IRRI), the Philippines</td>
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<td>Yunliang Peng</td>
<td>Sichuan Academy of Agricultural Sciences, China</td>
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<td>Qinghua Pan</td>
<td>South China Agricultural University, China</td>
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<td>Yukio Tosa</td>
<td>Kobe University, Japan</td>
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<td>Gloria Mosquera</td>
<td>International Center for Tropical Agriculture (CIAT), Colombia</td>
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<td>You-Liang Peng</td>
<td>China Agricultural University, China</td>
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<td>Naweed I. Naqvi</td>
<td>Temasek Life Sciences Laboratory, Singapore</td>
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<td>Yulin Jia</td>
<td>United States Department of Agriculture, USA</td>
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## Local Organizing Committee

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
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<tr>
<td>Yong-Hwan Lee</td>
<td>Seoul National University, South Korea</td>
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<td>Seong-Sook Han</td>
<td>National Institute of Crop Science, South Korea</td>
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<td>Kyu Young Kang</td>
<td>Gyeongsang National University, South Korea</td>
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<td>Sun Tae Kim</td>
<td>Pusan National University, South Korea</td>
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<td>Sang-Nag Ahn</td>
<td>Chungnam National University, South Korea</td>
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<td>Young-Chan Cho</td>
<td>National Institute of Crop Science, South Korea</td>
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<td>Woo Bong Choi</td>
<td>Dong-Eui University, South Korea</td>
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<td>Kyoung Su Kim</td>
<td>Kangwon National University, South Korea</td>
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<td>Ki Duk Kim</td>
<td>Korea University, South Korea</td>
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<td>Hongsik Shim</td>
<td>National Academy of Agricultural Science, South Korea</td>
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<td>Nam-Soo Jwa</td>
<td>Sejong University, South Korea</td>
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<td>Jong-Seong Jeon</td>
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<td>Sook-Young Park</td>
<td>Sunchon National University, South Korea</td>
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<td>Jaehyuk Choi</td>
<td>Korea Atomic Energy Research Institute, South Korea</td>
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<td>Junhyun Jeon</td>
<td>Seoul National University, South Korea</td>
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GENERAL INFORMATION

Venue

Ramada Plaza Jeju Hotel, 66, Top-dong-ro Jeju-si, Jejudo, South Korea

From Jeju International Airport

Toward Yongmoon Rotary → Hancheon Bridge → Seomoon Market → Turn left at the Market Intersection → Go straight toward Tap-dong-ro (distance: 3.8 Km)

From Jeju Port

Turn left at Imhang-ro → Drive toward Yongdoom / Tap-dong (distance 2.2 Km)

Shuttle Bus Information

From Ramada Plaza Jeju hotel to Jeju internation airport

August 24 (Sat) : Depart at 16:00 and 19:00 from hotel entrance (1F)
August 25 (Sun): Depart at 11:10 and 13:40 from hotel entrance (1F)

Registration

The registration desk is located at the Ballroom Lobby. All conference attendees are requested to register and collect the conference materials at the registration desk. Please do not hesitate if you need any help.

Registration Fee

<table>
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<tr>
<th>Activity</th>
<th>Early registration (USD)</th>
<th>On-site registration (USD)</th>
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<tr>
<td>Regular Member</td>
<td>$300</td>
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<td>Student</td>
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* Early registration deadline is June 30, 2013.
* All credit cards will be accepted.
Instructions for Oral Presentations

1. Presenters must gather in their session room **15 minutes prior to the session start** time to conduct a brief meeting to coordinate the session. The session room will provide PC for your presentation, however, presenters may use their own laptop for the presentation. If you want to use your laptop, please inform to our staff. Please refer to the following image for a layout of session rooms and confirm the PC operation booth.

2. If you bring your own computer, please ensure that your computer is equipped with the proper monitor connector (**D-sub 15 pins**), as shown below. If your computer does not have this connection, please bring an appropriate converter with you.

3. During a meeting, moderators work with presenters to make sure the presentations are ready for projection onto a screen.

Poster Presentation and Hours

1. Posters will be displayed in the **Ballroom Lobby** of Ramada Plaza Jeju Hotel.

2. Posters are numbered as indicated in the PDF file of the abstract book, and a corresponding numbered poster board is available for attaching your poster.
3. Posters still displayed after the removal time has passed will be disposed of by the Congress Secretariat.

4. Please make your poster fit the space within the display panel. The panel size is 90 x 136 cm (Width x Height).

**Poster Hours**

Authors standby: **21, August (Wed) 16:00 - 18:00**

For more information about authors, titles, poster numbers, and poster sessions, please refer to Poster Session Information in this book.

**Accomodation**

**Ramada Plaza Jeju Hotel**, 66, Top-dong-ro, Jeju-si, Jejudo, Korea (+82-64-729-8100)

**Jeju Palace Hotel**, 1192-18 Samdo-2-dong, Jeju-si, Jejudo, Korea (+82-64-753-8811)

**Welcome Reception**

All registrants are invited to Welcome Reception at 18:00 – 20:00 on Aug. 20th (Tue) at Ramada Ballroom Lobby (standing buffet, please refer to the map).

**Conference Dinner and Award Ceremony**

All registrants are invited to Conference Dinner and Award Ceremony (with Korean traditional music and Raffle) at 18:30 – 20:00 on Aug. 23rd (Fri) at Ramada Ballroom.

**Photo Time**

Group photo will be taken before Poster Session on Aug. 21st (Wed) outside of Ramada Hotel.

**Field Trip to Rice Paddy Field**

Field trip to rice paddy field will be from 15:30 to 20:00 on Aug. 22nd (Thu).
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<tr>
<td>08:30-10:10</td>
<td>Genomics and Proteomics</td>
<td>Effectors of the Pathogen</td>
<td>Diversity and Population Biology</td>
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<td>Post Conference Tour</td>
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<td>10:10-10:30</td>
<td>Coffee Break</td>
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<td>10:30-12:10</td>
<td>Genomics and Proteomics</td>
<td>Effectors of the Pathogen</td>
<td>Name for the Rice Blast Fungus</td>
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<td>12:10-13:00</td>
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<td>Post Conference Tour</td>
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<td>13:00-13:20</td>
<td>Opening Ceremony</td>
<td>Lunch</td>
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<td>13:20-13:30</td>
<td>Plenary Lecture</td>
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<td>13:30-14:45</td>
<td>Plenary Lecture</td>
<td>Host Resistance</td>
<td>Country Reports</td>
<td>Disease Management and Breeding</td>
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<td>15:30-16:00</td>
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<td>Host Resistance</td>
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<td>Disease Management and Breeding</td>
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<td>16:00-16:50</td>
<td>Plenary Lecture</td>
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<td>Field Trip to Rice Paddy Field</td>
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<td>18:30-20:00</td>
<td>Welcome Reception</td>
<td>Policy Committee Meeting</td>
<td>Conference Dinner &amp; Award Ceremony</td>
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DAILY SCHEDULE

August
20
TUE
2013

10:00 - 18:00 **Registration**

13:00 - 13:20 **Opening Ceremony**
   Local Organizing Committee Chair
   President of Korean Society of Plant Pathology

**Plenary Lectures**

13:20 - 14:00 Nicholas J. Talbot, *University of Exeter, UK*
   Septin-mediated plant tissue invasion by the rice blast fungus *Magnaporthe oryzae*

14:00 - 14:40 Barbara Valent, *Kansas State University, USA*
   Towards understanding how the rice blast fungus invades living rice cells

14:40 - 15:20 Kyu Young Kang, *Gyeongsang National University, Korea*
   *In planta* secretome analysis during rice-rice blast fungus interactions

15:20 - 16:00 Coffee break

16:00 - 16:40 Sophie Kamoun, *The Sainsbury Laboratory, UK*
   From pathogen genomes to host plant processes

16:40 - 17:20 Guo-Liang Wang, *Ohio State University, USA*
   Molecular dissection of rice innate immunity to *Magnaporthe oryzae*

18:00 - 20:00 **Welcome Reception** (in Ramada Plaza Jeju Hotel)

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**Scientific Session**
**Genomics/Proteomics and Molecular Biology of the Pathogen**
(Co-Chairs: Thomas Mitchell and Kyoung Su Kim)

August
21
WED
2013

08:30 - 08:55 Wei Tang, *Nanjing Agricultural University, China*
   The bZIP transcription factor MoHac1 modulates growth, conidiogenesis, and pathogenicity of the rice blast fungus *Magnaporthe oryzae*

08:55 - 09:20 Kyoung Su Kim, *Kangwon National University, Korea*
   Functions of a Yippee-like (YPEL) gene family in development and pathogenicity in *Magnaporthe oryzae*

09:20 - 09:45 Naweed Naqvi, *Temasek Life Sciences Laboratory, Singapore*
   Cell signaling involved in appressorium development and host interaction in the rice-blast pathosystem

09:45 - 10:10 Ralph Dean, *North Carolina University, USA*
   Post translational modification of the proteome during appressorium formation in the rice blast fungus

10:10 - 10:30 Coffee break

10:30 - 10:55 Fucheng Lin, *Zhejiang University, China*
   Working on the molecular machinery of autophagy in *Magnaporthe oryzae*
10:55 - 11:20 Junhyun Jeon, Seoul National University, Korea
DNA methylation dynamics during pathogenic development in the rice blast fungus

11:20 - 11:45 Urayama Syunichi, Tokyo University of Agriculture and Technology, Japan
Magnaporthe oryzae chyrosivirus 1 has potential ability to change the host virulence and to infect via extracellular route

12:10 - 13:30 Lunch

**Scientific Session**
**Host Resistance, Signaling and Defense Responses**
(Co-Chairs: Guo-Liang Wang and Nam-Soo Jwa)

13:30 - 13:55 Jong-Seong Jeon, Kyung Hee University, Korea
Differential requirement of OsRAR1 in immune receptor-mediated resistance of rice to Magnaporthe oryzae

13:55 - 14:20 Bo Zhou, International Rice Research Institute, Philippines
Cloning of AvrPi9 by genome comparison of a pair of putative wild and mutant strains: an important step toward the understanding of the mechanism underlying the broad-spectrum resistance mediated by the rice blast resistance gene Pi9

14:20 - 14:45 Houxiang Kang, Chinese Academy of Agricultural Sciences, China
Molecular dissection of the complex genetic architecture of rice immunity to the blast fungus Magnaporthe oryzae using genome-wide association

14:45 - 15:10 Coffee break

15:10 - 15:35 Yoji Kawano, Nara Institute of Science and Technology, Japan
Elucidation of mechanisms of small GTPase OsRac1 activation by R protein Pit through OsSPIKE1

15:35 - 16:00 Nam-Soo Jwa, Sejong University, Korea
Physical mapping of rice MAP kinase interactome and functional analysis of OsMEK2 in early innate immunity

16:00 - 18:00 **Poster Session**

18:30 - 20:30 **Policy Committee Meeting**

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**Scientific Session**
**Effectors of the Pathogen**
(Co-Chairs: Chang Hyun Khang and Jong-Seong Jeon)

08:30 - 08:55 Ryohei Terauchi, Iwate Biotechnology Research Center, Japan
Toward understanding evolution and function of Magnaporthe oryzae effectors AVR-Pia, AVR-Pii and AVR-Pik

08:55 - 09:20 Zonghua Wang, Fujian Agriculture and Forest University, China
Evolution of AvrPiz-1 in the rice blast fungus

09:20 - 09:45 Chang Hyun Khang, University of Georgia, USA
Delivery of blast effector proteins into rice cells
09:45 - 10:10 You-Liang Peng, China Agricultural University, China
Alpha-1,3-mannosyltransferase mediated N-glycosylation of effector proteins is required for the rice blast fungus to evade host innate immunity

10:10 - 10:30 Coffee break

10:30 - 10:55 Yogesh K. Gupta, University of Exeter, UK
Role of exocyst complex in effector protein delivery during host colonization by Magnaporthe oryzae

10:55 - 11:20 Qinghua Pan, South China Agricultural University, China
Co-evolutionary stepwise relationships between AvrPik and Pik alleles in the rice blast pathosystem

11:20 - 11:45 Valerie Mogga, RWTH Aachen University, Germany
Comparative analysis of candidate effector genes in different Magnaporthe species

12:10 - 13:30 Lunch

Country Reports (Co-Chairs: Seong-Sook Han and Bo Zhou)

13:30 - 13:45 Cailin Lei, Chinese Academy of Agricultural Sciences, China

13:45 - 14:00 Necmi Beser, Trakya Agricultural Research Institute, Turkey

14:00 - 14:15 Bui Chi Buu, Institute of Agricultural Science for Southern Vietnam, Vietnam

14:15 - 14:30 Erwina Lubis Suwarno, Indonesian Center for Rice Research, Indonesia

14:30 - 14:45 Hira Kaji Manandhar, Nepal Agricultural Research Council, Nepal

14:45 - 15:00 Seong-Sook Han, National Institute of Crop Science, Korea

15:30 - 18:00 Field Trip to Rice Paddy Field

Scientific Session
Diversity and Population Biology of the Pathogen
(Co-Chairs: Didier Tharreau and Sook-Young Park)

08:30 - 08:55 Yanli Wang, Zhejiang Academy of Agricultural Sciences, China
Genetic diversity of Magnaporthe oryzae isolates from Zhejiang province, China over the past thirty years

08:55 - 09:20 Didier Tharreau, CIRAD, France
Origin, structure and migration of Magnaporthe oryzae populations pathogenic on rice

09:20 - 09:45 Izumi Chuma, Kobe University, Japan
Various species of Pyricularia constitute a robust clade distinct from Magnaporthe salvinii and its relatives in the Magnaporthaceae

09:45 - 10:10 Marc-Henri Lebrun, INRA, France
Molecular taxonomy of Magnaporthe and Pyricularia species

10:10 - 10:30 Coffee break
Scientific Session
Which Name for the Rice Blast Fungus, Pyricularia or Magnaporthe?

10:30 - 10:45 Introduction (Barbara Valent, Kansas State University, USA)
10:45 - 11:45 Discussion
11:45 - 12:00 Vote
12:10 - 13:30 Lunch

Scientific Session
Disease Management and Breeding
(Co-Chairs: Sang-Nag Ahn and Young-Chan Cho)

13:30 - 13:50 Sang-Nag Ahn, Chungnam National University, Korea
Mapping and candidate gene analysis of blast field resistance in an advanced backcross population in rice

13:50 - 14:10 Mary Jeanie Telebanco-Yanoria,
Japan International Research Center for Agricultural Sciences, Japan
Development of near-isogenic lines for blast resistance with an indica-type rice variety IR64 genetic background

14:10 - 14:30 Kumar Vasudevan, ETH Zurich, Switzerland
Exploring rice genetic diversity for identification of novel alleles of rice blast resistance genes

14:30 - 14:50 Young-Chan Cho, National Institute of Crop Science, Korea
Enhancing for broad-spectrum resistance to blast using Korean weedy rice

14:50 - 15:10 Nguyen Thi Lang, Cuu Long Delta Rice Research Institute, Vietnam
Using molecular markers identified from blast resistance genes and gene pyramiding for selection of durable resistant cultivars

15:10 - 15:30 Coffee break

15:30 - 15:50 Takashi Kamakura, Tokyo University of Science, Japan
Magnaporthe oryzae as a sensitive search tool for drug targets

15:50 - 16:10 Mathilde Sester, CIRAD, France
The impact of conservation agriculture on blast disease epidemics in upland rice

16:10 - 16:30 Alfredo Seiti Urashima, Universidade Federal de Sao Carlos, Brazil
Effect of oat blast on wheat blast disease

16:30 - 16:50 Hongsik Shim, National Academy of Agricultural Sciences, Korea
Damage analysis of rice panicle blast on disease occurrence time and severity

18:30 - 20:00 Conference Dinner and Award Ceremony (with Korean traditional music and Raffle)

Post Conference Scientific Activities
Visit to Research Institutes of Agricultural Biotechnology
Exploring Historic Agricultural Field and Machinery
International Rice Blast Conference

2013 Jeju, Korea

Invited Lecture Abstracts

Plenary Lectures
Scientific Sessions
Country Reports
ABSTRACTS OF INVITED LECTURE

Abstracts for invited speakers presentation at the International Rice Blast Conference 2013 in Jeju, Korea, August 20-24.

LIST OF ABSTRACT

### Plenary Lectures

**20, August (Tue) 13:20 – 17:20**

**PL-1**
- Nicholas J. Talbot, *University of Exeter, UK*
  - Septin-mediated Plant Tissue Invasion by the Rice Blast Fungus *Magnaporthe oryzae*

**PL-2**
- Barbara Valent, *Kansas State University, USA*
  - Towards Understanding How the Rice Blast Fungus Invades Living Rice Cells

**PL-3**
- Kyu Young Kang, *Gyeongsang National University, South Korea*
  - *In planta* Secretome Analysis during Rice-Rice Blast Fungus Interactions

**PL-4**
- Sophien Kamoun, *The Sainsbury Laboratory, UK*
  - From Pathogen Genomes to Host Plant Processes

**PL-5**
- Guo-Liang Wang, *Ohio State University, USA*
  - Molecular Dissection of Rice Innate Immunity to *Magnaporthe oryzae*

### Genomics/Proteomics and Molecular Biology of the Pathogen

**21, August (Wed) 8:30 – 12:10**

**GP-1**
- Wei Tang, *Nanjing Agricultural University, China*
  - The bZIP Transcription Factor MoHac1 Modulates Growth, Conidiogenesis, and Pathogenicity of the Rice Blast Fungus *Magnaporthe oryzae*

**GP-2**
- Kyung Soo Kim, *Kangwon National University, Korea*
  - Functions of a Yippee-like (YPEL) Gene Family in Development and Pathogenicity in *Magnaporthe oryzae*

**GP-3**
- Naweed Naqvi, *Temasek Life Sciences Laboratory, Singapore*
  - Cell Signaling Involved in Appressorium Development and Host Interaction in the Rice-Blast Pathosystem

**GP-4**
- Ralph Dean, *North Carolina University, USA*
  - Post Translational Modification of the Proteome during Appressorium Formation in the Rice Blast Fungus

**GP-5**
- Fucheng Lin, *Zhejiang University, China*
  - Working on the Molecular Machinery of Autophagy in *Magnaporthe oryzae*
GP-6 Junhyun Jeon, Seoul National University, Korea
DNA Methylation Dynamics during Pathogenic Development in the Rice Blast Fungus

GP-7 Urayama Syunichi, TUAT, Japan
Magnaporthe oryzae chrysovirus 1 Has Potential Ability to Change the Host Virulence and to Infect via Extracellular Route

HR

Host Resistance, Signaling and Defense Responses
21, August (Wed) 13:30 – 16:00

HR-1 Jong Seong Jeon, Kyunghee University, Korea
Differential Requirement of OsRAR1 in Immune Receptor-Mediated Resistance of Rice to Magnaporthe oryzae

HR-2 Bo Zhou, International Rice Research Institute, Philippines
Cloning of AvrPi9 by Genome Comparison of a Pair of Putative Wild and Mutant Strains: an Important Step toward the Understanding of the Mechanism Underlying the Broad-spectrum Resistance Mediated by the Rice Blast Resistance Gene Pi9

HR-3 Houxiang Kang, China Academy of Agricultural Sciences, China
Molecular Dissection of the Complex Genetic Architecture of Rice Immunity to the Blast Fungus Magnaporthe oryzae Using Genome-wide Association Study

HR-4 Yoji Kawano, Nara Institute of Science and Technology, Japan
Elucidation of Mechanisms of Small GTPase OsRac1 Activation by R Protein Pit through OsSPIKE1

HR-5 Nam Soo Jwa, Sejong University, Korea
Physical Mapping of Rice MAP Kinase Interactome and Functional Analysis of OsMEK2 in Early Innate Immunity

EP

Effectors of the Pathogen
22, August (Thu) 8:30 – 12:10

EP-1 Ryohei Terauchi, Iwate Biotechnology Research Center, Japan
Toward Understanding Evolution and Function of Magnaporthe oryzae Effectors AVR-Pia, AVR-Pii and AVR-Plk

EP-2 Zonghua Wang, Fujian Agriculture and Forest University, China
Evolution of AvrPiz-t in the Rice Blast Fungus

EP-3 Chang Hyun Khang, University of Georgia, USA
Delivery of Blast Effector Proteins into Rice Cells
EP-4 You-Liang Peng, China Agricultural University, China
Alpha-1,3-Mannosyltransferase Mediated N-Glycosylation of Effector Proteins Is Required for the Rice Blast Fungus to Evade Host Innate Immunity

EP-5 Y Gupta, University of Exeter, UK
Role of Exocyst Complex in Effector Protein Delivery During Host Colonization by Magnaporthe oryzae.

EP-6 Qinghua Pan, South China Agricultural University, China
The Co-evolutionary Stepwise Relationships between AvrPik and Pik Alleles in the Rice Blast Pathosystem

EP-7 Valerie Mogga, RWTH Aachen University, Germany
Comparative Analysis of Candidate Effector Genes in Different Magnaporthe Species

DP Diversity and Population Biology of the Pathogen
23, August (Fri) 8:30 – 10:10

DP-1 Yanli Wang, Zhejiang Academy of Agricultural Sciences, China
Genetic Diversity of Magnaporthe oryzae Isolates from Zhejiang Province, China over the Past Thirty Years

DP-2 Didier Tharreau, CIRAD, France
Origin, Structure and Migration of Magnaporthe oryzae Populations Pathogenic on Rice

DP-3 Izumi Chuma, Kobe University, Japan
Various Species of Pyricularia Constitute a Robust Clade Distinct from Magnaporthe salvinii and Its Relatives in the Magnaporthaceae

DP-4 Marc-Henri Lebrun, INRA, France
Molecular Taxonomy of Magnaporthe and Pyricularia species

DM Disease Management and Breeding
23, August (Fri) 13:30 – 16:50

DM-1 Sang Nak Ahn, Chungnam National University, Korea
Mapping and Candidate Gene Analysis of Blast Field Resistance in an Advanced Backcross Population in Rice

DM-2 Mary Jeanie Telebanco-Yanoria, JIRCAS, Japan
Development of Near-isogenic Lines for Blast Resistance with an Indica-type Rice Variety IR64 Genetic Background
Kumar Vasudevan, *ETH Zurich, Switzerland*
**Exploring Rice Genetic Diversity for Identification of Novel Alleles of Rice Blast Resistance Genes**

Young Chan Cho, *National Institute of Crop Science, Korea*
**Enhancing for Broad-spectrum Resistance to Blast Using Korean Weedy Rice**

Nguyen Thi Lang, *Cuu Long Delta Rice Research Institute, Vietnam*
**Using Molecular Markers Identified from Blast Resistance Genes and Gene Pyramiding for Selection of Durable Resistant Cultivars**

Takashi Kamakura, *Tokyo University of Science, Japan*
**Magnaporthe oryzae as a Sensitive Search Tool for Drug Targets**

Mathilde Sester, *CIRAD, France*
**The Impact of Conservation Agriculture on Blast Disease Epidemics in Upland Rice**

Alfredo Seiiti Urashima, *Universidade Federal de Sao Carlos, Brazil*
**Effect of Oat Blast on Wheat Blast Disease**

Hongsik Shim, *National Academy of Agricultural Sciences, Korea*
**Damage Analysis of Rice Panicle Blast on Disease Occurrence Time and Severity**

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**Country Reports**
22, August (Thu) 13:30 – 15:00

Cailin Lei, *Chinese Academy of Agricultural Sciences, China*
**Rice Blast Disease and Resistance Breeding in China**

Necmi Beser, *Trakya Agricultural Research Institute, Turkey*
**Rice Cultivation and Blast *(Pyricularia oryzae)* Management in Turkey**

Bui Chi Buu, *Institute of Agricultural Science for Southern Vietnam, Vietnam*
**Current Status for Blast Disease in Rice at Vietnam**

Erwina Lubis Suwarno, *Indonesian Center for Rice Research, Indonesia*
**Breeding Upland Rice for Resistance to Diverse Blast Pathogen in Indonesia**

Hira Kaji Manandhar, *Nepal Agricultural Research Council, Nepal*
**Rice Blast and Its Management in Nepal**

Seong Sook Han, *National Institute of Crop Science, Korea*
**Pathogenic Population Study of Magnaporthe oryzae and Novel Screening System for Durable Rice Blast Resistance in Korea**
Plenary Lectures

PL-1

Septin-mediated Plant Tissue Invasion by the Rice Blast Fungus *Magnaporthe oryzae*

Dagdas, Y.F., Ryder, L.S., Kershaw, M.J., and Talbot, N.J.

School of Biosciences, University of Exeter, Geoffrey Pope Building, Exeter EX4 4QD, United Kingdom. email: N.J.Talbot@exeter.ac.uk

*Magnaporthe oryzae* is the causal agent of rice blast, one of the most serious diseases affecting rice production. During plant infection, *M. oryzae* develops a differentiated infection structure called an appressorium. This unicellular, dome-shaped structure generates cellular turgor, which is translated into mechanical force to cause rupture of the rice cuticle and entry of the fungus into plant tissue. We have shown that a hetero-oligomeric septin GTPase complex is necessary for re-organisation of a toroidal F-actin network at the base of the appressorium which allows re-establishment of polarised fungal growth. Re-modeling of F-actin is necessary for cortical rigidification and localisation of proteins associated with membrane curvature to the appressorium pore. Septin-mediated cytoskeletal re-modeling is necessary formation of a penetration hypha that breaches the host cuticle and leads to plant tissue colonization by biotrophic invasive hyphae of *M. oryzae*. We will present evidence that septin-mediated plant infection is regulated by NADPH oxidase activity and a regulated burst of reactive oxygen species. A specialised Nox2 NADPH oxidase-tetraspanin complex is necessary for septin-mediated control of actin dynamics. We will also describe the potential operation of a pressure-mediated checkpoint, mediated by a cell wall mechanosensor protein that is necessary for initiation of septin activation and the re-orientation of the cortical F-actin cytoskeleton to facilitate plant tissue invasion.
Towards Understanding How the Rice Blast Fungus Invades Living Rice Cells

Valent, B.1, Giraldo, M.C.1, Yi, M.1, Khang, C.H.2, Mosquera, G.3, Kankanala, P.4, Berruyer, R.5, and Dalby, M.1

1Dept. of Plant Pathology, Kansas State University, Manhattan, Kansas 66506, USA
2Dept. of Plant Biology, University of Georgia, Athens, Georgia 30602, USA
3International Center for Tropical Agriculture (CIAT), Cali, Colombia
4Dept. of Grain Science and Industry, Kansas State University, Manhattan, Kansas USA
5Research Institute of Horticulture and Seeds, Université d'Angers, France

*Magnaporthe oryzae* secretes both apoplastic and cytoplasmic effectors during biotrophic invasion of rice. Apoplastic effectors remain extracellular in the extrainvasive-hyphal matrix surrounding biotrophic invasive hyphae that grow inside rice cells. In contrast, cytoplasmic effectors are translocated into the cytoplasm of invaded rice cells, and some move ahead into surrounding rice cells before fungal invasion. Known cytoplasmic effectors and many biotrophy-associated-secreted (BAS) proteins preferentially accumulate in biotrophic interfacial complexes (BICs) located outside the tips of filamentous hyphae that enter rice cells. BICs remain subapically beside the first bulbous invasive hyphal cells after hyphal differentiation. Subapical BIC-associated hyphal cells continue to express protein secretion components after they stop growing. Additionally, cytoplasmic effectors are continuously secreted into BICs using a secretory pathway that is insensitive to Brefeldin A (BFA), an inhibitor of conventional Golgi-mediated secretion. Pathogen mutants that fail to express exocyst complex components or a t-SNARE are defective in secretion of cytoplasmic effectors and in pathogenicity. In contrast, secretion of apoplastic effectors is blocked by BFA treatment and is not impaired in the exocyst- or t-SNARE defective mutants. Therefore, it appears that *M. oryzae* possesses distinct secretion mechanisms for cytoplasmic and apoplastic effectors. We previously reported that the fungus appears to seek out rice pit fields, with clustered plasmodesmata, for sending effectors into neighboring cells and for its cell-to-cell spread. We are currently performing correlative light and electron microscopy and time-course, live-cell imaging to decipher roles for six Bas proteins that accumulate around IH where they cross the rice cell wall, and to understand how plasmodesmata might play a role.

References:


In planta Secretome Analysis during Rice-Rice Blast Fungus Interactions

Kang, Kyu Young

Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju 660-701, Korea. Division of Applied Life Science (BK21 program), Gyeongsang National University, Jinju 660-701, Korea.

The in vivo apoplastic fluid (APF) secretome of rice-blast fungus interactions remains uncharacterized. Here, we report proteomics investigation of in vivo secreted proteins isolated from AFP of rice leaves infected with incompatible (KJ401) and compatible (KJ301) races of Magnaporthe oryzae (M. oryzae) along with appropriate controls using two widely used techniques, two-dimensional gel electrophoresis (2-DE) and multidimensional protein identification technology (MudPIT) coupled with MALDI-TOF-MS and/or liquid chromatography mass spectrometry (LC-MS/MS). Total 720 apoplastic proteins, 291 from rice and 429 from M. oryzae during rice-rice blast interactions, were identified.

Among them, Magnaporthe oryzae sondport1 homolog (MSP1) is recognized in a rice cultivar and triggers host cell death and defense responses. Exogenous treatment of recombinant MSP1 protein induced autophagic cell death in both suspension cultured rice cells and leaves. Protein kinase(s) play an important role for triggering cell death, and cell death was enhanced by the treatment with jasmonic acid and abscisic acid but suppressed by salicylic acid. We demonstrated that secretion of MSP1 into apoplast is necessary for triggering cell death and activating defense gene expression, implying that it is recognized in the host plasma membrane. M. oryzae mutants deficient in MSP1 failed to trigger strong cell death response and caused more severe disease compared to a wild-type strain, while its over-expression enhanced cell death and defense gene activation. Furthermore, pre-treatment of rice with a low concentration of MSP1 primed and strengthened resistance against the pathogen. Taken together, our data suggest that a rice cultivar has evolved a recognition mechanism of the secreted protein MSP1, resulting in triggering strong host innate immunity.

One of those GH family protein alpha-L-arabinofuranosidase B (MoAbfB) belongs to the GH43 subfamily. Leaf blotting and Western blot analysis showed that MoAbfB was expressed and secreted to the apoplastic region of M. oryzae infected sites. Biochemical analysis indicated that MoAbfB contains arabinofuranosidase activity. Exogenous treatment and transient expression indicated that MoAbfB triggers host cell death both in vitro and in planta, and its secretion is essential for the cell death activation. Gene deletion mutant of MoAbfB provoked more severe disease symptom, but over-expression of MoAbfB transformants significantly reduced fungal virulence comparing with recipient fungal strain ΔKU70. Protein dynamic analysis of MoAbfB pro::mCherry transformants exhibited that MoAbfB was closely expressed in appressorium, penetration peg and invasive hyphal tip. MoAbfB::GFP fusion protein was secreted into the apoplastic region during infection. Furthermore, RT-PCR data suggests that MoAbfB triggered priming effects to host immune response.
Infectious plant diseases cause havoc to world agriculture and threaten to slow laudable efforts to launch a second green revolution to meet the food security needs of a booming world population. Pathogens such as the rice blast fungus, wheat stripe and stem rust, the Irish potato famine pathogen, and many others continue to trigger recurrent epidemics with far reaching consequences. When faced with opponents like these, we need to know our adversary. The genome sequence of a plant pathogen is a deep look into its soul. From important and often unexpected insights into the biology of the pathogen to the tools needed to develop surveillance and diagnostic DNA markers, the genome is an invaluable resource that accelerates research and output delivery. With the cost of genome sequencing decreasing even faster than Moore’s law, the cost-benefit calculation is evident. In this talk, I will discuss some of ways in which genome biology impacts plant pathology. In particular, I will address how pathogen genomics can drive basic and applied plant pathology, and how state of the art findings on pathogen biology can be exploited to drive the development of new approaches to breeding disease resistant crops. In particular, I will focus on effector biology. I will discuss how pathogen genomes have revealed complex repertoires of effector genes, and how the study of these genes has resulted in major conceptual advances in plant pathology. I will conclude with a discussion of how novel methods of sequence-based gene mapping and cloning as well as targeted plant genome engineering are ushering the era of next-generation disease resistance breeding in plants.

References:
Molecular Dissection of Rice Innate Immunity to *Magnaporthe oryzae*

Wang, Guo-Liang

*Department of Plant Pathology, Ohio State University, Columbus, Ohio 43210 USA and Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China*

Because of rapid broken down of rice resistance to the blast fungus *Magnaporthe oryzae* in the field, increasing resistance durability is one of the key goals of blast disease management in rice production. However, molecular mechanism underlying the stable and broad-spectrum resistance to *M. oryzae* has not been fully understood. In the last several years, we have applied integrated approaches to dissection of the rice innate immunity using molecular, biochemical and bioinformatic techniques. We combined the genome-wide-association mapping study (GWAS) with the RNAi silencing method to rapidly pinpoint the resistance genes in the rice genome. Using the next-generation sequencing techniques, we isolated a large number of *in planta* expressed *M. oryzae* effectors and identified over 10 cell-death inducing apoplastic and nuclear or chloroplast-localized effectors. We identified 12 AvrPiz-t interacting proteins (APIPs) using the Avr effector AvrPiz-t as the bait. Among the characterized APIPs, we found that the E3 ligase gene *APIP6* is a positive regulator of PAMP-triggered immunity (PTI), the E3 ligase gene *APIP10* is not only a positive regulator of PTI but also a negative regulator of Piz-t-mediated resistance and the transcription factor gene *APIP5* negatively regulates cell death and disease resistance to *M. oryzae*. We also found that the histone H4 deacetylase gene *HDT701* negatively regulates the resistance to both *M. oryzae* and *Xanthomonas oryzae pv. oryzae* by modulating histone H4 acetylation of defense-related genes, suggesting the importance of epigenetic regulations in rice immunity. Finally, the inoculation results from the transgenic plants transformed with the *M. oryzae* host-induced gene silencing (HIGS) constructs will be discussed.

