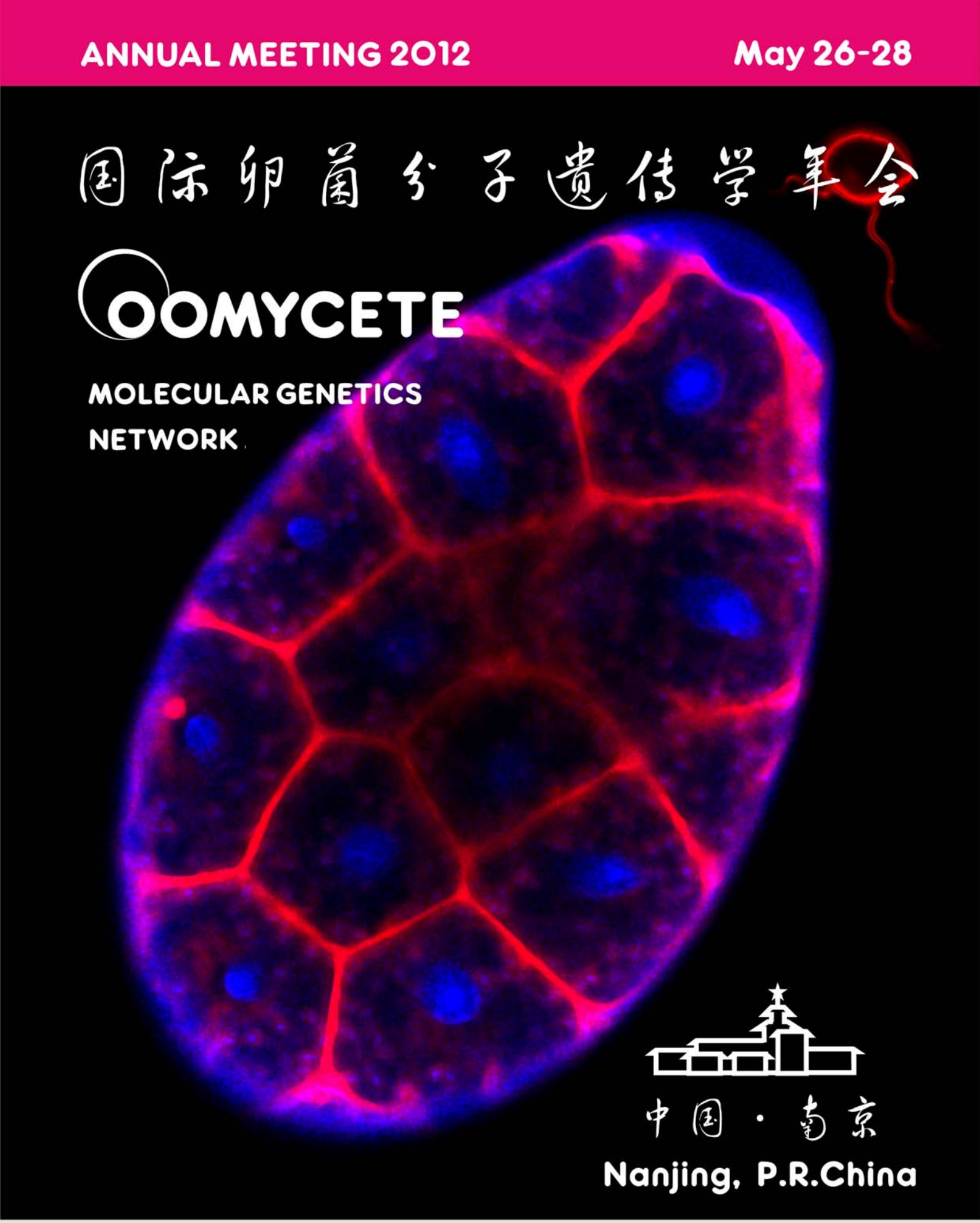
ANNUAL MEETING 2012 May 26-28

国际卵菌分子遗传学年会

OOMYCETE

MOLECULAR GENETICS NETWORK



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Nanjing, P.R.China

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Oomycetes are a fascinating group of eukaryotic microbes within the kingdom Stramenopila, that occupy a wide array of ecological niches, most commonly as destructive pathogens of plants and animals. They have serious impacts on agriculture, forestry, aquaculture and natural ecosystems. Our understanding of the physiology, pathology and epidemiology of oomycetes at the molecular level has rapidly expanded over the last 25 years, especially with the availability of genome sequences over the last 10 years. Oomycetes have become cutting edge model systems for understanding the molecular basis of host-microbe interactions and eukaryotic microbiology.

The Oomycete Molecular Genetics Research Collaboration Network (OMGN) was established in 2001. Since then OMGN has grown from around 20 investigators to more than 140 investigators from 18 countries. Much of the success of the oomycete molecular genetics research community has built upon its long culture of collaboration and communication, and sharing of techniques and resources. The network has facilitated the integration of new investigators into the community, especially junior faculty. Network activities include funds for community members to travel to the annual meeting, to participate in training internships and in bioinformatics training workshops to gain familiarity with bioinformatics tools and the oomycete genome resources.

This conference in Nanjing, China is the 12th annual meeting supported by network funding and the first to be held outside the US and Europe. On behalf of the community and the meeting organizers I welcome you all, especially our new colleagues from China, to another exciting meeting filled with cutting edge science.

Committee Chairs:

Dr. Xiaobo Zheng
Professor in Plant Pathology Department
Nanjing Agricultural University

Dr. Brett Tyler
Director, Center for Genome Research and Biocomputing
Stewart Professor of Gene Research
Oregon State University
Coordinator, Oomycete Molecular Genetics Research Collaboration Network

May 2012.

Oomycete Molecular Genetics Network

Annual Meeting 2012

Committee Chairs:

Xiaobo Zheng (Nanjing Agricultural University, China)
Brett Tyler (Oregon State University, USA)

Local Committee:

Yuanchao Wang (Nanjing Agricultural University, China)
Daolong Dou (Nanjing Agricultural University, China)
Suomeng Dong (Nanjing Agricultural University, China)
Zhijian Zhao (Nanjing Agricultural University, China)
Xiuguo Zhang (Shandong Agricultural University, China)

Academic Committee:

Brett Tyler (Oregon State University, USA)
Xiaobo Zheng (Nanjing Agricultural University, China)
Paul Birch (Scottish Crop Research Institute, UK)
Francine Govers (Wageningen University, Netherlands)
Weixing Shan (N&W A&F University, China)

Meeting Program

Friday, 25 May 2012

Arrival in Nanjing

8:30~22:30	Registration	Hanyuan Hotel

Saturday, 26 May 2012

8:30~9:00	Opening Session	
9:00~9:40	Photos and break	
9:40~11:40	Communications	Keynote speakers
11:40~13:30	Lunch	
14:00~15:30	Communications	Ⅰ - Plant immunity
15:30~16:00	Break	
16:00~18:15	Communications	Ⅰ - Plant immunity
18:15~20:00	Dinner	

Sunday, 27 May 2012

8:30~10:30	Communications	Ⅱ - Oomycete effectors
10:30~11:00	Break	
11:00~12:15	Communications	Ⅲ- Genomics - I
12:15~13:30	Lunch	

14:00~17:00	Guided Tour of Dr. Sun Yat-sen's Mausoleum	
17:30~19:30	Dinner	
20:00~21:00	Poster Session	

Monday, 28 May 2012

8:15~10:00	Communications	Ⅲ- Genomics - II
10:00~10:30	Break	
10:30~12:15	Communications	Ⅳ - Cell biology and population genetics
12:15~13:30	Lunch	
14:00~16:00	Communications	Ⅴ-Functional genomics
16:00~16:30	Break	
16:30~17:30	Conclusion of the meeting	
17:30~19:30	Dinner	

Tuesday, 29 May 2012

Departure

Key notes

Meeting Room : Conference hall, 6 floor in Hanyuan Hotel

Lunch / Dinner : Zhongshan hall, 2 floor in Hanyuan Hotel

Program of talks

Saturday, 26 May 2012

Opening Sessions

8:30~9:00	Welcome address	
9:00~9:40	Photos and break	

Morning Session I : Invited Key Talks

Session Chair: Sophien Kamoun

9:40~10:20	Kasturi Haldar	Red cells and plant cells with and without PIPs
10:20~11:00	Jianmin Zhou	Bacterial type III effectors and plant immunity
11:00~11:40	Youliang Peng	A novel component of the Prp19-associated complex is essential to safeguarding efficient intron splicing of pathogenicity genes in the rice blast fungus
11:40~13:30		*Lunch*

Afternoon Session II : Plant Immunity

Session Chairs: Brett Tyler and John McDowell

14:00~14:30	Sophien Kamoun	Suppression of plant immunity by *Phytophthora* effectors
14:30~14:45	Susan Breen	The recognition of the *P. infestans* RxLR effector *AVR2* and its interaction with a Ser/Thr phosphatase from the brassinosteroid pathway
14:45~15:00	Gilroy Eleanor	Linking plant immunity, development and abiotic stress responses in the *Solanaceae*?
15:00~15:15	Arnaud Bottin	New insights into recognition of *Aphanomyces euteiches* by *Medicago truncatula*
15:15~15:30	Stefan Engelhardt	Re-localisation of late blight resistance protein *R3a* to pre-vacuolar compartments is associated with effector recognition and required for the immune response
15:30~15:45	Cao Hua	Genetic and Molecular Mechanism of an

		Arabidopsis thaliana Mutant Susceptible to *Phytophthora sojae* Infection
15:45~16:00	Jack Vossen	Isolation, functional characterisation and exploitation of potao late blight resistance genes
16:00~16:15		*Break*

Afternoon Session III : Plant Immunity

Session Chairs: Francine Govers and Susan Breen

16:15~16:45	John McDowell	How do obligate pathogens survive inside hostile hosts?
16:45~17:15	Wenbo Ma	*Phytophthora* Produces RNA Silencing Repressors (PSRs) to Promote Infection
17:15~17:30	Xiaoli Yu	The RxLR effector Avh241 from *Phytophthora sojae* requires plasma membrane localization to induce plant cell death
17:30~17:45	Xiangzi Zheng	Identification of *Phytophthora infestans* RXLR effectors suppressing flg22-triggered early signalling in both *Arabidopsis* and tomato
17:45~18:00	Tianqiao Song	Two avirulence genes in *Phytophthora sojae* determine soybean *Rps1-k* mediated disease resistance
18:00~20:00		*Dinner*

Sunday, 27 May 2012

Morning Session IV : Oomycete Effectors

Session Chairs: Wenbo Ma and Suomeng Dong

8:30~9:00	Brett Tyler	Structural basis for interactions of the *Phytophthora sojae* RXLR effector Avh5 with phosphatidylinositol 3-phosphate and for host cell entry
9:00~9:15	Remco Stam	Characterisation of Crinkler (CRN) effector proteins from *Phytophthora capsici* and their putative host targets
9:15~9:30	Lars Lobach	The recombinant putative RxLR effector protein Htp3 from the fish pathogenic oomycete *Saprolegnia parasitica* is translocated into fish cells

9:30~9:45	Weixiao Yin	The *Phytophthora sojae* Effector *Avr1d* Encodes an RXLR-dEER Protein
9:45~10:00	Shan Lu	Phosphatidylinositol 3-phosphate produced by *Phytophthora sojae* is important for infection by binding to RXLR effectors
10:00~10:30		*Break*