References:
The bZIP transcription factor MoHac1 was investigated. Our results showed that the ΔMohac1 mutant had a severe growth defect with thinner and lacked aerial hyphae. Moreover, MoHAC1 deletion led to a significant reduction in conidiation and pathogenicity on both barley and rice leaves. Microscopic observation found that the ΔMohac1 mutant could form appressoria on the barley leaf surface but failed to penetrate into the cells. In addition, the mutant was more sensitive to ER stress induced by the tunicamycin (TM) and dithiothreitol (DTT) treatment, which consistent with the situation in *Saccharomyces cerevisiae*, suggesting MoHac1 was involved in the UPR signaling pathway. Similar to the *S. cerevisiae* Hac1, a 23 bp unconventional intron was also identified in the MoHAC1 mRNA, indicating the UPR was involved in the unconventional splicing of MoHAC1 mRNA. Further analysis revealed that MoHac1 was localized in the nucleus during the development stages. Together, we concluded that the bZIP transcription factor MoHac1 is critical for growth, conidiogenesis, and pathogenicity of the rice blast fungus *Magnaporthe oryzae* and have roles in the UPR signaling pathway.
Functions of a Yippee-like (YPEL) Gene Family in Development and Pathogenicity in *Magnaporthe oryzae*


Department of Applied Biology, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon, 200-701, Korea

*Magnaporthe oryzae* is an ascomycete fungus and the causal agent of rice blast, one of the most destructive diseases of rice. This pathogen is considered as a good model organism to study phytopathogenic fungal development. Like most fungal pathogens, conidia (asexual spores) of *M. oryzae* play a key role in the disease cycle. Transcriptional regulation is an important process for development and pathogenicity in response to environmental factors. Therefore, transcription factors (TFs) is critical for understanding regulation of fungal development and pathogenicity. Yippee-like (YPEL) proteins exist in eukaryotes including fungi, plants and animals, and are highly homologous across different taxa. YPEL contains a putative zinc-finger binding domain, which has diverse functions such as binding DNA and RNA, and mediating protein folding and protein-protein interaction. For humans, five YPEL proteins were found. Analysis of human YPELs suggests their distinct functions in regulation of cell division and development. However, functions of YPEL proteins are still unknown in most organisms including fungi. Recently, we identified two YPEL genes in the genome of *Magnaporthe oryzae*. The two *M. oryzae* loci MGG_06263 and MGG_00255 share a significant sequence homology with humans YPEL proteins, and therefore named as MoYPEL1 and MoYPEL2, respectively. To elucidate function of MoYPEL1 and MoYPEL2 genes, we generated deletion mutants for each gene via homology-dependent gene replacement. In brief, the deletion mutant ΔMoypel1 showed a remarkable reduction in conidiation, reduced mycelial growth and no appressorial formation on hydrophobic slide glass, compared to the wild-type. In addition ΔMoypel1 showed a little appressorial formation and penetration *in planta*. On the other hand, the deletion mutant ΔMoypel2 showed no phenotypes in germination and appressoria formation, except for a reduced pathogenicity, compared to the wild-type. Also, ΔMoypel2 appears to increase conidiation and produce multiple appressorium. These data indicate that two YPEL proteins are important in development and pathogenicity.
In *Magnaporthe oryzae*, the conidial germ tube tip senses and responds to a wide array of requisite cues from the host in order to switch from polarized to isotropic growth during appressorium initiation. Although the role of G-protein mediated Cyclic AMP signaling was first identified almost two decades ago, little is known about the spatio-temporal dynamics of the cascade and how the signal is transmitted through the intracellular network during cell growth and morphogenesis in *Magnaporthe*. In this study, we demonstrate that the late endosomal compartments, comprising of a PI3P-rich (Phosphatidylinositol 3-phosphate) highly dynamic tubulo-vesicular network, scaffold active MagA/GαS, Rgs1 (a GAP for MagA), Adenylate cyclase and Pth11 (a non-canonical GPCR) in the likely absence of AKAP-like anchors during early pathogenic development in *M. oryzae*. Loss of HOPS component Vps39 and consequently the late endosomal function caused a disruption of adenylate cyclase localization, cAMP signaling and appressorium formation. Remarkably, exogenous cAMP rescued the appressorium formation defects associated with VPS39 deletion in *M. oryzae*. We propose that sequestration of key G-protein signaling components on dynamic late endosomes and/or endolysosomes, provides an effective molecular means to compartmentalize and control the spatio-temporal activation and rapid down-regulation (likely via vacuolar degradation) of cAMP signaling amidst changing cellular geometry during pathogenic development in *M. oryzae*. 
GP-4

Post Translational Modification of the Proteome during Appressorium Formation in the Rice Blast Fungus

Franck, W.L.¹, Gokce, E.², Oh, Y.¹, Muddiman, D.C.², and Dean, R.A.¹

¹Center for Integrated Fungal Research, North Carolina State University, Raleigh, NC 27695, United States
²W.M. Keck FT-ICR Mass Spectrometry Laboratory, Department of Chemistry, Raleigh, NC 27695, United States

Here, I present the first comprehensive view of the Magnaporthe oryzae proteome and phosphoproteome during early infection-related development and highlight biological processes important for pathogenicity. A total of 3200 unique proteins were identified by nanoLC-MS/MS in a temporal study of conidial germination and cAMP-induced appressorium formation. Using spectral counting based label free quantification, observed changes in relative protein abundance during the developmental process revealed changes in the cell wall biosynthetic machinery, transport functions and production of extracellular proteins in developing appressoria. One hundred and sixty-six up-regulated and 208 down-regulated proteins were identified in response to cAMP treatment. Proteomic analysis of a cAMP-dependent protein kinase A mutant revealed proteins whose developmental regulation is dependent upon cAMP signaling. A comparison of the proteome and transcriptome showed little correlation between transcript and protein regulation, with the exception of many of the most strongly regulated proteins indicating a central role in appressorium formation. Following the optimization of a phosphopeptide enrichment strategy, a total of 4984 phosphorylation sites from 1514 phosphoproteins were identified from 12 distinct biological conditions. Gene ontology analysis revealed GO categories related to signal transduction, protein phosphorylation and transcriptional regulation are enriched in the phosphoproteomics dataset. Phosphorylation sites whose occupancy changes during appressorium formation and sites that show CPKA dependent phosphorylation will be presented. Finally, the status of network analysis integrating transcriptomic, proteomic, and phosphoproteomic datasets will be described.
Magnaporthe oryzae is an important plant pathogenic fungus that greatly threatens the world’s food security. Autophagy is necessary for the formation of conidia and appressoria and for normal development and pathogenicity of M. oryzae. Progress in understanding the mechanistic basis of autophagy has been greatly facilitated by the discovery of the ATG genes and characterization of the encoded proteins. Evidence for the involvement of 24 autophagy-related genes in the pathogenicity of M. oryzae has been provided by genome-wide functional analysis. Recently, we identified and characterized a novel ATG protein, MoATG14, which has a coiled-coil domain in its N-terminals and is essential for autophagy in M. oryzae. Furthermore, we analyzed the coiled-coil domain in MoAtg14 and detected it is sufficient to interact with MoAtg6. Vesicle docking and fusion with a vacuole rely on a SNARE (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor)-dependent reaction. In yeast, the Mon1-Ccz1 complex is regarded as a guanine nucleotide exchange factor (GEF) of Ypt7, which triggers endosomal maturation by activating Ypt7 in late endosomes. In our research, MoYpt7 and MoMon1 are involved in vacuolar morphology and autophagy, and are required for homotypic vacuole fusion in M. oryzae. The ΔMoYpt7 and ΔMoMon1 mutants show lots of small and fragmented vacuoles in hypha, blocked in autophagy process, a breach in cell wall integrity and more sensitive to ions stress. And the mutants lost their ability to penetrate and infect the two tested host plants, rice and barley. There are still many questions that need to be answered concerning the autophagy machinery in M. oryzae. To identify novel ATG genes and their related functions in M. oryzae is imperative.
DNA Methylation Dynamics during Pathogenic Development in the Rice Blast Fungus

Jeon, J.¹², Choi, J.¹³, Lee, G.-W.⁴, Park, S.-Y.⁵, Huh, A.¹, Dean, R.A.²⁶, and Lee, Y.-H.¹³⁵⁷

¹Department of Agricultural Biotechnology, College of Agriculture and Life science, Seoul National University, Seoul 151-921, Korea; ²Functional Genomics, North Carolina State University, Raleigh, NC 27607, USA; ³Fungal Bioinformatics Laboratory, Seoul National University, Seoul 151-921, Korea; ⁴Department of Bioinformatics and Life Science, Soongsil University, Seoul 156-743, Korea; ⁵Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea; ⁶Center for Integrated Fungal Research Department of Plant Pathology, North Carolina State University, Raleigh, NC 27607, USA; ⁷Center for Fungal Genetic Resources, Seoul National University, Seoul 151-921, Korea

A key to evolutionary success of fungal pathogens is their ability to undergo morphological changes in response to host or environmental factors. A growing body of evidences suggests implication of epigenetic mechanisms in regulating such developmental processes of fungal pathogens. However, contribution of one such epigenetic component as DNA methylation to fungal development has been largely unappreciated at genomic scale to date. Here we used genetic manipulations and high-throughput bisulphite sequencing on the model plant pathogenic fungus, Magnaporthe oryzae to elucidate the dynamics and mechanics of DNA methylation at single-nucleotide resolution during pathogen development. Our genome-wide profiling of DNA methylomes in developmental samples including infection-specific structure appressorium revealed that genome-wide reprogramming of DNA methylation in and around genes occurs during progression of fungal development. Analysis of DNA methylation deficient mutants indicated that such reprogramming is required for normal development in the fungus. Furthermore, RNA-seq analysis showed that DNA methylation is associated with transcript abundance of genes in context-dependent manner. This comprehensive approach suggests that DNA methylation in fungi can be a dynamic epigenetic entity that has assumed new roles in developmental processes other than genome defense.
Magnaporthe oryzae chrysovirus 1 (MoCV1) is the quintuple dsRNA mycovirus found in the Vietnamese isolate, S-0412-II 1a, of *M. oryzae* (Urayama et al. 2010, Le M et al. 2010). Phylogenetic analysis based on the putative RNA-dependent RNA polymerase sequence showed that MoCV1 forms a unique sister clade with chrysoviruses such as *Penicillium chrysogenum* virus (Urayama et al. 2012). By curing with single spore isolation and cycloheximide treatment of MoCV1-infesting S-0412-II 1a, MoCV1-free strains of the fungus were isolated. The virus-free strains had normal mycelial growth and rich pigmentation, indicating that this mycovirus impairs growth of host cells (Urayama et al. 2010). MoCV1 was detected not only in host cells but also in culture supernatant when cultured in liquid media. When the virus-free trains were cultured with the supernatant containing the mycovirus, the mycelia were abnormally aggregated and the MoCV1 dsRNAs were detected in the aggregated mycelia (Urayama et al. 2010). These data suggest that the released MoCV1 might be able to infect to the other host fungi. Inoculation tests with MoCV1-infesting and -free strains to international differential rice varieties showed that the virus-free strains had more virulence to increase the lesion numbers and to enlarge the lesion sizes on infected leaves, and that, surprisingly, the infection with MoCV1 resulted in changes of pathogenic races in host blast fungus. Now we have developed new method to detect MoCV directly from blast lesions by attaching them with toothpick. By using this method, more strong MoCV strains in rice blast fungus will be found.
Host Resistance, Signaling and Defense Responses

HR-1
Differential Requirement of OsRAR1 in Immune Receptor-Mediated Resistance of Rice to *Magnaporthe oryzae*

Jeon, J.S., Lee, S.K., Han, M., Kim, C.Y., and Lee, J.

*Graduate School of Biotechnology and Crop Biotech Institute, Kyung Hee University, Yongin 446-701, Korea*

The RAR1 (required for Mla12 resistance) protein is essential for the plant immune response. In rice, a model monocot species, the function of OsRAR1 (*Oryza sativa* RAR1) has been little explored. In our current study, we characterized the response of a rice osrar1 T-DNA insertion mutant to infection by *Magnaporthe oryzae*, the causal agent of rice blast disease. osrar1 mutants displayed reduced resistance compared with wild type rice when inoculated with the normally virulent *M. oryzae* isolate, PO6-6, indicating that OsRAR1 is required for an immune response to this pathogen. We also investigated the function of OsRAR1 in the resistance mechanism mediated by the immune receptor genes Pib and Pi5 that encode nucleotide binding-leucine rich repeat (NB-LRR) proteins. We inoculated progeny from Pib/osrar1 and Pi5/osrar1 heterozygous plants with the avirulent *M. oryzae* isolates, race 007 and PO6-6, respectively. We found that only Pib-mediated resistance was compromised by the osrar1 mutation and that the introduction of the OsRAR1 cDNA into Pib/osrar1 rescued Pib-mediated resistance. These results indicate that OsRAR1 is required for Pib-mediated resistance but not Pi5-mediated resistance to *M. oryzae*. 
Cloning of *AvrPi9* by Genome Comparison of a Pair of Putative Wild and Mutant Strains: an Important Step Toward the Understanding of the Mechanism Underlying the Broad-spectrum Resistance Mediated by the Rice Blast Resistance Gene *Pi9*

Bao, J.\(^1,3\), Wu, J.\(^2\), Tang, M.\(^1\), Zhu, X.\(^1\), Wang, H.\(^1\), Jeon, J.S.\(^4\), Han, S.S.\(^5\), and Zhou, B.\(^1,3\)

\(^1\)State Key Laboratory Breeding Base for Zhejiang Sustainable Pest and Disease Control, Institute of Virology and Biotechnology, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China; \(^2\)China National Hybrid Rice R&D Center, Hunan Hybrid Rice Research Center, MaPoLing, Changsha, 410125, China; \(^3\)International Rice Research Institute, DAPO Box 7777, Metro Manila 1301, Philippines; \(^4\)Graduate School of Biotechnology, Kyung Hee University, Suwon 446-701, Korea; \(^5\)Crop Environment Research Div., National Institute of Crop Science, RDA, Suwon 441-707, Korea.

Cloning of *AvrPi9* by genome comparison of a pair of putative wild and mutant strains: an important step toward the understanding of the mechanism underlying the broad-spectrum resistance mediated by the rice blast resistance gene *Pi9*.

The rice blast resistance gene *Pi9* has been reported to control broad-spectrum resistance in different rice growing areas worldwide. To understand the mechanism underlying the *Pi9*-mediated broad-spectrum resistance, we have initiated the cloning of *AvrPi9* by genome comparison of a pair of putative mutant/wild strains. A virulent strain RO1-1, which was previously isolated in susceptible lesions from *Pi9*-harboring varieties in a sequential planting assessment for the durability of resistance genes, was genome resequenced. An avirulent strain R88-002, which exhibited the most similar PCR amplification pattern of RO1-1, was further identified from the pool of 26 avirulent strains used for the sequential planting, providing an ideal pair of putative mutant/wild strains for the isolation of *AvrPi9*. Comparative analyses of genome sequences revealed that RO1-1 and R88 are more related to each other than to other sequenced strains, suggesting that RO1-1 most likely emerged from genome mutations in the sequential planting assessment. We further classified the predicted genes of R88-002 and RO1-1 into five groups, i.e., identity (ID), SNP (SNP), small InDel (SI), large InDel (LI), and presence/absence (PA) based on their sequence relatedness to each other. Interestingly, only two gene models encoding predicted secreted proteins were identified in groups of LI and PA and one of these two candidate genes R55 were further found to be associated with avirulence of *AvrPi9*. Gene complementation tests finally confirmed that R55 can restore the avirulence of RO1-1 to the *Pi9* plants, suggesting that R55 is the *AvrPi9* gene. R55 encodes a small predicted secreted protein and is 279 bp in length of its coding sequence. An Mg-SINE element was identified in the coding sequence of R55 in RO1-1, suggesting that the insertion of Mg-SINE might involve in the loss of function of *AvrPi9* in the mutant strains. The putative function of *AvrPi9* in the fitness of rice blast pathogen and its distribution in worldwide strain collection will be discussed in the presentation.
HR-3
Molecular Dissection of the Complex Genetic Architecture of Rice Immunity to the Blast Fungus Magnaporthe oryzae Using Genome-wide Association Study

1State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, China; 2College of Agronomy, Hunan Agricultural University, Changsha, Hunan, 410128, China; 3Department of Plant Pathology, Ohio State University, Columbus, OH, 43210, USA; 4 Institute of Virology and Biotechnology, Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang, 310021, China; 5Department of Plant Breeding and Genetics, Cornell University

Rice blast is the most severe disease of rice that causes 10-30% rice yield loss annually. Although there is an untapped blast resistance (R) gene repertoire in the rice germplasm collection, mapping and cloning of the resistance genes are still time-consuming and challenging. In this study, we developed an integrated strategy to rapidly map and clone new R genes against Magnaporthe oryzae by combining the genome-wide association study (GWAS) and RNAi gene-silencing techniques. Five diverse blast isolates collected in five countries were used to inoculate a world-wide collection of 413 diversity rice varieties that are genotyped with high-density SNP chips. GWAS revealed that 66 loci \((p<7.6\times10^{-5})\) are associated with leaf blast disease resistance to the five isolates. Among them, 53 are new loci and the other 13 loci are located in the known R gene regions. Among the 85 candidate genes identified in the 66 loci, about 50% encode NBS-LRR-like proteins and receptor-like protein kinases. Three major R loci with the highest contributions to the resistance phenotypes are located in the R gene clusters on chromosome 1, 4 and 9, respectively. RNAi constructs targeting the candidate genes on chromosome 4 and 9 were transferred to the resistant cultivars to validate gene function. Resistance of the transgenic plants is being evaluated to appropriate blast isolates. Our results demonstrate that GWAS is a highly efficient method for dissecting the complex genetic architecture of blast resistance in rice.
Elucidation of Mechanisms of Small GTPase OsRac1 Activation by R Protein Pit through OsSPIKE1

Kawano, Y. and Shimamoto, K.

Laboratory of Plant Molecular Genetics, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0101, Japan

Plant disease resistance (R) proteins act as intracellular receptors for sensing pathogen invasion, and trigger a variety of immune responses. In plants, the molecules that are activated by R proteins, and how these downstream molecules induce immune responses, are largely unknown. We have recently found that the small GTPase OsRac1 is activated by NB-LRR-type R protein Pit, and this activation is involved in a critical role in R protein-mediated immunity in rice. However, mechanisms underlying how Pit activates OsRac1 remain largely unclear. Here, we found that OsSPIKE1 carrying a GDP/GTP exchange factor (GEF) domain which induces an activation of small GTPases is an interactor of Pit and that OsSPIKE1 was associated with OsRac1. Moreover, OsSPIKE1 RNAi plants showed the attenuation of Pit-mediated resistance to the blast fungus Magnaporthe oryzae as compared with the wild-type plants. Taken together, these results suggest that Pit activates OsRac1 through OsSPIKE1 during pathogen attack, and this activation may play a critical role in OsRac1-mediated immunity in rice. We are going to validate this model by further studies.
HR-5

Physical Mapping of Rice MAP Kinase Interactome and Functional Analysis of OsMEK2 in Early Innate Immunity

Singh, R., Choi, J., Dangol, S., and Jwa, N.-S.

Department of Molecular Biology, College of Life Sciences, Sejong University, Seoul, South Korea.

Mitogen-activated protein kinase (MAPK) cascades support the flow of extracellular signals to intracellular target molecules and ultimately drive a diverse array of physiological functions in cells, tissues, and organisms by interacting with other proteins. Yet, our knowledge of global physical MAPK interactome in plants remains largely fragmented. Here, we utilized the yeast two-hybrid system and co-immunoprecipitation, pull-down, bimolecular fluorescence complementation, subcellular localization, and kinase assay experiments in the model crop rice (Oryza sativa L.) to systematically map the first plant MAPK-interacting proteins. We identified 80 non-redundant interacting protein pairs (74 non-redundant interactors) for rice MAPKs and elucidated the novel proteome-wide network of MAPK interactors. The established interactome contains four membrane-associated proteins, seven MAP2Ks, four MAPKs and 59 putative substrates, including 18 transcription factors. Several interactors were also validated by experimental approaches (in vivo and in vitro) and literature survey. Our results highlight the importance of OsMPK1, an ortholog of tobacco SIPK and Arabidopsis AtMPK6, among the rice MAPKs as it alone interacts with 41 unique proteins (51.2% of the mapped MAPK interaction network). Additionally, gene ontology (GO) classification of interacting proteins into 34 functional categories suggested MAPKs participation in diverse physiological functions. Together, obtained results essentially enhance our knowledge of the MAPK-interacting protein network and provide a valuable research resource for developing a near complete map of the rice MAPK interactome.

Furthermore, pathogen induced plant innate immune response consists of diverse components although most of them are not known yet. The rice mitogen-activated protein kinase kinase, OsMEK2 have been transcriptionally induced by various defense related hormones like methyl-jasmonic acid (MeJA), salicylic acid (SA), ethephone, ABA indicating its common roles in defense responses. Here, we report that the overexpression of the pathogen inducible mitogen-activated protein kinase kinase, OsMEK2, functions as a positive regulator against virulent fungal pathogen KJ401 (89-010) in rice through involvement of the two MAP kinases OsMPK1 and OsMPK6. OsMEK2 overexpression (OsMEK2 OX) plants showed early disease resistance phenotype upon virulent fungal KJ401 infection in compare to wild type. Furthermore, OsMEK2 OX plants showed high expression pattern of defense related marker genes like OsPR1b, OsPBZ, OsPAL1 and OsAPX1 upon infection than wild type plants. We previously showed that OsMEK2 interacts with OsMPK1 and OsMPK6 by yeast-two hybrid, in vivo and in vitro assays. Upon pathogen infection, both OsMPK1 and OsMPK6 transcript level increased in OsMEK2 OX plants suggesting the involvement of either one or both of MAP kinases in OsMEK2 induced immune response. Our data indicate that OsMEK2 plays positive role in defense response in rice.
Effectors of the Pathogen

**EP-1**
Toward Understanding Evolution and Function of *Magnaporthe oryzae* Effectors AVR-Pia, AVR-Pii and AVR-Pik

Terauchi, R.¹, Saitoh, H.¹, Kanzaki, H.¹, Fujisaki, K.¹, Takagi, H.¹, Yoshida, K.², and Kamoun, S.²

¹Iwate Biotechnology Research Center, 22-174-4 Narita, Kitakami, Iwate 024-0003, Japan ²The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, UK

Using *M. oryzae* whole genome sequence information and association genetics approaches, we isolated the genes encoding three avirulence effectors, AVR-Pia, AVR-Pii and AVR-Pik, as well as several other effector candidates (Yoshida et al. 2009). Cognate R-genes encoding NB-LRR immunoreceptors for these three AVR effectors have also been reported; *Pia* (Okuyama et al. 2010), *Pii* (Takagi et al. 2013) and *Pik* (Ashikawa et al. 2010). Each of these NB-LRR receptors function as a pair of two proteins. The three sets of AVR-R genes provide an excellent system to understand commonalities and differences in the recognition of rice blast fungus by rice. We showed that Pik directly binds AVR-Pik. The binding specificity determines the recognition specificity of *AVR-Pik* alleles by Pik alleles and these genes are likely involved in arms-race co-evolution (Kanzaki et al. 2012). Our recent studies revealed candidate host interactors of AVR-Pii and AVR-Pik, some of which could be virulence targets. In this paper, I will use the AVR-Pik and AVR-Pii examples to present our latest findings on the interactions between the three players, AVR effector, immune receptor and host interactor proteins.
Effectors of the Pathogen

EP-2

Evolution of AvrPiz-t in the Rice Blast Fungus*

Chen, M.1, Zhong, Z.1, Zhou, B.1, Lu, G.1, Ebbole, D.2, Mitchell, T.K.3, Wang, B.1*, and Wang, Z.1*

1Key Laboratory of Bio-pesticide and Chemistry Biology, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou,350002, China; 2Department of Plant Pathology and Microbiology Texas A&M University College Station, TX 77843 USA; 3Department of Plant Pathology, The Ohio State University, Columbus, OH, 43210, USA

The Magnaporthe oryzae avirulence gene AvrPiz-t activates rice immunity mediated by Piz-t in a gene-for-gene fashion. It also acts to suppress pathogen-associated molecular pattern-triggered immunity in rice by targeting the RING E3 ubiquitin ligase APIP6. Durable resistance to rice blast has been difficult to achieve because the genome of M. oryzae evolves rapidly, resulting in mutation of Avr genes. We found that the virulent allele frequency to Piz-t increased rapidly from early 1990 in Fujian province of southeast China. Therefore, further characterization of AvrPiz-t evolution and its associated regions will enhance our understanding with regards to the evolution of Avr genes and aid in designing a better and sustainable strategy to manage the rice blast disease.

By screening field isolates consisting of 800 isolates obtained countrywide in China, we found 76.8% of the isolates were avirulent to rice cultivars with Piz-t. In those virulent isolates, mutation of AvrPiz-t was due to transposable element (TE) insertion into the gene. The insertion events exhibited distinct patterns of geographical distribution, for example, the isolates from Taiwan all contained TE insertions in the AvrPiz-t coding region. Interestingly, complete gene deletion was detected in a few isolates obtained from Sichuan. By comparing the genomic region of the AvrPiz-t locus from 10 rice blast isolates and 2 wheat blast isolates, we observed that the AvrPiz-t locus was present and highly conserved in both sequence and microsynteny. Our results indicate that TE insertion, rather than deletion and point mutation, could be the main mechanism controlling AvrPiz-t allelic diversity, and that regional clones are responsible for the rapid evolution of virulent populations. Our future research will focus on detailed analysis of the AvrPiz-t locus by using high-throughput pool-seq technology to find mutational hotspots and elucidate the evolutionary changes at the whole genome level when AvrPiz-t is under the influence of natural selection.

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**Corresponding authors: zonghuaw@163.com; abaowang@msn.com
Effectors of the Pathogen

EP-3
Delivery of Blast Effector Proteins into Rice Cells

Khang, C.H.1, Hernández-Rodríguez, Y.1, Kim, D.W.1, Jones, K.1, and Valent, B.2

1Department of Plant Biology, University of Georgia, Athens, Georgia 30602, USA; 2Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506, USA

Magnaporthe oryzae delivers cytoplasmic effector proteins into living rice cells, where these proteins manipulate host cellular processes. Because the delivery of effectors is pivotal for successful infection, understanding mechanisms underlying this process may provide a novel target for disease control. In addition, this may lead to identification of all effectors that share the same delivery mechanism.

During biotrophic invasion, ‘biotrophic interfacial complexes (BICs)’ have been proposed as a portal for effector delivery into host cells. BICs are plant derived and membrane-rich interfacial structures. When growing in the first invaded cells, M. oryzae produces only one BIC that initially forms adjacent to the tip of a primary hypha that develops from a penetration peg and then later remains on the side of the first bulbous invasive hypha that differentiates from the primary hypha. Transmission electron microscopy showed that BICs contain aggregations of lamellar membranes immediately outside the fungal cell wall. These membranes were associated with an electron-dense region, presumed to be lipid-rich, which appeared to be encapsulated by a cap-like structure.

Using a quantitative host translocation assay, we found a major role of effector promoters in accounting for preferential BIC localization and host translocation of cytoplasmic effectors such as Pwl2p and Bas1p. Transcriptional fusion of the PWL2 upstream regulatory sequence to the gfp reporter gene showed strong upregulation of the gene in BIC-associated cells compared to lower basal levels of expression throughout the invasive hyphae at later infection stages. We determined that the PWL2upstream regulatory sequence and signal peptide-encoding sequence were sufficient for both preferential BIC localization and delivery into host cells. Taken together, our results suggest that blast effector delivery requires precise spatiotemporal regulation of effector gene expression in BIC-associated cells.

References:
Alpha-1,3-Mannosyltransferase Mediated N-Glycosylation of Effector Proteins Is Required for the Rice Blast Fungus to Evade Host Innate Immunity


*State Key Laboratory of Agrobiotechnology and Ministry of Agriculture Key Laboratory of Plant Pathology, China Agricultural University, Beijing 100193, China*

Protein N-glycosylation is important for growth and development of higher eukaryotes but remains largely unknown in filamentous fungi. Here, we show that MoALG3 mediates N-glycosylation of the effector protein Slp1 in the rice blast fungus *Magnaporthe oryzae*. MoALG3 encodes an α-1,3-mannosyltransferase involved in N-glycan biosynthesis of proteins. Deletion of MoALG3 in *Magnaporthe oryzae* results in a significant reduction in virulence and arrest of the primary infectious hyphae. The ΔMoalg3 mutant induced massive production of reactive oxygen species (ROS) in host cells, which is similar to the SLP1 deletion mutant. Slp1 sequesters chitin oligosaccharides from recognition by the rice chitin elicitor binding protein (CEBiP) for inducing innate immune responses including ROS production. Invasive growth of the ΔMoalg3 mutant was recovered in plant cells that were pretreated with the NADPH oxidase inhibitor diphenyleneiodonium. We found that Slp1 is an N-glycosylated protein with three N-glycosylation sites (Asn48, Asn104 and Asn131). Simultaneous N-glycosylation of these three sites is required for Slp1 to be functional. More importantly, MoALG3 is required for the full N-glycosylation of Slp1. These results indicate that MoAlg3 mediated N-glycosylation of effector proteins is a novel strategy for the rice blast fungus to evade host innate immunity, which may be widely deployed by fungal pathogens.
Effectors of the Pathogen

EP-5

Role of Exocyst Complex in Effector Protein Delivery During Host Colonization by Magnaporthe oryzae.

Gupta, Y.K.¹, Dagdas, Y.¹, Giraldo, M.², Valent, B.², and Talbot, N.J.¹

¹School of Biosciences, University of Exeter, EX4 4QD, UK; ²Department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA.

Effectors are required to be secreted by pathogen during host colonization to suppress host immune responses and may also mediate invasive growth. In rice blast fungus, Magnaporthe oryzae, effectors have been shown to localize at the appressorium pore prior to plant infection, at the tips of primary invasive hyphae and in a specialized plant-derived, membrane-rich structure called the Biotrophic Interfacial Complex (BIC). Secretion mechanism of the effectors is not well defined in M. oryzae. The exocyst plays a crucial role in polarized exocytosis. It is an octameric protein complex (composed of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84) that appears to be evolutionary conserved in fungi and to play a crucial role in vesicle tethering to the plasma-membrane. We are currently characterizing components of exocyst complex during infection related development of M. oryzae. We have shown that the exocyst localizes to hyphal tips as in other fungi during vegetative hyphal growth. Interestingly, during appressorium development exocyst components are localized around the appressorial pore, which suggests the pore is an active site for secretion at the point of plant infection. Recently we have shown a hetero polymeric septin network is required for the organization of the appressorium pore and we are showing here that localization of the exocyst at the appressorium pore is septin dependent. The exocyst is furthermore involved in secretion of symplastic (host cell-delivered) effectors but not apoplastic effectors. Targeted gene deletion of exocyst components Exo70 and Sec5 causes significant virulence defects because of impaired secretion. We will present new information on the role of the exocyst during appressorium mediated plant invasion of M. oryzae.
The rice blast *AvrPik* alleles correspond to the rice resistance gene *Pik* alleles in a gene-for-gene fashion. The genetic diversity of the non-signal domain of *AvrPik* was higher than that in its signal peptide domain. Positive selection for particular *AvrPik* alleles in the northeastern region of China was stronger than in the South, reflecting the dominance of fewer cultivars in the former region. The perfect relationships between the functional lineages and *AvrPik* allele specific pathotypes were established by ruling out the non-functional lineages derived from additional copies of target gene. All 16 possible *AvrPik* alleles based on combinations of four major SNPs were reconstructed, in which 24 pathways get through the wild type (0000) to the most virulent form (1111) via 4 evolutionary phases. Only four alleles conditioning stepwise pathotypes were detected in the natural populations, which were likely created by only one evolutionary pathway with three recognizable mutation steps. Assembling single SNP pairs responsible for avirulence or virulence against the relevant *Pik* alleles was informative for the sequence basis of *AvrPik* allele specificity. Assuming that *AvrPik* evolution has been largely driven by host selection, the co-evolutionary stepwise relationships between *AvrPik* and *Pik* were established. The data confirmed the centrality of stepwise mutation in the co-evolution of a plant pathogen and its host.
Effectors of the Pathogen

EP-7
Comparative Analysis of Candidate Effector Genes in Different Magnaporthe Species

Mogga, V.¹, Delventhal R.², Kroj T.², and Schaffrath, U.¹
¹Department of Plant Physiology, RWTH Aachen University, D-52074 Aachen, Germany; ²INRA, UMR Biologie et Génétique des Interactions Plante-Parasite, F-34398 Montpellier, France

While isolates of Magnaporthe oryzae establish a host interaction with barley or wheat, isolates of Magnaporthe grisea which are virulent on Digitaria sanguinalis establish a nonhost interaction with both crop plants (Zellerhoff et al., 2006). Transcript profiling in barley epidermal peels after inoculation with host- or nonhost Magnaporthe isolates led to a list of hypothetical effector genes (HEGs) which are up-regulated in the host isolate. Time course studies revealed that HEGs can be grouped according to their maximal transcript abundance. Expression of the first group of HEGs was up-regulated during the initial infection process when the fungus had not yet entered a host cell. Transcripts of those early HEGs could also be detected in fungal infection structures formed in vitro and partially in vegetative mycelium as well. HEGs of the second group showed expression maxima during the biotrophic infection stage and were down-regulated possibly correlated with the switch of the pathogen to necrotrophy. Homologues of M. oryzae HEGs could be identified in M. grisea. Interestingly, time course studies revealed a co-regulation of most homologues during interaction of M. grisea with the immune-compromised barley cv. Nigrate. This points to a developmental control of HEG expression during the infection process and led to the speculation that sequence differences among homologous HEGs condition the success of the pathogen. Currently functional analyses and localization studies of HEGs are in progress.

Nep1 (necrosis- and ethylene-inducing protein 1)-like proteins (NLPs) are highly conserved among pathogenic bacteria, fungi and oomycetes and are believed to be protein toxins rather than elicitors in dicotyledonous plants. Although there are no dicotyledonous hosts known for Magnaporthe sp., isolates of M. oryzae possess four NLP genes (MoNLPs). Interestingly expression of two of them could be detected during colonization of barley, especially during necrotrophy. Furthermore Agrobacterium-infiltration assays revealed the capacity of three MoNLPs to induce cell death in tobacco. Remarkably, this cell death could be suppressed by co-expression of the Colletotrichum higginsianum (Ch) effector candidate 3 (ChEC3) that suppresses ChNLP1-mediated cell death as well (Kleemann et al., 2012). Further experiments addressing the possible function of MoNLPs will be presented.

References:
1. Zellerhoff, N. et al. (2006). Nonhost resistance of barley is successfully manifested against Magnaporthe grisea and a closely related Pennisetum-infecting lineage but is overcome by Magnaporthe oryzae, Mol Plant Microbe Interact 19(9): 1014-22.
 Genetic diversity in *Magnaporthe oryzae* isolates is relatively common and closely related to the loss of rice resistance. Many studies show that strains isolated from different areas and in different years differ greatly in terms of genetic structure. Changes in the genetic structure of field isolates may suggest the alteration of rice varieties with different resistance genes. In this paper, ITS-5.8S rDNA sequencing technology was applied to detect the genetic diversity of *M. oryzae* strains isolated from 11 different areas in Zhejiang Province, China, from 1978 to 2009. Sequencing analysis showed 199 mutant sites in the rDNA region and 9 independent haplotypes. Haplotype H1 was found in all populations, with an average haplotypic diversity ($H_d$) of 0.337 and average nucleotide diversity ($P_i$) of 1.415%. Among the populations studied, the Li Shui population had the highest $H_d$ and $P_i$. The phylogenetic tree of the 9 haplotypes showed two branches. Haplotypes H5, H6, and H9 formed one branch and the rest of the haplotypes clustered into another branch. The small inter-population fixation index and high level of genetic exchange indicated a high degree of gene flow among the populations. Analysis of molecular variance revealed significant genetic variation in both inter- and intra-populations. However, genetic variations were mostly confined within the populations. Geographical distances showed no significant correlation with genetic distance, and genetic differentiation in the *M. oryzae* population was not consistent with a simple model of isolation based on distance.
Inferring invasion routes and identifying reservoirs of diversity of plant pathogens are essential to propose new strategies for their control. *Magnaporthe oryzae*, the fungus responsible for rice blast disease, has invaded all rice growing areas. Virulent genotypes regularly (re)emerge, causing rapid resistance breakdowns. However, the worldwide genetic subdivision of *M. oryzae* populations on rice and its past history of invasion have never been elucidated. To investigate the centers of diversity, of origin and of migration of *M. oryzae* on rice, we analyzed the genetic diversity of fifty-five samples from fifteen countries. Three genetic clusters were identified worldwide. Asia was the center of diversity and the origin of most migrations to other continents. In Asia, two centers of diversity were revealed in the Himalayan foothills: South China-Laos-North Thailand, and Western Nepal. Sexual reproduction persisted only in the South China-Laos-North Thailand region, which was identified as the putative center of origin of all *M. oryzae* populations on rice. Our results suggest a scenario of early evolution of *M. oryzae* on rice that matches the past history of rice domestication. This study confirms that crop domestication may have considerable influence on the pestification process of natural enemies.
Various Species of *Pyricularia* Constitute a Robust Clade Distinct from *Magnaporthe salvinii* and Its Relatives in the Magnaporthaceae

Chuma, I., Murata, N., Aoki, T., Kusaba, M., and Tosa, Y.

Graduate School of Agricultural Sciences, Kobe University, Kobe 657-8501, Japan; Genetic Resources Center, National Institute of Agrobiological Sciences, Tsukuba 305-8602, Japan; Laboratory of Plant Pathology, Faculty of Agriculture, Saga University, Saga 840-8502, Japan.

Blast fungi have been designated as *Pyricularia* (as its anamorph) or *Magnaporthe* (as its teleomorph or holomorph). According to the new nomenclatural code for algae, fungi, and plants (the Melbourne Code), one fungus should have only one correct generic name (Hawksworth 2011). To discuss which should be adopted for the generic name of blast fungi, we examined phylogenetic structure of the family Magnaporthaceae using 39 isolates belonging to nine *Pyricularia* species along with other five Magnaporthaceous species employed by Zhang et al. (2011). Maximum parsimony, maximum likelihood, and Bayesian interface trees were constructed based on nucleotide sequences of the rDNA-ITS region and the *RPB1* gene. In all trees, isolates of Magnaporthaceous species tested were divided into two clusters. One was composed of *Pyricularia* spp. producing pyriform conidia, while the other was composed of *M. rhizophila*, *M. poae*, *G. graminis*, and *G. incrustans* etc. *Magnaporthe salvinii*, the type species of the genus *Magnaporthe*, was included in the latter group. The high bootstrap supports at the nodes of the two clusters suggest that each of them forms a monophyletic clade derived from a common ancestor. From these results we conclude that *Pyricularia* spp. constitute a large, but distinct fungal population that is not congeneric with *Magnaporthe salvinii*. Based on these results, we propose that the clade of the blast fungi should be divided from the genus *Magnaporthe*, and be designated as *Pyricularia* simply based on its priority. The name *Magnaporthe* should be left for the type species and its closely related species in the *M. salvinii* clade. If *Magnaporthe* is to be adopted for the generic name of the blast fungi, the type species of *Magnaporthe*, *M. salvinii* must be replaced with some species in the *Pyricularia* clade by taking an exceptional measure beyond the ordinary nomenclatural procedures.