Morning Session V : Genomics - I

Session Chairs: Rays Jiang and Laura J. Grenville-Briggs

10:30~11:00	Francine Govers	In silico identification of transcription factor binding sites in *Phytophthora*
11:00~11:30	Mark Gijzen	Transgenerational gene silencing of Avr effectors in *Phytophthora sojae*
11:30~11:45	Tripathy Sucheta	Integrating Oomycete Structural and Functional Genomics Datasets into FungiDB
11:45~12:00	Theerapong Krajaejun	Whole Genome Gene Discovery in the Pathogenic Oomycete *Pythium insidiosum* by 454 Sequencing Technology
12:00~12:15	James Wong	Differentially expressed microRNAs in soybean during *Phytophthora sojae* infection
12:15~13:30		*Lunch*
20:00~21:00		**Poster Session**

Monday, 28 May 2012

Morning Session VI : Genomics - II

Session Chairs: Mark Gijzen and Tripathy Sucheta

8:15~8:45	Rays Jiang	Computational biology drives the understanding of animal pathogenesis processes
8:45~9:00	Wenkui Dai	Bioinformatics analysis of fungal genomes
9:00~9:15	Brad Day	The cucurbit killer, *Pseudoperonospora cubensis*: splicing and transcriptome analysis of virulence and susceptibility
9:15~9:30	Xiuguo Zhang	The genomic study of the pathogenicity from *Phytophthora capsici*
9:30~9:45	Qinhu Wang	Identification and characterization of tRNA-derived small RNAs in the oomycete

		Pathogen *Phytophthora sojae*
9:45~10:00	Danyu Shen	Gene duplication and fragment recombination drive functional diversification of a superfamily of cytoplasmic effectors in *Phytophthora sojae*
10:00~10:30		*Break*

Morning Session VII : Cell Biology and Population Genetics

Session Chairs: Arnaud Bottin and Zhijian Zhao

10:30~10:45	Laura J. Grenville-Briggs	Cell wall biochemistry to illuminate mechanisms of pathogenicity in *Phytophthora infestans*
10:45~11:00	Tofazzal Islam	Molecular mechanisms of zoosporogenesis, motility, chemotaxis and differentiation of *Peronosporomycete* zoospores
11:00~11:15	Kai Tao	Live-cell Image of Compatible and Incompatible Interaction between *Phytophthora sojae* and Host
11:15~11:30	Jian Hu	Genetic Diversity of *Phytophthora capsici* in China
11:30~11:45	Mei Guo	Potato late blight in Heilongjiang
11:45~12:00	Yuee Tian	Phenotypic and genotypic characterization of potato late blight pathogen *Phytophthora infestans* in northern Shaanxi of China
12:00~12:15	Liangyu Jiang	Reserching on races of *Phytophthora sojae* and their virulences on soybean cultivars in Heilongjiang Province, and resistance related gene function of soybean
12:15~13:30		*Lunch*

Afternoon Session VIII : Functional Genomics

Session Chairs: Daolong Dou and Marcia Saraiva

14:00~14:15	Marcia Saraiva	Tyrosinase is required for melanin production in zoosporangia of the fish pathogenic oomycete *Saprolegnia parasitica*
14:15~14:30	Zhili Pang	Resistance genetic mechanisms of *Phytophthora capsici* to Iprovalicarb and Mandipropamid
14:30~14:45	Chenlei Hua	Characterization of a novel G protein-coupled receptor with a phosphatidylinositol phosphate kinase domain in *Phytophthora infestans*
14:45~15:00	Brian D Rutter	A yeast one-hybrid assay identifies a novel DNA-binding domain in *Phytophthora sojae*
15:00~15:15	Xin Zhang	Identification of target proteins associated with

		Phytophthora sojae G-protein alpha subunit PsGPA1
15:15~15:30	Zhijian Zhao	Functional analysis of pathogenicity related genes from SNARE family in secretion pathways from *Phytophthora sojae*
15:30~15:45	Yuting Sheng	A *Phytophthora sojae* heat shock transcription factor PsHSF1 is required for oxidative stress tolerance and suppression of plant immunity
15:45~16:00	Meixiang Zhang	*PnPMA1*, an atypical plasma membrane H+-ATPase, is Involved in zoospore development in *Phytophthora parasitica*
16:00~16:30		*Break*
16:30~17:30		**Conclusion of the meeting**

Session Chair: Brett Tyler

ABSTRACTS

Oomycete Molecular Genetics Network (OMGN) Meeting
Nanjing, CHINA
May 25-28, 2012

Plant Immunity

The recognition of the *P.infestans* RxLR effector *AVR2* and its Interaction with a Ser/Thr Phosphatase from the Brassinosteroid pathway

Susan Breen[1,2], Eleanor M. Gilroy[1], Diane Saunders[3], Miles R. Armstrong[2], Ingo Hein[1], Petra C. Boevink[1], Leighton Pritchard[1], Stephen C. Whisson[1], Sophien Kamoun[3] & Paul R. J. Birch[1,2]

([1] Cell and Molecular Science, The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK; [2] Division of Plant Sciences, College of Life Sciences, University of Dundee at JHI, Invergowrie, Dundee DD2 5DA, UK; [3] The Sainsbury Laboratory, John Innes Centre, Norwich, NR4 7UH)

An important research goal in the fight against potato late blight, caused by the oomycete *Phytophthora infestans,* is to identify and characterise the host targets of key pathogen avirulence effector proteins that are likely to be delivered into host cells and to assess their contribution to the mechanism of disease resistance.

We have identified the RxLR-dEER effector gene *PiAVR2* from the sequenced isolate t30-4, the product of which is recognised inside host cells by the potato R2 protein. Cloning *PiAVR2* from virulent isolates revealed an additional, variant form, *PiAVR2-like,* which evades recognition by *R2-like* genes. *PiAVR2* and *PiAVR2-like* encode proteins that differ in 13 amino acids; one or more of these specifies recognition by R2. In addition, both presence/absence polymorphism and transcriptional differences explain virulence of *P. infestans* isolates on *R2* plants.