References:
Molecular Taxonomy of *Magnaporthe* and *Pyricularia* species

Klaubauf, S.¹, Tharreau, D.², Fournier, E.³, Groenewald, J.Z.¹, Crous, P.W.¹, de Vries, R.P.¹, and Lebrun, M.-H.⁴

¹CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
²UMR BGPI, CIRAD, Campus International de Baillarguet, F-34398 Montpellier, France
³UMR BGPI, INRA, Campus International de Baillarguet, F-34398 Montpellier, France
⁴UR1290 INRA BIOGER-CPP, Campus AgroParisTech, Avenue Lucien Brétignières, F-78850 Thiverval-Grignon, France

*Magnaporthe* species cause serious diseases on grasses. *Magnaporthe oryzae* (previously named *Pyricularia oryzae* by Cavara in 1891, *Pyricularia grisea* and *Magnaporthe grisea* by Rossman in 1990) is responsible for rice blast disease and foliar diseases of wheat, barley, *Panicum*, *Eleusine* and *Setaria*. *Magnaporthe grisea* is responsible for foliar diseases of *Digitaria*. *Magnaporthe poae* causes summer patch of turf grass. The asexual genus *Pyricularia* encompasses species isolated from various monocots that produce pyriform, tri-cellular conidia from conidiophores. *Pyricularia higginsii* was isolated from infected *Cyperus*, while *P. zingiberi* was isolated from ginger, *P. pennisetii* from *Pennisetum*, *P. zizaniicola* from *Zizania*, *P. juncicola* from *Juncus* and *P. commelinicola* from *Commelina*. Here we present the phylogenetic relationships among isolates from a wide range of *Magnaporthe/Pyricularia* species based on partial DNA sequences of multiple genes including LSU, ITS, RPB1, actin and calmodulin. Our extensive analysis reveals a large diversity within the genus *Pyricularia*. Almost all *Pyricularia* species belong to a clade that includes all *Magnaporthe oryzae/M. grisea* isolates tested. This clade is clearly distinct from *Gaeumannomyces/Magnaporthiopsis* clades for all the sequences tested. A few *Pyricularia* species (e.g. *P. parasitica, P. lauri*) are unrelated to the *Magnaporthaceae*. This strongly suggests that the conidium morphology defining this genus is polyphyletic and cannot be used as taxonomical criterion without phylogenetic data. *Magnaporthe oryzae/M. grisea* isolates cluster in numerous related clades, one of which corresponds to *M. oryzae*. This clade includes isolates from rice (specific to this clade), wheat (specific), *Setaria* (specific) and *Eleusine* (specific) as well as isolates from other plants found as hosts also represented in other clades such as *Echinochloa, Cenchrus* or *Lolium*. Another clade corresponds to *M. grisea* and consists of isolates from *Digitaria* (specific to this clade). In addition, we identified four clades that could correspond to novel species, two of which were already described as distinct clades in previous phylogenetic analyses of this genus. These novel species are more related to *M. oryzae/M. grisea* clades than *Pyricularia* species clades. A re-evaluation of generic and species concepts within the *Magnaporthe/Pyricularia* clade is suggested and potential novel names will be proposed for discussion.
Disease Management and Breeding

DM-1

Mapping and Candidate Gene Analysis of Blast Field Resistance in an Advanced Backcross Population in Rice

Kim, D.M.1, Fabreag, E.R.1, Ju, H.G.2, Han, S.S.3, Roh, J.H.3, and Ahn, S.N.1*

1Department of Agronomy, Chungnam National University, Daejeon 305-333, Korea
2Agricultural Department, Agricultural Coll. of Yanbian Univ., Longjing, Jilin 133400, China
3National Institute of Crop Science, RDA, Suwon 441-857, Korea

A QTL for field resistance to rice blast was mapped using an advanced backcross population of 117 BC3F5 lines. These lines were evaluated in blast nurseries at four locations for two years. QTL analysis identified two QTLs on chromosomes 4 and 7 for resistance to blast nursery tests. One QTL, bn4 on chromosome 4 was detected at all locations in both years explaining from 14.5% to 35.9% of the phenotypic variance. Genetic analysis of the blast phenotypic data of the F2 and F3 population from a cross between an NIL harboring the target region on chromosome 4 and the recurrent parent, indicated that a major dominant gene designated as Pi45(t), was conferring resistance to blast nursery test. Linkage analysis indicated that Pi45(t) was located in the interval RM17511-RM3687, a region of approximately 409-kb. Seven lines with/without Pi45(t) were assayed in the greenhouse using a sequential planting method in seven cycles using 29 virulent isolates. Lines with the Pi45(t) gene showed less than 20% diseased leaf area, which was significantly below the threshold leaf of 40% considered for durable blast resistance. Five promising lines nearly isogenic to the recurrent parent except for blast resistance were evaluated at the preliminary yield trial. The Pi45(t) locus landed on three BAC clones, OSJNa0058K23, OSJNb0085C12 and OSJNa0053K19 in the Gramene and IRGSP database. According to sequence annotation database, 70 possible ORFs were found in the target region. These included six resistance gene analogs with an NBS-LRR domain. We are screening the segregating population to further narrow down the target region using DNA markers newly designed based on candidate genes sequence.

*Corresponding author: ahnsn@cnu.ac.kr
DM-2

Development of Near-isogenic Lines for Blast Resistance with an Indica-type rice variety IR64 Genetic Background

Telebanco-Yanoria, M.J.¹, Fukuta, Y.¹, Koide, Y.¹, and Kobayashi, N.²

¹Japan International Research Center for Agricultural Sciences, 1-1 Ohwashi, Tsukuba, Ibaraki 325-8686, Japan; ²National Institute of Crop Science, 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8517, Japan

Rice blast is one of the major diseases of rice (Oryza sativa L.) caused by Pyricularia grisea (Cooke) Sacc. that considerably affect rice production in most rice growing areas worldwide. It is economical to use resistant variety but the cultivation of it in large areas promotes vulnerability to rice blast disease. This is due to the unpredictable variation of rice blast population overtime that eventually leads to breakdown of resistance. To prevent this scenario, a multiline variety is maybe more practical and effective to use than a single variety. Multiline variety is composed of near-isogenic lines (NILs) in the same genetic background but contained several resistance genes that can be deployed simultaneously according to the current population structure of the fungus.

We tried to develop a multiline variety using IR64 as the genetic background, because it is one of the most popular rice varieties for irrigated lowland in tropical Asia. It was estimated to include six resistance genes, one of these three genes (Pi20, Pita, or Pita-2), Piz-t, Pib, Pik-s, Pia, and one unknown gene (Ebron et al. 2004). But in 2005, the resistance of IR64 was breakdown in Indonesia and Philippines.

To improve the resistance level of IR64, 22 NILs with eleven resistance genes—Pish, Piz, Piz-5, Pi9, Pii, Pi3, Pik, Pik-h, Pik-p, Pi1, and Pi7(t)—derived from 21 donor varieties were developed by six times recurrent backcrossing and selfing. Segregation analysis using BC6F3 family lines confirmed that the inheritance of resistance in each NIL was controlled by single gene using blast isolates avirulent to the targeted resistance genes. Each NIL showed a reasonable reaction pattern to 19 standard blast isolates from Philippines and Japan as compared to those of IR64 and monogenic lines, and these results confirmed that the targeted resistance genes were successfully introduced in IR64. The genome wide survey revealed that 95.2-99.1 % of 334 SSR markers showed polymorphism between NILs and IR64. It means that the NILs were genetically similar to those of IR64. The introgressed segments of each targeted resistance genes were detected near the regions where those genes were mapped previously. The morphological traits of all NILs, such as days to heading, culm length, and panicle length and panicle number were comparable to those of IR64. The spikelet fertility and 1000 seed weight of the NILs were significantly bigger than those of IR64. These NILs are important materials for breeding to improve resistance to rice blast under irrigated lowland condition in tropical Asia. And the multiline variety can be one of the alternative approaches to use for the durability of resistance in rice against blast disease in tropical areas.

Reference:
Disease Management and Breeding

**DM-3**

Exploring Rice Genetic Diversity for Identification of Novel Alleles of Rice Blast Resistance Genes

Vasudevan, K.¹, Vera Cruz, C.M.², Gruissem, W.¹ and Bhullar, N.K.¹

¹Plant Biotechnology, Swiss Federal Institute of Technology, Zurich, Switzerland; ²International Rice Research Institute, Philippines

Rice blast is one of the most widespread and devastating diseases of rice crop, and the very fast evolving fungus *M. oryzae* has overcome the resistance of many of the blast resistance varieties. The present study aims at studying the allelic diversity at major rice blast resistance loci.

International Rice Genebank at IRRI holds more than 117274 accessions of rice, originating from different geographical regions of the world (129 countries). In this large scale allele mining project, a total of 4347 germplasm accessions were selected from 13 major rice growing countries. The selection also applied a focused identification of germplasm, on the basis of documented rice blast resistance in their country of origin (IRGCIS). These 4347 germplasm were screened for blast disease resistance at two levels including natural and artificial disease screening methods i.e., at uniform blast nursery as well as under greenhouse conditions using at least 5 different blast isolates (conducted at IRRI, Philippines). Over 3000 accessions found resistant in the nursery screening were subjected to molecular screening using PCR based gene specific markers for Pi2/Pi9/Pizt, Pi54 and Pib. Based on phenotypic screening and molecular pre-screening determining the presence or absence of Pi2/Pi9/Pizt, Pi54 and Pib genes, candidate rice accessions for full coding sequence amplification of respective alleles have been identified. The amplified sequences are currently being sequenced. The alleles with interesting variations particularly in the NBS-LRR domains will be studied further for functional validation.

Utilization of such natural diversity of blast resistance genes in breeding programs will contribute to sustainable blast disease management.
DM-4
Enhancing for Broad-spectrum Resistance to Blast Using Korean Weedy Rice

National Institute of Crop Science, RDA, Iksan 570-080, Republic of Korea

Rice blast caused by the fungal pathogen, *M. grisea*, is a serious disease affecting yield and decreasing its quality in rice production. Rice breeders in Korea have developed many *japonica* rice varieties with blast resistance. However, the resistance in most varieties has broken down within a few years after they were released to farmers because of the spread of new races of *M. grisea*. It is necessary to look for novel resistance gene(s) for blast that can express a broad-spectrum of resistance in diverse environmental conditions. We identified a major QTL *qLB4.1* on chromosome 4 related to the resistance for blast nursery and isolates from Korean weedy rice GL33. It was explained 45.3~53.1% and 26.1~28.6% of total phenotypic variation by the allele of GL33 for resistance to blast nursery and isolates, respectively. The QTL *qLB4.1* was tightly linked to RM6352 and RM3643 of 52.6cM region. These markers were comprised into a BAC clone OSJNBb0012E08 consisting of 14 candidate ORFs. An ORF Os04g32940 would be related to the putative candidate *R* gene. A QTL-NIL, SR30058-BC3F2(52)-1-1 (Suweon545) was developed by marker-assisted backcross method. This line showed resistant reactions at blast nursery across regions and years, and also showed resistance to neck blast in Jecheon field. 'Suweon545' had durable resistance of lower 10% of diseased leaf areas (DLA) in sequential planting method.
Rice (Oryza sativa L.) is one of the most important cereals worldwide. Although attempts have been made to introduce chemical prevention by spraying crops with fungicides, the use of resistant varieties is still considered the most feasible and economical strategy to control rice blast (Magnaporthe oryzae). Blast fungus Magnaporthe grisea Barr caused which one of the most serious diseases in rice due to wide distribution and incidence under favorable conditions. In 2005, more than 70% of rice varieties in the Mekong Delta (MD) had blast infection caused great loss to yield. The optimal method was selected to prevent the blast found resistant varieties. However, resistant varieties exist only few crops production in the MD, farmers should replace new cultivars because the fungi isolate more virulence diseases had formed.

So, “Using molecular markers identified blast disease resistance gene in rice (Oryza sativa L.) and gene pyramiding method for selection of durable resistant cultivars” was conducted in order to release many blast durable resistance varieties, high yield and good quality.

- Evaluate the standard of international differential varieties bearing know blast resistance gene in the Mekong Delta. Four resistance genes Pi9, Pik-p, Piz-t và Pik were found which broad spectrum resistance (>75%) of 13 isolates blast disease which isolated presenting from provinces in Mekong Delta River.
- Te Tep, Nang Huong (Acc550) which had spectrum resistance with 13 isolates Mekong Delta River. The phenogram of the phenotype was constructed based on the matrix similarity using program of the NTSYS-pc in order to found same genetic subgroup with the same resistance genes Pi-k, Pik-p, Piz- t and Pi9.
- Exploiting of the high yielding rice lines with blast fungus isolates identify varieties selected as parents: OM2514, P40 had resistant phenotype corresponding resistance gene Pi-k and Pik-p; C53 resistance phenotype corresponding Pi9 and Piz-t resistance gene.
- Assess the interaction of rice varieties with 13 isolates identified as race 12 and 13 blast fungus capable of causing virulence were common in the Mekong Delta.
- Developing 4 blast gene pyramiding populations of IR64/Te Tep, IR64/OM2514, IR64/C53 and IR64/P47.
- Development of 215 individual plants F2 12844/12845 populations, of which 38 individual plants carrying the three resistance genes identified Pi2, Pik, and Pi47 through the marker STS-RG64, RM21, RM206.
- 2 promising lines G18 and G21 combined high yield, good quality and resistant to blast from scale (2-3). Continue to evaluate of the potential yield of two promising lines G18 and G21, testing on multiple ecological zones, advanced to national testing and developmental production areas.
DM-6

*Magnaporthe oryzae* as a Sensitive Search Tool for Drug Targets

Kamakura, Takashi

Department of Biological Science, Tokyo University of Science, Japan

Appressorium formation of *Magnaporthe oryzae* is one of the simplest models of cellular differentiation. In general, many proteins are involved in the process of differentiation and they could be inhibition targets of various chemicals. Thus, we regarded appressorium formation of *M. oryzae* as a very sensitive and useful tool to identify the molecular target(s) of chemicals. This time, I will talk about some actual search for the unknown molecular target(s) of authentic antibiotics.

We found that some well-known antibiotics had inhibitory effects on appressorium formation, germ tube elongation and conidial germination in *M. oryzae*. Among the antibiotics, roxithromycin (RXM) and chloramphenicol (Cm) specifically inhibited the appressorium formation and it is strongly suggested that the target molecule(s) of RXM or Cm may play some important roles in appressorium formation. These antibiotics are broad-spectrum antibiotics effective against bacteria by inhibiting translation on the 50S ribosomal subunit. However, both of those antibiotics show various beneficial or harmful side effects to human. These facts indicate there are unknown secondary targets of these antibiotics in eukaryotic cells. Therefore we aimed to identify the target protein(s) of RXM and Cm that cause side effect(s) by using *M. oryzae*.

To screen for binding peptide(s) to antibiotics, we employed T7 phage display method using a phage library constructed from the genomic DNA of *M. oryzae* and identified the candidates of target proteins.

Understanding the molecular targets and the mode of action of chemicals could be a strong base on drug discovery and development. We here propose the appressorium formation of *M. oryzae* as a search tool for novel molecular targets of drugs and drug candidates.
Disease Management and Breeding

DM-7
The Impact of Conservation Agriculture on Blast Disease Epidemics in Upland Rice

Sester, M.1, Auzoux, S.2, Gozé, E.2, Lugassy, L.3, Maminantenaina, H.4, Michellon, R.5, Raveloson, H.4, Tharreau, D.6, and Dusserre, J.1

1CIRAD, UPR SCA, BP 230, Antsirabe 110, Madagascar; 2CIRAD, UPR SCA, F-34398 Montpellier, France; 3MNHN, CERSP, F-75005 Paris, France; 4Fofifa, BP 230, Antsirabe 110, Madagascar; 5CIRAD, UPR SIA. BP 230, Antsirabe 110, Madagascar; 6CIRAD, UMR BGPI, F-34398 Montpellier, France

Madagascar is one of the biggest rice producers in Africa. About 20% of the production is rainfed rice. Since the 1990’s, cold tolerant cultivars adapted to high altitude have been successfully proposed to farmers and adopted in the central highlands of the country. But rice blast pressure on the hillsides is important and is the second constraint (after cold) breeders have to take into account. In the same time, hillsides were more and more put under cultivation, conservation agriculture cropping systems have been proposed to farmers (Scopel et al., 2013), to limit erosion and to ensure the sustainability of rainfed crops. In experimental studies, these conservation agriculture cropping systems were shown to limit blast epidemics (Sester et al., in press). We hypothesized that the specificities of the conservation agriculture cropping systems (no tillage, permanent soil cover and rotations) induced changes in plant nutrition and plant nutritional balance, especially nitrogen assimilation, and that it was the reason of a differential expression of tolerance to blast.

To test this hypothesis, pluriannual factorial experiments were conducted in two sites to measure blast disease severity on leaves and on panicles in two cropping systems (conservation agriculture vs conventional) and three nitrogen fertilization levels. Crop development and nitrogen assimilation were measured at four stages and yield components were assessed at harvest. A mineral diagnosis was performed at booting stage on the flag leaf. A relational database has been created to manage agronomic and disease severity rating data from the project. For statistical analysis (performed using Sas) the proportion of damaged leaf area and damaged panicles were fitted to a generalized linear model.

We will present the results of the respective impacts of the cropping system environment, the nitrogen fertilization and the crop density on the leaf and panicle blast level. In some particular conditions, a significant impact of the cropping system appeared in addition to the effects explained by crop density, confirming the hypothesis of a plant susceptibility modification. We present then the way these results could be used to adapt cropping systems and to improve upland rice tolerance to blast in a sustainable way.

References:
Wheat blast disease caused by *Magnaporthe oryzae* (*Pyricularia oryzae*) has been confined to a few countries in South America. Due to severe yield loss, absence of resistant varieties and inefficacy of fungicides to protect wheat spikes the disease is considered a global threat to wheat production. The lack of basic knowledge on many aspects of the epidemiology of the wheat blast disease is one reason for the low efficacy of currently employed measures of control. The most conspicuous symptom of disease in nature is on wheat spikes, on leaves is rarely seen. The same phenomenon occurs in blast of other winter crops. The only exception is black oat (*Avena strigosa*) where symptom is easily observed on leaves at early stages of host development. The aim of the present work was to examine the role of oat blast on the epidemiology of wheat blast disease.

Nine isolates of *M. oryzae* of wheat from three localities and ten of black oat from six municipalities were selected and genotyped at 11 microsatellite loci. The binary matrix data generated were analyzed by Simple Matchin’s similarity coefficient of NTSYS software package 2.2 and cluster analysis performed with UPGMA. Bootstrap analysis was carried out with WINBOOT program with 5000 replications.

Data of the present work showed wheat isolates clustered in two groups with 57% of genetic similarity, suggesting two distinct populations are causing wheat blast disease in Brazil. One wheat subpopulation grouped with oat blast isolates with 88% relatedness demonstrating that *Magnaporthe* from oat is a potential primary source of inoculum for wheat blast disease.
Rice blast disease attacked by *Pyricularia grisea* causes severe yield losses in temperate region. Some recent surveys confirm that blast remains among the most serious biotic constraints to yield in Korea. Infection of panicle base, branches and spikelet pedicels may occur together, or they may occur separately under some different conditions. In this study, we try to estimate rice yield loss according to timing of panicle blast occurrence in the field and to estimate rice yield loss and quality according to severity of panicle blast in the field and to set up economic threshold level for control of panicle blast based on disease severity.

When the neck blast symptom developed early after heading, the yield loss was very high. Infection of panicle base by blast until 20 days after heading caused more than 50% of yield loss in both Jinmibyeo and Chucheongbyeo. There was positive correlation between incidence of the panicle blast and rice yield losses. The regression equations between incidence of the panicle blast and yield losses were $y=-3.61+496.7(R^2=0.70)$ in Jinmibyeo and $y=-3.93+520.2(R^2=0.82)$ in Juanbeo. The panicle blast caused deterioration of grain quality. Healthy grain rate was reduced by increase of panicle blast infection. Meanwhile, the percentage of damaged grain increased according to the increase of the panicle blast incidence. There was negative correlation between the panicle blast incidence and the healthy grain rate and correlation coefficient were $r=-0.74$ on Jinmibyeo and $r=-0.74$ on Juanbeo.

Meanwhile, damaged grain ratio was increased according to the increase of the panicle blast incidence. The correlation coefficients between disease incidence and percent of damaged grains were $r=0.97$ and $r=0.92$, and the regression equations were $y=0.20x+4.8(R^2=0.94)$ in Jinmibyeo and $y=0.31x+7.6(R^2=0.84)$ in Juanbeo. Crop losses assessment can be used to establish economic thresholds. The information can provide a better understanding of the relative severity of disease in rice. Economic threshold level of the panicle blast was calculated to determine chemical application or not using yield losses model of Jinmibyeo and Juanbeo. Economic threshold level of the panicle blast in the two rice cultivars was 2%. As a conclusion chemical application to control the panicle blast should be carried, when the panicle blast incidence become higher than 2%. Economic threshold level can changes with changes in crop growth, level and cost of fertilizer, pesticide and labor, yields and price of products.

References:
Rice production has largely relied on the utilization of heterosis since 1970’s especially in the southern rice-growing areas in China. Hybrid rice including three-line and two-line hybrid rice accounts for 55.1% of the total rice planting area of around 30 million hectares. Rice blast occurred at different extent in China every year, causing 3-6 million hectares of rice field infected and 0.7-1.25 million tons of loss of grain yield. Main strategies applied in control of the blast disease covered the following four aspects: breeding for new resistant varieties/crosses (allele mining and gene pyramiding), chemical control, cultivation management and administrative measurement (regional trials and official release of new variety). Among them, breeding for new resistant variety appears to be the most effective approach for blast control. Late in 1970s, 7 differential varieties including Tetep, Zhenlong 13, Sifeng 43, Dongnong 363, Kanto 51, Hejiang 18 and Lijiangxintuanheigu (LTH), were screened and then widely used for pathotyping of blast fungus in China. Six Chinese near isogenic lines (NILs) for blast resistance with LTH genetic background were first reported in the mid 1990s, and by now more than 30 LTH-NILs are developed under the support of the Special Fund for Agro-scientific Research in the Public Interest Program of China. These LTH-NILs will be applied in monitoring the avirulence genotypes of *Magnaporthe oryzae* (*M. oryzae*) populations and guiding the rational deployment of blast resistance genes in main rice-growing regions. Chinese researchers have identified/cloned some avirulence genes in *M. oryzae*, mapped/cloned more than 20 blast resistance genes in rice, and developed a series of elite resistant hybrids and cultivars through intense research. Molecular breeding techniques such as marker-assisted selection and transgenic approach have been widely applied in the development of rice cultivars with broad-spectrum and durable blast resistance, and will contribute significantly to rice blast control in China.
Rice Cultivation and Blast (*Pyricularia oryzae*) Management in Turkey

Beser, N. and Surek, H.
*Trakya Agricultural Research Institute- P. Box. 16, Edirne, Turkey*

The most important cereal is wheat and it is grown on 8,103,400 ha, followed by barley (3,040,000 ha), maize (590,000 ha), rye (141,000 ha), and rice (99,000 ha). Rice can be grown in nearly all regions of Turkey. Edirne is the most important rice growing city and nearly 50% of Turkey rice production is coming from Edirne. Direct seeding is used as a rice sowing method and Japonica rice varieties are grown in Turkey. Blast was observed before 1995 but in 1995 first time it became big problem, and than 1997, It was seen very big blast epidemic. Since than farmers begun to use chemical to control blast. But, chemical application is difficult, expensive and some times not effective, thus we started joint project with IRRI to improve blast resistant rice varieties in Turkey. Pi40 gene and some other genes were found as resistant genes for Turkey blast races, and studies to transfer of these genes to Turkish rice cultivars was started with this project.
Blast fungus *Magnaporthe grisea* Barr caused which one of the most serious diseases in rice due to wide distribution and incidence under favorable conditions. Although attempts have been made to introduce chemical prevention by spraying crops with fungicides, the use of resistant varieties is still considered the most feasible and economical strategy to control rice blast (*Magnaporthe oryzae*). In 2006, more than 50% of rice varieties in the Mekong Delta (MD) had blast infection caused great loss to yield. The optimal method was selected to prevent the blast found resistant varieties. However, resistant varieties exist only few crops production in the MD, farmers should replace new cultivars because the fungi isolate more virulence diseases had formed. Therefore, With five keys management for the blast: the first: selected rice resistant variety, especially in fields with a history of blast breeding for disease resistance is of special importance in rice, to prevent or reduce yield losses. Furthermore, genetic resistance allows the reduction of agrochemical applications and thereby greatly contributes to environment and consumer protection. Resistance breeding has already been very successful in the past and provided many resistant varieties highly adapted to adverse growing conditions. The genetic basis of such resistance depends on the respective pathogen: Many cases of major gene(s) resistance have been broken down, but polygenic types of resistance are also widespread and appreciated by breeders and growers due to their superior durability. The second: control the time for direct seedling to avoid the likelihood of heavy blast pressure late in the season. The third: use the recommended N fertilizer rate. Avoid high N rates, especially on susceptible varieties in blast-prone fields. The fourth: maintain a consistent, deep flood after the drain and dry period for straight-head prevention growing season. Avoid losing the flood or shallow flood depths, especially on susceptible varieties. And final: cropping system with crop and natural resources management in blast affected areas also contributed substantially to overall yield and stability.
Blast is the most important disease constraining to high yield of upland rice in Indonesia. The pathogen has genetically and pathologically diverse population comprising many pathotype or races. The population of the pathogen is also dynamic enabling it to rapidly break down varietal resistance. The resistance of improved varieties was broken down and the varieties became susceptible to the disease after wide cultivation for 3-4 consecutive planting seasons. Farmers in blast endemic areas cultivate traditional varieties maintaining genetic diversity and stable blast resistance though the yield is low. Breeding rice for blast resistance in Indonesia has been directed to develop improved varieties with diverse blast resistance. Many different genetic sources for blast resistance, mostly not genetically identified yet, were used in the breeding program. Many crosses to incorporate the blast resistance with other desirable characteristics have been made every planting season. A modified bulk method was applied to the early generations up to F5 where individual or pedigree selection was initiated. Evaluation and selection for blast resistance were conducted in the field of the endemic area as well as in green house simultaneously with the observation and yield trials of the conventional breeding procedure. Ten improved varieties of upland rice with blast resistance were officially released in Indonesia since 2001. The varieties had different other desirable characteristics including tolerance to Al toxicity and drought, aroma, and purple colored rice. Some collaboration researches with IRRI, JIRCAS, and Washington State University to develop durable blast resistant varieties through pyramiding resistance genes, developing multi lines, and introducing wide spectrum resistance genes, are in progress.
Rice Blast and Its Management in Nepal

Manandhar, H.K., Chaudhary, B., Parajuli, G.P., and Jha, P.

Nepal Agricultural Research Council, P.O. Box 5459, Kathmandu, Nepal

Rice blast caused by *Pyricularia oryzae* is one of the major crop damaging diseases of rice and occurs throughout the diverse rice growing environments from 60 to 3050 masl in Nepal. The disease was recorded for the first time during 1966 in Nepal. Since then several blast epidemics have been reported in the country. Efforts have been concentrated on blast management with more emphasis on varietal resistance. Each year hundreds of national and exotic rice genotypes are being evaluated for resistance to the blast fungus at different testing sites. As a result, several resistant varieties have been deployed for terai (plain), mid hill and high hill environments. Still farmers are growing blast susceptible varieties in various areas as these varieties have been the choice of farmers for some qualities and also because blast resistant improved varieties have not reached to them yet. There have been great variations in blast pathotypes within and between rice production environments. In addition, blast management has become difficult as many rice genotypes showing resistance to leaf blast are found susceptible to neck blast.

Epidemiological studies indicate that the disease may be transmitted from seed. Leaf blast in the seedbed has caused complete loss of seedling while leaf blast and panicle blast in the transplanted field cause yield reduction up to 80 percent. Grain yield of up to 76 kg/ha with one percent increase in neck blast has been estimated. Seed treatment with effective fungicides (carbendazim and tricyclazole) has been recommended to reduce leaf blast. Use of salt-sorted rice seeds has a significant influence on lowering blast disease and increasing number of grains per panicle and increased kernel weight. Foliar spray with fungicides (edifenphos, tricyclazole, kasugamycin) is also effective in minimizing leaf blast and panicle blast. Some cultural practices like water management and planting of wider spacing are also recommended to reduce disease development. Foliar application of non-pathogenic or non-rice pathogens and non-pesticidal chemicals also minimizes blast development. However, all these practices are not often followed by the rice growers due to several reasons. Use of biological control agents and major blast resistant genes alone in rotation or in combination are getting priority for blast management in collaboration with national and international organizations. Studies done in the past showed that combination of Pi-1 and Pi-2 genes had complementary resistance, conferring resistance to most of the blast isolates in Nepal.

Reference:
Country Reports

CR-6

Pathogenic Population Study of *Magnaporthe oryzae* and Novel Screening System for Durable Rice Blast Resistance in Korea

Goh, J., Roh, J.H.¹, Kim, B.R.¹, Shin, D.B., Cho, Y.C.¹, Kang, H.W., Lee, E.J., and *Han, S.S.¹

¹ Crop Environment Division, National Institute of Crop Science, RDA, Suwon, 441-857, Korea

Rice cultivation area in Korea have decreased from 1.2 million ha in 1970s to 0.85 million ha in 2012, and rice self-sufficiency ratio have declined from about 100% in 1980s to 83.0% in 2011. Fortunately, rice blast in Korean fields has rarely occurred under 0.1% disease occurrence ratio since rice blast epidemic in 1977 due to resistance breakdown of Tongil cultivar. In order to control rice blast in fields, Korean researchers and government have tried to apply several methods such as suitable chemical utilization, management practices and breeding of blast-resistant cultivar. Especially, breeding of blast resistant cultivar is the most important and efficient methods for rice blast management. For this purpose, it is required to examine distribution of pathotypes in rice blast fungus and reveal relationship of those and rice cultivars. Thus, population studies about pathogens have been done using Korean differential rice cultivars since 1981. From 1980 to 2010, almost 10,000 field isolates of rice blast were collected and categorized by more than 60 races in Korea. This data shows that complex and diverse pathogen races have existed since 2000s while two main races such as KJ-301 and KJ201 remained until 1990s. This fact indicates that distribution of resistance genes in rice might have been more complex and diverse compared to past years. Therefore, it is required to design novel Korean differential system and screening resistant rice cultivars.

For the resistant of rice lines, we have been used the three kinds of resistance screening methods in blast resistance breeding programs. The methods are artificial inoculation of rice seedling in greenhouse, nursery screening (hot spot) in many different locations, and field test. These methods are very effective in screening of qualitative resistance but those are hard and the examination of the durability of rice cultivars takes a very long time. Since these methods have chiefly targeted genes displaying great influence, it has been observed that duration of resistance was either extremely short or not significant occasionally. For the evaluation of durable resistance to blast disease in rice, we conducted the sequential planting method, which allows for the screening of durable resistance under artificial conditions in a short period of time. This research was carried out to evaluate the effectiveness of sequential planting method in determination durable resistance of monogenic resistant lines and some Korean elite lines.

A sequential planting method was developed to identify durable blast resistance in Korean rice cultivars. Two types of rice cultivars were investigated by a sequential planting after inoculation of mixed 30 rice blast isolates. These blast isolates are selected as representative strains according to their genotypes and pathotypes among 2,000 blast isolates during 15 years in Korean rice fields. Daesanbyeo and Gihobyeo, showing initial high resistance in farmer's field followed by rapid breakdown of the resistance in very short periods, showed low disease until the third and fourth planting but later showed higher than 40% DLA (diseased leaf area), respectively. In contrast, Palgongbyeo and Seomjinbyeo, showing low disease occurrence and sustainable field resistance during the last 20 years in farmers' field, showed less than 20% of DLA until the seventh planting. Results from the sequential planting method in greenhouse were significantly similar to the farmers' field data. This suggests that the current sequential planting method is effective to evaluate durable resistance of rice blast. This work has been applied to resistant breeding program to rice blast during 7 years.
International Rice Blast Conference

2013 Jeju, Korea

Poster Abstracts

A. Molecular and Cellular Biology and Effector of the Pathogen
B. Genomics and Proteomics of the Pathogen
C. Host Resistance, Signaling and Defense Responses
D. Diversity and Population Biology of the Pathogen
E. Disease Management and Breeding
F. Resources for Microbe Researches
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1Research Institute of Phytopathology, B. Vyazemy, Moscow region, 143050 Russia; 2Russian People’s Friendship University, 8 M. Maklay str., Moscow 117813, Russia; 3Agricultural Research Service USDA, Beltsville, Maryland 20705, USA

Solar light is known to directly suppress early development of rice blast fungus (Magnaporthe grisea) and somewhat protect rice plants from the disease. Photo damages of any cells often involve reactive oxygen species (ROS), which, as a counteraction, may mobilize antioxidants. The purpose of the work was to determine whether a preliminary, short-term intense illumination of spores might attenuate the inhibition of their development by ROS and change their aggressiveness against the host.

Germination of fungus spores was suppressed under 5-hour intense light. The effect was diminished by antioxidants added to germination media and, therefore, depended on ROS. One-hour light did not affect germination. But it stimulated superoxide (O2-) production (assayed with exogenous epinephrine) in spore diffusates.

The illuminated spores were more tolerant (than non-illuminated ones) to artificially generated O2-, H2O2, or hydroxyl radical. They were less vulnerable to ROS-dependent toxicities of diffusates of infected rice leaves of resistant cultivars. Besides, such spores caused more severe disease symptoms if applied to leaves of the susceptible rice cultivar at low concentration. These changes might be due to increased spore antioxidant capacity in response to the light-induced oxidative burst. Meanwhile, if spore inocula were applied to leaves at concentrations optimal for the disease the illuminated samples turned to be less aggressive than the darkness-incubated ones. We suppose that, under light, the denser spore suspension produced more O2- than dilute suspension. The excessive extracellular superoxide appeared to induce plant defense responses, and this action outweighed the increased aggressiveness.

Part of spore antioxidant activity was extracellular. Actually, spore diffusates decomposed hydrogen peroxide assayed with TiCl4. They detoxified exogenous H2O2 and O2- as well as leaf diffusates; spore illumination increased some of these protective effects.

It is suggested that short-term light led to mild oxidative stress, which adaptively induced spore antioxidant capacity, enhancing spore tolerance to subsequent stronger oxidative stress and its aggressiveness in planta. Such tolerance depends partly on the antidotal action of spore exo-metabolites, which may also be light-stimulated. Therefore, environmental factors may modulate pathogenicity of the blast causal agent through its pro-/antioxidant balance.
Genome-scale Analysis of ABC Transporter Genes and Characterization of the ABCC Type Transporter Genes in *Magnaporthe oryzae*

Kim, Y.¹, Park, S.-Y.², Kim, D.¹, Choi, J.², Lee, Y.-H.², Lee, J.¹, and Choi, W.¹

¹Department of Biotechnology and Bioengineering, Dong-Eui University, Busan 614-714, Korea; ²Department of Agricultural Biotechnology, Fungal Bioinformatics Laboratory, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea

Rapid adaptation to various environmental stresses is a prerequisite for successful infection in fungal pathogens. ABC transporters are responsible for regulating intracellular levels of cytotoxic or xenobiotic compounds, suggesting a crucial role in pathogenesis. Here, we report genome-scale identification of putative ABC transporter genes in *Magnaporthe oryzae*. A total of 50 ABC transporter genes were predicted and phylogenetic analysis divided them into 11 subfamily groups: ABCA, ABCB, ABCC-1, ABCC-2, ABCD, ABCE, ABCF, ABCG-1, ABCG-2, ABCI, and YDR061W-like. In the 11 ABCC subfamily genes, the transcript levels were elevated during infection stages and after exposure to various abiotic stresses. Based on expression pattern, three representative genes, MoABC5, MoABC6 and MoABC7, were selected. Functional analysis of MoABC5, MoABC6, MoABC7 revealed that the genes may be responsible for virulence, abiotic stress tolerance, and conidiation, respectively. Our data will be providing valuable information to examine the role of ABC transporter genes in *M. oryzae*. 
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DNA Double-strand Breaks Induce Genetic Variations in the Rice Blast Fungus

Arazoe, T.1, Ohsato, S.1, Arie, T.2, and Kuwata, S.1

1Meiji University, Graduate school of Agriculture, Japan; 2Tokyo University of Agriculture and Technology, Graduate school of Agriculture, Japan

The rice blast fungus *Pyricularia oryzae* (synonym *Magnaporthe grisea*) is widely recognized as a genetically variable pathogen and many races have been identified. Recently, genetic variations such as duplication, translocation and deletion of the *Avr* gene, or chromosome rearrangement have been observed in field isolated *P. oryzae*. However, the mechanisms underlying these genetic variations have not been clearly clarified. In our study, we focused on DNA double-strand breaks (DSBs) that cause genome instability in many organisms. It is known that DSBs can be repaired by homologous recombination (HR) and non-homologous recombination (NHEJ) during asexual cell division. Therefore, we constructed a system to introduce an artificial DSB in the site of a marker gene in the genome of *P. oryzae*, and analyzed patterns of somatic HR and NHEJ repairs in the genome of *P. oryzae*.

We developed a novel detection/selection system for DSBs-mediated somatic HR. The system consists of two nonfunctional yellow fluorescent protein (YFP)/blasticidin S deaminase (BSD) fusion genes, a donor repair template and recipient gene containing I-Sce I site, and also a yeast endonuclease I-Sce I gene that was used as a DSB-inducer. In this system, ectopic HR can be detected and selected by restoration of YFP fluorescence and blasticidin S (BS) resistance at a single cell level. These donor and recipient genes were simultaneously integrated into the *P. oryzae* genome and the transformed lines were isolated. The frequency of DSB-mediated ectopic HR was around 40% in transformed lines with the integrated DSB-inducer gene, compared with only around 2% in the lines without any DSB inducer. This result clearly showed that DSBs in a sequence of *P. oryzae* induced ectopic HR events between the sequence and its homologs.

We further analyzed the NHEJ repair mechanism, which is not dependent on the homologous sequence. In this case only the recipient gene was integrated into the *P. oryzae* genome, by agrobacterium-mediated transformation, and the transformants harboring the single copy gene were isolated. The integration of the DSB-inducer gene into the transformed lines caused small deletions and point mutations to occur in the recipient gene, and we also detected entire deletions of the recipient gene in the genome.

Taken together, these results strongly suggest that DSBs can drive genomic rearrangement and genetic variation in the genome of *P. oryzae*, through the DSBs repair processes.

Reference:
Macroautophagy During Appressorium Morphogenesis in *Magnaporthe oryzae*.

Inoue, K., Ikeda, K., and Nakayashiki, H.
Graduate School of Agricultural Science, Kobe Univ., Japan

*Magnaporthe oryzae* is known as a causal agent of blast disease in cultivated gramineous crops. This fungus takes various differentiation patterns depending on the environmental condition. On the leaf surfaces, the spores elongate germ tubes and differentiate appressoria. However, on the root surfaces, immature appressoria known as hyphopodia were differentiated at the tip of the germ tubes. Recent molecular biological studies suggested that autophagy induction and metabolism of storage substance in the spores were important for functional appressoria. Although cytological analyses were examined, these studies were targeted to not spore but hyphae because of technical difficulty and never captured autophagosome that was typical features of macroautophagy. Cytological report of hyphopodia differentiation was also rare. In this study, we evaluated cellular dynamics by transmission electron microscopy (TEM) during infection process both on hydrophobic (leaf) and hydrophilic (root) surfaces, especially focused on autophagy machinery. Furthermore, we examined the correlation between the autophagy and metabolism of the storage substance in the spores. TEM observation in the spores producing appressoria at 12 hours post inoculation (hpi) revealed that many autophagosome-like vesicles were accumulated at the adjacent regions of enlarged vacuoles. At 24 hpi, most of the organella in the spores disappeared. In contrast, on the root surfaces, such vesicles had never observed during the infection process. Moreover, we found that transfer of storage substance in spores hardly occurred toward the hyphopodia. These results suggested that autophagic machinery and relevant metabolic pathways is important switching determining organ specific pathogenicity in *M. oryzae*. 
A Rice Blast Fungus Alpha-N-Arabinofuranosidase B Degrades Rice Host Cell Wall, which Elicits Host Defense

Wu, J.1, Lee, D.Y.1, Wang, Y.2, Kim, S.G.3, Kim, S.T.4, and Kang, K.Y.1,3*

1Division of Applied Life Science (BK21 program), Gyeongsang National University, Jinju, 660-701, South Korea; 2Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, 50829, Germany; 3Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju, 660-701, South Korea; 4Department of Plant Bioscience, Pusan National University, Miryang, 627-706, South Korea.

Rice blast disease caused by *M. oryzae* is the most devastating fungal disease in rice. *M. oryzae* secretes a large number of glycosyl hydrolase (GH) proteins into apoplastic region to digest host cell wall and aids to pathogen ingress into tissues. In this study, we identified an *M. oryzae* Aarabinofuranosidase protein, MoAbfB, which is secreted during infection. Live-cell imaging exhibited that fluorescent labeled MoAbfB was accumulated in appressorium, and tips of penetration peg as well as invasive hyphae, which were closely related to invasive structures. However, deletion of MoAbfB facilitated extending biotrophic phase of pathogen followed by enhanced disease severity. When MoAbfB was over-expressed, host defense genes were strongly induced and hence less disease symptom were occurred. The extract MoAbfB degraded rice cell wall fragments induced host defense activation, suggesting that not MoAbfB itself but oligosaccharides (OGs) derived from MoAbfB dissolved rice cell wall elicited rice defense response.
Efficient Method for Secreted Proteins from Phylloplane of Rice Leaves Free from Cytosolic Proteins: Application to Study Rice-M. oryzae Interaction

Hwang, J.S.¹, Wu, J.¹, Lee, D.Y.¹, Wang, Y.²,⁴, Hwang, D.H.², Kim, S.T.⁴, Kim, S.G.², and Kang, K.Y.¹,²*

¹Division of Applied Life Science (BK21 Program), Gyeongsang National University, Jinju, 660-701, South Korea; ²Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju, 660-701, South Korea; ³Department of Plant Bioscience, Pusan National University, Miryang, 627-706, South Korea; ⁴Department of Plant Microbe Interaction, Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, 50829 Köln, Germany.

The rice blast disease caused by Magnaporthe oryzae (M. oryzae) is the most serious disease of cultivated rice (Oryzae sativa) in most rice-growing regions of the world. To identify secreted proteins involved in rice blast fungus, M. oryzae without cytosolic contamination, we applied proteomics analysis with naturally dropped proteins from rice leaves combined with water-saturated phenol extraction method. The extracted proteins were applied to 2-DE analysis and 45 secreted proteins were identified by MALDI-TOF mass spectrometry. The proteins were classified as relating to metabolism (47%), stress/defense (36%), and proteolysis (13%). Most of secreted proteins were predicted by SignalP or SecretomeP as retaining a signal peptide or secretory through other pathways, and highly expressed in incompatible interaction. Among them, an apoplastic protein, with domain unknown function 26 (DUF26) proteins were secreted drastically after treatment of rice-blast fungus in rice leaves. OsDUF26 promoter transgenic rice and Arabidopsis has been generated to assess the function of OsDUF26 in the biotic stress response. The results show that OsDUF26 promoter was activated in response to Xanthomonas oryzae (X. oryzae), M. oryzae, and fungal elicitor. These results indicate that plant apoplastic proteins may have important roles in the plant biotic stress responses.
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Subcellular Localization and Functional analysis of Blast Effectors in Rice Cells

Xie, X.¹, Vamgnul, P.¹, Liu, W.¹, and Wang, G.L.¹²

¹State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, China; ²Department of Plant Pathology, Ohio State University, Columbus, OH, 43210, USA

Elucidating of the molecular mechanism of effector-mediated suppression of plant immunity is an emerging topic and challenging task in plant-pathogen interaction research. Rice blast disease, caused by the fungus Magnaporthe oryzae, is one of the most damaging factors in rice production worldwide, affecting global food security. To cause rice blast disease, the biotrophic invasive hyphae of M. oryzae will secrete cytoplasmic effectors, which preferentially accumulate in biotrophic interfacial complexes (BICs), and eventually are translocated into the rice cytoplasm to facilitate disease development by suppressing plant defence responses. Although the M. oryzae genome consist of a large number of secreted genes, only few of them have been functionally characterized. Bioinformatic analysis of the in planta expressed secretome of M. oryzae revealed that about 90 secreted proteins are predicted to contain conserved plant organelle target motifs. Our preliminary data confirmed that several of the putative secreted proteins with a nuclear-target motif are indeed localized in the plant nucleus. Any targets to chloroplast or mitochondria? Further survey of the localization and functionally studies of those candidate effector genes will provide insight into the function of blast effector targets in regulation of plant immunity.
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Multiple Host Adhesion Factors in *Magnaporthe oryzae* -Potential Target for Disease Control

Ikeda, K., Kitagawa, H., Inoue, K., Shimoï, S., Osaki, Y., Park, P., and Nakayashiki, H.

*Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai, Nada, Kobe, Japan*

The germlings of *Magnaporthe oryzae* are tightly attached on the host surface producing the extracellular matrix (ECM) from germ tubes and appressoria. ECM constitutes fibrous and amorphous materials, which were positively reacted with antibodies of animal cell adhesion factors and concanavalin A lectin. We also evaluated the effects of hydrophobins, the fungal surface protein, on adhesion and pathogenicity. Gene knockdown and knockout experiments of hydrophobin genes revealed that class I *Mpg1* was involved in adhesion and pathogenicity but class II *Mhp1* was not. Moreover, we found that treatment with natural nutrients such as beef and yeast extract suppressed the appressorium formation and the adhesion that was irrespective of yeast α-factor. By biochemical study, the ECM of *M. oryzae* could be degraded by collagenolytic/gelatinolytic enzymes. We screened gelatinolytic bacteria from rice leaves and soil to establish a novel biological control agent inhibiting germling adhesion on the host plant surface. The selected bacteria were identified as *Acidovorax*, *Sphingomonas*, *Chryseobacterium*, and *Pseudomonas* sp. Based on the treatment with EDTA, most isolates produced metalloproteinase. The screened bacterial culture showed inhibitory effects on spore adhesion on the plastic cover glass and disease protective effects on rice. However, the selected bacteria could not fix on leaf within 1 week using transgenic bacteria conferring chloramphenicol resistance. We improved bacterial fixation supplemented with 0.3% gelatin and disease protection effect lasted 2 week after bacterial incubation. This study suggests that gelatinolytic bacteria inhibiting germling adhesion may have promise as a biological agent.
Expression Analysis of AVR-Pia, Avirulence Gene of Magnaporthe oryzae

Sornkom, W., Takeuchi, S., Miki, S., Sato, Y., and Sone, T.
Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan

Rice blast disease is one of the most devastating diseases of rice over the world. To cause rice blast disease, the fungus Magnaporthe oryzae produces biotrophic invasive hyphae that secrete effectors at the host–pathogen interface. Effectors facilitate disease development, but some (avirulence effectors) also trigger the host resistance gene-mediated hypersensitive response and block disease. Recently, the effector genes have been cloned from the fungus, some of them are expressed during infection, but detail of its expression control is unknown.

AVR-Pia is one of avirulence genes that be recognized by the rice Pia gene (resistance gene) via the gene-for-gene relationship between rice blast fungus and rice plant. RT-PCR analysis revealed that expression of AVR-Pia is induced only during infection, at 18-24 hours after inoculation (hai). AVR-Pia-eGFP fusion protein indicated AVR-Pia is accumulated in BIC (biotrophic interfacial complex), secreted, and then probably delivered to plant cytosol. However, it was not clear about the timing of induction, because appressorium formation is usually found in 18-24 hai, but no clear GFP signal was found in appressorium, probably due to localization of GFP signal by the AVR protein. In order to study the expression of AVR-Pia in Magnaporthe oryzae, PPR (putative promoter of AVR-Pia) was attached with eGFP gene and introduced into M. oryzae strain Ina168m95-1, the Δavr-pia mutant, to observe GFP signal in the fungus during infection on rice sheath via fluorescent observation. GFP signals were expressed by PPR in the appressorium and invasive hyphae of M. oryzae, indicating that AVR-Pia induction is started in appressorium. In order to clarify the precise timing of AVR-Pia induction, mutants of appressorium development were used. Mac1 is adenylate cyclase gene, which is responsible for appressorium formation, and the defective mutant of Mac1 was identified to be unable to develop appressorium. PIsl encodes tetraspanin-like protein which is required for penetration peg formation to invade rice cell, the defective mutant of PIsl is able to develop appressorium but unable to develop penetration hypha. So GFP expression in these mutants with PPR-eGFP will clarify whether AVR-Pia starts to be expressed at appressorium formation, appressorium maturation or penetration.

Expression analysis of PPR-eGFP will clarify the relationship between AVR-Pia induction, appressorium formation and penetration. Knowledge of AVR-Pia expression is expected to provide us the clear understanding on molecular basis of rice blast fungus interaction, which is beneficial to control the rice blast disease.
**Study on Mutation Mechanism of AVR-Pia and AVR-Pik in Magnaporthe oryzae**

Funabiki, M., Takeuchi, S., Sato, Y., and Sone, T.

*Graduate School of Agriculture, Hokkaido University, Sapporo, JAPAN*

*Magnaporthe oryzae* is the causal agent of rice blast disease, a devastating problem worldwide. In order to control the disease, many strategies have been developed, but often such attempts are negated by mutations in the genome of the pathogen. One approach is the usage of blast-resistant cultivars that carry major resistance genes (*Pi* genes), which interact specifically with fungal avilulence genes (*AVR* genes) to trigger the hypersensitive reaction. Mutations of *AVR* genes are responsible for breakdown of resistance in the field. Recently, mechanism of *AVR-Pia* deletion in the spontaneous mutant Ina168m95-1 was revealed to be homologous recombination between two copies of DNA-type transposon (Sone *et al.* *FEMS Microbiol Lett* 339, 102–109, 2013). In this study, *Magnaporthe oryzae* isolate Ina168 and the spontaneous *avr-Pik* mutants, Ina168m95-2, 3, 4, 5, and 6, were used for elucidation of the mutation mechanisms of *AVR-Pik* gene. These mutants were isolated from lesions appeared on rice cultivar Kanto51 (*Pik*) or Tsuyuake (*Pikm*), after spray inoculation of Ina168.

Presence of *AVR-Pik* in Ina168 and Ina168 m95-2–6 was checked by PCR and Southern hybridization. One copy of *AVR-Pik* was conserved in Ina168 genome, but *AVR-Pik* has been deleted in Ina168 m95-2–6. A cosmid clone, named 45-1, was screened from Ina168 cosmid library for the sequence analysis of *AVR-Pik* flanking region. Sequence analysis of the cosmid 45-1 revealed a sequence contig, 13 kb in size, containing *AVR-Pik* and repetitive elements (*Transposon Pot2*, *retrotransposon MGR583*, and *solo-LTRs of retrotransposons*) in its flanking region. Another contig, named fragment u-4, was identified from the other end of the cosmid clone. The fragment u-4 was 5.6 kb in length, and predicted to contain non-repetitive region. PCR and Southern hybridization revealed that the region was conserved in m95-5, but not in the other mutants. These results indicated that one end of the deleted region was present between the fragment u-4 and *AVR-Pik* in m95-5. Further sequence in the cosmid clone is currently under investigation.
Important Amino Acid Region for Multimerization of AVR-Pia Protein of *Magnaporthe oryzae*

Higuchi, Y.\(^1\), Sato, Y.\(^1\), Ose, T.\(^2\), and Sone, T.\(^1\)

\(^1\)Graduate School of Agriculture, Hokkaido University, Sapporo, Japan; \(^2\)Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

AVR-Pia is one of the *Magnaporthe oryzae* AVR genes and was cloned from strain Ina168 (Miki et al., 2009). This gene is 255 bp encoding 85 amino acids, including N-terminal 19 amino acids which are predict to be signal peptides. As the result of protein-protein interaction to AVR-Pia by using Matchmaker Yeast Gold Two-Hybrid system (Clontech), the interaction between AVR-Pia without signal peptide and itself was investigated. In order to clarify the relationship between AVR-Pia multimerization and the induction of host hypersensitive reaction, yeast two-hybrid assay was used to identify the amino acid region which is required for multimerization. The yeast two-hybrid (Y2H) system utilizes 4 genes (*HIS3, ADE2, AUR1-C* and *MEL1*) as reporter genes which confer auxotrophy and antibiotic resistance. If there is the protein-protein interaction of inserted gene products, the interaction is detected by the expression of 4 reporter genes on selective media.

Six types of *AVR-Pia* partial 10 amino acids deletion mutants (AVR-Piad1-10, 11-20, 21-30, 31-40, 41-50 and 51-60) and AVR-Pia without signal peptide were used in Y2H analysis. The interactions of all 49 \((7*7)\) possible combinations of mating and Negative control were analyzed. The combinations including AVR-Piad11-20, AVR-Piad31-40 and AVR-Piad41-50 did not express all 4 reporter genes as strongly as wild-type, indicating decreased protein-protein interactions. Based on the result, 5 amino acids deletion *AVR-Pia* mutants of the 3 regions (11-20, 31-40, 41-50) were then constructed and named AVR-Piad11-15, 13-17, 16-20, 31-35, 33-37, 36-40, 41-45, 43-47 and 46-50, and these protein-protein interactions were analyzed. Among these 5 amino acids deletion mutants, combination of two AVR-Piad36-40 did not express all 4 reporter genes, indicating decreased interaction. These results suggest that amino acids region (36-40) is important for the protein-protein interaction between AVR-Pia and itself. Further analysis to identify an amino acid residue important for the multimerization of AVR-Pia is currently under investigation.
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Functional Analysis of Cbp1 in Appressorium Differentiation

Tokyo University of Science, Japan; *Tokyo University of Agriculture and Technology, Japan

We previously reported that a gene CBP1 (chitin-binding protein 1) seemed to play important roles in appressorium formation of Magnaporthe oryzae. CBP1 was specifically expressed in the germ tubes and null mutants Δcbp1 showed significant reduction of appressorium differentiation on hydrophobic polyvinyl chloride surface (PHOB-PS) that mimicked rice leaf surface at 8 h after germination. The Δcbp1 could not differentiate pre-invasive hyphae on hydrophilic glass slide (PHIL-GS) that mimicked rice root surface. However, Δcbp1 maintained ability to cause disease symptoms on both leaves and roots of susceptible rice cultivars. The germlings of Δcbp1 treated with cutin monomers such as 1,16-hexadecanediol (HDD) could form appressoria on PHOB-PS at 8 h after germination.

First, in this study, we investigated how CBP1 worked on induction of appressorium formation. Cbp1 contains a highly conserved domain of chitin-deacetylase (CDA) of several species. To examine whether Cbp1 acts as CDA in appressorium formation, we compared the chitosan contents of Δcbp1 with that of wild type by estimating the fluorescence intensities of eosinY which can stain chitosan selectively. Fluorescence intensity of germ tube undifferentiating appressorium in Δcbp1 was similar to wild-type. However, induction of appressorium by HDD caused significant increase of fluorescence intensity of appressorium in wild-type, but not in Δcbp1 mutants. These results indicated the possibility that CBP1 worked as CDA and the activity of CDA was supportive but not essential for appressorium formation.

Next, we investigated which signal transduction pathway is carrying the information downstream of Cbp1. Appressorium formation of M. oryzae is induced by environmental signals including surface properties. In studies until now, it has been revealed that cutin monomers, hardness and/or hydrophobicity are seemed to be elements to induce appressorium formation. The signals are transmitted by a variety of intracellular signal transduction pathways, such as a cAMP pathway, calcium signaling, and MAPK cascade. However, how the signals are recognized by this fungus in the first place has not been understood completely. We performed appressorium assays in the presence or absence of the signals influencing the induction of appressorium formation. On PHOB-PS, appressorium formation ratios of Δcbp1 mutants were lower than wild-type without HDD, but it rose to the same as wild-type by adding HDD. These results suggested that Cbp1 does not involve in appressorium formation induced by HDD treatment. In contrast, on PHIL-GS, appressorium formation ratios of wild type were decrease, but they restored by adding HDD. Δcbp1 mutants treated with HDD could not form appressorium on PHIL-GS, unlike on PHOB-PS. These data suggested that appressorium formation was not induced by only adding HDD.

Therefore, we presumed that Cbp1, cutin monomer and hydrophobicity act independently of each other. Moreover, we found that induction of appressorium formation required the presence of at least two of the following conditions: hydrophobicity, application of cutin monomer and preservation of functional Cbp1. Further analyses are in progress.
Hydrophobic Surface Binding Proteins in *Magnaporthe oryzae* Suppress Defense Responses in Barley and Wheat

Marie Nishimura  
*National Institute of Agrobiological Sciences, Japan*

Hydrophobic surface binding (HSB) proteins are broadly found in filamentous fungi. In *M. oryzae* genome, eight HSB proteins (MoHSBs) were annotated. These MoHSBs were dispensable for the fungal growth, infectious structure development and rice infection. However, *M. oryzae* mutants incapable of secreting of MoHsb1 and MoHsb2, two of the eight MoHSBs, became avirulent to some Pooideae hosts e.g., wheat, barley and Italian ryegrass. Consistently, *M. oryzae* lacking MoHsb1 and MoHsb2 evoked intense defense responses in barley but not in rice. Fluorescently labeled MoHsb1 accumulated in plant epidermal layer around penetration pore, whereas MoHsb2 outlined the infectious hyphae developed in plant cells. Taken together, MoHsb1 and MoHsb2 facilitated the fungal infection to the specific hosts via suppressing of the hosts’ defense responses.
A Multiple-KH Domain Protein Is Important for Infection-Related Morphogenesis in the Rice Blast Fungus

State Key Laboratory of Agrobiotechnology and Ministry of Agriculture Key Laboratory of Plant Pathology, China Agricultural University, Beijing, P. R. China

KH domain-containing proteins are widely conserved and play diverse roles in post-transcriptional gene control. However, few studies deal with their roles in pathogenesis of plant pathogenic fungi. Here, we identified a novel virulence gene \( PCG6 \) in the rice blast fungus \( \textit{Magnaporthe oryzae} \) that encodes a protein with multiple KH domains. Deletion of \( PCG6 \) led to severely attenuated virulence toward host plants. Microscopic observation showed that the deletion mutant was reduced in appressorium formation, penetration peg formation, and invasive growth of infection hyphae during infection process. In addition, the deletion mutant was also significantly reduced in vegetative hyphal growth and conidiation. \( PCG6 \) encodes a cytoplasmic protein that is expressed in mycelia, conidia, appressoria, and infection hyphae. Interestingly, the expression level of \( GLT1 \), a gene for glutamate synthase, was markedly increased in the \( pcg6 \) null mutant as compared with the wild-type strain. Consistently, over-expression of \( GLT1 \) in the wild-type strain exhibited retarded growth of vegetative hyphae. Taken together, \( PCG6 \) for a multiple KH domain protein in \( M. oryzae \) is involved in the infection-related morphogenesis and asexual developments possibly by regulating mRNA level of \( GLT1 \).
Where Blast Effectors Go during Biotrophic Invasion by the Rice Blast Fungus, *Magnaporthe oryzae*

Yi, M.¹, Wang, X.², Lee, J.-Y.², and Valent, B.¹

¹Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506, USA
²Department of Plant and Soil Sciences, University of Delaware, Newark, Delaware 19711, USA

To understand the effector repertoire of the rice blast fungus, secreted effector candidates highly expressed in a previous biotrophic interactome analysis were fluorescently-labeled and screened using a high-throughput strategy. The candidate effectors were tagged with red fluorescent protein as C-terminal translational fusions under control of their native promoters and tracked over key infection stages by live cell imaging. Dozens of candidate effectors accumulated in the biotrophic interfacial complex, a critical structure that appears associated with translocation of effectors into the rice cytoplasm. The putative effectors showed diverse patterns of temporal and spatial regulation. Host translocation and cell-to-cell movement were clearly demonstrated, and confirmed with plasmolysis, for a group of the cytoplasmic effectors. Many of the cytoplasmic effectors exemplified and supported the presence of a Golgi-independent secretion mechanism different from the classical secretion pathway followed by apoplastic effectors, as reported in a recent study. In-depth characterization of one group of effectors localized at the cell wall crossing points highlighted finely tuned expression patterns. These distinct localization patterns implicated a role for these effectors in invasion into neighboring cells with maintaining the biotrophic nature of invasive hyphae. Taken together, these results provide initial genome-wide understanding of blast effector dynamics during biotrophic invasion and lead to novel approaches to control the most crucial disease on the staple crop rice worldwide.
SRR: A Novel Strategy Using the Sulfonylurea Resistant Reconstruction for Locus-specific Ectopic Integration in *Magnaporthe*

Yang, F. and Naqvi, N.I.

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

A sulfonylurea resistant allele of the *Magnaporthe oryzae* ILV1 gene has been used as a dominant selectable marker conferring resistance to chlorimuron ethyl (CE), a sulfonylurea herbicide, for fungal transformation (Sweigard et al., 1997). The ILV1 gene encodes an acetolactate synthase (ALS) enzyme, which catalyzes the first step in the synthesis of the branched-chain amino acids (valine, leucine, and isoleucine). Most of the wild type *Magnaporthe* strains contain CE sensitive alleles of the ILV1 gene (MGG_06868.6). We analyzed the CE sensitive and resistant alleles of ILV1 and found that they share a very high sequence similarity. The sequences of the two alleles showed only a few nucleotide differences, which indicate the potential switch of the critical residue(s) of the ALS enzyme at its CE binding site(s). Therefore, the integration of foreign DNA into the targeted ILV1 locus could be achieved by homologous recombination, through replacing the CE sensitive allele with the resistant variant.

We demonstrate the construction of a novel ILV1-locus integration vector for fungal transformation. Resultant transformants revealed the correct in-locus integration with a very high percentage (>95%). This vector has been successfully and routinely used in our lab. A series of *Magnaporthe* strains with different single or dual-organelle fluorescent labeling have been also generated through this strategy. Unlike the traditional way to introgress foreign gene into the target genome via random insertions, the targeted integration at a defined locus eliminates position/orientation effects, unnecessary mutations and/or variation in gene expression. Our data advise the development of a highly efficient ectopic integration strategy with an in-built selection system. Our further study revealed that the biosynthesis of branched-chain amino acids is essential for proper conidiation and pathogenesis in *Magnaporthe*.

Reference:
Deployment of the *Burkholderia glumae* Type III Secretion System as an Efficient Tool for Studying *Magnaporthe oryzae* Effector Functions in Plant Cells

Saitoh, H.¹, Sharma, S.¹, Hirabuchi, A.¹, Sharma, S.¹, Yoshida, K.¹, Fujisaki, K.¹, Uemura, A.¹, Kamoun, S.², Sohn, K.H.², Jones, J.D.G.² and Terauchi, R.¹

¹Iwate Biotechnology Research Center, 22-174-4 Narita, Kitakami, Iwate 024-0003, Japan; ²The Sainsbury Laboratory, John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK

Genome sequences of *Magnaporthe oryzae* have enabled the identification of effectors that suppress host defense and cooperatively modulate the cellular environment for successful fungal growth. Identification and characterization of novel effector proteins are crucial for understanding pathogen virulence and host-plant defense mechanisms. Previous reports indicate that the *Pseudomonas syringae* pv. *tomato* DC3000 type III secretion system (T3SS) can be used to study how non-bacterial effectors manipulate dicot plant cell function using the effector detector vector (pEDV) system. Here we report a pEDV-based effector delivery system in which the T3SS of *Burkholderia glumae*, an emerging rice pathogen, is used to translocate the AVR-Pik and AVR-Pii effectors of *M. oryzae* to rice cytoplasm. The translocated AVR-Pik and AVR-Pii showed avirulence activity when tested in rice cultivars containing the cognate R genes. AVR-Pik reduced and delayed the hypersensitive response triggered by *B. glumae* in the non-host plant *Nicotiana benthamiana*, indicative of an immunosuppressive virulence activity. AVR proteins fused with fluorescent protein and nuclear localization signal were delivered by *B. glumae* T3SS and observed in the nuclei of infected cells in rice, wheat, barley and *N. benthamiana*. Our bacterial T3SS-enabled eukaryotic effector delivery and subcellular localization assays provide a useful method for identifying and studying effector functions in monocot plants.
Recombinant AVR-Pia Protein: Structure, Biological activity, and Application for Immunological Detection of Native AVR-Pia in Magnaporthe oryzae

Satoh, Y.1, Ose, T.2, Kamiya, M.3, Terauchi, R.4, and Sone, T.1

1Graduate school of Agriculture, Hokkaido Univ., Sapporo, Hokkaido, Japan; 2Research faculty of Pharmacology, Hokkaido Univ., Sapporo, Hokkaido, Japan; 3Research Faculty of Advanced Life Sciences, Hokkaido University, Sapporo, Hokkaido, Japan; 4Iwate Biotechnology Research Center, Kitakami, Iwate, Japan.

The avirulence gene AVR-Pia, which induces hypersensitive reaction (HR) of rice cultivars with the resistance gene Pia was isolated from Magnaporthe oryzae strain Ina168 (Miki et al., Molecular Plant Pathology, 10, 361–374, 2009). In order to analyze the function of AVR-Pia protein, the recombinant AVR-Pia (rAVR-Pia) protein was produced in E. coli. rAVR-Pia was purified from inclusion body, by denature-refolding, affinity chromatography and gel filtration. Structural feature and its biological activity were investigated in this study.

Structural analysis by crystallography was not successful. Finally AVR-Pia structure was solved by nuclear magnetic resonance and revealed the core region composed of beta sheet. Biological activity of rAVR-Pia was revealed to induce HR-like browning spots when it was infiltrated into Pia rice leaf, suggesting the activity to trigger the host’s resistance reaction. An anti-AVR-Pia antibody was prepared with the recombinant protein, and its validity was investigated by Western blotting. Native AVR-Pia was detected from total soluble protein extracted from inoculated pia rice leaf sheath, and the MW of AVR-Pia was estimated as 7.4 kDa, corresponding to AVR-Pia w/o signal peptide. This result suggested that rAVR-Pia has similar structure to the native AVR-Pia, and confirmed the affinity of antibody to native AVR-Pia. Quantification of secreted AVR-Pia by M. oryzae during infection was performed with anti-AVR-Pia. It was revealed that approx. 1.5 ppm (relative to total soluble protein of Ina168-inoculated rice sheath) of AVR-Pia was secreted during infection. These facts suggest that the Pia rice can recognize AVR-Pia at lower concentration than 1.5 ppm and trigger the immune response at the early stage of blast infection.
Mitophagy is Necessary for Rice Blast Development

He, Y., Deng, Y.Z., and Naqvi, N.I.

Temasek Life Sciences Laboratory and Department of Biological Sciences, 1 Research Link, National University of Singapore, Singapore 117604.

Although, macroautophagy-mediated glycogen catabolism was shown to be essential for asexual differentiation in *Magnaporthe oryzae*, the function(s) of selective subtypes of autophagy is (are) still unknown. Here, we report that mitophagy, autophagic delivery of mitochondria to the vacuoles for degradation, occurs during conidiation and *in-planta* growth. During early stage of conidiation, mitophagy was found to occur in the foot cells while being undetectable in aerial hyphae and/or conidiophores. And during *in-planta* growth, mitophagy was evident and required during biotrophic/necrotrophic switch. We show that loss of MoAtg24, disrupts mitophagy and consequently leads to highly reduced conidiation and highly reduced invasive hyphal growth, suggesting that mitophagy in the foot cells plays an important role during asexual development and mitophagy in invasive hyphae might be essential during biotrophic/necrotrophic switch in *Magnaporthe*. Interestingly, MoAtg24 was also required for oxidative stress response in *Magnaporthe*. 

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Two Ypt5 Homologs are Essential for Development and Pathogenesis of *Magnaporthe oryzae*

Dang, X.1, Zhang, D.M.1,2, Li, G.P.1,2, Lu, G.D.1, Wang, Z.H.1, and Zhou, J.1**

1Key Laboratory of Bio-pesticide and Chemistry Biology, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou, 350002, PR. China; 2Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, 73104, OK. USA

The rice blast fungus, *Magnaporthe oryzae*, infects many economically important cereal crops, particularly rice. It has emerged as an important model organism for studying the growth, development and pathogenesis of filamentous fungi.

Rab GTPases belong to the Ras superfamily and are important molecular switches in regulation of intracellular membrane trafficking. There are three Rab5-like GTPases in the budding yeast *Saccharomyces cerevisiae*, Ypt51p, Ypt52p, and Ypt53, which control endocytic trafficking and vacuole biogenesis.

MoYpt51 (MGG_06241) and MoYpt52 (MGG_01185) in *Magnaporthe oryzae* are orthologs of the yeast Ypt51p and Ypt52p. To investigate the biological functions of MoYpt51/52, we constructed both the dominant negative form (DN) and the constitutively active form (CA) of MoYpt51/52 alleles and transformed them into the wild-type strain Guy11. The resulting DN and CA mutants were analyzed for their development and pathogenesis in rice. Both MoYpt51/52-CA and MoYpt51/52-DN mutants caused significant reduction in hyphal growth, and more sensitivity to CaCl₂. Furthermore, we found that conidium formation was completely blocked in all mutants, suggesting that MoYpt51/52 are essential for conidiogenesis. No disease symptoms were observed when mycelia plugs of MoYpt51/MoYpt52 CA/DN mutants were used to inoculate wounded rice and barley leaves and intact rice roots, indicating that ectopic expression of dominant negative and constitutively active MoYpt51/52 alleles results in defects in the fungal pathogenicity. To further understand the biological function of MoYpt51/52, we generated MoYpt51/52 RNAi mutants and they exhibited similar defects as the MoYpt51/52-DN mutants.

Our results suggest that MoYpt51/52 play an important role in the development and pathogenesis of *Magnaporthe oryzae*.

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** Corresponding author email: yxxc19204@126.com
Rice blast, caused by the fungal pathogen *Magnaporthe oryzae*, is one of the most devastating diseases of rice worldwide. A highly diversified specific virulence results in the short life of many released blast-resistant rice cultivars. So, it is very important to understand the mechanism of blast resistance and cognate avirulence. *Pi9* confers broad-spectrum resistance to more than 75 blast isolates from 15 countries. Identification of avirulence gene *AvrPi9*, which prevents the fungus from infecting rice cultivars carrying *Pi9*, may shed new light on such resistance pathways. To identify *AvrPi9*, pull-down assays and comparative proteomics approach was used to compare avirulent and virulent isolates. We identified 4 putative *AvrPi9* candidates. Further complementation and allele swapping analyses suggested that *R55* is *AvrPi9*. *R55* encodes a predicted secreted protein in avirulent strains. Insertion of Mg-SINE into the exon of *R55* or loss of *R55* caused the gain of virulence toward rice cultivar containing *Pi9*. Preliminary studies suggest that *R55* is expressed only during *in planta* growth and localizes to the BIC and host cells.
Rice Proteins that Interact with the *Magnaporthe oryzae* Avirulence Effector AVR-Pik

Kanzaki, K.¹, Saitoh, H.¹, Fujisaki, K.¹, Kobayashi, M.¹, Ito, K.¹, Kanzaki, E.¹, Mitsuoka, C.¹, Banfield, M.², Kamoun, S.³, and Terauchi, R.¹

¹Iwate Biotechnology Research Center, 22-174-4 Narita, Kitakami, Iwate 024-0003, Japan; ²John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK; ³The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, UK

We have previously isolated three avirulence (AVR) effectors, AVR-Pia, AVR-Pik and AVR-Pii from the rice blast fungal pathogen, *Magnaporthe oryzae* (Yoshida et al. 2009. Plant Cell. 21: 1573). We are currently focusing on identification of rice proteins that interact with the three effectors. Recently we showed that AVR-Pik of *M. oryzae* binds rice Pik-1, its cognate NB-LRR immune receptor (Kanzaki et al. 2012. Plant J. 72:894). This interaction involves the coiled-coil (CC) domain of Pik-1. Recognition specificity of AVR-Pii alleles by different Pik alleles is mainly determined by the binding specificity between AVR-Pik and CC-domain of Pik-1. In order to understand the virulence effector function of AVR-Pik, we carried out yeast 2-hybrid (Y2H) screen of rice interactors using AVR-Pik as bait, and identified several low molecular proteins belonging to Heavy Metal Associated domain protein family (sHMAs). We found that 6 different sHMAs interact with AVR-Pik in Y2H, co-immunoprecipitation or BiFC methods. The N-terminal HMA domain of sHMA proteins shares a high similarity with the CC domain of the rice R-protein Pik-1. We are currently addressing the functions of rice sHMA proteins by generating sHMA RNAi and overexpressing lines in rice.
Pks1-Mediated Secondary Metabolism is Essential for Host Penetration and Colonization during Rice Blast Disease

Qu, Z. and Naqvi, N.I.
Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, Singapore

*Magnaporthe oryzae* differentiates a specialized infection structure, the appressorium, to penetrate the rice leaves. A layer of condensed melanin is formed in the appressorium to maintain a high turgor pressure therein. The turgor is then translated to physical force and facilitates the host penetration. However, other mechanisms that are related to the appressorial penetration remain elusive. In this study, we describe a PKS deletion mutant, *pks1* mutant, which is completely nonpathogenic and fails to penetrate the host surface and artificial membranes. Interestingly, the *pks1* mutant showed defects in septin localization and failed to elaborate a proper penetration pore. *PKS1* encodes a highly-reducing iterative polyketide synthase which is predicted to produce linear and cyclic non-aromatic polyketides. The Pks1-GFP fusion protein was mainly expressed in the appressorium and was localized in the cytoplasm and vacuoles. Transmission electron microscopy analysis showed that the *pks1* mutant forms appressoria with abnormal melanin layer. Inhibition of melanin synthesis affects Pks1-GFP expression and localization. Therefore it is likely that a substrate of Pks1 is likely shared with the melanin biosynthesis pathway, and more importantly, host penetration requires the secondary metabolite(s) synthesized by Pks1 in *Magnaporthe*
Specific Expression of the \textit{CBP1} gene during Appressorium Differentiation is Controlled by a Limited 16 Base-Paired Region on the 5'-Upstream

Harashima, S.\textsuperscript{1}, Kusunoki, K.\textsuperscript{1}, Saitoh, K.\textsuperscript{1}, Izumikawa, K.\textsuperscript{2}, Takeuchi, M.\textsuperscript{2}, Kamakura, T.\textsuperscript{3}, Arie, T.\textsuperscript{1}, and Teraoka, T.\textsuperscript{1}

\textsuperscript{1}Laboratory of Plant Pathology, \textsuperscript{2}Laboratory of gene function and regulation, Graduate School of Agriculture, Tokyo University of Agriculture and Technology (TUAT), Tokyo 183-8509, Japan; \textsuperscript{3}Faculty of Science and Technology, Tokyo University of Science, Chiba 278-8510, Japan

The rice blast fungus, \textit{Magnaporthe oryzae}, differentiates a specialized infection structure called an appressorium, which is essential to penetrate into the host plant. From our differential cDNA library including ESTs strongly expressed in appressorium formation by subtracting the cDNA in vegetative mycelia, \textit{CBP1} (Chitin Binding Protein 1) gene was found to be involved in the surface recognition on which conidia attach (Kamakura et al., 2002). The \textit{CBP1} gene is specifically expressed at early stage of the appressorium differentiation, but not in vegetative mycelial growth at all. The \textit{CBP1} 5'-upstream region was analyzed to identify the regulatory domain using the \textit{eGFP} reporter gene. And the region around -854 to -696 bp of the 5'-upstream, CUR159, was assigned to be important to regulate the expression. Probably \textit{CBP1} expression is repressed in vegetative growth by a transcriptional factor (TF). Here, the TF(s) of the \textit{CBP1} gene was searched from the nuclear fraction of vegetative mycelia by using an electrophoretic mobility shift assay (EMSA). The candidate TFs to shift the CUR159 up in EMSA were fractionated by heparin affinity chromatography from the nuclear fraction, but still contaminated by many other proteins. When the CUR159 was divided into smaller parts, the region from -854 to -806 bp and -829 to -782 bp had the shift-up ability in EMSA. In the overlapping region, some known motifs to bind TFs were found in the region from -832 to -817 bp (CUR16TF). As the motifs were substituted to the nonsense sequences or deleted, the shift-up ability was lost. Additionaly in \textit{eGFP} reporter assay \textit{in vivo}, as the similar substitution or deletion were introduced in the 5'-upstream region, \textit{eGFP} was always expressed not only in appressoria but also in vegetative mycelia. So this 16 base-paired region was confirmed to be the key domain. Now using streptavidin magnetic beads and the biotin-labeled DNA probe including CUR16TF, the proteins to bind specifically with the region are purified and identified by LC-MS/MS after electrophoretic analyses. And some candidates such as ASC1 and EF1 were nominated for TFs to control \textit{CBP1}. 