Yeast-2-hybrid analysis was used to identify a family of host proteins as candidate interactors of PiAVR2. One of these, BSU1-like 1 (BSL1), was cloned and the interaction with PiAVR2 confirmed using Bimolecular fluorescence complementation (BiFC). BSL1 is a Ser/Thr Phosphatase from the brassinosteroid hormone signal transduction pathway. In addition, PiAVR2-like was also found to interact with BSL1, suggesting that both forms share a similar function. In the model plant *Arabidopsis thaliana* BSL1 is an activator of the BR signal transduction pathway. The components upstream and downstream of BSL1 in this pathway have been found within the *S. tuberosum* cv *phurjea* genome suggesting that the BR pathway is intact within potato. Evidence has emerged from Virus-Induced Gene Silencing (VIGS) and BiFC experiments that BSL1 mediates indirect recognition of PiAVR2 by R2.

Linking Plant Immunity and Development in the *Solanaceae*

Eleanor M. Gilroy[1], Susan Breen[1,2], Dionne Turnbull[1,2], Miles R. Armstrong[2], Leighton Pritchard[1], Eva Randall[1], Stephen C. Whisson[1], Ingo Hein[1] & Paul R. J. Birch[1,2]

([1] Cell and Molecular Science, The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK; [2] Division of Plant Sciences, College of Life Sciences, University of Dundee at JHI, Invergowrie, Dundee DD2 5DA, UK)

The oomycete *Phytophthora infestans* is a devastating pathogen of tomato and potato and is a global problem for crop production and food security. *P. infestans* possess large numbers of rapidly evolving effector proteins. The oomycete effectors that are recognised in host species by *Resistance* (*R*) gene products have all belonged to the RxLR class. RxLR effectors are secreted from haustoria, delivered into plant cells and are thought to manipulate host processes to aid pathogen disease progression and combat defence responses. Identifying the host proteins that are targeted by RxLR effectors is revealing connections to host pathways that were previously thought to be distinct from plant defence.

Two forms of one RxLR effector have been identified, PiAVR2 which is recognised by R2 ortholgues and PiAVR2-like which evades recognition. Following their identification we have discovered through yeast-2-hybrid analysis that both interact with all three members of a kelch repeat containing Ser/Thr Phosphatase family (StBSL1, StBSL2a and StBSL2b) which are associated with the brassinosteroid (BR) signal transduction pathway. Many components of the BR pathway described thus far in the model plant *Arabidopsis* have been found within the *S. tuberosum* cv *phurjea* genome. We have been trying to determine if PiAVR2 is manipulating this pathway? Data will be presented that demonstrates the conservation and possible differences between the BR signalling in *Arabidopsis* and the Solanaceae. Is the BR pathway as well defined in the model plant as current literature would have you believe?

Re-localisation of late blight resistance protein *R3a* to pre-vacuolar compartments is associated with effector recognition and required for the immune response

Stefan Engelhardt
(University of Dundee)

An important objective of plant-pathogen interactions research is to determine how and where resistance proteins detect pathogen effectors to mount a strong immune response. Many NB-LRR resistance proteins have been shown to accumulate in the plant nucleus following effector recognition, where they initiate defence signalling leading to programmed cell death. Here we show a novel behaviour of potato resistance protein R3a, which re-locates from the cytoplasm to pre-vacuolar compartments (PVCs) only when co-expressed with recognised effector forms AVR3a^{KI} and Pex147-3, but not unrecognised forms AVR3a^{EM} or Pex147-2, from *Phytophthora infestans*. We also show that Avr3a^{KI} relocalises to PVCs before HR development. Although yeast-2-hybrid analyses did not support direct interaction between R3a and AVR3a^{KI}, both proteins co-localised into close physical proximity at PVCs *in planta*. Treatment with brefeldin A (BFA) and wortmannin, inhibitors of the endocytic cycle, attenuated both the re-localisation of R3a to PVCs, and the R3a-mediated hypersensitive response (HR). No such effect with these inhibitors was observed on HR triggered by the gene-for-gene pairs Rx1/PVX-CP or Sto1/ipiO1. An R3a(D501V) autoactive MHD mutant, which triggered HR in the absence of AVR3a^{KI}, failed to localise to PVCs. Moreover, BFA and wortmannin did not alter cell death triggered by this mutant form. We conclude that effector recognition and consequent HR signalling by NB-LRR resistance protein R3a occur in association with its re-localisation to vesicles in the endocytic pathway.

Genetic and Molecular Characterization of an *Arabidopsis thaliana* Mutant Susceptible to *Phytophthora sojae* Infection

Hua Cao[1], Fangyuan Teng[2], Gang Wang[1], Jinbu Jia[2] Biao Gu[1], Meixiang Zhang[2], Weixing Shan[1*]

([1] State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, China; [2] State Key Laboratory of Crop Stress Biology in Arid Areas and College of Life Science, Northwest A&F University, Yangling, Shaanxi 712100, China. * Correspondence to wxshan@nwsuaf.edu.cn)

The nonhost resistance of plant is defined as the resistance of entire plant species to all isolates of a microbial species, which is the most common phenomenon and is likely a durable form of plant resistance against pathogens. Nonhost plant resistance is considered to be closely related with the basal defense responses. The genetic basis of nonhost resistance is still not clear though it was proposed that simultaneous recognition of a number of pathogen-derived molecules by multiple resistance genes is involved. In many nonhost resistance cases, the active defense responses play an important role, which is similar to gene-for-gene resistance and single genes are therefore likely involved. *Phytophthora* species represent a unique group of plant pathogens called oomycetes that are evolutionally distant from true fungi and probably possess distinct genetic and biochemical mechanisms for interacting with host and nonhost plants. In this study, *Phytophthora sojae* and *Arabidopsis thaliana* were employed to understand genetic and molecular mechanism of nonhost resistance in plants against oomycete pathogens. In previous work, we identified an *A. thaliana* T-DNA insertional mutant susceptible to *P. sojae* infection. The boundary sequences of all four T-DNA insertion sites (*nhr-1,2,3,4*) were identified by TAIL-PCR. Genetic analysis reveals a single recessive mutation. We created gene-silencing transformants which target individual or combination of candidate genes (*nhr-1,2,3,4*). Infection assay showed that over half of the *nhr2*-silenced T_1 plants were susceptible to *P. sojae*. Furthermore, the T-DNA insertional lines *nhr-2.5 and nhr-2.8* showed similar level of susceptibility to *P. sojae* infection under our experiment conditions.