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The Role of a Serine/threonine Protein Kinase, MoYAK1 in Conidiation and Pathogenicity of *Magnaporthe oryzae*


Department of Applied Biology, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon 200-701, Korea

Conidia are the major source for disease development in most plant pathogenic fungi. The rice blast fungus *Magnaporthe oryzae* also infects the host plant in the form of conidia. Recently we profiled expression changes of genes during conidiation based on microarray analysis. As a result, we found many putative regulators, which may play a role in conidiation and disease development. Among those, we first focused on MGG_06399 encoding a serine/threonine-protein kinase since several protein kinases such as a mitogen-activated protein (MAP) kinases have critical roles in regulating plant infection processes of *M. oryzae*. MGG_06399 encoding a serine/threonine-protein kinase was similar with Yak1 in *Saccharomyces cerevisiae*. *S. cerevisiae* Yak1 kinase was originally identified as a suppressor of the lethality associated with the loss of either RAS function or the Tpk1, Tpk2, and Tpk3 catalytic subunits of the cAMP-dependent protein kinase A (PKA). Also, *S. cerevisiae* Yak1 is a key regulator of a regulatory cascade affecting adhesion growth and stress resistance. However, the regulatory mechanisms for Yak1 activity and localization are largely unknown. Furthermore, functions of Yak1 protein are still unknown in most organisms including fungi. In this study, we focused on the functions of MoYak1. To investigate functional roles of MGG_06399 gene, named MoYAK1, a deletion mutant was obtained via homology-dependent gene replacement. The deletion mutant for MoYAK1 showed a remarkable reduction in conidiation. A microscopic analysis revealed that ΔMoyak1 mutants produced conidia with an abnormal shape and septum formation. The conidia form ΔMoyak1 were able to adhere to a hydrophobic surface and develop a germ tube, but failed to form appressoria. These data indicate that MoYAK1 play a role in conidiation, appressorial formation and pathogenic development in *Magnaporthe oryzae*. Detailed characterization of MoYAK1 will be presented.
Identification of Rice Proteins Interacting with *Magnaporthe oryzae* Effector Proteins

Giraldo, M.C.\(^1\), Dalby, M.\(^1\), Zhou, X.\(^2\), Jayasundera, K.B.\(^3\), Tao, A.\(^3\), Xu, J.\(^2\), and Valent, B.\(^1\)

\(^1\)Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506, USA; \(^2\)Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907, USA; \(^3\)Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907, USA

*Magnaporthe oryzae* exhibits a broad range of host and geographical distribution, ranking it worldwide as one of the most serious diseases in rice and other cereals. The biotrophic phase of the fungus requires a close plant-pathogen interaction. This interaction is mediated by a finely tuned system, where the fungus takes control of the host defense by regulated deployment of effector proteins. These effector proteins are critical for the pathogen to either avoid recognition by the plant resistance proteins or to turn off the plant mechanism of defenses. In order for the fungus to succeed these effector proteins need to be deployed inside the host in a dosage dependent manner at the right time and place. *M. oryzae* hypha throughout invasion displays a dimorphic switch which seems to be related to the successful formation of an interface between the host plasma membrane and the fungal hypha. This interface includes the biotrophic interfacial complex (BIC) where secreted cytoplasmic effector proteins are preferentially accumulated, and the extra-invasive hyphal membrane (EIHM) compartment where secreted apoplastic effectors are localized. The invasive hyphal development and the special localization of deployed effector proteins implicate a plant signaling dependent mechanism. The molecular mechanisms and the plant targets involved are still unknown. Since many pathogenicity genes encode unique proteins or proteins of unknown biochemical functions, few AVR genes and effector proteins have been identified in *M. oryzae*. Here we used immunoprecipitation to identify host or fungal proteins *in planta* that are associated with known effector proteins. We established a protocol using affinity purification and mass spectrometry to identify interacting proteins during invasion. This study will lead to the identification of rice blast *in planta* protein-protein interaction networks and their biological functions, and provide tools to achieve durable disease control.
Characterization of Genes Encoding a GRAM Domain-containing Protein in the Rice Blast Fungus Magnaporthe oryzae


Department of Applied Biology, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon, 200-701, Korea

The GRAM domain is commonly found in many glucosyltransferases, myotubularins and other membrane-associated proteins. This domain consists of 70 conserved amino acid residues and is predicted to form four β-strands and one α-helix. The physiological and biochemical functions of GRAM domain-containing genes, however, have not almost been determined at either the cellular or molecular levels. Generally, this domain is likely to be involved in membrane-associated processes such as intracellular protein or lipid binding signaling pathways. GRAM domains were first identified by computational analysis of proteins from fungi, plants and animals. To investigate functional roles of this domain, deletion mutants for the GRAM domain-containing genes (MGG_11211, and MGG_03459) were obtained via homology-dependent gene replacement. The two deletion mutant \( \Delta Mogram1 \) and \( \Delta Mogram2 \) showed significant reduction in conidiation and failure in appressorial formation on the surface of hydrophobic glass. Interestingly, the degree of melanization in \( \Delta Mogram1 \) mutant was higher while the \( \Delta Mogram2 \) was lower, compared to the wild-type. Pathogenicity of the both mutants was reduced while the complemented transformants were recovered to the level of the wild-type. To reveal functional roles of the GRAM domain-containing genes in \( M. oryzae \), we have been performing other analyses, including promoter analysis, protein localization, and identification of GRAM domain-interacting proteins.
MYSTery HAT in the Rice Blast Fungus is Essential for Pathogenic Development

Department of Agricultural Biotechnology, Seoul National University, Seoul, 151-742, Korea

Histone acetylation emerged as one of the major epigenetic mechanisms controlling gene expression and various cellular processes in eukaryotes. Plant pathogenic fungi, however, remain a relatively unexplored territory in epigenetics. One of the idiosyncrasies in fungi that have not been examined in the model organisms is a fungal-specific group of MYST family histone acetyltransferases (HATs) with a characteristic domain organization. Here we set out investigate the role of such HAT, MoHAT10, in a model plant pathogenic fungus, *Magnaporthe oryzae*. Identified among 10 putative *M. oryzae* HATs predicted by the profile hidden Markov model, targeted deletion of *MoHAT10* rendered the fungus completely non-pathogenic due to defects in radial growth and appressorium formation. This is in stark contrast to the lack of obvious defective phenotypes in the mutant for SAS3 gene, an orthologue in *Saccharomyces cerevisiae*, and suggests functional divergence associated with differences in domain architecture. Detailed biochemical and genetic analysis are underway to reveal how *MoHAT10*-mediated histone modifications are implicated in the regulation of fungal development and pathogenesis.
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Characterization of Genes Encoding Putative Secreted Proteins during Pathogenesis in *Magnaporthe oryzae*

Kim, S.1, Kim, K.1, Park, S.-Y.1, Choi, J.2, Jeon, J.1, Jeon, J.1, Huh, A.1, Lee, D.1, and Lee, Y.-H.1,2,3,4

1Department of Agricultural Biotechnology, 2Fungal Bioinformatics Laboratory, 3Center for Fungal Genetic Resources, and 4Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea

The secreted proteins define the nature of interaction between microbes and their hosts at the molecular level. Thus, characterizing the set of secreted proteins from a given pathogen is a essential step in understanding pathogenic mechanisms. Unlike bacterial and oomycete phytopathogens, only a limited number of secreted proteins has been identified and analyzed in plant pathogenic fungi. Here new secreted proteins were identified and characterized in the rice blast fungus. SingalP program predicted a total 1,573 putative secreted proteins in *M. oryzae*. Fourteen genes, which have T-DNA mutants already available, were prioritized for functional analysis. To reveal relation with pathogenicity, knockout mutants were generated and their functionalities characterized. Deletion of *MoSPE1, MoSPE3, MoSPE6*, and *MoSPE15* resulted in non-pathogenic or reduction of virulence. There were developmental defects in the deletion mutants; Δ*Mospe1*, which showed reduced conidiation and Δ*Mospe3*, which showed reduced growth on complete media. No observable developmental defects (vegetative growth, conidiation, germination, appressoria formation) in other deletion mutants. Rice sheath inoculation of Δ*Mospe15* showed that defects in pathogenicity could be attributed to the obstruction to grow inside plant tissues, suggesting their implication in interaction with rice. Furthermore, proteins encoded by *MoSPE1, MoSPE6* and *MoSPE15* were shown to be secreted in the yeast secretion trap system. This work would reveal novel function of these secreted proteins, providing new insight into fungal pathogenesis.
A-30

Functional Characterization of Histone Demethylases in the Rice Blast Fungus, *Magnaporthe oryzae*

Huh, A.¹, Jeon, J.¹, Park, S.-Y.⁴, Choi, J.², Kim, S.¹, Jeon, J.¹, and Lee, Y.-H.¹,²,³,⁴

¹Department of Agricultural Biotechnology, ²Fungal Bioinformatics Laboratory, ³Center for Fungal Genetic Resources, and ⁴Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea

The post-translational modification of histones plays important roles in regulating chromatin dynamics and transcription. It has been shown that disruption of proper modifications can lead to developmental defects and cancer in plants and mammals, respectively. Despite the generality of histone modifications as epigenetic mechanisms in eukaryotes, implication of histone modifications in fungal pathogenesis is beginning to emerge. Here research plan was set out to identify and characterize putative histone demethylases in the model plant pathogenic fungus, *Magnaporthe oryzae*. To date, two classes of histone demethylases have been identified: LSD and JmjC domain-containing family proteins. Combining BLAST and HMMER, we identified 8 genes encoding putative JmjC domain-containing histone demethylases and named them as *MoJMJ1* to *MoJMJ8*. Phylogenetic analysis showed that six of them belong to JARID, JHDM2, JMJD2, and JmjC-only domain families, while two proteins are orphans. Deletion of *MoJMJ1*, which is the orthologue of *AtREF6* in *Arabidopsis thaliana*, resulted in defects in vegetative growth, asexual reproduction, appressorium formation and pathogenicity. Introduction of native *MoJMJ1* gene into ∆*mojmj1* recovered all the defects, indicating the importance of regulating the steady-state level of histone methylation during fungal development and pathogenesis. We are currently undertaking deletion of remaining putative histone demethylase genes. These genetic approaches will be followed by biochemical approaches to examine the demethylase activity and identify the genes whose expression is regulated by steady state level of histone methylation. It is anticipated that this work would provide not only the insight into epigenetic regulation of fungal pathogenesis but also the knowledge that can be used in devising new control strategies against rice blast.
A-31

Functional Analysis of the bZIP Transcription Factor Family in the Rice Blast Fungus

Kong, S.1, Park, S.-Y.3, and Lee, Y.-H.1,2,3

1Department of Agricultural Biotechnology, 2Center for Fungal Genetic Resources, and 3Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea

Lifestyle of fungi depends largely on their adaptability in environments. It is therefore crucial to elucidate the transcriptional programs operating under different environmental conditions such as physical and chemical stresses, and host-dependent constraints. Regulatory roles of the basic leucine zipper (bZIP) transcription factors (TFs) in fungi have been identified in diverse cellular processes such as nitrogen metabolite repression, iron supply, sulfur metabolism, and other various stress responses. In this study, genes encoding bZIP (MoZIPs) TF family in the rice blast fungus, *Magnaporthe oryzae* has been systemically characterized. bZIP TF sequences from 36 fungal species were identified and analyzed for their phylogenetic relationship. In total, 12 clades encompassing MoZIPs and conserved orthologs were identified only in phylum Ascomycota. Quantitative RT-PCR analysis for all MoZIPs on 32 different conditions showed dynamic expression profiles, suggesting their involvement in various stress responses and during pathogenesis. To link phylogenetic and expression data to phenotypes, gene deletion mutants were generated for 9 MoZIPs having orthologs, and 4 *Magnaporthe*-specific ones. Among total 13 deletion mutants, 3 show functional conservation with their characterized orthologs and detectable phenotype changes on growth in several stress conditions, developments and/or pathogenicity were observed from other 6 mutants. Deletion of other 4 genes does not make any distinguishable changes compared to the wildtype. Taken together, MoZIPs play critical roles in adapting environmental changes, fungal development and pathogenicity, especially highly conserved members reflecting their functional importance.
A-32

Functional Conservation of MoCRZ1 in *Magnaporthe oryzae* and its Orthologs in *Fusarium graminearum* and *Neurospora crassa*

Lee, D.¹, Choi, J.², Jeon, J.¹, Park, S.-Y.³, and Lee, Y.-H.¹,²,³,⁴

¹Department of Agricultural Biotechnology, Seoul National University, ²Fungal Bioinformatics Laboratory, Seoul National University, ³Center for Fungal Genetic Resources, Seoul National University, ⁴Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea

Calcium signaling is one of the most common and important signal transduction cascades present in any living organism and is said to be highly conserved in throughout evolution. In *Magnaporthe oryzae*, a rice blast pathogen, calcium signaling pathway is required for conidiation and infection-related development, which contributes to the pathogenicity of the fungus. *M. oryzae* gene (*MoCRZ1*) encoding calcineurin-responsive zinc finger 1 is involved in the calcium-dependent signaling pathway of *M. oryzae*, acting as a transcription factor. Deletion mutants of *MoCRZ1* (∆MoCRZ1) showed drastic reduction in conidiation, as well as loss of pathogenicity. In this study, we planned to identify and phylogenetically analyze the orthologs of this gene in *Fusarium graminearum* (necrotrophic cereal pathogen) and *Neurospora crassa* (saprophyte). Orthologs of *MoCRZ1* in *F. graminearum* (*FgCRZ1*) and *N. crassa* (*NcCRZ1*) were identified using BLASTP. Domain analyses showed that *MoCRZ1* and its orthologs possess DNA binding-C2H2 zinc finger domains and conserved PxIxIT motifs, suggesting that the orthologs may be transcription factors involved in calcium signaling. In addition, when *FgCRZ1* and *NcCRZ1* were introduced into ∆MoCRZ1 mutants, these transformants restored conidiation and pathogenicity on rice. Further analysis on conservation and/or evolution of gene function among different species will be presented.
Two Conidiation-related Zn(II)$_2$Cys$_6$ Transcription Factors in Rice Blast Fungus

Chung, H.\textsuperscript{1}, Choi, J.\textsuperscript{2}, Park, S.-Y.\textsuperscript{2}, Jeon, J.\textsuperscript{1}, and Lee, Y.-H.\textsuperscript{1,2,3}

\textsuperscript{1}Department of Agricultural Biotechnology, \textsuperscript{2}Center for Fungal Pathogenesis, \textsuperscript{3}Center for Fungal Genetic Resources, Seoul National University, Seoul 151-921, Korea

Transcription factors (TFs) play important roles in regulation of gene expression during cellular processes. One of the largest group of TFs in all fungi, Zn(II)$_2$Cys$_6$ TF has been exclusively identified in fungi and reported as multifunctional regulator. The rice blast fungus, \textit{Magnaporthe oryzae} undergoes morphological changes during the infection cycle. To elucidate the roles of the TF in pathogenic development of rice blast disease, two Zn(II)$_2$Cys$_6$ TF genes, \textit{MoCOD1} and \textit{MoCOD2}, were characterized. Both $\Delta$Mocod1 and $\Delta$Mocod2 mutants showed defects in conidiation and pathogenicity. Reduced pathogenicity of the $\Delta$Mocod1 mutant was resulted from defects in invasive growth and appressorium development while the $\Delta$Mocod2 mutant showed no pathogenicity. In contrast to the $\Delta$Mocod1 mutant, restricted invasive growth and accumulation of dark brown granules around infection hyphae were frequently observed in the $\Delta$Mocod2 mutant. The granulation is considered as a plant defense response. Genetic complementation with the wild type alleles restored the defects in conidiation and pathogenicity. Taken together, both \textit{MoCOD1} and \textit{MoCOD2} are responsible for conidia development and pathogenicity in the rice blast fungus. This is the first report of the role of Zn(II)$_2$Cys$_6$ TF in pathogenesis of fungal plant pathogens.
Functional Characterization of PAP2 Encoding Lipid Phosphate Phosphatases and Diacylglycerol Pyrophosphate Phosphatase Genes in *Magnaporthe oryzae*


*Department of Agricultural Biotechnology, Fungal Bioinformatics Laboratory, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea*

Type 2 phosphatidic acid phosphatases (PAP2) catalyze dephosphorylation of phosphatidic acid (PA) to produce diacylglycerol (DAG). PAP2 genes have been reported to play significant roles in a variety of cellular processes in both prokaryotic and eukaryotic organisms. However, molecular functions of PAP2 genes in plant pathogenic fungi including *Magnaporthe oryzae* remain unclear. We have identified eight PAP2 encoded genes in *M. oryzae* containing three lipid phosphate phosphatase (*MoLPP1*, *MoLPP2* and *MoLPP3*) and one diacylglycerol pyrophosphate phosphatase (*MoDPP1*). Expression analyses of four genes indicating the involvement of early infection stage. In this article, we showed that PAP2 encoded genes are essential for appressorium initiation and for early stage host-pathogen interaction. *MoLPP1* and *MoLPP2* deletion mutants showed similar phenotypes including disease development with wild type. *ΔMoLpp3* and *ΔModpp1* showed defect in delayed appressorium formation and also showed significant reduction of mycelial growth on media containing sodium propionate as a sole carbon source during early stage of colonization. Expression profile of knock-out mutants showed that both genes also regulate the activity of reported genes of lipid signaling pathway. We found PAP2 genes encoded *MoLPP3* and *MoDPP1* have an important role in phosphatidic acid mediated lipid signaling pathway for fungal development and pathogenicity in *M. oryzae.*
A Non-HEAM Peroxiredoxins (Moprxn1) is Critical for ROS Detoxification and Full Virulence of *Magnaporthe oryzae*


*Department of Agricultural Biotechnology, Fungal Bioinformatics Laboratory, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea*

Peroxidases, the oxidoreductase enzymes, occur in all eukaryotes cell, and catalyze the oxidation of substances by using ROS (Reactive oxygen species). The distribution pattern of all peroxidase genes were observed in 33 different genomes. Thirty seven (37) putative peroxidase genes have been identified in *Magnaporthe oryzae* genome through bio-informatics analysis, sequence alignment analysis and domain search according to peroxidase enzyme classes. Expression of all peroxidase genes were observed in different developmental, infectious and oxidative stress conditions. Peroxiredoxins, the non-HEAM peroxidase ubiquitously present in plants, fungus, mammals and bacteria, were predicted as a good scavenger of Intra and extracellular ROS. From the qRT-PCR result, 1-Cys Peroxiredoxins (Moprxn1) expressed highly in infectious stage among the six non-HEAM peroxidases of *M. oryzae*. MoPrxn1 can complement yeast prx1p mutant successfully and the defective phenotypes of Δprx1p was restored by MoPrxn1. The deletion mutant of Moprxn1 showed defective mycelial growth in MM and reduced growth and melanization compare to wild type in CM. ΔMoprxn1 was highly sensitive to oxidative stress condition and also reduced and delayed penetration were observed in both onion epidermis and rice sheath assays with mutant conidia than KJ201 and complement strains. ΔMoPrxn1 shows less virulence to rice plant with reduce number of small black lesions and also less pathogenic in wound inoculation and in rice roots. ΔMoPrxn1 blocked by higher level of plant generated ROS and failed to proliferate properly for successful colonization inside rice sheath cells. Our study detect that Moprxn1 has role in intracellular ROS homeostasis as well as detoxify extracellular ROS and necessary for full virulence and during plant-microbe interaction Moprxn1 is indispensable for successful colonization. Extracellular peroxidase, laccase activity and melanization were drastically reduced in absence of Moprxn1 and normal mycelial growth in nutrient stress condition was found to influence by Moprxn1. The 1-Cys Peroxiredoxins, MoPrxn1 in *Magnaporthe* has multifunctional role as it is critical for peroxidase and laccase activity and excellent penetration; detoxify extracellular ROS and ultimately reduced the level of virulence of *Magnaporthe oryzae*. 
In silico Identification of Putative Effectors in *Magnaporthe oryzae*

Kim, K.-T., Choi, J., Jeon, J., and Lee, Y.-H.

1Fungal Bioinformatics Lab, 2Department of Agricultural Biotechnology, 3Center for Fungal Pathogenesis, 4Center for Fungal Genetic Resources, 5Plant Genomics and Breeding Institute and 6Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea

The rice blast fungus *Magnaporthe oryzae* and its host *Oryza sativa* interplay each other in molecular level with secretion of variety of proteins including effectors, cell wall degrading enzymes, receptors and resistant proteins. Among the interacting molecules from the fungus, effectors are the major pathogenicity factors that induce the disease on the host plant. The effector proteins can be grouped into two according to their target site either apoplastic interface between the pathogen and host or inside the host cytoplasm. To date, many apoplastic effectors such as LysM proteins and Cys rich proteins are characterized for many other fungal pathogens, but not for *M. oryzae*. Moreover, despite the importance of cytoplasmic effectors as manipulating and disrupting host physiology, only a few cytoplasmic effectors of *M. oryzae* are characterized including AVR-pita and BAS3 (Stergiopoulos and de Wit, 2009).

Many previous studies have attempted to predict a set of secreted proteins. Fungal Secretome Database (FSD) is one such example that predicted 3,262 secreted proteins of *M. oryzae* with a number of signal peptide detection programs (Choi et al., 2010). This is generalized number of secretome which may have place to be improved and specialized for effector prediction. In order to reveal proteins with higher probability of being effectors, the FSD pipeline is improved. The *in silico* predicted proteins are validated with a number of experimental data and RNA-seq analysis (unpublished data). As a result, a total of 1,364 secreted proteins of *M. oryzae* are predicted to be refined secretome and after removing the putative and characterized apoplastic secretome and *in vitro* expressed proteins, the remaining proteins expressed *in planta* and their gene expressions are mostly up-regulated during the infection process. Most of them are hypothetical proteins with short length and includes BAS3 (MGG_11610T0) effector protein, suggesting their roles as possible cytoplasmic effectors. The updated pipeline is also applied to the other 28 fungal and *Phytophthora infestans* genomes for comparative analysis and the relationship between lifestyle and fungal secretome is analyzed. In addition, fraction of intrinsically unstructured regions is calculated to investigate if refined secretome has differential distribution of structural compositions. Taken together, this in silico study would provide more accurate resource for studying fungal effectors and facilitate identification of novel effectors in *M. oryzae*.

Reference:
Characterization of Glycosylation-related Genes in Pathogenesis of *Magnaporthe oryzae*

Jeon, J.¹,², Park, S.-Y.², Choi, J.², Choi, J.³, Kim, S.¹, and Lee, Y.-H.¹,²,³,⁴,⁵,⁶

¹Department of Agricultural Biotechnology, ²Center for Fungal Pathogenesis, ³Fungal Bioinformatics Laboratory, ⁴Center for Fungal Genetic Resources, ⁵Plant Genomics and Breeding Institute, and ⁶Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea

Rice blast caused by *Magnaporthe oryzae* is one of the most devastating diseases in rice-growing regions worldwide. It is also known as a model system to understand plant-microbe interactions. Proper functioning of the secretory machineries in the ER is important for successful disease development of *M. oryzae*. One of the secretory machineries is glycosylation involved in protein stability and quality control although little is known about roles of glycosylation during disease development of plant pathogens. Comprehensive BLASTP analysis revealed that 37 homologous genes were identified in *M. oryzae*, based on the glycosylation related genes of *Saccharomyces cerevisiae*. Two of 13 mutants found in the T-DNA mutant library showed defects in pathogenicity. These two mutants have T-DNA insertions near the *MoANP1* and *MoALG8* genes, respectively, and they are known to play roles in adding glycan during N-glycosylation. To characterize the functions of the genes, additional deletion mutants were generated. Both ΔMoanp1 and ΔMoalg8 mutants showed significantly reduction in pathogenicity and invasive growth. The ΔMoanp1 mutant grew slowly and produced 60% fewer conidia. The mutant was also susceptible to Congo red, indicating cell wall defects. The ΔMoalg8 mutant showed no defect in the tested phenotypes compared to wild-type. However, in the ΔMoalg8 mutant without the treatment of tunicamycin, expression of six genes involved in the UPR pathway was strongly triggered. Similarly, when treated with tunicamycin, an inhibitor of protein glycosylation, the genes were also up-regulated. Taken together, this study suggests that glycosylation is important in the pathogenicity of *M. oryzae*. 
A Novel Approach for Functional Analysis of Genes in the Rice Blast Fungus

Park, S.Y.¹, Choi, J.¹, Kim, S.¹, Jeon, J.¹, Choi, J.¹, Kwon, S.¹, Lee, D.¹, Huh, A.¹, Shin, M.¹, Jeon, J.¹, Kang, S.² and Lee, Y.-H.¹

¹ Dept. of Agricultural Biotechnology, Fungal Bioinformatics Laboratory, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea; ² Dept. of Plant Pathology & Environmental Microbiology, The Pennsylvania State University, University Park, PA 16802, USA

Null mutants generated by targeted gene replacement are frequently used to reveal function of the genes in fungi. However, targeted gene deletions may be difficult to obtain or it may not be applicable, such as in the case of redundant or lethal genes. Constitutive expression system could be an alternative to avoid these difficulties and to provide new platform in fungal functional genomics research. Here we developed a novel platform for functional analysis genes in Magnaporthe oryzae by constitutive expression under a strong promoter. Employing a binary vector (pGOF), carrying EF1 promoter, we generated a total of 4,432 transformants by Agrobacterium tumafaciens-mediated transformation. We have analyzed a subset of 54 transformants that have the vector inserted in the promoter region of individual genes, at distances ranging from 44 to 1,479 bp. These transformants showed increased transcript levels of the genes that are found immediately adjacent to the vector, compared to those of wild type. Ten transformants showed higher levels of expression relative to the wild type not only in mycelial stage but also during infection-related development. Two transformants that T-DNA was inserted in the promotor regions of putative lethal genes, MoRPT4 and MoDBP5, showed decreased conidiation and pathogenicity, respectively. We also characterized two transformants that T-DNA was inserted in functionally redundant genes encoding alpha-glucosidase and alpha-mannosidase. These transformants also showed decreased mycelial growth and pathogenicity, implying successful application of this platform in functional analysis of the genes. Our data also demonstrated that comparative phenotypic analysis under over-expression and suppression of gene expression could prove a highly efficient system for functional analysis of the genes. Our over-expressed transformant library would be a valuable resource for functional characterization of the redundant or lethal genes in M. oryzae and this system may be applicable in other fungi.
**Magnaporthe Atlas: a Web-based Resource for Studying Intra-species Comparative Genomics in Magnaporthe spp.**

Choi, J.\textsuperscript{1,2}, Kim, K.-T.\textsuperscript{1,2}, Jeon, J.\textsuperscript{1,2}, and Lee, Y.-H.\textsuperscript{1,2,3,4,5,6}

\textsuperscript{1}Fungal Bioinformatics Lab, \textsuperscript{2}Department of Agricultural Biotechnology, \textsuperscript{3}Center for Fungal Pathogenesis, \textsuperscript{4}Center for Fungal Genetic Resources, \textsuperscript{5}Plant Genomics and Breeding Institute and \textsuperscript{6}Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea

As fully sequenced genomes have released rapidly, online resources for genomics have been developed and widely used, that usually support analysis between different species, not within a species. According to experimental data, some genes or genomic elements are absent or variable among the strains or isolates, suggesting that single genome sequence cannot represent one species. In the rice blast fungus, *Magnaporthe oryzae*, for example, the distribution of avirulence genes were different from one isolate to another, according to host specificity. To redeem this matter, sequencing multiple isolates of model organisms had been recently conducted. For example, a total of seventy isolates of *Saccharomyces cerevisiae* and *S. paradoxus* were sequenced to study population genomics of domestic and wild yeasts (Liti et al. 2009). In addition, eighteen natural *Arabidopsis thaliana* accessions were sequenced to set a group standard of the species (Gan et al. 2011). However, there had been only one reference genome sequence from *M. oryzae*, the lab strain 70-15 (Dean et al. 2005). Although a few field isolates were additionally sequenced, it is inadequate to represent the whole gene catalog of this species. Here, we developed *Magnaporthe* Atlas (http://www.magnaporthe.org/), a genomics platform which provides the genome sequences and predicted gene models of 39 *M. oryzae* and two *M. grisea* isolates from world-wide as well as closely related species including *M. poae* and *Gaeumannomyces graminis*. Performing homology searches by BLAST and BLASTMatrix against the set of *Magnaporthe* genomes would provide representative distribution of homology in the genus or species level. Furthermore, Seoul National University Genome Browser is implemented to navigate multiple genomes. Collectively, the *Magnaporthe* Atlas will facilitate intra-species comparative and evolutionary genomics, which had been hardly possible in fungal genomics field.
pHnome: a Novel Platform for High-throughput Phenotype Analysis in *Magnaporthe oryzae*

Park, J. and Lee, Y.-H.

*Department of Agricultural Biotechnology, Center for Fungal Pathogenesis, Center for Fungal Genetic Resources, Plant Genomics and Breeding Institute, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, South Korea.*

To unravel the underlying mechanisms of pathogenicity, there is a demand for a large-scale and systematic gene characterization assay in the rice blast fungus, *Magnaporthe oryzae*. However, phenotype observation upon mutant libraries has been met with considerable difficulty because it entails time-consuming and labor-intensive works. Although several microplate-based phenotype assays are frequently used in unicellular organisms, its application in filamentous fungi is hindered by uneven distribution of cells. Here, we developed pHnome, a new high-throughput phenotype screening platform in filamentous fungi. This platform assesses the ability of fungus to change its ambient pH in optimized amino acid medium, which could reflect the vitality of cellular respiration. Using a microplate and two colorimetric pH indicators, bromocresol purple and phenol red, up to 384 cultures could be monitored in a single experiment by a microplate spectrophotometer. To validate applicability of pHnome system, we confirmed that pH changing activity in *M. oryzae* was altered by various stresses or nutrient conditions and thus we could compare the cellular responses among different strains within 24 hours. In Conclusion, pHnome will facilitate rapid progress in functional genomic studies and phenotype standardization for further comparative analysis.
Transcriptomics Analysis of Early Responsible Genes in Rice during *Magnaporthe oryzae* Infection

Wang, Y.¹, Kwon, S.J.², Kim, S.G.³, Wu, J.¹, Choi, J.Y.⁴, Lee, Y.-H.⁴, Agrawal, G.K.⁵, Rakwal, R.⁵,⁶,⁷, Jung, K.-H.⁸, Kang, K.Y.², and Kim, S.T.²

¹Department of Plant Microbe Interaction, Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, 50829 Köln, Germany; ²Department of Plant Bioscience, Pusan National University, Miryang, 627-706, South Korea; ³Plant Molecular Biology and Biotechnology Research Center, Division of Applied Life Science (BK21 Program), Gyeongsang National University, Jinju, 660-701, South Korea; ⁴Department of Agricultural Biot

The rice blast disease caused by *Magnaporthe oryzae* (*M. oryzae*) is one of the most serious diseases of cultivated rice (*Oryza sativa* L.) in most rice-growing regions of the world. In order to investigate early responsible genes in rice, we analyzed transcriptomics analysis using 300 K tilling microarray chip. The quality of RNA samples was initially validated by 4 defense related genes and phytoalexins measurement using RT-PCR and HPLC, respectively, which are well known defense markers. We determined that accumulation of 608 genes showed statistically significant changes in the level of transcription (>2 fold change, P<0.05). Among them, 261 genes were more up-regulated in incompatible interaction than that of compatible one. We further analyzed GO enrichment analysis of the 41 and 231 which were 2 fold up-regulated genes at 12 h and 48 h in incompatible interaction, respectively. MapMan analysis (http://mapman.gabipd.org/) revealed that 21 and 85 genes including 18 receptor-like genes which were more induced in incompatible interaction compared to compatible interaction were found to be involved in biotic stress. Expression patterns of them were validated by RT-PCR. Those early inducible genes including receptor-like protein kinases in incompatible interaction may play a key role in disease resistance against *M. oryzae* attacks.
Genome Analyses of Magnaporthe Species Complex

Meghana, D.S.¹, Mahesh, H.B.², Pinal Chandrana³, Bharat Chattoo³, and Malali Gowda¹

¹Genomics Laboratory, Centre for Cellular and Molecular Platforms, National Centre for Biological Sciences, GKV campus, Bangalore-560065, India; ²Department of Genetics and Plant Breeding, University of Agricultural Sciences, GKV, Bangalore-560065, India; ³Centre for Genome Research, M.S. University of Baroda, Gujarat-390002

Correspondence: Malali Gowda, malalig@ncbs.res.in

Rice, wheat and millets are the most important food crops in India. Blast disease caused by Magnaporthe species is a major factor affecting productivity of these important food crops. The Magnaporthe species complex comprises of many phylogenetic species that cause diseases on over 50 grass species. Rice blast disease caused by Magnaporthe oryzae, is a well known model system to study plant-fungal interactions. Genomic information is lacking for non-rice infecting Magnaporthe species. This study is aimed to generate genomic information from rice and non-rice isolates from Southern India. We have sequenced, assembled and annotated the genomes of Magnaporthe isolates from rice and non-rice species. The whole genome information is available at Magnaporthe database; www.ccamp/genomes/fungi/magnaporthe. Results will be presented in conference. This is the first whole genome study on tropical Magnaporthe (rice and non-rice) isolates from India, which will hasten the understanding of fungal virulence spectrum.
The Fungal Effector AvrPiz-t Suppresses Host Innate Immunity by Targeting Two RING Finger E3 Ligases in Rice


1Department of Plant Pathology, Ohio State University, Columbus, Ohio 43210 USA; 2Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China; 3Department of Plant Pathology, The Kansas State University, Manhattan, KS, 66506 USA. * These authors contribute equally to this work.

Although the function of effector proteins of plant bacteria and oomycete pathogens has been elucidated in the recent years, the information for plant fungal effectors is still lacking. We found that the avirulence effector AvrPiz-t from the rice blast fungus Magnaporthe oryzae preferentially accumulates in the biotrophic interfacial complex (BIC), and is translocated into rice cells. Ectopic expression of AvrPiz-t in transgenic rice causes suppression of the flg22- and chitin-induced reactive oxygen species (ROS) generation and enhances susceptibility to M. oryzae, indicating that AvrPiz-t has virulence function to suppress the PAMP triggered immunity (PTI) in rice. Interaction analyses show that AvrPiz-t suppresses the E3 ligase activity of two rice RING E3 ligases, APIP6 and APIP10, and in return, the two E3 ligases ubiquitinate AvrPiz-t in vitro. We also found that AvrPiz-t promotes the degradation of APIP6 and APIP10 in vivo. Silencing of APIP6 and APIP10 in the non-Piz-t background leads to a significant reduction of flg22-induced ROS generation, suppression of defense gene expression and enhanced susceptibility to M. oryzae. Interestingly, silencing of APIP10 in the Piz-t plants causes strong cell death, accumulation of Piz-t and enhanced resistance to virulent isolates. Taken together, our results demonstrate that AvrPiz-t suppresses PTI through manipulating APIP6 and APIP10 in the non-Piz-t plants but in the Piz-t plants, instability of APIP10 leads to the accumulation of Piz-t and activation of downstream defense responses.
Identification and Mapping of a Hidden Resistance Gene in Tetraploid Wheat Using Laboratory Strains of *Pyricularia oryzae* Produced by Backcrosses


*Crop Protection Cluster, College of Agriculture, University of the Philippines Los Baños, College, Laguna 4031 Philippines; Graduate School of Agricultural Science, Kobe University, Kobe, 657-8501 Japan; RIKEN Yokohama Institute, Tsurumi-ku, Yokohama, 230-0045, Japan*

*PWT3* is a gene involved in the avirulence of *Avena* isolates of *Pyricularia oryzae* on wheat. Molecular mapping revealed that the *PWT3* locus is located on chromosome 6. In the process of backcrosses (BC$_3$F$_1$ generation) for producing *PWT3*-segregating populations, color mutants with white mycelia were obtained. These white mutants lost virulence on all hexaploid and most tetraploid wheat lines. In a BC$_4$F$_1$ population white and black cultures segregated in a 1:1 ratio, suggesting that the mutant phenotype is controlled by a single gene. Furthermore, the mycelial color was perfectly linked with avirulence in the BC$_4$F$_1$ population; white cultures were all avirulent on common wheat (*Triticum aestivum*) cultivar ‘Norin 4’ (N4) while black cultures were all virulent. The white cultures in the BC$_3$F$_1$ and BC$_4$F$_1$ generations were also avirulent on tetraploid wheat (*T. dicoccoides*) accession ‘KU109’ (Tat4) which were susceptible to all cultures from parental wild isolates through the BC$_2$F$_1$ generation. A cross between Tat4 and a susceptible tetraploid (*T. paleocolchicum*) accession ‘KU196’ (Tat14) produced resistant and susceptible F$_2$ seedlings in a 3:1 ratio against the white cultures. In the F$_3$ generation all resistant: segregating: susceptible lines segregated in a 1:2:1 ratio. These results suggest that the resistance of Tat4 to the white cultures is controlled by a single major gene. This gene, tentatively designated as *RmgTd(t)*, is considered a hidden resistance gene because it was not detected with Br58, F$_1$, BC$_1$F$_1$, and BC$_2$F$_1$ cultures. Molecular mapping showed that *RmgTd(t)* is located on chromosome 7B. Cytological analysis revealed that the moderate resistance controlled by *RmgTd(t)* was associated with hypersensitive reaction of mesophyll cells.
C-3

The Rice Blast Fungus Secretory Protein MSP1 Triggers Host Cell Death and Defense Responses

Wang, Y.1,2, Wu, J.3, Kim, S.G.1, Kim, S.T.4, Tsuda, K.2, and Kang, K.Y.1,3*

1Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju 660-701, Korea; 2Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, Carl-von-Linne Weg 10, Cologne, 50829, Germany; 3Division of Applied Life Science (BK21 program), Gyeongsang National University, Jinju 660-701, Korea; 4Department of Plant Bioscience, Pusan National University, Miryang 627-706, Korea.