How do biotrophic pathogens survive inside hostile hosts?

R. G. Anderson[1], D. Deb[1], K. Fedkenheuer[1], H. Wise[1], T. How-Yew-Kin[1], M. Casady[1], R. Fee[1], Reed Bryant[1], B. M. Tyler[2], and J. M. McDowell[1]

([1] Department of Plant Pathology, Physiology and Weed Science, Virginia Tech; [2] Center for Genome Research and Biocomputing, Department of Botany and Plant Pathology, Oregon State University)

We investigate the molecular interplay and co-evolution between *Arabidopsis thaliana* and the oomycete pathogen *Hyaloperonospora arabidopsidis* (*Hpa*, downy mildew disease). *Hpa*, along with other downy mildew pathogens, is an obligate pathogen that extracts nutrients only from living plant tissue and cannot exist apart from its host. Recent sequencing of the *Hpa* genome and comparison to genomes of facultative parasites in the related *Phythophthora* genus revealed several genomic signatures of evolution towards an obligate life style. For example, almost every gene family encoding secreted pathogenicity proteins is downsized in *Hpa* compared to *Phytophthora*, presumably to facilitate "stealth" inside the host. We are particularly interested in RXLR effectors, which are exported to the interior of plant cells where they promote host susceptibility or are recognized as signals of invasion. Hpa contains 134 candidate RXLR genes, compared to ~370-550 in *Phytophthora* genomes. There is very little evolutionary conservation between the predicted RXLR proteins in *Hpa* and *Phytophthora*, suggesting that oomycetes must continually invent or re-invent RXLR weaponry. However, some RXLR genes do appear to be conserved, suggesting that they play a general role in oomycete pathogenicity. We are focusing on functional analysis of these conserved genes from *Hpa* and their homologs in the soybean root/stem rot pathogen *Phytophthora sojae*. We will present current results from these experiments, which demonstrate that these effectors can suppress plant immune responses across a broad range of host plants, from different locations within the plant cell. We will also introduce a new project employing effectoromics to screen for new resistance genes against *P. sojae*.

Phytophthora Produces RNA Silencing Repressors (PSRs) to Promote Infection

Yongli Qiao[1,2], Lin Liu[2,3], Cristina Flores[1,4], James Wong[1,4], Qijun Xiang[1,2], Xianbing Wang[1,2], Xigang Liu[2,3], Howard Judelson[1,2], Xuemei Chen[2,3], Wenbo Ma[1,2] *

([1] Department of Plant Pathology and Microbiology, [2] Center for Plant Cell Biology, [3] Department of Botany and Plant Sciences, and [4] Graduate Program of Cell, Molecular, Developmental Biology, University of California, Riverside, CA 92521. * Presenting author)

Phytophthora sojae is an oomycete pathogen that causes the severe soybean stem and root rot disease. Over 400 *P. sojae* proteins are predicted to contain the N-terminal RxLR-dEER motif, which mediates the entry of these effector proteins into the host cytoplasm. So far, the functions of RxLR effectors during infection remain largely unknown. In this work, we identified two *P. sojae* RxLR effectors with RNA silencing suppression activity. These effectors are thereby designated Phytophthora RNA Silencing Repressors (PSRs). PSR1 strongly suppresses the biogenesis of both microRNA and small-interference RNA in plants. Unlike PSR1, PSR2 specifically inhibits the biogenesis of small-interference RNA. Importantly, *Nicotiana benthamiana* plants over-expressing PSRs, as well as two viral RNA silencing repressors, are more susceptible to the infection of *Phytophthora infestans*, suggesting that PSRs significantly promotes Phytophthora infection. This work represents the first report of non-viral effectors with gene silencing suppression activity. Our data suggest that Phytophthora have evolved PSRs to target the small RNA pathway in plant hosts and promote infection.

Function of an RXLR Effector Avh241 from *Phytophthora sojae*

Xiaoli Yu, Junli Tang, Qunqing Wang, Wenwu Ye, Shuyi Duan, Chenchen Lu, Xinyu Yang, Suomeng Dong, Xiaobo Zheng, Yuanchao Wang*

College of Plant Protection, Key Laboratory of Integrated Management of Crop Diseases and Pests (Ministry of Education), Nanjing Agricultural University, Nanjing 210095, China

The *Phytophthora sojae* genome encodes hundreds of RxLR effectors predicted to manipulate various plant defense responses, however the molecular mechanisms involved are largely unknown.

A high-throughput screening in our lab was carried out to identify the functions of the RxLR effectors of *P. sojae*, showing most could suppress the plants cell death and several could induce the plant cell death. The most strongly expressed immediate-early effectors could suppress the cell death triggered by several early effectors, and most early effectors could suppress INF1-triggered cell death, suggesting the two classes of effectors may target different functional branches of the defense response. It is very important for *P. sojae* to transcript effectors exactly. These effectors can fight as a team to interrupt the plant immunity.

The localization assays of 15 RxLR candidate effectors in plant cells indicated that the effectors suppressing both ETI and PTI almost accumulate in the nucleus of the plant cells, while the effectors only suppressing ETI localized in the cytoplasm. Avh241, an RxLR effector from *P. sojae*, was intensively studied. In transient overexpression of Avh241-induced cell death on tomato and *Arabidopsis thaliana*, Avh241 localized at the plant plasma membrane. Also, an N- terminal motif of Avh241 was important for membrane localization and cell death-inducing activity, suggesting that full effector functions are closely associated with subcellular localization. Two MAP kinases, NbMEK2 and NbWIPK, were required for the cell death triggered by Avh241 in *Nicotiana benthamiana*, suggesting involvement of a receptor-mediated MAPK-dependent signaling pathway. Silencing of *Avh241* in *P. sojae* impaired the pathogen's virulence on soybean, indicating that it played an essential role in *P. sojae* pathogenicity. When expressed in *N. benthamiana* without causing cell death, *Avh241* could promote infection by *Phytophthora capsici*. Motif deciding localization was not required to promote infection, suggesting that *Avh241* interacts with the plant immune system via at least two different mechanisms. Studies are needed to identify and characterize signaling components targeted by Avh241 and provide further insights into the mechanisms regulating host cell death, pathogenesis, and virulence.

Identification of *Phytophthora infestans* RXLR Effectors Suppressing flg22-triggered early signalling in both *Arabidopsis* and Tomato

Xiangzi Zheng[1], Malou Fraiture[1], Liu Xiaoyu[1], Hazel McLellan[2], Eleanor M. Gilroy[3], Ying Chen[1], Paul R.J. Birch[2,3], Frédéric Brunner[1]

([1] Department of Biochemistry, Centre for Plant Molecular Biology, Eberhard Karls University, Auf der Morgenstelle 5, D-72076 Tübingen, Germany; [2] Division of Plant Sciences, University of Dundee (at James Hutton Institute), Errol Rd, Invergowrie, Dundee DD2 5DA, UK; [3] Cell and Molecular Sciences, The James Hutton Institute, University of Dundee , Errol Rd, Invergowrie, Dundee DD2 5DA, UK)

The genome of *Phytophthora infestans*, the causal agent of potato and tomato late blight, is encoding for several hundreds of so-called RxLR effectors which are translocated inside the host cells during the infection (1). In order to elucidate the biological function of *P.infestans* RxLR effectors (PiRxLR) *in planta*, we used a protoplast-based system to assess their potential for subverting MAMP-triggered early signalling pathways (2,3). Fourty-five PiRxLR effectors were tested for their ability to suppress the activation by flg22 of a reporter gene under control of a typical MAMP-inducible promoter (pFRK1::Luc).

Seven PiRxLR effectors blocked significantly reporter gene activation by flg22 in tomato protoplasts. Further, three of them affected post-translational MAP Kinase activation, suggesting an interference with MAMP signalling at- or upstream of the MAP kinase cascade.

As MAMP-signalling pathways appear to be conserved across the plant kingdom, we hypothesized that a set of PiRXLR effector candidates may target proteins/mechanisms that are highly conserved in both host and non-host plants. From the aforementioned seven candidate PiRxLR effectors that were transiently expressed in *Arabidopsis thaliana* protoplasts, five were confirmed to strongly inhibit flg22-induced pFRK1::Luc reporter gene activity but none of them were able to affect post-translational MAP kinase activation. Three PiRxLR candidates appeared to share similar functions in both *Arabidopsis* and tomato by suppressing transcriptional activation of MAMP-marker genes.

Altogether, our results suggest that a subset of *P.infestans* RxLR effectors co-evolved with their cognate targets in tomato to achieve efficient PTI suppression. Non-host resistance in *Arabidopsis* can be partially explained by the existence of such PiRxLR effectors that are inefficient in suppressing PTI signalling. Still, *P.infestans* disposes of a core set of RxLR effectors that are obviously targeting ubiquitous plant proteins/mechanisms involved in PTI and the considerable genetic resources existing in the model plant *Arabidopsis* offer a great opportunity to study the molecular mechanism underlying the mode of action of these “unspecific” PiRxLR effectors with the aim to guide future work in crop plants.

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Two avirulence genes in *Phytophthora sojae* determine soybean *Rps1-k* mediated disease resistance

Tianqiao Song*, Shiv D. Kale‡, Felipe D. Arredondo‡, Danyu Shen*, Liming Su*, Li Liu*, Yuanchao Wang*, Daolong Dou*‡ and Brett M. Tyler‡

*, College of Plant Protection, Nanjing Agricultural University, Nanjing, 210095, China

‡ Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

Monogenic resistance encoded by *Rps* (resistance to *Phytophthora sojae*) genes has been widely used for soybean against root and stem rot diseases caused by this oomycete pathogen. Among 15 soybean *Rps* genes that have been identified, *Rps*1k has been the most widely used and most durable in the past three decades. Here, we found that two different RXLR effectors, encoded by closely linked, were equally recognized by Rps1k. One effector is encoded by the avirulence gene *Avr1b*-1, the first cloned oomycete avirulence gene. Three independent lines of evidence, including over-expression or gene silencing of *Avr1b* in stable *P. sojae* transformants and transient expression of this gene in soybean, indicated that Avr1b could trigger *Rps*1k mediated "gene-for-gene" resistance. We also identified another RXLR effector (Avh331, designated as Avr1k), which is 5 kb away from Avr1b that could trigger *Rps*1k mediated resistance. Silencing or over-expression of *Avr1k* in *P. sojae* stable transformants resulted in the loss or gain of the avirulence phenotype on *Rps*1k plants, respectively. These results were supported by transient expression of Avr1k in soybean leaves. Avr1k and Avr1b exhibit no sequence similarity at all. Only isolates of *P. sojae* with the virulence alleles of *Avr1b* and frameshift alleles of *Avr1k* could evade the perception by the soybean *Rps*1k gene product. Those results suggest that soybean *Rps*1k gene provides stable and broad-spectrum resistance against *P. sojae* by surveilling two avirulence genes.