Rice blast fungus secretes proteins into plant apoplast in favor of their infection. Some plants may recognize secreted proteins to induce defense responses. Here, we report that a small secreted protein, Magnaporthe oryzae sordport1 homolog (MSP1), is recognized in a rice cultivar and triggers host cell death and defense responses. Exogenous treatment of recombinant MSP1 protein induced autophagic cell death in both suspension cultured rice cells and leaves. Protein kinase(s) play an important role for triggering cell death, and cell death was enhanced by the treatment with jasmonic acid and abscisic acid but suppressed by salicylic acid. We demonstrated that secretion of MSP1 into apoplast is necessary for triggering cell death and activating defense gene expression, implying that it is recognized in the host plasma membrane. M. oryzae mutants deficient in MSP1 failed to trigger strong cell death response and caused more severe disease compared to a wild-type strain, while its over-expression enhanced cell death and defense gene activation. Furthermore, pre-treatment of rice with a low concentration of MSP1 primed and strengthened resistance against the pathogen. Taken together, our data suggest that a rice cultivar has evolved a recognition mechanism of the secreted protein MSP1, resulting in triggering strong host innate immunity.
Molecular Dissection of the E3 Ubiquitin Ligase APIP6-mediated Defense Signaling Pathway against Magnaporthe oryzae


1State Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China; 2Department of Plant Pathology, The Ohio State University, Columbus OH 43210, USA

Ubiquitination-regulated protein degradation is a common regulatory mechanism that controls a range of cellular processes in eukaryotes. Recent research suggests that ubiquitination plays an important role in plant disease resistance. We isolated and characterized the rice E3 ubiquitin ligase APIP6 (Park et al., 2012), which was a target of the Magnaporthe oryzae effector AvrPiz-t (Li et al., 2009). APIP6 ubiquitinates AvrPiz-t in vitro and promotes its degradation in vivo, while AvrPiz-t suppresses APIP6 E3 ligase activity in vitro. Silencing of APIP6 reduces the flg22-triggered ROS generation and enhances the susceptibility to a virulent blast strain. To identify other components involved in the APIP6-mediated signaling pathway, we used APIP6 as the bait in the yeast-two hybrid screens and identified 22 PAS (Protein Associated with APIP6) proteins. Real-time PCR analysis indicated that most of the PAS genes are up- or down- regulated during the rice and rice blast interaction, suggesting that PASs might play a role in defense responses in rice. The over-expression and RNAi lines of selected PAS genes have been generated and are being used to evaluate their functions in response to the rice blast fungus. In-depth analysis of these PAS genes will provide new insight into the molecular mechanism of the E3 ubiquitin ligase c-mediated signaling during rice and rice blast interactions.

References:
C-5
Recognition of *Magnaporthe oryzae* AVR-Pii Effector by rice NB-LRR Immune Receptor Pii Requires OsExo70: a Guardee, Decoy or Helper?

Fujisaki, K.¹, Abe, Y.¹, Ito, A.¹, Yoshida, K.¹, Kanzaki, H.¹, Saitoh, H.¹, Kanzaki, E.¹, Utsushi H.¹, Yamashita, T.², Kamoun, S.³, and Terauchi, R.¹

¹Iwate Biotechnological Research Center, 22-174-4 Narita, Kitakami, Iwate 024-0003, Japan; ²Iwate University, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan; ³The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, UK

We have previously isolated an avirulence effector, AVR-Pii from *Magnaporthe oryzae* (Yoshida et al. 2009). To understand AVR-Pii virulence and avirulence activities, we first used live cell imaging to demonstrate translocation of AVR-Pii into rice cells during *M. oryzae* infection. To identify AVR-Pii interactors inside rice cells, we then used co-immunoprecipitation (Co-IP) and mass-spectrometry analysis. These experiments identified two rice Exo70 proteins (OsExo70-F2 and OsExo70-F3) that associate with AVR-Pii via their C-terminal regions. AVR-Pii co-immunoprecipitates with OsExo70-F2 and OsExo70-F3 but not with other members of the Exo70 protein family.

Exo70 is known as a member of the exocyst complex that regulates exocytosis, a process that is presumably involved in plant defense responses. To determine the virulence functions of AVR-Pii and possible defense functions of Exo70, we generated transgenic rice lines overexpressing AVR-Pii and lines silenced for Exo70 genes. When these lines were challenged with a compatible race of *M. oryzae*, we could not detect any alterations in susceptibility and so far we have no evidence that these interactions modulate pathogen virulence and host susceptibility.

On the other hand, we found that OsExo70-F2/F3 double knock-down completely abolished Pii-dependent Effector-Triggered Immunity (ETI) against AVR-Pii. This requirement of OsExo70-F2/F3 is specific to the Pii-AVR-Pii interaction, since Pia-dependent ETI was not affected by OsExo70-F2/F3 gene silencing. We conclude that OsExo70-F2 and/or F3 are involved in the recognition of AVR-Pii by Pii. These rice OsExo70 proteins could function as guardee and/or decoy. Alternatively, they could function as “helpers” that contribute to AVR-Pii trafficking inside rice cells. We are currently pursuing more detailed analysis of the complex involving the three players: AVR-Pii, Exo70 and Pii.
C-6

Differential Induction of Defense-Related Enzymes in Suspension-Cultured Cells of Blast Resistant and Susceptible Genotypes of Rice in Response to Treatment with *Magnaporthe grisea* Elicitor and Toxin

Malathi, S., Madhavan, S., Rabindran, R., Paranidharan, V., and Velazhahan, R.

*Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore- 641 003, Tamil Nadu, India*

Rice blast caused by *Magnaporthe grisea* is a major threat to rice production worldwide. Many genes for blast resistance have been identified. The mechanisms underlying resistance of rice to *M. grisea* are not yet fully understood. The fungus produces α-picolinic acid as one of the toxins that helps in pathogenesis. On the other hand elicitors have been isolated from *M. grisea* that induces resistance responses in rice. The aim of this work was to study the effect of cell wall crude elicitor from *M. grisea* and α-picolinic acid on induction of peroxidase (PO), polyphenol oxidase (PPO), chitinase and β-1,3-glucanase in suspension cultured rice cells. Suspension-cultured cells of blast resistant (Usen) and susceptible (CO 39) rice genotypes were treated with crude elicitor from mycelial walls of *M. grisea* (50 µg of glucose equivalents per ml) or α-picolinic acid (400 ppm). The cells were harvested at different time intervals and analyzed for the induction of defense-related enzymes. Peroxidase (PO) isozyme analysis indicated that the elicitor strongly induced the activities of PO-3 and PO-4 in suspension cultured cells of Usen 3 days after treatment. In the Usen, toxin also induced the activities of PO-3 and PO-4. However similar levels of activities corresponding to these isozymes were recorded 7 days after treatment. In CO 39, the activities of PO-1 and PO-2 were induced 3 days after elicitor treatment. In contrast, the toxin suppressed the activity of PO-2.

PPO isozyme analysis revealed constitutive expression of PPO-3 in Usen. The elicitor induced the activities of PPO-1, PPO-2 and PPO-3 in both Usen and CO 39. In Usen, steady increase of PPO-3 was observed and higher level of activity was recorded 5 days after treatment. In CO 39, higher level of PPO-3 was observed 1 day after treatment and declined thereafter. However the activities of PPO-1 and PPO-2 increased 3 days after treatment in CO 39. In toxin treated cells of Usen, higher level of activity of PPO-3 was observed 3 days after treatment. Western blot analysis revealed that a β-1,3-glucanase (30 kDa) and a chitinase (35 kDa) were strongly induced in rice cells 1-3 days after treatment with elicitor. No difference was observed in the protein pattern between control and toxin-treated rice cells. The above results will be presented.
C-7

A Rice Mannose-Binding Lectin-Like Protein May Function as the Receptor of *Magnaporthe oryzae* Chitinase I to Trigger Plant Immunity

Han, Y.J.¹, Liu, L.H.¹, Zhou, J.¹, Wang, Z.H.¹, Wang, S.H.², Shim, W.B.³, and Lu, G.D.¹*¹

¹Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou 350002, China; ²School of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China; ³Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, USA

Plants can initiate immunity responses against fungal pathogen invasion by recognizing pathogen associated molecular pattern (PAMP), such as chitin released from invading hyphae. Here we show that *Magnaporthe oryzae* chitinase I (MoChi1) plays a reversible role in the rice-*M. oryzae* interaction. The deletion of *MoChi* hampered the virulence of the pathogen on rice, likely due to the aberrant delay in germination, appressorium formation and penetration. However, the mutation did not affect fungal vegetative growth. Intriguingly, over-expression of *MoChi* also reduced virulence on plants and rendered white colony growth, which is indicative of mycelia autolysis. We used yeast two hybrid (Y2H) screen to identify rice proteins interacting with Mochi1. Subsequent GST-pull down and BiFC demonstrated that rice OsMBL1 interacts with Mochi1 both *in vitro* and *in vivo*, respectively. OsMBL1, a putative mannose-binding lectin-like protein, accumulates everywhere in rice, with the highest level found in roots and when stimulated by SA and JA. The loss of *Mochi* activated pathogen-related (PR) genes and *OsMBL1* in rice. Significantly, *OsMBL1* over-expression in rice led to enhanced resistance against *M. oryzae*, similar to the resistance observed in *Mochi1*-transgenic rice. Coincidentally both can activate defense response to compatible *M. oryzae*. However, *OsMBL1* transcription level in *Mochi1*-transgenic rice is much lower than the wild-type rice when challenged with *M. oryzae Guy11* and *Mochi1* strains. Taken together, we postulate that Mochi1 may block the down-stream signaling by suppressing the expression of *OsMBL1*, and that the deletion of mochi1 can relieve this suppression thus leading to enhanced defense in rice. Conversely, elevated Mochi1 level has detrimental impact on its hyphae, making it difficult for the fungus to grow and invade rice.

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*E-mail: guodonglu@yahoo.com
Function, Evolution, and Interaction of the Coupled Genes Responsible for the Pik-h Encoded Blast Resistance of Rice

Zhai Chun¹, Zhang Yu¹, Hua Lixia¹, Yao Nan², Lin Fei¹, Liu Zhe³, Dong Zhongqiu¹, Wang Li¹, Wang Ling¹, and Pan Qinghua¹

¹National Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, Rice Blast Research Center, South China Agricultural University, Guangzhou, China; ²National Key Laboratory of Biocontrol, College of Life Sciences, Sun Yat-sen University, Guangzhou, China

Pik-h, which is an allele of Pik, confers resistance against certain races of rice blast. Its positional cloning showed that it comprises a pair of NBS-LRR genes, Pikh-1 and Pikh-2. The allele is distinguishable from other known blast resistance genes on the basis of key variable nucleotides, and SNP diagnosis among the five rice populations implies that it appears to be the most recently evolved of the set of Pik alleles. Comparisons between the sequences of Pik-h and other Pik alleles showed that the functional K haplotype exists as two sub-haplotypes, KM and KH, which both evolved prior to the domestication of rice. While Pikh-1 appears to be constitutively transcribed, the transcript abundance of Pikh-2 responds to pathogen challenge, suggesting that while Pikh-1 may well be involved in elicitor recognition, Pikh-2 is more likely to be responsible for downstream signalling. In vitro, the CC domain of Pikh-1 was shown interact directly with both AvrPik-h and Pikh-2. Transient expression assays demonstrated that Pikh-2 mediates the initiation of the defence response. Moreover, nucleocytoplasmic partitioning of both Pikh-1 and Pikh-2 is required for the functionality of Pik-h. In the proposed Pik-h resistance pathway, it is suggested that Pikh-1 acts as an adaptor between AvrPik-h and Pikh-2, while Pikh-2 transduces the signal to trigger Pik-h-specific resistance.
Ectopic Expression of a Leucine Rich Protein Isolated from *Oryza sativa* Confers Enhanced Resistance to *Xoo*


*National Academy of Agricultural Science, Rural Development Administration, Suwon, 441-707, Korea*

Bacterial blight, caused by *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*), is one of the most serious diseases of rice. To obtain useful resistance genes against *Xoo*, we performed a differential screening from rice and have isolated a novel gene encoding a leucine rich repeat (LRR) protein, which was expressed highly upon *Xoo* infection. Expression of the *OsLRP* was highly induced under biotic and abiotic stress conditions. The over-expression of the *OsLRP* gene in transgenic rice plant confers disease resistance in response to *Xoo* infection, and was shown the up-regulated expression of various pathogen related genes, such as peroxidase and chitinase. These results suggest that over-expression of the *OsLRP* in rice modulates the expression of several pathogenesis related genes, and thereby affects resistances to *Xoo* in rice. Amounts of free SA and expression of SA synthesis related-genes were induced in *OsLRP* over-expressing transgenic lines compared to non-transgenic plant, meaning *OsLRP* plays an important role in up-stream of SA mediated disease responses in rice. In previous reports, *LRR1* had interacted with *HIR1* induced cell death in *Arabidopsis* and rice. The complex of *OsLRP* and *OsHIR1* by the analysis of BiFC was located in plasma-membrane by the rice protoplast method. In addition, it was confirmed that the most co-transfected protoplasts with *OsLRP* and *OsHIR1* were shown cell-death after crushing quickly. The induction of cell death in protoplast was correlated with *OsLRP* over-expressing transgenic lines showing legion mimic phenomenon, but not shown in non-transgenic plant. In our previous report, over-expression of *OsLRP* was shown enhanced resistance to bacterial soft rot disease in Chinese cabbage and up-regulation of PR genes. However, it was not clear yet how *OsLRP* located in extra-cellular region recognizes external signals and transfers them into cell. Now, we are trying to figure out the mechanism of *OsLRP*-mediated signal transduction in response to *Xoo* infection.
Identification of Resistant Related Metabolites to Discriminate the Resistance in Rice against Blast Disease

Madhavan, S., Velazhahan, R., and Paranidharan, V.

Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore – 641003, Tamil Nadu, INDIA

Rice blast caused by Magnaporthe grisea (Hebert) Barr, well known most destructive diseases of rice in both tropical and temperate areas of the world causing 10–40 % yield losses. Metabolomics is a technology geared to identify and quantify all the metabolites in an organism or a biological system. Metabolomics has the potential to make a large impact on areas of plant microbe interactions that extend far beyond the scope of this study. Metabolites have been linked to specific genomic positions, and a set of co-localized genes/QTLs have been proven to regulate certain metabolic pathways leading to the production of a series of metabolites that are in turn linked to phenotypes.

The ultimate goal of this study, is to understand and to predict the behavior of Rice- Magnaporthe grisea interaction systems by using the results to be obtained from data mining tools from metabolomic studies for subsequent modeling and simulation. For that purpose, Seeds of resistant cultivar Usen having resistant genes, Pia and CO39, susceptible line were sown in the pots and kept at 25°C with 70% RH. M. grisea spores @ 10^5 ml^-1 with 0.02% tween 20 were sprayed on two weeks old seedlings and kept at 25°C with > 90% relative humidity. Control and mock treated plants were also kept at same environmental conditions. Leaf samples collected at 0 hr, 24 hr, 48 hr and 72 hrs after inoculation were immediately frozen in liquid nitrogen and kept at -80°C till extraction of metabolites. Development of M. grisea infection on plants was confirmed by observing symptom on CO39 leaves. Metabolites were extracted by following the method described by Lisec et al., (2006) with the minor modification. Metabolites extracted from the cultivars, CO39 and Usen were analyzed using GC-EI-TOF-MS analyzer and the compounds were identified by using TagFinder software with NIST library and Golms database. With use of TagFinder several resistance-related metabolites, including constitutive and induced with some common metabolites were identified. Canonical discriminant analysis and independent component analysis were used to classify treatments and to identify the associated metabolic functions.

Resistance related metabolites will be identified in the present study can be used as biomarkers to screen breeding lines, after validation using more cultivars against the blast pathogen of rice. Screening of breeding lines under field conditions requires the inoculation of plants with the pathogen, which requires maintenance of free water for at least 24 h and often this is a limiting factor for field testing. Use of the metabolomic tools can overcome such a problem, and will prove this potential tool for screening for resistance of rice cultivars.
D-1

Secondary Hosts as Potential Inoculum Source for Rice Blast Pathogen in Argentina


Estación Experimental Agropecuaria Concepción del Uruguay, INTA. Ruta Provincial 39, km 143.5, pedraza.virginia@inta.gob.ar. mariavirginiapedraza@yahoo.com.ar. Tel. +54 03442 438075

Concepción del Uruguay, Entre Ríos, Argentina. CC Nº 6 - C.P. 3260.

Rice Blast is the main disease of this crop in Argentina. It occurs sporadically at different rice growing areas, mainly in Santa Fe and Chaco provinces. However, when environmental conditions are favorable to disease development, Rice Blast can cause damages in other rice growing areas, such as Entre Ríos or Corrientes provinces. Inoculum survival and pathogen population specificity are important issues to understand Rice Blast epidemiology. *Pyricularia grisea* isolates from *Phalaris canariensis*, *Stenotaphrum secundatum*, *Lolium perenne* and *Digitaria sanguinalis* were collected in Entre Ríos fields in 2007. Greenhouse assays were performed. The isolates were artificially inoculated to rice genotypes, including differential lines with resistant genes *Pi1*, *Pi2*, *Pita* or *Pi33*; and commercial varieties as Cambá INTA-Proarroz, Puitá INTA-CL, or Supremo-13. Re-isolations of the pathogen from foliar symptoms were done. Any contamination was prevented. All isolates caused typical Rice Blast foliar symptoms in different rice genotypes. Differential line with *Pi2* showed symptoms with all the isolates evaluated. The other genotypes showed susceptible or no reactions, according to the isolate. Molecular characterization of original and re-isolated strains will be done. This work enhances the study of secondary hosts’ potential role in Rice Blast epidemiology in Argentina.

References:
D-2

Use of Microsatellite Markers in Genetic Variability Studies of *Magnaporthe oryzae* Brazilian Isolates

Goncalves, F.J.¹, Silva, G.B.², Araujo, L.G.¹, Coelho, A.S.G.¹, Silva-Lobo, V.L., and Filippi, M.C.

¹IUniversidade Federal de Goiás (UFG), Goiania, GO, Brasil; ²Universidade Federal Rural da Amazonia (UFRA), Belem, PA, Brasil

Throughout its lifecycle, the rice plant is subjected to the attack of some diseases that reduce productivity and affect grain quality. Among them rice blast, the most devastate one is caused by *Pyricularia oryzae* (*Magnaporthe oryzae*, teleomorph) and has an accentuate variability. The known origins of *M. oryzae* variability are mutation, parassexual recombination, Avr gene deletions and the movement of transposition elements (transposons and retrotransposons).

Eighteen microsatellite markers (Adreit et al., 2007) were used in order to search the genetic variability of *M. oryzae*, collected from different rice producing areas, located in distinct Brazilian geographical regions. Eighty-nine isolates from the upland cultivar BRS Primavera and seventy-five from irrigated cultivar BR IRGA 417 were selected based on its ability to infect its origin cultivars. The selected isolates were grown in liquid culture medium, followed by mycelium filtration, frozen and lyophilized for DNA extraction, which quantified, amplified and separated by electrophoresis. The polymorphism data were analyzed with Structure Version 2.3 software in order to determine the existence of any population structure and the analysis of molecular variance was calculated with the Arlequin software.

The best value found for grouping 164 isolates was K = 2. According to the AMOVA the variability among subpopulations was 36.55% and 63.45% within subpopulations. Among the 18 locos studied 16 were polymorphic, markers Pyrms 233-234 e Pyrms 063-064 were the best ones for detecting differences among the subpopulations with 66.95% and 66.03% of variation, respectively. The value of genetic differentiation of populations (FST) was 0.36451 (p < 0.001) calculated by 10,000 random permutations, indicating that the genetic flow among isolates was very low. The SSR markers used in this study indicated a grouping considering the cultivar that originated the isolate and were efficient to detect 2 subpopulations, organizing 164 Brazilian isolates collected from two different rice systems.

Reference:
D-3

Unravelling the Blast ‘R’ Genes in Popular Rice Cultivars of Karnataka (India)

Prasanna Kumar, M.K.\textsuperscript{1}, Mahesh, H.B.\textsuperscript{2}, Shailaja Hitalmani\textsuperscript{2}, P. Mahadevu\textsuperscript{1} and Malali Gowda\textsuperscript{3}

\textsuperscript{1}Zonal Agricultural Research Station, V.C. Farm, Mandya; \textsuperscript{2}Department of Genetics and Plant Breeding, GKVK, UAS, Bangalore. \textsuperscript{3}C-CAMP, National Centre for Biological Sciences, GKVK Campus, Bangalore

Rice blast is one of the major limitations in rice production. Plant breeders have evolved several high yielding blast resistant varieties with limited knowledge on the resistant genes incorporated in the released varieties. In this study we selected 45 popular improved cultivars grown in different agro-climatic zones of Karnataka state, India. Our main focus of the study was to survey the resistant genes present in the cultivars and their phenotypic reactions to \textit{Magnaporthe oryzae} infection. The phenotypic evaluation was done in high disease pressure Uniform Blast Nursery (UBN) condition in the field. The scoring was done as per Standard Evaluation System (SES), IRRI with 0-9 scale. Most of the varieties were found either moderately resistant or susceptible. However, variety BR2655 which is grown as blast resistant variety in irrigated ecosystem was found to be resistant in the field evaluation with score 1. DNA Finger printing was done for 45 commercial varieties along with Co-39, HR12 (universal susceptible varieties) and Tetep, Tadukan (Universal Resistant varieties) using 23 validated blast R gene linked markers. The blast Resistance (R) genes selected for evaluation are Pi27(t), Pi35(t) Pi37, Pish, Pit, Pitp(t) Pib, Piz, Pii, Pi3(t), Pi5(t), Pi38, Pia, Pik, Pik-h, Pi20(t), Pita, Pi5, Pi9, Pi40(t), Pi33 and Pita. Reference genotypes were used as positive control to compare the test entries. Genotypic data revealed that most of the varieties have more than one blast R gene e.g., BR2655 resistant cultivar has Pita/Pita2, Pi40 and Pi2 genes. Similarly, a popular variety Tunga grown in hilly ecosystem which was released as blast resistant variety is now susceptible in this region inspite having four different R genes. This could be attributed to existence of new \textit{Magnaporthe oryzae} races. We have observed the genetic diversity among varieties for many of the ‘R’ genes. Varietal development in the background of evolution of new pathotypes in blast fungus is a continuous process. The present work will help the breeders to select the parental lines for subsequent breeding programme and selection of breeding material with combination of suitable resistant genes.
Genetic Diversity Analyses of *Magnaporthe* Species Complex using Multi-Marker System

Mahesh, H.B.¹, Meghana, D.S.², Shailaja Hittalmani¹, and Malali Gowda²

¹Department of Genetics and Plant Breeding, University of Agricultural Sciences, GKVK, Bangalore-560065, India; ²Genomics Laboratory, Centre for Cellular and Molecular Platforms, NCBS, GKVK campus, Bangalore- 560065, India

Rice and small millets play a prominent role in Indian diet. However, blast diseases caused by Ascomycetous fungus, *Magnaporthe* is a serious constraint in crop production. *Magnaporthe* species complex is consists of several species and sub-species such as *M. oryzae*, *M. grisea*, *M. poae*, *M. salvinii*, *M. rhizophila*, etc (Couch and Kohn, *Mycologia*. 94:683-693, 2002) which infects food crops (rice, wheat, and millets) and grasses. In real-cropping system, the food crops and grasses are grown together in a geographical region. There will be possibility of migration of these isolates from one crop to another. In this study, we aimed to sample and characterize *Magnaporthe* species complex from rice, millets and grasses in Southern India. We have isolated 160 isolates (monoconidia) from infected leaf samples of rice (*Oryza sativa*), fingermillet (*Eleusine coracana*), foxtail millet (*Setaria italica*) and grasses (*Cenchrus* spp.). DNA was isolated from these strains and fingerprinted using multi-markers system such as transposons (Pot2 – DNA transposon, and Grasshopper - retroelement), microsatellites, avirulence genes and mating locus (MAT1-1 and MAT1-2). From Rep-PCR profiling, we detected upto 30 bands (500bp to 5kb) and 4 bands of Pot2 transposon integration signatures in the rice and fingermillet isolates, respectively. In contrary we did not observe Pot2 Rep-PCR amplification from foxtail millet and grass isolates. Rep-PCR profiling of Grasshopper element showed 10 to 12 bands in fingermillet isolates, but not in rice, foxtail millet and grass isolates. These transposons are effective markers to distinguish rice and non-rice isolates. We surveyed for cloned Avr-genes (*PWL-1, PWL-2, PWL-3, PWL-4, Avr-Pizt, Avr-Pia, Avr-Pii, Avr-Pik, Avr-Pita* and *Avr-Co39*) to understand virulence pattern in rice and non -rice isolates. In rice isolates, we observed amplification (presence or absence) for all Avr-genes except *PWL-3*. In fingermillet isolates, *PWL-2* and *Avr-Pita* were absent. Interestingly all Avr genes were amplified from isolates of foxtail and grasses. Using highly polymorphic SSR markers (Zheng et al., *Fungal Genetics and Biology*. 45, 1340-1347, 2008), we observed 2 to 8 alleles and average polymorphic information content of 0.74 from our isolates. Mating locus revealed presence of mixture of male (MAT1-1) and female (MAT1-2) isolates in rice, fingermillet and foxtail millet. Whereas MAT locus amplification was absent in Cenchrus isolates. By combining the data of multi-markers system, we constructed a dendrogram based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm considering Jaccard coefficient of similarity using NTSYSpc 2.02i software. The results revealed distinct groups of isolates that were specific to crop, cultivar and geographical regions. In addition, this study provided the DNA markers to distinguish rice and non-rice isolates. The possible migration of isolates from one crop to another will be verified by cross-infectivity and cross-mating assays of *Magnaporthe*. 
D-5
Pathogenic Population Study of *Magnaporthe oryzae* and Novel Screening System for Durable Rice Blast Resistance in Korea


*Crop Environment Division, National Institute of Crop Science, RDA, Suwon, 441-857, Korea*

Rice cultivation area in Korea have decreased from 1.2 million ha in 1970s to 0.85 million ha in 2012, and rice self-sufficiency ratio have declined from about 100% in 1980s to 83.0% in 2011. Fortunately, rice blast in Korean fields has rarely occurred under 0.1% disease occurrence ratio since rice blast epidemic in 1977 due to resistance breakdown of Tongil cultivar. In order to control rice blast in fields, Korean researchers and government have tried to apply several methods such as suitable chemical utilization, management practices and breeding of blast-resistant cultivar. Especially, breeding of blast resistant cultivar is the most important and efficient methods for rice blast management. For this purpose, it is required to examine distribution of pathotypes in rice blast fungus and reveal relationship of those and rice cultivars. Thus, population studies about pathogens have been done using Korean differential rice cultivars since 1981. From 1980 to 2010, almost 10,000 field isolates of rice blast were collected and categorized by more than 60 races in Korea. This data shows that complex and diverse pathogen races have existed since 2000s while two main races such as KJ-301 and KJ201 remained until 1990s. This fact indicates that distribution of resistance genes in rice might have been more complex and diverse compared to past years. Therefore, it is required to design novel Korean differential system and screening resistant rice cultivars.

For the resistant of rice lines, we have been used the three kinds of resistance screening methods in blast resistance breeding programs. The methods are artificial inoculation of rice seedling in greenhouse, nursery screening (hot spot) in many different locations, and field test. These methods are very effective in screening of qualitative resistance but those are hard and the examination of the durability of rice cultivars takes a very long time. Since these methods have chiefly targeted genes displaying great influence, it has been observed that duration of resistance was either extremely short or not significant occasionally. For the evaluation of durable resistance to blast disease in rice, we conducted the sequential planting method, which allows for the screening of durable resistance under artificial conditions in a short period of time. This research was carried out to evaluate the effectiveness of sequential planting method in determination durable resistance of monogenic resistant lines and some Korean elite lines.

A sequential planting method was developed to identify durable blast resistance in Korean rice cultivars. Two types of rice cultivars were investigated by a sequential planting after inoculation of mixed 30 rice blast isolates. These blast isolates are selected as representative strains according to their genotypes and pathotypes among 2,000 blast isolates during 15 years in Korean rice fields. Daesanbyeo and Gihobyeo, showing initial high resistance in farmer's field followed by rapid breakdown of the resistance in very short periods, showed low disease until the third and fourth planting but later showed higher than 40% DLA (diseased leaf area), respectively. In contrast, Palgongbyeo and Seomjinbyeo, showing low disease occurrence and sustainable field resistance during the last 20 years in farmers' field, showed less than 20% of DLA until the seventh planting. Results from the sequential planting method in greenhouse were significantly similar to the farmers' field data. This suggests that the current sequential planting method is effective to evaluate durable resistance of rice blast. This work has been applied to resistant breeding program to rice blast during 7 years.

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Genetic and Population Structure of *Magnaporthe grisea* (*sensu lato*) the Causal Agent of Blast Disease Obtained from Rice and Other Hosts in Iran, Japan and USA

Zarrinnia, V., Javan-Nikkhah, M., Zamani Zadeh, H.R., and Mehrabi, R.

Zarrinnia, V. Department of Plant protection, Science and Research Branch, Islamic Azad University, Tehran, Iran; Javan-Nikkhah, M. Department of Plant Pathology and Entomology, College of Agriculture, University of Tehran, Karaj. Zamani Zadeh, H.R. Department of Plant pathology, Science and research Branch, Islamic Azad University, Tehran, Iran; Mehrabi, R. Pathology Lab., Seed Research Dept., Seed and Plant Improvement Institute (SPII). Karaj, Iran.

Rice blast, caused by *Magnaporthe grisea* (*sensu lato*), is one of the most important and destructive disease of rice in Iran. Genetic and population structure of *M. grisea* s.l was examined using data derived from 7 primer pairs of polymorphic minisatellite loci by Nei’s test for population differentiation, $G_{ST}$, and analysis of molecular variation (AMOVA). In this research 92 isolates of the fungus were collected from diverse geographical locations and hosts such as *Digitaria sanguinalis*, *Setaria italica*, *Echinochloa* sp., *Eragrostis curvula*, *Festuca arundinacea*, *Stenotaphrum secundatum*, *Panicum miliaceum*, *Triticum aestivum*, *Lolium perenne* from Japan, USA and most important rice growing regions in north of Iran.

The results revealed that *M. grisea* s.l populations were composed of eight lineages designated as A to H. Among the lineages, lineage C with 25 (22.7%) isolates were considered as dominant lineage. All isolates collected from *Digitaria sanguinalis* established a discrete lineage. Also isolates from rice were closely related to those from *Setaria italica*, *Echinochloa* sp., *Eragrostis curvula*, *Festuca arundinacea*, *Stenotaphrum secundatum*, *Panicum miliaceum*, *Triticum aestivum* and *Lolium perenne*. Widely distributed isolates in different lineages suggested that gene flow occurs across the regions. The highest level of gene flow ($Nm = 0/7$) were observed between USA and Japan populations and maximum inter population differentiation ($G_{ST} = 0/5$) were observed among rice and weeds populations of Iran. Analysis of Molecular Variance (AMOVA) revealed that variation within populations (88%) was significantly higher than among populations (12%).

It seems that selection due to host species was the primary factor determining population structure according to analysis of molecular variance.
Genetic Diversity, Mating Type and Presence of Avirulence Genes in *Magnaporthe oryzae* Populations from the Philippines.

Lopez, A.L.¹, Milazzo, J.², Adreit, H.², Fournier, E.³, Cumagun, C.J.R.¹, and Tharreau, D.²

¹University of the Philippines Los Baños, Laguna 4031, The Philippines; ²CIRAD, UMR BGPI, TA A 54 K, 34398 Montpellier, France; ³INRA, UMR BGPI, TA A 54 K, 34398 Montpellier, France

To keep abreast of the current genetic diversity of the populations of *Magnaporthe oryzae*, the rice blast disease pathogen, we characterized the genetic diversity of 453 monoconidial isolates recently collected from different geographic areas in the Philippines in experimental plots and in farmer fields. With 13 microsatellite markers, a total of 93 new genotypes were determined when compared to the reference isolates representing the genotypes previously published. Analysis revealed a weak geographic structuring of pathogen genotypes, with some of those collected from Luzon clustering with those from Visayas or Mindanao. Nevertheless, none of the strains from Mindanao clustered with those from the Visayas or vice versa. Significant differences were noted on isolates collected from distinct agroecosystems and isolates collected from the upland tended to be more diverse when compared to isolates from lowland. This structure likely results from limited natural migration and from some events of long distance migration, probably through the transport of infected seeds.

A subset of 30 isolates representative of the genotypic diversity were tested for fertility status. Of these, eight induced the production of perithecia, of which six also were only male-fertile and two were also female-fertile. These fertile isolates were mostly found in the upland agroecosystem. In addition, the same isolates and 23 additional isolates were subjected to PCR test for mating type. MAT1.1 isolates represented 36% of our sample whereas it represented only 5% in the collection of reference strains. This result confirms that this new sampling improves our knowledge of the blast population in the Philippines.

Pathogenicity test of a subset of 24 isolates inoculated to 16 differential rice varieties demonstrated that most isolates were avirulent to varieties C101Lac and C104Lac which carry the Pi1 resistance gene. The very popular Pi33 gene, integrated into the varieties IR 64, Bala, and IR1529 also conferred resistance to most of the isolates. In addition to Pi1 and Pi33, other possible candidate resistance gene for plant breeding effort to manage the rice blast disease in the Philippines are Pi9, Pikm, and the combination of Piz and Pish.

PCR amplifications of cloned avirulence gene were also carried out. Data analysis is in progress and results will be presented.
Investigation on Mating type Distribution and Fertility Status of *Magnaporthe oryzae (sensu lato)* Populations from Rice in North of Iran

Zarrinnia, V., Javan-Nikkah, M., Zamani Zadeh, H.R., and Mehrabi, R.

1Department of Plant protection, Science and Research Branch, Islamic Azad University, Tehran, Iran; 2Department of Plant Pathology and Entomology, College of Agriculture, University of Tehran, Karaj, Iran; 3Department of Plant pathology, Science and research Branch, Islamic Azad University, Tehran, Iran. 4Pathology Lab., Seed Research Dept., Seed and Plant Improvement Institute (SPII). Karaj, Iran.

Rice blast caused by the heterothallic ascomycete *Magnaporthe oryzae (sensu lato)* is one of the most destructive diseases in major rice growing regions of Iran.

One hundred monoconidial isolates were collected from infected rice plants showing typical blast symptoms on leaf, neck, or panicle in Guillan and Mazandaran provinces of Iran. Crossing experiments were performed with two standard hermaphrodite isolates GUY-11 (*MAT1-2*) and KA-7 (*MAT1-1*) on Rice Polish Agar (RPA) to determine the mating behavior and fertility status.

Among 100 isolates, only 55 (55%) isolates produced mature perithecia in crosses with GUY-11 and the remaining isolates did not produce prethecia in crosses with both tester isolates and, thus, were completely sterile. As a result, all fertile isolates were male fertile (female sterile) and determined as *MAT1-1*. Isolates were considered as fertile only if their perithecia produced viable ascospores. The mating type idiomorph of each isolate was determined through a PCR-based assay by using mating type specific primers. Mating type of all isolates was determined as *MAT1-1*.

The presence of only one mating type and the absence of female fertile isolates indicate that sexual reproduction is absent in *M. grisea s.l* populations from rice in North of Iran where approximately 75% of rice growing areas are located.
Rice Blast in Colombia: An Interesting Population That Implies Challenges and Opportunities for Breeders and Pathologists

International Center for Tropical Agriculture, CIAT Km 17 Recta Cali-Palmira, Cali, Colombia

Rice blast is the most important disease affecting rice production in Latin America and the Caribbean (LAC). The selection for germplasm resistant to Blast for this region relays on the field evaluation in the blast “hot spot” Santa Rosa located in Colombia. This site is known for having environmental conditions that favors the development of blast infection, which combined to the diversity of germplasm planted there year after year creates the ideal scenario for pathogen diversification. As expected, blast population in Santa Rosa is characterized by its high level of complexity in terms of pathogenicity. However, in the last years it has been noticed that the resistance of certain rice genotypes is compromised when they are planted in sites nearby Santa Rosa. These findings induced the development of Blast population analyses using recent isolates collected in Santa Rosa and other places in Colombia where the disease is endemic. Results obtained so far using 121 isolates confirmed the high level of variation at pathogenicity level using the monogenic lines system hosting 23 different resistance genes. We found that the analyzed population belongs to 59 races, and that this diversity contrasts with the low level of differentiation observed at genetic level using Pot2-based fingerprinting. The percentage of virulence of the races ranged from 0 to 97% and the effectiveness of the tested R genes against the different races evidenced the strength of Pi-9 and Pi-ta2, with incompatible reaction to the 89 and 80% of the races respectively, and other genes with lower but still potential performance like Pi-40, Pi-km, Pi-z5, Pi-zt in which effectiveness was estimated in 78, 60, 64, y 70% of to all tested races, respectively. On the other hand we also found that there are genes which their effectiveness is completely defeated by the races present in Colombia like Pi-a and Pi-b, with only 2% of incompatible reaction against all tested races. Results from this research provided critical information to define potential gene combinations to tailor effective blast resistance on new developed germplasm based on the blast structure found in Colombia. Additionally, having such diversity at CIAT’s host country confirms the relevance of Colombian fields as ideal places to test breeding germplasm for blast resistance not only for Colombia but also for LAC countries where the blast population is predicted to be less complex and limited to few races.

Future research activities include: to have a better estimation of the blast diversity found in LAC countries, establishment of a regional blast nursery, and extend this evaluation to other resistance genes that are not present in the monogenic line system.
Comparative Analysis of Diverse *Magnaporthe* Isolates from Different Hosts in Korea

Goh, J.\(^1\), Park, S.-Y.\(^2\), Lee, Y.-H.\(^2\), Shin, D.B.\(^1\), Lee, B.C.\(^1\), Kang, H.W., and Han, S.S.\(^1\)

\(^1\)Crop Environment Division, National Institute of Crop Science, Rural Developmental Administration, Suwon, Korea; \(^2\)Department of Agricultural biotechnology, College of Agriculture and Life Science, Seoul National University, Seoul, Korea

*Magnaporthe* species are well-known pathogen fungi for Gramineae plants including rice, barley, wheat, and other weeds. In this study, 462 *Magnaporthe* species from rice and weeds are collected all over the country. There are 106 isolates from rice, 253 isolates from crabgrass, 63 isolates from foxtails, 20 isolates from millet, 9 isolates from tall fescue, 5 isolates from maize, 4 isolates from barnyard grass and 2 isolates from dayflower. Among 356 isolates from weeds, 90 isolates showed dual virulence on their original host and rice. We performed cross-infection assay with these isolates to 6 Gramineae hosts - rice, barley, wheat, millet, crab grass and foxtails. Results of cross-infection assay showed that only 4% of *Magnaporthe* isolated from rice are capable of infection to crab grass while 16% of *Magnaporthe* isolates from crabgrass have virulence on rice cultivar Hopyeongbyeo. This fact suggested that rice infection pathogens have specialized to rice cultivar rather than other hosts. Moreover, re-isolation of mixed pathogens from rice and crab grass showed that incompatible pathogens can remain coexisting with other compatible pathogens in fields. DNA fingerprinting using Pot-2 rep PCR and URP PCR showed that some of the isolates showed high similarity according to their original hosts regardless of pathotypes. And, ITS sequencing showed that there are a few exceptions although many *Magnaporthe* species from rice and crab grass showed clear different sequences. These results suggested that some of *Magnaporthe* species could shift hosts in nature, so that contribute to virulence evolution through maintaining diversity of pathogen population.
Comparison of Marker System for Genetic Diversity of *P. oryzae*

Manjunath Hubbelli, Rabindran, R., Velazhahan, R., and Ganapathy, T.

Division of Plant Biosecurity National Institute of Plant Health Management Rajendrnagar, Hyderabad-500030 AND Department of Plant Pathology, TNAU, Coimbatore-641003 Tamil Nadu, India

Rice being the most cultivated cereal in the world and a staple food for the majority of the world’s inhabitants earned a synonym to food. As many as 70 diseases known to hamper the production reflecting yield losses of staggering dimension. Rice blast disease incited by *M. oryzae* having widespread threatens rice production across the globe. The disease has gain special emphasis as it is extremely difficult to manage and yield losses of cent per cent has been recorded under congenial conditions. Thus disease is considered as humanitarian problem. Further, the ability of fungus to overcome resistance within a short time after the release of a resistant cultivar made breeding for resistance as a never ending challenge. Thus analysis of genetic variation in plant pathogen populations is an important pre-requisite for understanding co-evolution in the plant pathosystem (McDonald et al., 1989). In the history, endurous march has been made to understand the diversity of pathogen using a vast number of marker. However, very few studies given impetus on comparison of these marker for their efficacy in assessing the diversity of test pathogen. In this study, an effort was made to compare RAPD and REMAP for their efficacy in assessing diversity of *M. oryzae* population. Twelve RAPD and seven REMAP primers were used to obtain polymorphic profiles. The polymorphism in each case was documented and grouping was done using Jaccards similarity coefficient. The two marker systems were compared for their efficacy in assessing the genetic diversity of *P. oryzae* isolates from different geographical locations. Various parameters viz., observed number of alleles, effective number of alleles, Nei's gene diversity, Shannon's Information index, fraction of polymorphic loci, polymorphic information content (PIC) value and assay efficiency index (AEI) were recorded as criteria to differentiate their efficacy. The result indicated REMAP was superior to RAPD in all the parameters mentioned apart from this grouping of isolates based on REMAP was in accordance with geographical origin of isolates. In addition to this, the overall topology of dendrogram in RAPD was not in agreement with clustering based on principal component analysis (PCA); however, dendrogram based on REMAP perfectly matched with PCA. Thus REMAP marker was concluded to be efficient and specific enough to differentiate *P. oryzae* isolates.
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Diversity Analyses in Mini Core Collection of Rice Blast Fungus Isolates in Laos

Hayashi, N.1*, Inthapanya, P.2, Thiravong, K.2, Xangxayasan, P.2, Kawasaki-Tanaka, A.3, and Fukuta, Y.3
1National Institute of Agrobiological Sciences, Tsukuba 305-8602, Japan; 2Rice and Cash Crop Research Center, Vientiane, Laos; 3Japan International Research Center for Agricultural Sciences, Tsukuba 305-8686, Japan
*nhayash@affrc.go.jp

Genetic variation within rice blast pathogen Pyricularia oryzae in Laos was determined in mini core collection consisting 35 isolates, which was selected from cluster analysis based on infection types to 26 differential LTH monogenic lines, different ecosystem such as irrigated lowland, rainfed lowland, upland, burnt field, and different geographic locations among 200 isolates collected from 2007 through 2009 in whole country. Three and two haplotypes were identified in ribosomal DNA ITS and Histon H3, respectively. In SSR markers, two and seven haplotypes were identified in Mgms01 and Mgms07 loci (Suzuki et al., 2009, 2012). The combined data set of the two gene and the two SSR loci enabled the identification of 12 haplotypes with a high haplotype diversity. Haplogroup H_4 was largest and contained isolates of only MAT1-1 mating type from different geographic locations and different ecosystems except burnt field. Interestingly, H_4 isolates were avirulent to Pik locus LTH monogenic lines except Pik-s line. Haplogroup H_3, H_5, H_7, H_9, H_11 isolates were distributed in upland or burnt field. Genetic diversity was higher in upland and burnt field (H' = 2.197 and 2.322, respectively) than in irrigated and rainfed lowland (H' = 0.918 and 1.669, respectively). This may be relevant to the existence of both mating type fungus in upland and burnt field. PCR products by AvrPia primers were detected in five isolates, which were coincident with avirulent to IRBLa-A having Pia. On the other hand, PCR products by AvrPii primers were detected in 14 isolates, which were coincident with avirulent to IRBLi-F5 having Pii except three isolates.

References:
Rice Blast Control by Release of Resistant Varieties

Zelensky, G.L. and Zelenskaya, O.V.

Kuban State Agrarian University, Krasnodar, Russian Federation

Blast is the most noxious and common in the world among rice diseases [1, 2]. It is caused by imperfect fungus Pyricularia oryzae Cav. Rice is susceptible to blast at all vegetation stages and all above ground plant organs (leaves, stem nodes and panicles) are affected. Practically in all rice growing countries the yield losses according to different estimates reach 3% to 25% during normal years; up to 60% and even 100% during years with blast epiphytoty. The damage caused by blast increases significantly due poor grain quality received from affected plants. Rice yields are damaged by blast practically in all countries.

Over the 80-year period of rice cultivation in the Krasnodar Territory a 10-12 year cycle of blast epiphytoties has been observed. Chemicals are widely used all over the world to control blast. In the Krasnodar Territory over 40% of rice fields are situated close to built-up areas and water reservoirs where the aerial application is forbidden and the nomenclature of allowed pesticides is very limited. The allowed pesticides are to be surface applied and only in an emergency. Permanent application of fungicides may result in mutant, fungicide resistant forms of P. oryzae. Thus introduction of high yielding and immune to pathogen rice varieties should be the main methods of blast control. Therefore the relevance of breeding for blast resistance is constantly increasing. And it is impossible without reliable infectious background and joint research of plant breeders and plant pathologists.

Search for rice varieties and samples resistance to blast disease started in All-Russian Rice Research Institute in the 60-ies of XX century. Assessment of varieties and breeding samples was continued in the years to follow. These tests brought to the conclusion that the majority of varieties grown at that period in Krasnodar Territory and / or being under state trials had weak resistance to blast. In many of them even under natural inoculation up to 85-100% plants were infected by P. oryzae. This can be explained by the fact that practically all varieties were bred from blast susceptible initial material.


References:
E-2

Selection of Antagonistic Microorganisms for Rice Blast Control from Reclaimed Land Soil


1Department of Rice and Winter Cereal Crop, National Institute Crop Science (NICS), Rural Development Administration (RDA), 67, Osan-ro, Iksan, Jeollabukdo, 570-080, Korea; 2National Institute Crop Science (NICS), Rural Development Administration (RDA), Suwon 441-857, Korea

Rice blast disease caused by *Magnaporthe grisea* was serious problems in rice cultivation area. In this study, total of 102 indigenous antagonistic bacteria were isolated from reclaimed land soil in Saemangeum, Korea. Among these, 60 strains were able to inhibited *M. grisea*. The 16s rDNA genes of the selected 16 strains were amplified and sequenced. The strains has strong antagonistic ability against rice fungal pathogens was achieved HS023-11 (*Bacillus subtilis*), HS022-6 (*B. amyloliquefaciens*), RS1914 (*B. vallismortis*), HS027-2 (*Bacillus* sp.). The selected 4 strains tested for investigation of antifungal mechanisms further analyses; four strains of these validated for production of siderophore and chitinase using chrome azurol S blue agar, CMC-congo red agar and DNS method. The four strains were able to utilized insoluble phosphate as determined by vanado-molybdate method. The four strains verified for production of auxin and gibberellic acid using Salkowski test and holdbrook test. The four strains were able to effectively suppress *M. grisea* causing blast diseases on the *in vitro* test.
Development of a Host-Induced Gene Silencing System to Engineer Resistant Transgenic Rice to the Rice Blast Fungus *Magnaporthe oryzae*

Wang M.Y.\(^1\), Liu J.\(^1\), Wang X.L.\(^1\), and Wang G.L.\(^1,2\)

\(^1\)State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, China; \(^2\)Department of Plant Pathology, Ohio State University, Columbus, OH, 43210, USA

Rice blast, caused by the ascomycete fungus *Magnaporthe oryzae*, is the most devastating disease of rice worldwide. Frequent loss of resistance in newly released rice cultivars in the field has prompted us to look for alternative strategies to engineer resistant rice plants. Host induced gene silence (HIGS) has already been described as a potential disease control approach in improving host resistance against fungal pathogens in other crop plants. In this study, we selected 20 genes that play an essential role in appressorium formation, post penetration or other infection processes. Two hairpin RNAi constructs targeting two fragments of each *M. oryzae* gene were generated and transformed into Nipponbare plants. More than 20 independent transgenic lines for each construct were generated. The punch inoculation results showed that enhanced resistance phenotype against *M. oryzae* was observed in the transgenic rice plants that were transformed with the genes important for the post penetration compared to the control plants. These results suggest uptake of RNA molecules by the rice blast fungus from infected plant cells may interfere the function of the target fungal genes in invasive hyphae, which may lead to reduced disease severity on rice plants.
A Eukaryotic Molecular Target Candidate of Roxithromycin: Fungal Differentiation as a Sensitive Drug Target Analysis System

Akira, I., Mayu, K., Megumi, N., and Kamakura, T.
Tokyo Univ. of Science, Japan

Discovery of drug target provides new opportunities for understanding the mechanisms of diseases in humans and for drug development. Novel methods are needed to identify the drug target, since many drugs have targets that are still unclear.

Roxithromycin (RXM), a 14-membered macrolide antibiotic which was originally active against the 50S ribosomal subunit of prokaryote, has beneficial activities to eukaryotes. RXM reported to show anti-tumor and anti-inflammatory activities, but the precise mechanisms of these effects remain uncertain. In this study, we aimed to determine the alternative targets of RXM in eukaryotes and to establish a novel approach to identify new targets for drugs using fungi. Since the developmental stage of the appressorium is sensitive to various chemicals, we utilized cellular differentiation of *Magnaporthe oryzae* as a sensitive indicator of various inhibitory effects caused by chemicals. We screened molecules that might interact with the drugs by the T7 phage display method using the genomic DNA library of *M. oryzae* instead of cDNA library, because the genomic DNA library composes whole genes equally, and the ratio of the possession of CDS in *M. oryzae* can reach nearly 50%.

We identified the MoCdc27 (*M. oryzae* cell division cycle 27 homolog) as a candidate target of RXM by the T7 phage display method. MoCDC27 was highly homologous to human CDC27 that is considered to be a component of an E3 ubiquitin ligase, anaphase promoting complex/cyclosome, which controls cell-cycle events. We generated *mocdc27* knockdown mutants expressing MoCdc27 lower than that of wild type during the differentiation of appressoria. The appressoria formation was less affected by RXM. To investigate whether lower expression of MoCdc27 causes the less sensitivity to RXM, we generated a MoCDC27 complemented strain. A complemented mutant restored sensitivity against RXM to the level of the wild type. These results suggested that MoCDC27 was involved in the inhibition of appressorium formation by RXM, and that the complex of RXM-MoCDC27 affected another molecule involved in appressorium formation. In recent years, APC/C was reported to play an important role in cellular differentiation in addition to the cell-cycle regulation. Hence, CDC27 could be a possible target for anti-cancer and anti-inflammatory effect of RXM in eukaryotes. The T7 phage display method with fungal genomic DNA library can be a useful tool in the quest for drug target.
Screening and Evaluation of Growth Promoting Rhizobacteria and Resistance Inducer against Rice Pathogens in Upland Rice

Pereira Filho, C.R.¹, Araujo, L.G.¹, Cortes, M.V.B.², Silva, G.B.³, Silva-Lobo, V.L., and Filippi, M.C.

¹Universidade Federal de Goias (UFG), Goiania, GO, Brasil; ²Embrapa Arroz e Feijão; ³Universidade Federal Rural da Amazonia (UFRA), Belem, PA, Brasil

Plant growth-promoting rhizobacteria (PGPR), also play a role as inducers of disease resistance against plant pathogens. This study was conducted to select bacterial isolates that promote rice plants growth, suppress rice leaf blast (Magnaporthe oryzae) and other rice pathogens, and to determine the altered enzymatic activity during these two processes.

Initially, 25 bacterial isolates were evaluated for growth promotion and leaf blast severity suppression. Eight isolates, which showed potential for disease control and growth promotion, were selected for quantification of nitrate reductase activity and total soluble sugars.

Based on these results, four bacterial isolates (82R, 20.7, 138.7 and 235) were selected for further investigations on: 1) the most efficient method of application of bacterial isolates to suppress rice leaf blast; 2) their ability to increase activity of key enzymes (peroxidase, lipoxygenase, phenyl-alanin-ammonialase, β-1,3-glucanase) for resistance induction; 3) their ability to increase salicylic acid levels. The treatments consisted of: 1) seed microbiolization; 2) drenching the trial soil at seven days after sowing; 3) seed microbiolization and drenching the trial soil at seven days after sowing.

Of the four isolates tested, 82R applied to the seed by microbiolization was found to be most efficient in suppressing affected leaf area and reducing the AUDPC by 76.8% as compared to the control. However, the same strain did not suppress other diseases of rice sheath blight (Rhizoctonia solani), leaf scald (Monographella albescens) and brown spot (Bipolaris oryzae). The isolate 235, showed 183.33% increase in relation to nitrate reductase activity, in root extracts and 35.71% in leaf extracts. The same isolate promoted root length (9.0%), plant height (13.47%) and total plant height including root length (16.66%), when compared to control. All isolates, including 82R increased the enzymatic activity, but each one of them showed different mode of action, indicating that the method of application was greatly influenced by the origin of the isolate and its elicitors (MAMPs).
Pomecin B™ - A Novel Class of Compound for Controlling Rice Blast Caused by *Magnaporthe grisea*

Arunan Gomathi, V., Hirasker Sanjay, K., Raghavan Shriram, S., and Panchapagesa Murali, M.
Evolva Biotech Pvt Ltd, 401, TICEL BIOPARK, CSIR Road, Taramani, Chennai 600113 INDIA

Rice blast caused by *Magnaporthe grisea* is a devastating disease and is a serious constraint in the rice growing regions throughout the world. A novel class of compound, Pomecin B with a novel mode of action was tested against mycelial growth and spore germination of *M. grisea*. EC$_{50}$ of Pomecin B for spore germination was found to be 1.0 - 2.5 ppm depending on the test medium.

Field evaluation was carried out at disease hot spot for rice blast at Hybrid Research Evaluation Centre, Gudalur, India, where epidemics of rice blast disease occurs annually. The trial was laid out in a randomized block design (RBD) with nine treatments and four replications. A Percent disease index (PDI) of 15.97 was recorded for leaf blast at the start of the experiment in the susceptible rice cultivar, Bharathy. Pomecin B was formulated as microemulsion (ME) and sprayed at 50, 100, 200, 400 and 600 ppm (a.i.). Tricyclazole at 300 and 600 ppm (a.i.) was sprayed as positive control. Observations recorded 10 days after 2nd spray of Pomecin B-ME revealed that the disease intensity was reduced to 38% and 36% at 200 and 400 ppm respectively compared to untreated control. Pomecin B-ME treatment at 200 and 400 ppm showed 2.6 and 2.5 fold higher inhibition than Tricyclazole at 600 ppm on leaf blast. After fourth spray, Pomecin B-ME at 100 ppm demonstrated better inhibition than all other treatments and was 1.3 fold higher than 200 and 400 ppm. Leaf blast inhibition by Pomecin B-ME at 100 ppm was also 2.0 fold higher than the inhibition by Tricyclazole at 600 ppm. Pomecin B-ME treatment at 200 ppm a.i. recorded 23.6% increase in yield over untreated control. Phytotoxicity symptoms were not visualized in the Pomecin B-ME treated plants.

Plants sprayed with Pomecin B-ME exhibited resistance reaction with 1-2mm size blast lesions which were brown in colour. Post third spray of Pomecin B ME at 100 and 200 ppm, rice leaves exhibited 30% higher resistance reaction to leaf blast with reference to control. Immune priming by Pomecin B is under investigation.

References:
Elucidating Slow Blasting Phenomenon in Rice Genotypes

Rabindran, R., Manjunath Hubballi, Robin, S., and Velazhahan, R.

Department of Plant Pathology, Centre for Plant Protection Studies Department of Rice, Centre for Plant Breeding and Genetics Tamil Nadu Agricultural University, Coimbatore-641003

Among the diseases that hamper the production, rice blast incited by Pyricularia oryzae (perfect stage Magnaporthe oryzae) previously called as P. grisea (perfect stage M. grisea) is the most destructive and notorious disease worldwide inflicting yield losses of staggering dimension (Marta et al., 2011). Despite decades of research towards its management, blast has remained and continued to be as a challenge for plant pathologists. Of the management practices adopted utilization of resistance genes is most practical approach. In the study various genotypes having different gene combinations were screened under epidemic conditions and slow blasting phenomenon in these lines were assessed. The slow blasting resistance in twenty five genotypes was evaluated in two seasons namely Kharif 2011 and 2012 at Paddy Breeding Station, Coimbatore under artificially bombarded conditions with spore suspension. In order to assess the slow blasting resistance in genotypes, infection rate (r) and area under disease progress curve (AUDPC) were used as measure of indicators of slow blasting resistance. To ascertain the mechanism of rate of reducing resistance, per cent leaf area covered, lesion number, size of lesion and spores per lesion were recorded at the peak of epidemic in all the entries (Villareal et al., 1981). Further correlation and path analysis of components of slow blasting resistance was studied. The result indicated that In the present study, twenty five genotypes were assessed for the slow blasting resistance through multipoint assessment in two seasons. The per cent disease index at fifteen days interval was taken and apparent rate of infection and AUDPC was calculated. Further, various components of slow blasting namely per cent leaf area covered, lesion number, size of lesion and spores per lesion were documented once at the peak of epidemic. In general, the results of first season agreed well with second season data. From the result it was observed that AUDPC and other components except apparent rate of infection was increasing as the PDI was increased in both seasons. However, the intensity and magnitude of increase in various parameters with PDI was varied significantly. This leads to difficulty in relying on the particular parameter to classify the genotypes as slow blasters. In order to overcome this problem correlation analysis of various parameters under study with PDI was studied. The extent and nature of relationship prevalent between the parameters and PDI was obtained by simple correlation. Results of correlation revealed that except apparent rate of infection all the other parameters under study had significant positive correlation with PDI in both seasons. In addition to this, correlation among the parameters itself was also significant and positive in both seasons. As all the parameters had significant positive correlation the importance of these parameters while selecting slow blasting genotypes could be appreciable. PDI is dependent on several component parameters which are mutually associated. These will in turn impair the true association existing between a component parameters and PDI and a change in any one component is likely to disturb the whole network of cause and effect. Thus each component has two paths of action viz., (1) the direct influence on PDI (2) Indirect effects through components which are not revealed from the correlation studies. In order to obtain the development relations, the cause and effect of relationship between PDI per se, six components was studied in rice through path coefficient analysis. From the path analysis, it was observed that among the parameters, AUDPC, lesion number and lesion size had maximum direct effect on PDI in both seasons which was further supported by maximum correlation of these parameters with PDI in both seasons. Thus these parameters were concluded as the reliable parameters which can be used to classify genotypes as slow blasters. From two season data, genotypes having lesser AUDPC, lesion number and smaller lesion in both seasons were categorized as slow blasters. The genotypes IR64, Tadukan, Rasi, Tetep and Ramanad Str 3 were having lower AUDPC value, lesser number of lesion and also small lesions in both seasons and hence, these were grouped as slow blasters among the twenty five genotypes. Further, various parameters of yield loss assessment was documented in all the genotypes in both seasons. It was clear that the genotypes classified as slow blasters recorded least per cent loss in ear head weight, per cent loss in test weight and had minimum per cent of black seeds compared to other genotypes.
Identification of Novel Qtls for Leaf Blast Resistance in Rice Under Aerobic Condition

Uday, G. and Shailaja Hittalmani*

Marker assisted selection laboratory, Dept. of Genetics and Plant Breeding, UAS, GKV, Bangalore
*Corresponding author e-mail: Shailaja_maslab@rediffmail.com

Rice blast caused by Magnaporthe grisea (anamorph: Pyricularia grisea) limits rice yield in most major rice-growing regions. It affects the crop at all stages of the crop, at seedlings, vegetative stage and maturity causing significant yield losses. Resistance to blast may be conditioned by major genes or by quantitative trait loci (QTLs). QTLs reduce the sporulation of the pathogen within a compatible interaction. Genetic studies indicate that partial resistance is under oligo or polygenic control and resistance provided by QTL is durable and useful to control the disease at field level. An RIL population of 2000 was developed from cross between BPT5204 (Susceptible) and HPR 14 (Moderately resistant) and a subset of population of 281 lines were evaluated for blast disease. The standard protocol for evaluation disease response in blast nursery at hotspot was followed. Both resistance and susceptible genotypes were identified. Genotyping of population was done with 112 Single Sequence Repeat (SSR) markers across 12 chromosomes. Mapping was carried out using composite interval mapping. A total of four QTL were identified on two chromosomes. The QTLs qLB 4-1, qLB 4-2 qLB 12-1 qLB 12-2 at a LOD score 5.9, 2.46, 3.16 and 5.17 showed resistance variation of 7.9, 5.2, 4.7 and 7.3 percent respectively. Of these two QTL were novel and not reported earlier in that chromosome region. This indicates that they are new genes and the possibility of a different race of pathogens existing in the location. These QTLs explained relatively high phenotypic variance for blast resistance and could be dissected for resistance genes using rice genome sequence and deployed for introgression into popular and new rice varieties.
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Screening for the Protein Target(s) of Chloramphenicol by Using Magnaporthe oryzae

Narukawa-Nara, M.1, Nishiwaki, A.1, Inoue, M.1, Takeuchi, T.2, Takakusagi, Y.3, Sugawara, F.1, and Kamakura, T.1

1Tokyo University of Science, Japan, 2University of Chicago, USA, 3National Institute of Health, USA

Appressorium formation of Magnaporthe oryzae is one of the simplest models of cellular differentiation. In general, many proteins are involved in the process of cell differentiation; there are many inhibition sites by small molecules during forming appresoria. Thus, we regarded appressorium formation in M. oryzae as a very sensitive and useful tool to identify the target protein(s) for many chemicals; we used the appresorium assay to search for the novel molecule target(s) of authentic chemicals.

We investigated the effects of many chemicals on appressorium formation, germ tube elongation and conidial germination in M. oryzae. Among the chemicals, chloramphenicol (Cm) showed specific inhibition on appressorium formation. This data suggested that the target protein(s) of Cm work in appresorium formation. Cm is a broad-spectrum antibiotic that is effective against rickettsiae, gram-positive and gram-negative bacteria by inhibiting translation on the 50S ribosomal subunit at the peptidyltransferase step. However Cm is used to treat certain types of serious infections caused by bacteria when other antibiotics cannot be used, because of its toxicity and side effects such as bone marrow depression, aplastic anemia and gray syndrome. The molecular mechanisms of side effects by Cm have remained unclear. Therefore we aimed to identify the target protein(s) of Cm that cause serious side effect(s) by using M. oryzae.

To screen for binding peptide(s) to Cm, we synthesized biotinylated Cm and used T7 phage display method exploiting quartz crystal microbalance system. The phage display library was constructed from the genomic DNA of M. oryzae. As a result, we obtained several peptides that were the portions of candidate target proteins for Cm. Overexpression of the one candidate protein, which has 87% homology in its amino acid sequence with peptide 1-3, led to tolerance for Cm on appressorium formation in M. oryzae. Further analyses are in progress.
Enhancing Blast Resistance of Rice Cultivars by Marker Assisted Multiple Trait Pyramiding suitable for Drought and Aerobic Conditions

Shailaja Hittalmani, Chandrashekar B. Haradari,Venkatesh Gandhi R., and Shilpa Reddy B.

Marker Assisted Selection Laboratory, Department of Genetics and Plant Breeding University of Agricultural Sciences, GKVK, Bengaluru, India

Author for correspondence E-mail: shailajah_maslab@rediffmail.com

With the global water shortage gaining paramount importance, breeding for drought tolerant rice cultivars become a priority. Rice cannot be cultivated in the present form of irrigation in future. Hence, growing rice under aerobic cultivation, saves water to an extent of 60 per cent. However, the shift in cultivation from irrigated to aerobic situation has also observed change in dynamics of pathogens. Blast is one such disease that affects rice. Normally drought tolerant rice varieties developed do not have insulation from blast. In this study rice genotypes pyramided with root morphological traits, Water Use Efficiency (WUE), osmotic adjustment genes, seedling vigour trait and bacterial leaf blight resistance genes were further crossed with rice blast resistant gene pyramids with Pi-1 and Pi-2 genes in IR 64 background. The F2 pyramids were evaluated in blast hotspots and scored. Pyramids with Pi-1 and Pi-2 with roots traits QTL performed superior and showed resistant reactions. The combination with 5-7 genes / QTL had epistatic effect between biotic and abiotic genes. However, the pyramids with 2-4 QTL for abiotic traits, one Xa-4 and Pi-2 genes had additive effect for blast disease resistance and grain yield under aerobic condition. These superior performing genotypes for blast as well as drought will be progressed for developing new varieties for cultivation under less water scarce situations.
Identification of Resistance Sources for Blast Disease in Indian Traditional Rice Varieties Using SSR Markers

Nandini, B.1, Gangappa, E.1, Rajanna, M.P.2, Mahadevu, P.3, Prasanna Kumar, N.K.2, Devaraja.4, Ramesh, S.1, Mahesh, H.B.1, and Shailaja Hittalmani1

1Department of Genetics and Plant Breeding, UAS, GKVK, Bangalore-560 065, India; 2Zonal Agricultural Research Station, V.C. Farm, Mandya, India; 3Department of Genetics and Plant Breeding, Collage of Agriculture, Hassan, India; 4Agricultural Research Station, Ponnampet, Karnataka, India
*Email:nandu.gpb@gmail.com

Traditional rice varieties (TRV’s) are highly valued genetic resources because of their wide adaptability and reservoirs of several desirable untapped genes including those controlling biotic and abiotic stresses resistance. Blast disease is one of the major production constraints in rice causing grain yield losses up to 30%. Under this premise, a study was undertaken during 2012 to screen 324 TRV’s involving 62 photoperiod sensitive and 262 photoperiod insensitive types for reaction to blast disease under natural infection condition at Agricultural Research Station (ARS), Ponnampet, Karnataka, India which is a hot-spot for rice blast disease expression. The TRV’s were also screened under artificial disease infection pressure at field level at Zonal Agricultural Research Station (ZARS), Mandya, Karnataka, India. The inoculum was prepared using the extract of blast disease infected leaves and sprayed to 25 days old seedlings. The response of TRV’s and disease reaction were scored on 45 DAS. The disease severity was scored on a 0 to 9 IRRI scale. A wide variability for responses of TRVs to both natural and artificial blast disease infection was documented. A total of 18 TRV’s elicited moderate resistance reaction with a score of 3 under artificial infection condition at ZARS, Mandya. Two TRV’s viz., Bangara sanna and Neer IET were found resistant under natural infection condition at Agricultural Research Station (ARS), Ponnampet. Four TRVs such as ‘Bilidoddi budda’, ‘Kotayam’, ‘Mysore sanna’ and ‘Coimbattur’ were found moderately resistant to blast disease under both natural and artificial blast disease infection conditions. Twenty-two reported linked SSR markers of mapped genes identified blast disease resistant TRVs. The results revealed the presence of seven resistant genes- ‘Pish’ and ‘Pi37’ located on chromosome #1, ‘Pib’ on chromosome #2, ‘Pi 38’ and ‘Pik’ on chromosome #11 and ‘Pi 20(t)’ on chromosome #12 in the blast disease resistant TRVs. The identified resistant TRVs serve as donors for breeding blast resistant rice varieties.
The Effect of Physical Environmental Factors and Airborne Concentration of Pyricularia oryzae on the Development of In-field Panicle Blast Disease

Allicia, J.¹, Shahida, H.¹, Siti Norsuh, M.¹, Habibuddin, H.¹, Azlina, M.², and Mohd. Fadil, Y.³

¹Rice and Industrial Crops Research Centre, MARDI Seberang Perai, P. O. Box, 13200 Kepala Batas, Penang, Malaysia; ²Biotechnology Research Centre, MARDI HQ, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia; ³Promotion and Technology Development Centre, MARDI Seberang Perai, P. O. Box, 13200 Kepala Batas, Penang, Malaysia
E-mail: allicia@mardi.gov.my

Rice blast caused by Pyricularia oryzae Cav. is the most destructive rice disease in Malaysia as well as other rice growing regions in the world. In Malaysia alone, the estimated yield loss caused by the disease is about 90 000 t/season which is valued at about RM 72 million. Although P. oryzae may infect at any parts of the aboveground parts of the plant, but infection on the panicles are the most damaging. Currently, the defence strategies against the disease is still mainly based on varietal resistance with the support of chemical control. However, the issue is when is the appropriate time and situation that require chemical treatment intervention to ensure its effectiveness in controlling the disease. Usually disease incidence and severity need to be above a certain level (the threshold level) to cause significant yield loss. Therefore investigation involving predicting when the disease is likely to exceed this threshold level need to be conducted.

A study was carried out at MARDI Seberang Perai research station during main season 2012/2013. Quantitative air sampling was carried out using Burkard automatic volumetric spore trap. The spore trap was placed at the centre of the study site. Airborne spores were sampled daily before rice heading phases up to ripening stage to determine the airborne concentration of P. oryzae. Mobile blast nursery which consist of eight differential varieties namely Mahsuri, Setanjung (MR1), Bahagia, Engkatek, Seribu Gantang, Tadukan, Pankhari 203 and Pongsu Seribu were sowed in trays and placed along the sides of the study plot. These differential varieties produced different degrees of blast disease reactions and have been used to differentiate pathotypic variability of local blast isolates to monitor blast pathogen population structure in the fields and to predict the existence or emergence of new blast races. The mobile blast nurseries were replaced every week. Climate data was collected from the meteorological department facilities and by using mobile temperature and humidity recorder.

The MR 219 seeds were sowed on 2nd November 2012 and leaf blast symptoms were visible on 28th November 2012. The infection were predicted to be happen on 24th November as the temperature on the particular day was the lowest. The maximum temperature recorded was 31.0 °C, minimum temperature: 23.0 °C and the relative humidity was more than 95% during the night. There were also rain for two consecutive night before that. The pattern of P. oryzae spore dispersal indicated that lesions on leaves released conidia soon after dark and reached maximum in a few hours and slowly decreased and ceasing at dawn. After 18 weeks of observation, P. oryzae pathotypes recovered out of 114 trays of mobile nurseries were P₇,₀ (79.8%), P₁₅,₀ (12.3%) and P₃,₀ (7.0%). These results conclude that P₇,₀ was the most prevalent pathotype in the area.
Molecular Cloning of the Partial Resistance Gene Pi34 and Early Response of Partial Resistant Rice Cultivar Chugoku IL1 to Infection of Magnaporthe oryzae

Kito, H. and Zanbayashi-Sawata, K.

NARO Tohoku Agricultural Research Center, Shimofurumichi3, Yotsuya, Daisen, Akita, Japan

Durable resistance to rice blast disease is needed for the sustainable usage of genetic resources. Partial resistance is considered as to be durable because breakdown of partial resistance genes have never been reported. In this study, we cloned and characterized the partial resistance gene Pi34 to utilize for breeding. As results of the QTL analysis and the fine mapping (Zenbayashi-Sawata et al. 2007), Pi34 was mapped on the chromosome 11 and its region was covered by two BAC clones Ch21121 and Ch46F14. To specify candidate genes, we screened for genes expressing in a Pi34-carrying rice cultivar during infection of M. oryzae. SuperSAGE method (Matsumura et al. 2002) was carried out using mRNA collected from infected Chugoku IL1, which is a Koshihikari near isogenic line carrying Pi34 (Maeda et al. 2012), and susceptible control cultivar Koshihikari. Four differentially expressed tags were aligned to two BAC sequences and OMG02 was selected as the principal candidate of Pi34. Chugoku IL1 transformant suppressing OMG02 showed decreased partial resistance to M. oryzae. Comparison of nucleotide sequences of OMG02 between Chugoku IL1 and Nipponbare alleles showed low and high similarity in 5' and 3' region, respectively. We conducted phenotypical analyses by fungal inoculation to Chugoku IL1. The number of early lesions on Chugoku IL1 was less than on Koshihikari. On the other hand, the frequency of fungal penetration into rice showed no significant difference between them. Therefore, we concluded the function of Pi34 is inhibition of fungal growth after penetration. Reactive oxygen substrates (ROS) accumulation of Chugoku IL1 was also compared to Koshihikari and a NIL carrying the complete resistance gene Pib (Pib-NIL) using 3,3'-Diaminobenzidine staining. In 24 hours post inoculation, Chugoku IL1 showed similarity to Pib-NIL. By contrast, in 48hpi, Chugoku IL1 showed similarity to Koshihikari. We supposed that low level or short period expression of resistance responses to arrest fungal infection resulted in the partial resistant phenotype of Pi34.

References:
Identification and Fine Mapping of Two Blast Resistance Genes in Rice Cultivar 93-11


Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China; Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, China

The blast pathogen, *Magnaporthe oryzae* (*M. oryzae*), has a high pathogenic variability, which often leads to the rapid breakdown of the resistant cultivars. One major strategy to develop effective resistance is to pyramid multiple *R* genes each recognizing overlapping isolates/races of *M. oryzae* into a single cultivar. Thus, continued identification of new *R* genes in broad-spectrum resistance germplasms is essential for pyramiding *R* genes.