Effectoromics to Improve Resistance against *Phytophthora sojae* in Soybean

Fedkenheuer, K and McDowell, JM

(Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, VA, USA)

Oomycete pathogens cause billions of dollars in crop losses each year. *Phytophthora sojae*, the causal agent of soybean root and stem rot, is responsible for soybean losses estimated at $1-3 billion worldwide and $420 million in the US (2008). These losses are rising because resistance (*R*) genes, which have been utilized for decades in soybean cultivars, are being defeated by pathogen co-evolution. In this project, we are using a high-throughput, effector-directed breeding strategy, pioneered against *P. infestans*, to identify and breed R genes against *P. sojae* into elite soybean cultivars. The disadvantages of *R* gene mediated resistance are that resistance is often race-specific and that avirulence loci often encode effectors that can be modified or discarded with little or no impact on virulence. To overcome these problems, we aim to identify *R* genes that recognize essential effectors which cannot be discarded without a fitness penalty to the pathogen. As a first step in this screen, we are developing a system to transiently deliver effector proteins to the interior of soybean cells. To test different delivery systems, we are delivering known *P. sojae* avirulence effectors into soybean leaves that contain a corresponding *R* gene. The goal is to produce a visual hypersensitive response (HR) to the delivered effector. Our most promising delivery system uses a strain of *Pseudomonas fluorescens* called EtHAN to deliver effectors through a genetically engineered type III secretion system. We have increased the consistency and intensity of macroscopic HRs by co-infiltrating soybean leaves with virulent *Pseudomonas syringe pvglycinea* (helper strain) at low concentrations. The helper strain increases growth of *P.f* EtHAN *in planta*, most likely by weakening soybean PTI. We are optimizing this system so that it can be seamlessly transitioned for use in a high-throughput screen for resistance genes against essential *P. sojae* effectors. Ultimately we will use this system to screen resistant soybean germplasm, as well as wild relatives of soybean and other legumes.

Insight into *Medicago truncatula* immunity and *Aphanomyces euteiches* PAMPs and effectors

Bottin A [1,2], Nars A [1,2], Rey T [1,2], Lafitte C [1,2], Brown C [3], Melida H [3], Ramirez D [1,2], Fliegmann J [1,2,4,5], Heux L [6], Bulone V [3], Jacquet C [1,2], Gaulin E [1,2], Dumas B [1,2]

([1] Université de Toulouse; UPS; UMR 5546, LRSV; BP 42617, F-31326, Castanet-Tolosan, France; [2] CNRS; UMR 5546; BP 42617, F-31326, Castanet-Tolosan, France; [3] Division of Glycoscience, School of Biotechnology, KTH, 106 91 Stockholm, Sweden; [4] INRA, LIPM, UMR441, 31326 Castanet-Tolosan, France; [5] CNRS, LIPM, UMR2594, 31326 Castanet-Tolosan, France; [6] CERMAV-CNRS, BP 53, 38041 Grenoble cedex 9, France)

Aphanomyces euteiches is a Saprolegniale oomycete responsible for significant pea and alfalfa crop losses worldwide. Large scale EST sequencing associated with biochemical analyzes revealed several distinctive features, such as the competency for sterol biosynthesis and the presence of structural cell wall chitosaccharides. *Medicago truncatula* is a legume model plant with a large collection of natural lines which show differential interactions with *A. euteiches*. To identify genes involved in quantitative resistance to this pathogen, we have initiated a genome-wide association genetics study. In parallel, we are analyzing the involvement of LysM receptor-like kinases (LysM-RLKs) in the perception of *A. euteiches* chitosaccharidic signals. We showed recently that mutation of the LysM-RLK *NFP*, which is necessary for the perception of rhizobial lipochitooligosaccharidic symbiotic signals, results in increased susceptibility to *A. euteiches.* This suggests that NFP is not only involved in the perception of symbiotic signals but possibly also of *A. euteiches* PAMPs, which might be derived from cell wall chitosaccharides. Therefore, we are investigating the biogenesis, structure, and activity of the *A. euteiches* chitosaccharides. First, we characterized a dual chitin synthase activity, producing both soluble and insoluble chitosaccharides. Next, chitosaccharide-enriched fractions have been generated from the cell wall, and their elicitor activity is being analyzed in *M. truncatula* WT and *nfp* mutant plants. In addition to the characterization of *A. euteiches* putative PAMPs and their receptors, a functional analysis of CRinkle and Necrosis (CRN) effectors is being conducted by heterologous protein expression in *M. truncatula* roots. Finally, sequencing of the *A. euteiches* genome has been completed and RNAseq data is being generated from various strains/species. The new sequence resources will allow a comprehensive analysis of the gene repertoires which are potentially involved in pathogenicity and host range determination.

Phytophthora capsici-Arabidopsis, a model pathosystem for studying the role of lectin receptor kinases in *Phytophthora* disease resistance

Yan Wang[1,2] , Klaas Bouwmeester[1], Weixing Shan[2], Francine Govers[1,*]

([1] Laboratory of Phytopathology, Plant Sciences Group, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; [2] College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China)

The oomycete pathogen *Phytophthora capsici* is able to infect a wide range of plants and causes extensive losses in Solanum and Cucurbita crop plants. To better understand the interactions between *P. capsici* and its host plants, information gained from model pathosystems will be instrumental. Here, we describe a novel pathosystem between *P. capsici* and Arabidopsis. We screened a large collection of Arabidopsis accessions with several isolates of *P. capsici*, and found interaction specificity among isolate-accession combinations. In compatible interactions, appressorium-mediated penetration was followed by formation of invasive hyphae, haustoria and sporulation on leaves and roots. In contrast, in incompatible interactions, Arabidopsis activated defense responses including callose deposition, strong accumulation of H_2O_2 and O^{2-} and cell death resulting in early pathogen encasement. Defense-related genes involved in camaelxin and *indole-glucosinolate* pathways were strongly induced upon infection by *P. capsici*. Moreover, mutants impaired in these pathways showed compromised resistance to *P. capsici*. Previously, an Arabidopsis legume-like lectin receptor kinase (LecRK), LecRK-I.9, was identified to be essential for *Phytophthora* resistance. *LecRK-I.9* is a member of a family consisting of 45 genes. How other family members contribute to oomycete disease resistance is not yet known. The *Phytophthora capsici*-Arabidopsis pathosystem will be used for functional characterization of *LecRK* genes.