The sequenced *indica* cultivar (cv.) 93-11 is a widely grown blast resistant cultivar and hybrid rice restorer in China. It is resistant to 86.5% of 215 *M. oryzae* isolates from northern China, and can be used as broad-spectrum resistance resource in *japonica* rice breeding programs. We identified and mapped two blast *R* genes, *Pi60(t)* and *Pi61(t)*, from cv. 93-11 using the F2 and F3 populations derived from a cross between susceptible cv. Lijiangxintuanheigu (LTH) and resistant cv. 93-11 by inoculating *M. oryzae* isolates from different geographic origins. *Pi60(t)* was delimited to a 274 kb region on the short arm of chromosome 11, flanked by Indel markers K1-4 and E12 and co-segregating with Indel markers B1 and Y10. *Pi61(t)* was delimited to a 200 kb region on the short arm (near the centromere) of chromosome 12, flanked by Indel markers M2 and S29 and co-segregating with Indel marker M9.

In the 274 kb region of *Pi60(t)*, 93-11 contains six NBS-LRR genes including the two *Pia/PiCO39* alleles (*BGIOSGA034263* and *BGIOSGA035032*) which are quite close to the two *Pia/PiCO39* alleles (*SasRGA4* and *SasRGA5*) in Sasanishiki and CO39, with only nine amino acid difference existing in the protein sequences of *BGIOSGA035032* and *SasRGA5*. In the 200 kb region of *Pi61(t)*, 93-11 contains four NBS-LRR genes which all show high-level identities with their respective corresponding NBS-LRR alleles of susceptible cv. Nipponbare in protein sequences. Comparison of resistance spectra and physical positions between the target genes and other *R* genes at the same chromosome regions indicated that *Pi60(t)* could be *Pia/PiCO39* or an allele, whereas *Pi61(t)* could be different from *Pita*, *Pita-2*, *Pi19(t)*, *Pi39(t)* and *Pi42(t)* in the same *R* gene cluster. The tightly linked flanking/ co-segregating markers for *Pi60(t)* and *Pi61(t)* should ensure rapid and accurate transfer of the two *R* genes from 93-11 into new breeding lines. The delineation of physical positions and the short-listed candidate genes of the two blast *R* loci have set solid foundations for positional cloning of *Pi60(t)* and *Pi61(t)*.
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Molecular Characterization of Rice Landraces for Blast Resistance

Mahesh, H.B.¹, Malali Gowda², Mahadevu, P.³, and Shailaja Hittalmani¹

¹Department of Genetics and Plant Breeding, University of Agricultural Sciences, GKV, Bangalore-560065, India; ²Genomics Laboratory, Centre for Cellular and Molecular Platforms, National Centre for Biological Sciences, GKV campus, Bangalore- 560065, India; ³University of Agricultural Sciences, Hassan-573225, India.
Correspondence: Shailaja Hittalmani: shailajahittalmani@gmail.com

Rice is a major food crop in India, contributes 22% to world rice production. Though, many high yielding varieties are available, the yield potential of these varieties is significantly threatened by various biotic and abiotic stresses. Among biotic stresses, rice blast disease, caused by Magnaporthe oryzae, has significant impact on yield loss. Rapid breakdown of blast resistance is the major cause yield instability in rice growing regions. Our main objective in this study is to characterize wild alleles exist in the native rice germplasm. We have collected over 250 rice landraces from Southern States of India. These landraces have been characterized for various phenotypic traits including blast disease. Genetic diversity of these landraces has been characterized using SSR markers. Further these landraces will be characterized by GBS/RAD-seq method using next generation sequencing technology. Genes/markers identified from this work can be used in blast resistance breeding programme.
Identification of a Novel Blast Disease Related Gene in *Oryza sativa*

Wang, X.¹, Lu, W.¹, Pan, L.¹, Jia, M.², and Jia, Y.²

¹China Jiliang University, Hangzhou, China; ²Dale Bumpers National Rice Research Center, Stuttgart, AR USA

Blast disease caused by *Magnaporthe oryzae* is one of the most serious diseases in rice fields worldwide. The resistance gene (*R*) in rice can recognize the corresponding *Avr* gene in *Magnaporthe oryzae* by direct or indirect interaction to active the expression of defense genes. Recently, a novel gene *Pi-katy*, related with blast disease resistance in rice, was fine mapped and identified. *Pi-katy* locates in a 8kb interval on the chromosome 12 by the positional cloning using a F2 population crossed between the resistance accession Katy and the susceptible accession L172. The candidate gene encodes a kinase-like protein, which contains 3 protein kinase C phosphorylation sites, 2 casein kinase II phosphorylation sites, 2 amidation sites and 1 N-myristoylation site. To verify the function of *Pi-katy*, a 5.5 kb fragment including the full length ORF (Open Reading Frame) of *Pi-katy* plus 2.9kb upstream and 2.1 kb downstream sequence was cloned into the pCAMBIA 1301 expression vector and transformed into the calli of L172 to perform the genetic complementation test. The T0 lines expressed increased disease resistance to the blast isolate C30A, which illustrates *Pi-katy* involves in rice resistance to the blast disease. The subcellular location of Pi-katy was determined using transient expression of *Pi-katy::YFP* in rice protoplast. The *Pi-katy* was predicted to locate in nucleus with a NLS at N terminal. We confirmed that *Pi-katy* was located in nucleus using DAPI staining. The expression level of *Pi-katy* in the rice leaves didn’t show significantly difference at 0, 24, 48, 72, 96 hpi, which suggests that *Pi-katy* may constitutively expressed. These results provide insight into the mechanisms of rice blast resistance.

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Identification and Mapping of a Novel Blast Resistance Gene *Pi57(t)* in *Oryza longistaminata*


1Food Crops Research Institute, Yunnan Academy of Agricultural Sciences (YAAS), 2Agricultural Environment & Resources Research Institute, YAAS, 650205, Kunming, Yunnan, China; 3CIRAD, UMR BGPI, 34398 Montpellier, France

Xu, P., Dong, L.Y. contributed equally to this work.

Correspondences: Yang, Q.Z., qzhyang@163.com; Tao, D.Y., taody12@yahoo.com.cn

*Oryza longistaminata* with strong resistance to biotic and abiotic stress was regarded as an excellent gene pool of wild rice, mining and utilization of favorable genes of *O. longistaminata* would be important in breeding of *O. sativa* by broadening its genetic basis. To explore blast resistant genes from *O. longistaminata*, RD23, an indica cultivar from Thailand, was crossed with an accession of *O. longistaminata* through embryo rescue, and a set of BC$_3$F$_7$ introgression lines (ILs) had been constructed. The ILs were tested for blast resistance in natural blast nursery, and three desirable ILs that exhibited a high level of resistance to rice blast was obtained. Using a BC$_4$F$_2$ population from a cross of IL-E1454/RD23, a novel dominant blast resistant gene, designated as *Pi57(t)*, was mapped on chromosome 12 of rice flanked by SSR markers RM27892 and RM28093. It was found that *Pi57(t)* conferred a broad-spectrum resistance to *Magnaporthe oryzae* isolates collected from Yunnan, and the resistant spectrum distinguished from 5 known blast R genes located on chromosome 12. Our results suggest that the *Pi57(t)* would be a promising gene for breeding blast-resistant rice in Yunnan, China.
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Cloning and Application of Rice Blast Resistance Gene Pi54 and its Alleles: A Success Story

Sharma, T.R.

National Research Centre on Plant Biotechnology IARI, Pusa Campus, New Delhi-110012

Rice is considered as a model crop for genetic and molecular biology studies largely because of its small genome size among cereals. Though, many high yielding varieties of rice are available, the yield potential of these varieties is considerably affected by various biotic and abiotic stresses. Among the various factors like bacterial leaf blight, sheath blight and stem borer limiting rice productivity, rice blast caused by *Magnaporthe oryzae* (*Pyricularia oryzae*) is a serious constraint in rice production at global level. Because of highly variable nature of the pathogen, management of rice blast using disease resistant cultivars has become a difficult task. Hence, most of the blast resistant varieties of rice succumb to the disease within 2-3 years of their introduction in the disease prone areas. To expedite the process of developing durable blast resistant cultivars, marker-assisted gene pyramiding and back cross breeding are considered very attractive approaches. Therefore, high resolution mapping of blast resistant genes with robust molecular markers like STMS and SSR is highly desirable. We extensively used computational methods for positional cloning and characterization of rice blast resistance gene *Pi54*. The predicted *Pi54* protein contains a nucleotide binding site -leucine rich repeat (NBS-LRR) domain in addition to a small zinc finger domain. Functional complementation of the gene has confirmed its stable and high-level of resistance against geographically diverse strains of *Magnaporthe oryzae*. We further showed by microarray analysis that, the single *Pi54* gene regulates a complex defense response mechanisms against *M. oryzae* in rice. We computationally analyzed proteins of a total of 70 resistance (R) genes sequences of various crops and concluded that *Pi54*, a rice blast resistance (R) protein have a small zinc finger domain of NFX type which is distinct amongst all other cloned resistance genes. The alleles of blast resistance genes were also mined in different wild species and land races of rice. The rice blast resistance gene *Pi54* was linked with specific DNA markers and transferred in mega varieties of rice using marker assisted selection. These rice varieties are at various stages of release in different parts of India.
Development of DNA Markers for Selecting Green Rice Leafhopper (GRH)-Resistant Variety


Department of Functional Crop, National Institute of Crop Science, RDA, Miryang, 627-803, Korea

The green rice leafhopper (GRH), *Nephotettix cincticeps* Uhler, is one of the most serious insect pests affecting cultivated rice (*Oryza sativa* L.) in temperate regions of East Asia. It also causes damage to rice by transmitting viral diseases such as rice dwarf virus (RDV) disease. GRH sucks sap from both the xylem and phloem of susceptible rice varieties, leading to yield loss. Development of GRH resistant rice varieties for reducing yield loss is an important objective in current breeding programs. Molecular markers are useful for selecting GRH-resistant varieties in a point of view reducing time and effort of bio-examination. In this study, we finely mapped *Grh1* locus using BC$_2$F$_2$ population derived from cross lines between susceptible Ilpum and resistant Singwang varieties. And we also developed SSR (RM18166, RM516 and RM18171) and Indel (Indel 15040) markers which could select *Grh1*-resistant varieties. The three SSR and one indel markers were co-segregating with the *Grh1* locus. The detailed genetic and physical maps of the *Grh1* locus will facilitate for marker assisted selection for the improvement of resistance to GRH in rice breeding program.
Rice blast is one of the serious disease affecting the quantity and quality of rice production throughout the entire growing season. There are several methods to protect the rice blast as cultural control, chemical control and using resistant cultivar, well-known as the most effective method. Though a lot of resistant rice cultivars were provided, breakdown of resistance were reported in 2 to 5 years after cultivation in real fields. To asses rice blast resistance in fields, there are several methods such as true resistance test, nursery test, field test. However, these methods are hard to examine durable resistance. Sequential planting method is the effective novel method to solve this problem in a short period of time. In this study, we examined durable resistance of novel resistant rice cultivars and analyzed results from sequential planting method and field test. According to sequential method results from 2008 to 2012, 14 rice cultivars were selected for field test and nursery test as durable resistant cultivars. Similar to sequential planting results, they showed low disease ratio in nursery test (resistance index 1.36~4.29) and field test (DLA: leaf 0~0.04%, panicle 0~4.83%). Especially, some rice cultivars such as Keumobyeo, Suwon545, Suwon535, Cheolwon81, Sangju44 and Suwon 534 showed high durable resistance in both sequential planting and nursery test. And, field test result in these cultivars shows no disease in leaves and panicles. These facts indicated that durable resistance determined by sequential planting is significantly valid in nursery and fields test.
A New Rice Variety ‘Hwaweon 5’ with Durable Resistance to Rice Blast

Kang, J.W.¹, Kim, D.M.¹, Ju, H.G.², Han, S.S.³, and Ahn, S.N.¹*

¹Department of Agronomy, Chungnam National University, Daejeon 305-333, Korea; ²Agricultural Department, Agricultural College of Yanbian University, Longjing, Jilin 133400, China; ³National Institute of Crop Science, RDA, Suwon 441-857, Korea

‘Hwaweon 5’ was developed from a cross between the African upland cultivar, ‘Moroberekan’ and ‘Ilpumbyeo’ based on marker-aided backcross selection. The recurrent parent ‘Ilpumbyeo’ is a high grain quality cultivar with medium to late maturity. ‘Hwaweon 5’ is nearly isogenic to ‘Ilpumbyeo’ except Moroberekan introgressed segments on chromosomes 1, 4 and 6. The Moroberekan segment on Chromosome 4 has the resistance gene for blast. The preliminary and replicated yield trial was conducted at Chungnam National University in 2006 and 2007. The local adaptability test was carried out by the National Seed Management Office (NSMO) from 2008 to 2009. This cultivar was registered to NSMO designated as ‘Hwaweon 5’. This cultivar averaged 78 cm in culm length and has medium growth duration. Milled rice of ‘Hwaweon 5’ is translucent and the grain quality traits are comparable to those of the recurrent parent. It has low protein content. The yield potential of ‘Hwaweon 5’ in grain was about 6.53 MT/ha at the ordinary fertilizer level for two years. This variety showed highly resistance reaction at the blast nursery test at four locations and also at the sequential planting method. This resistance is due to the gene designated as Pi45(t) on chromosome 4 from Moroberekan. The Pi45(t) gene would be useful in enhancing resistance to blast in rice breeding program.
Mapping and Race Specific Reaction of the Resistance Gene Pi45(t) in Rice

Kim, D.M.¹, Ju, H.G.², Han, S.S.³, Roh, J.H.³, and Ahn, S.N.¹*

¹Department of Agronomy, Chungnam National University, Daejeon 305-333, Korea; ²Agricultural Department, Agricultural College of Yanbian University, Longjing, Jilin 133400, China; ³National Institute of Crop Science, RDA, Suwon 441-857, Korea

QTL analysis for blast resistance was carried out using 140 BC3F3 lines derived from a cross between Ilpum as a recurrent parent and Moroberekan as a donor parent. 140 BC3F3 lines with the parents were inoculated with nine blast isolates. To identify QTLs for resistance to nine blast isolates, 134 SSR markers showing polymorphisms between the parents were genotyped for the 140 BC3F3 lines. A total of 17 resistance QTLs to nine isolates were detected on chromosomes 2, 3, 4, 6, 7, 9 and 10. The phenotypic variance explained by each QTL ranged from 8.2% to 26.4%. The Moroberekan alleles contributed the positive effect at these 17 QTL loci. In a previous study, the QTL, Pi45(t) for durable resistance to blast was identified using a sequential planting method. To know the relationship between Pi45(t) and the isolate-specific resistance gene, an F2 population was developed from a cross between Ilpum and an introgression line harboring Pi45(t). F3 lines segregating for the Pi45(t) were inoculated to three isolates. F3 lines from the F2 plants with the Moroberekan segment at the target region showed resistance to two isolates. This result seems to indicate that the Pi45(t) and the isolate-specific resistance gene are tightly linked or the resistance is controlled by the same gene(s). The markers linked to genes controlling blast resistance would be useful in developing blast resistance lines in the breeding program.
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Distribution of Blast Resistant Genes in Korean Local Variety Germplasm of Rice (Oryza sativa L.)


National Agrobiodiversity Center, NAAS, RDA, Suwon 441-707, Republic of Korea

Rice blast (Magnaporthe oryza B.) is one of the worst diseases in rice. Several genes of blast resistance were found that are effectively used to control rice blast disease in rice breeding and genetic studies. However, most of the resistance genes break down in a few years because of their race specificity and the rapid change in pathogenicity of the blast fungus. This study was conducted to get the blast resistance degree of Korean local variety and the information about the distribution of blast resistant genes using 10 major genes of blast resistance.

Blast resistant rice accessions were selected from more than 1,000 being field nursery screening. Pita/Pita-2, Piz-t, Pik, Pi-39 genes are abundantly located in selected high resistant accessions, while Piz and Pik-m genes were found in high susceptible accessions. We carried out the effect value of tested dominant genes to rice blast resistance by comparing with gene distribution in highly susceptible accession. Knowing the relatedness of these accessions will enable rice breeders to incorporate novel blast resistance from more diverse backgrounds into the new rice varieties being developed. This study will help to develop effective strategies for managing rice blast disease in japonica type rice germplasm.
Fine Mapping of a New Blast Resistance Gene $\text{Pi58(t)}$ in $\text{Oryza sativa}$ subsp. $\text{japonica}$ Cultivar, Yunxi 2


Agricultural Environment & Resources Research Institute, Yunnan Academy of Agricultural Sciences. Kunming, Yunnan province, P.R. China (650205)
Corresponding author: Qinzhong Yang, qzhyang@163.com

A new blast resistance gene was identified in $\text{Oryza sativa}$ subsp. $\text{japonica}$ cultivar Yunxi-2 of Yunnan. Using F$_2$ population from susceptible cultivar Lijiangxingtuanheigu and resistant cultivar Yunxi 2, this gene was mapped on the long arm of chromosome 1 flanked by 2 SSR markers RM2527 and RM11757. This gene was tentatively designated as $\text{Pi58(t)}$. Using a large F$_2$ mapping population, $\text{Pi58(t)}$ gene was finally mapped in an interval of ca. 108 kb, flanked by 2 markers, STS42-8 and STS42-4, and cosegregated with STS marker STS42-7. Based on the genomic sequences of Nipponbare flanked by STS42-8 and STS42-4, four genes of NBS-LRR class encoded by most plant disease-resistant genes were identified in this region. These four genes were considered as the candidate genes of $\text{Pi58(t)}$, named as $\text{Pi58(t)-1}$, $\text{Pi58(t)-2}$, $\text{Pi58(t)-3}$ and $\text{Pi58(t)-4}$, respectively. Comparison of allelic sequences of candidate genes among susceptible cultivars and resistant cultivar Yunxi 2 indicated that candidate genes $\text{Pi58(t)-1}$ and $\text{Pi58(t)-2}$ from Yunxi-2 are identical to those from susceptible cultivars; $\text{Pi58(t)-3}$ is absent in Yunxi 2; $\text{Pi58(t)-4}$ gene from Yunxi-2 has 6 amino acids different to that from susceptible cultivars, it suggests that $\text{Pi58(t)-4}$ would be considered as the candidate gene of new blast resistance gene $\text{Pi58(t)}$. This result provided an important information for further cloning and function analysis of $\text{Pi58(t)}$ gene.
Fifty one rice cultivars bred for special purpose were classified into 5 groups such as super yield rice (SYR), aromatic rice (AR), colored rice (CR), glutinous rice (GR), and other rice (OR) and characterized their blast resistance. Leaf blast reaction of 5 groups in the blast nursery, rice group CR and OR were showed commonly 6.2 high susceptible reactions whereas SYR group showed 3.2 resistant reaction. Leaf blast in the field test, CR group was showed 1.29% of diseased leaf area but AR and OR groups were commonly showed 0.09% of diseased leaf area. And CR and OR group were showed 9.1% and 8.1% panicle blast infection but AR group was only showed 2.8% of panicle blast infection. In the inoculation test with blast isolates, there are quite differences between rice groups. CR group was showed 60.4% of compatible isolates whereas OR group have 12.0% of compatible isolates. Generally CR group showed more susceptible reaction to rice blast than the other rice group. But most of special purpose rice cultivars showed susceptible reaction against rice blast so need to develop blast resistant rice cultivars for stable production of special purpose rice.
Trade Competitiveness of Rice in post-WTO Period in Andhra Pradesh

National Academy of Agricultural Research and management (NAARM/Hyderabad) and International Crops Research Institute for Semi-Arid Tropics (ICRISAT)

In India paddy is a major food crop, its competitiveness in indo-gangatic plains are reducing due to environmental and sustainability issues (Chand, 1999). Indian government is looking for expansion of area under paddy in southern states like Andhra Pradesh. In Andhra Pradesh, paddy is the major staple crop. There are significant changes in the macro policy environment and its implications for crop competitiveness since early 1990s after introduction of WTO. This paper examines the trends in production and competitiveness of paddy which is a major crop in Andhra Pradesh in pre and post-WTO period and its implications in producer and consumer surplus and social cost benefits at state level by using Policy Analysis Matrix (Yao S. 1997). The data on area, production and cost of cultivation were collected from comprehensive cost of cultivation scheme, government of India. Paddy registered impressive growth in production in post-WTO period. Trade competitiveness of paddy increased in post-WTO period as shown by DRC and NPC levels. The EPC shows that paddy production was fairly protected by the government even after post-WTO period. Overall, liberalization has positive impact on the welfare of the state. The distortion in domestic prices would result in a change in revenue to producers and consumers. Due to free trade of paddy, welfare gains were much larger than the respective welfare losses to the economy. The policy prescriptions from the study are (i) Reduced cost of production by education the farmers on improved crop management practices in paddy farming systems, (ii) Ensuring supply of quality inputs, replacement of low potential/ pest susceptible old varieties by new high yielding varieties with high yield potential. (iii) Encourage hybrid rice cultivation in suitable areas by conducting demonstrations and making seed available to the farmers, (iv) Providing Farm implements and farm machinery for improving efficiency in farm operations and cost of cultivation and lastly (v) Keep exports and Imports free. Only use tariffs as an adjusting instrument.

References:
F-1

Korea National Microorganisms Research Resource Center

Yeom, S.J.* and Lee, S.S.
Kyonggi University, Research Center #201 San 94-6 Iui, Yeongtong, Suwon, Gyeonggi, 443-760
Korea

The Korea National Microbiological Research Resource Center is the core center of the twelve microorganism banks designated by the Ministry of Education, Science and Technology. The KNMRRC supports microorganism banks with necessary guidelines, standards, training for efficient operation of the banks. It also provides with an effective forum to solve common issues of the related banks.

The ultimate goal of the KNMRRC is the followings: 1 construction of standardized and integrated management system, 2 construction of Core center and other organs network, 3 Quality Control (QC) of microbial resources in the member banks, 4 conservation of Resources in the member banks and the interrupted banks, 5 education for professionals in the member banks, 6 public Relations for raising people's awareness of the importance of microbiological resources.
Development of Genetic Resources for Blast Resistance of Hybrid Rice Breeding Programme in Egypt.

El-Mowafi, H.F., Abdallah, R.M., Reda, A.M., and El-Ekhtiar, A.

Rice Research & Training Center, Sakha, Kaf Elsheikh, Egypt

Different groups of breeding materials were evaluated for leaf blast infection at seedling stage under blast nursery conditions. Three groups i.e. Promising lines, observational materials and hybrid rice yield trials were tested.

For large scale adaption of hybrid rice technology the released hybrids should possess a fair degree of resistance to some of the major diseases/pests in the target areas, in addition to the distinct yield advantage over the existing varieties. Keeping this in view, the promising hybrids are regularly being evaluated for resistance to blast disease in glass house as well as under field conditions. Promising hybrids and some parental lines with resistance to blast have been identified.

The most important rice diseases in Egypt are blast, brown spot, false smut, as fungal diseases and white tip nematode as nematode disease. However, blast disease is considered a major constraint for maximizing yield production.
Fungi are eukaryotic organisms, growing in a wide range of habitats. Fungi are significantly important in a variety of ways. They play an essential role in the decomposition of organic matters. They have been used as a source of food, and agents for fermentation of food products and for the production of various antibiotics and enzymes that are used in a field of research, industry, medicine, etc. In contrary, impact of many fungi on animals and plants is economically and socially detrimental. For example, *Magnaporthe oryzae* causes the most destructive disease, “rice blast”. Annual yield loss of rice by rice blast is equivalent to rice that could feed about 60 million people. The Center for Fungal Genetic Resources (CFGR) was established to collect, maintain and distribute genetic resources mainly from plant pathogenic fungi, which are important for both educational and research purposes. This will contribute to development of new strategies for management of crop diseases and of new components for improvement of our lives. CFGR possesses important fungal species; a total of 42,000 isolates from 54 species of fungi including 20,902 T-DNA transformants of the rice blast fungus. In addition to the biological materials, CFGR has developed user-friendly databases to maintain genetic information of fungal stocks and help to solve questions about fungal pathogenicity, population genetics, development, and evolution. Also, CFGR seeks strategies for sustainable and scientific plant quarantine to better protect our ecosystem from invasive microorganisms.
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Address</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amarender Reddy</td>
<td>ICRISAT/CGIAR, ICRISAT, India</td>
<td><a href="mailto:anugu.amarender.reddy@gmail.com">anugu.amarender.reddy@gmail.com</a></td>
<td></td>
</tr>
<tr>
<td>Akira Ishii</td>
<td>Tokyo university of science, 2641, Yamazaki, Noda-shi, Tiba, Japan</td>
<td><a href="mailto:j6412701@ed.noda.tus.ac.jp">j6412701@ed.noda.tus.ac.jp</a></td>
<td></td>
</tr>
<tr>
<td>Albely Afifa Mir</td>
<td>Fungal Plant Pathology Lab, Seoul National University, Seoul, Korea, Korea</td>
<td><a href="mailto:albelymir@yahoo.com">albelymir@yahoo.com</a></td>
<td></td>
</tr>
<tr>
<td>Alfredo Seiiti Urashima</td>
<td>Federal University of Sao Carlos, Via Anhanguera km 174, 13600-000 Araras, Sao Paulo, Brazil</td>
<td><a href="mailto:alfredo@cca.ufscar.br">alfredo@cca.ufscar.br</a></td>
<td></td>
</tr>
<tr>
<td>Allicia Anak Jack</td>
<td>Malaysian Agricultural Research And Development Institute, MARDI Seberang Perai, Jalan Paya Keladi / Pinang Tunggal, 13200 Kepala Batus, Pulau Pinang, Malaysia</td>
<td><a href="mailto:allicia@mardi.gov.my">allicia@mardi.gov.my</a></td>
<td></td>
</tr>
<tr>
<td>Andrey Aver’yanov</td>
<td>Research Institute of Phytopathology, VNIIIF, B. Vyzemsky, Moscow region, 143050 Russia, Russian Federation</td>
<td><a href="mailto:aaveryanov@post.ru">aaveryanov@post.ru</a></td>
<td></td>
</tr>
<tr>
<td>Aram Huh</td>
<td>Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-921, Korea</td>
<td><a href="mailto:huharam@snu.ac.kr">huharam@snu.ac.kr</a></td>
<td></td>
</tr>
<tr>
<td>Barbara Valent</td>
<td>Kansas State University Dept. of Plant Pathology, 4024 Throckmorton Plant Sciences Center, United States</td>
<td><a href="mailto:bvalent@ksu.edu">bvalent@ksu.edu</a></td>
<td></td>
</tr>
<tr>
<td>Bo Zhou</td>
<td>International Rice Research Institute, DAPO Box 7777, Central Manila, Philippines, Philippines</td>
<td><a href="mailto:b.zhou@irri.org">b.zhou@irri.org</a></td>
<td></td>
</tr>
<tr>
<td>Chang Hyun Khang</td>
<td>University of Georgia, 1603 Miller Plant Sciences, Athens, GA 30602, United States</td>
<td><a href="mailto:ckhang@plantbio.uga.edu">ckhang@plantbio.uga.edu</a></td>
<td></td>
</tr>
<tr>
<td>Changhyun Choi</td>
<td>National Academy of Agricultural Science, RDA, 126 Suni-ro, Kweeonseong-gu, Suwon 441-707, Republic of Korea, Korea</td>
<td><a href="mailto:chchhy1789@gmail.com">chchhy1789@gmail.com</a></td>
<td></td>
</tr>
<tr>
<td>Christian Joseph R. Cumagun</td>
<td>University of the Philippines Los Banos, Crop Protection Cluster, College of Agriculture, UPLB, College, Laguna 4031, Philippines</td>
<td><a href="mailto:christian_cumagun@yahoo.com">christian_cumagun@yahoo.com</a></td>
<td></td>
</tr>
<tr>
<td>Da-Young Lee</td>
<td>Seoul National University 200-1053 College of Agriculture and Life Sciences, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul, Korea</td>
<td><a href="mailto:dy88lee@snu.ac.kr">dy88lee@snu.ac.kr</a></td>
<td></td>
</tr>
<tr>
<td>Didier THARREAU</td>
<td>CIRAD, UMR BGPI, TA A 54 K, 34398 Montpellier, France</td>
<td><a href="mailto:tharreau@cirad.fr">tharreau@cirad.fr</a></td>
<td></td>
</tr>
<tr>
<td>Dong Liying</td>
<td>Agricultural Environment &amp; Resources Research Institute, Yunnan Academy of Agricultural Sciences, Longtou Street, Kunming, Yunnan Province, P.R. China</td>
<td><a href="mailto:qzhyang@163.com">qzhyang@163.com</a></td>
<td></td>
</tr>
<tr>
<td>Dongmin Kim</td>
<td>Chungnam National University, 79 Daehagro, Yuseon-go, Daeyeon, 305-764, Korea</td>
<td><a href="mailto:acekdm@naver.com">acekdm@naver.com</a></td>
<td></td>
</tr>
<tr>
<td>Fucheng Lin</td>
<td>Zhejiang University Yuhangtang Road 866, C739, Hangzhou, China</td>
<td><a href="mailto:fuchenglin@zju.edu.cn">fuchenglin@zju.edu.cn</a></td>
<td></td>
</tr>
<tr>
<td>Gloria Mosquera</td>
<td>International Center for Tropical Agriculture, CIAT, Km 17 Recta Cali-Palmira, Colombia</td>
<td><a href="mailto:g.m.mosquera@cgiar.org">g.m.mosquera@cgiar.org</a></td>
<td></td>
</tr>
<tr>
<td>Gomathi Arunan</td>
<td>Evolva Biotech Pvt Ltd., 401, TICEL BIO PARK, Switzerland</td>
<td><a href="mailto:gomathiarunan@gmail.com">gomathiarunan@gmail.com</a></td>
<td></td>
</tr>
<tr>
<td>Guo-Liang Wang</td>
<td>Ohio State University, 2021 Coffey Road, Columbus OH 43235 USA, United States</td>
<td><a href="mailto:wang.620@osu.edu">wang.620@osu.edu</a></td>
<td></td>
</tr>
</tbody>
</table>
Koki Fujisaki
Iwate Biotechnology Research Centre,
22-174-4 Narita, Kitakami, Iwate
024-0003, Japan
k-fujisaki@ibrc.or.jp

Kouyanjun
Temasek Life Sciences Laboratory,
Temasek Life Sciences Laboratory, 1 Research Link,
National University of Singapore, Singapore
yanjun@tll.org.sg

Kumar Vasudevan
ETH Zurich, Switzerland,
LFW D13, IAS, Plant Biotechnology lab,
Universitatsstrasse 2, ETH Zurich, 8092, Switzerland
vkumar@ethz.ch

Kyoung su Kim
Kangwon National University,
Korea
kims@kangwon.ac.kr

Kyu Young Kang
Gyeongsang National University,
Korea, Jinju 660-701
Gyeongsang National University,
Korea
kykang@gnu.ac.kr

Lei Cailin
Institute of Crop Sciences,
CAAS,
12# Zhongguancun South street, 10081, Beijing, China
leicailin@caas.cn

Mahesh H B
University of Agricultural Sciences,
Ph. D scholar, Dept. of Genetics and Plant Breeding, MAS Lab,
UAS, GKVK, Bangalore-560065, India
maheshhbg@gmail.com

Mai Funabiki
Hokkaido University,
Kita9 Nish9 Kitaku Sapporo
Hokkaido 060-8589, Japan
mai-f@chem.agr.hokudai.ac.jp

Manjunath Hubballi
NIPHM,
Research Associate Division of Plant Biosecurity, National Institute of Plant Health Management, HYDERABAD INDIA 500030, India
manjunathabtnau@gmail.com

Maria Virginia Pedraza
INTA,
Ruta Prov. 39 km 143,5 Concepcion del Uruguay, Entre Ríos, Argentina
mariavirginiapdraza@yahoo.com.ar

Marie Nishimura
National Institute of Agrobiological Sciences,
Kan-non dai 2-1-2, Tsukuba, Ibaraki, 306-8602, Japan
marie@affrc.go.jp

Marta Cristina Corsi de Filippi
Embrapa,
Rodovia GO 462 Km 12, Zona Rural, Brazil
cristina.filippi@embrapa.br

Martha C. Giraldo
Kansas State University,
4024 Throckmorton Plant Science Center Manhattan KS 66502, United States
mcgiraldo@gmail.com

Mary Jeanie Telebanco-Yanoria
Japan International Research Center for Agricultural Sciences, 1-1 Ohwashii, Tsukuba, Ibaraki, 305-8636, Japan
mjeanie_telebanco@yahoo.com

Md Abu Sadat
Fungal Plant Pathology Lab,
Seoul National University, Seoul, Korea,
Fungal Plant Pathology Lab, Room no: Bio-5113, Dept. of Argro. Biotechnology (Plant Microbiology), College of Agriculture & Life Sciences, Seoul Nati, Korea
sadat@snu.ac.kr

Meghana
Centre for Cellular and Molecular Platforms, National Centre for Biological Sciences, Next Generation Genomics Laboratory, C-CAMP, NCBS, GKVK campus, Bangalore-560065, India
meghana@ncbs.res.in

MH Lebrun
INRA
UR1290 INRA BIOGER
Thiverval Grignon, France
marc-henri.lebrun@versailles.inra.fr

Mihwa Yi
Kansas State University,
4024 Throckmorton Plant Sci Ctr,
KSU, Manhattan KS66506, United States
mihwa@ksu.edu

Misa Kuroki
Tokyo University of Science,
2641, Yamazaki, Noda, Chiba,
278-8510, Japan
naru@rs.tus.ac.jp

Nagao Hayashi
NIAS,
2-1-1 Kannondai, Tsukuba, Ibaraki, Japan
nhayash@affrc.go.jp
Sester Mathilde
CIRAD,
BP 230 Antsirabe 110.
Madagascar, France
mathilde.sester@cirad.fr

Shailaja Hittalmani
University of Agricultural Sciences, Bangalore,
Marker Assisted Selection Laboratory, Department of Genetics and Plant Breeding, India
shailajah_maslab@rediffmail.com

Song Jae Young
National Agrobiodiversity Center, NAAS, RDA,
National Agrobiodiversity Center, National Academy of Agricultural Science, RDA, 88-20, Seodun-Dong, Suwon, Gyunggi-do, 441-707, Korea
jysong77@korea.kr

Soo-Kwon Park
National Institute of Crop Science, Rural Development Administration,
20th, Jeompiljaero, Miryang, Gyeongnam, Korea
sookwonpark@korea.kr

Sook-Young Park
Seoul National University,
200-1028, CALS, Seoul National University, Seoul 151-921, Korea
sookyp@gmail.com

Sornkom Worawan
Hokkaido University,
North Town House, 1123, Kita11, Nishi3, 2-23 ban chi, Kita-ku, Sapporo-shi, Hokkaido, 001-0011, Japan
wsornkom@gmail.com

Sun Tae Kim
Pusan national university,
1Department of Plant Bioscience, Pusan National University, Miryang, 627-706, Korea stkim5505@gmail.com

Sunghyung Kong
Seoul National University,
1 Gwanak-ro, Gwanak-gu, Seoul 151-921, Korea, Korea
n0212655@snu.ac.kr

Suwarno
ICRR,
KP Muara, JI. Raya Ciapus No. 25, Bogor, Indonesia, Indonesia
pakwarno@gmail.com

Takashi Kamakura
Tokyo University of Science
2641 Yamazaki, Noda, Chiba
278-8510, Japan
kamakura@rs.noda.tus.ac.jp

Takayuki Arazoe
Meiji University,
Laboratory of Plant cell technology, Division of Life sciences, Graduate school of Agriculture, Meiji University,
Japan
arazoet@gmail.com

Teruo Sone
Research Faculty of Agriculture, Hokkaido University,
Kita-9 Nishi-9 Kita-ku Sapporo Hokkaido 060-8589, Japan
sonet@chem.agr.hokudai.ac.jp

Tilak Raj Sharma
NRCPB, Indian Agricultural Research Institute, New Delhi,
LBS Centre Room No 38 Pusa Campus, IARI, New Delhi, India
trsharma@nrcpb.org

Tohru TERAOKA
Tokyo Univ. Agric. & Techn. (TUAT),
Saiwai-cho 3-5-8, Fuchu, Tokyo 183-8509, Japan
teraoka@cc.tuat.ac.jp

Uday.G
University Of Agricultural Sciences Bangalore,
1509 4th cross nes road sugappa layout yelahanka banglore
karntaka INDIA 560064, India
udaireddy7095@gmail.com

Urayama Syunichi
Tokyo University of Agriculture and Technology,
3-5-8, Saiwai-cho, Fuchu-shi,
Tokyo 183-8509, Japan
syun.ura@gmail.com

Vahid Zarrinnia
faculty member,
Plant Protection Department
Faculty of Agriculture and Natural Resource Scince and Research Branch Islamic Azad University Hesarak Av., Poonak Sq, Iran, Islamic Republic Of
zarrinnia@srbiau.ac.ir

Valerie Mogga
RWTH Aachen University,
Dep. of Plant Physiology (Bio III), Worringer Weg 1, Germany
mogga@bio3.rwth-aachen.de

Wang Yanli
Zhejiang Academy of Agricultural Sciences, Hangzhou, China,
Plant Protection and Microbiology Institute, Zhejiang Academy Of Agricultural Sciences 198, Shiqiao Rd, Hangzhou, Zhejiang, P, R, China
ylwang88@aliyun.com

Wei Tang
Nanjing Agricultural University,
Jiangsu Province, China
2010202023@njau.edu.cn