Surface-mediated response to elicitins is providing a novel layer of resistance to *Phytophthora infestans* in potato

Juan Du, Gerard Bijsterbosch, Evert Jacobsen, Richard G.F. Visser, Vivianne G. A. A. Vleeshouwers

(Wageningen UR Plant Breeding, Wageningen University)

Surface-mediated response is based on pattern recognition receptors (PRR) that recognize pathogen-associated molecular patterns (PAMPs). In contrast to resistance genes that are commonly used in resistance breeding and generally quickly defeated, PRR are reported to confer a broader type of recognition. Recently, it was shown that PAMP-triggered immunity can confer a broad-spectrum disease resistance in crop plants. This suggests that PRR have great promise for engineering effective and durable disease resistance. In this project, we study the *ELR1* gene, which encodes the first potato PRR that recognizes elicitins of the potato late blight pathogen *Phytophthora infestans*. Since elicitins are widely conserved and recognized as oomycete PAMPs, a defense response targeted to elicitins is expected to be generally broad spectrum. This hypothesis is confirmed by our results. We tested 16 elicitins from 8 different oomycete species including *P. infestans*, and found that most elicitins were recognized by ELR1. Besides, we are testing whether expression of *ELR1* in potato can enhance the resistance to *P. infestans* isolates. We will report on these data during the conference and discuss whether *ELR1* can potentially confer an enhanced broad-spectrum resistance to late blight.

Microbe Effectors

Structural basis for interactions of the *Phytophthora sojae* RXLR effector Avh5 with phosphatidylinositol 3-phosphate and for host cell entry

Furong Sun[1,2], Shiv Kale[3], Hugo Azurmendi[2], Dan Li[1], Brett Tyler[3,4], and Daniel Capelluto[1]

([1]Dept of Biological Sciences, [2]Dept of Chemistry, [3]Virginia Bioinformatics Institute, Virginia Tech, USA; [4]Center for Genome Research and Biocomputing, Oregon State University, USA)

Oomycetes, such as Phytophthora sojae, employ protein effectors that enter host cells to facilitate infection. Entry of some effector proteins into plant cells is mediated by conserved RxLR motifs in the effectors and phosphoinositides (PIPs) resident in the host plasma membrane such as phosphatidylinositol 3-phosphate (PtdIns(3)P). Recent reports differ regarding the regions on RxLR effector proteins involved in PIP recognition. To clarify these differences, we have structurally and functionally characterized the P. sojae effector, avirulence homolog-5 (Avh5). Using NMR spectroscopy, we demonstrate that Avh5 is helical in nature with a long N-terminal disordered region. Heteronuclear single quantum coherence titrations of Avh5 with the PtdIns(3)P head group, inositol 1,3-bisphosphate ($Ins(1,3)P_2$), allowed us to identify a C-terminal lysine-rich helical region (helix 2) as the principal lipid-binding site in the protein, with the N-terminal RxLR (RFLR) motif playing a more minor role. Mutations in the RFLR motif slightly affected PtdIns(3)P binding, while mutations in the basic helix almost abolished it. Avh5 exhibited moderate affinity for PtdIns(3)P, which increased the thermal stability of the protein. Mutations in the RFLR motif or in the basic region of Avh5 both significantly reduced protein entry into plant and human cells. Both regions independently mediated cell entry via a PtdIns(3)P-dependent mechanism. Our findings support a model in which Avh5 transiently interacts with PtdIns(3)P by specific electrostatic interactions mainly through its positively charged helix 2 region, enabling the RFLR domain to promote PI3P-mediated host entry. This study, including the identification of the PtdIns(3)P-binding site, provides an improved and updated model for how RxLR effector proteins recognize phosphoinositides and for the contributions of the RxLR motif and basic-rich C-terminal regions to the internalization process.

Characterisation of Crinkler (CRN) effector proteins from *Phytophthora capsici* and their putative host targets

Stam, R.[1,2], Howden, A.M.J.[1], Jupe, J.[1], Boevink, P.C.[2,] and Huitema, E.[1]

([1] Division of Plant Sciences, College of Life Sciences, University of Dundee, UK; [2] Cell and Molecular Sciences Programme, James Hutton Institute, Dundee, UK)

Phytophthora capsici (Pc) is a oomycete pathogen of many crops including tomato. Upon infection, Pc secretes an array of proteins that modify host cell processes and enable infection (effectors). The Crinkler (CRN) gene family encodes a complex family of intracellular effectors. CRNs feature conserved N-termini, required for translocation and diverse C-termini that carry functional effector domains. Despite their ubiquitous distribution amongst plant pathogenic oomycetes, little is known about their roles towards virulence. To unveil CRN effector function in Pc, we first used pre-existing models from other *Phytophthora* species, to identify 85 putative full-length CRN proteins. Collectively, the CRN complement carries 30 distinct effector domains, all of which are present in *P. infestans* and *P. sojae*. We used Microarrays, RT-PCR and sequencing to validate the gene models. To assess CRN effector functions, we cloned an array of C-terminal domains and localised GFP fusions *in planta*. All domains tested localised to the nucleus but distinct subnuclear localisation patterns were documented. To identify host targets, we are now employing yeast two hybrid and (Co-)immunoprecipitation approaches. These analyses are combined with nuclear protein enrichment experiments to unveil functions towards host targets. Here, we will present the latest results from our work.

The recombinant putative RxLR effector protein Htp3 from the fish pathogenic oomycete*Saprolegniaparasitica* is translocated into fish cells

Löbach L[1], Wawra S[1], DeBruijn I[1], Secombes CJ[2],Van West P[1]

[1] Aberdeen Oomycete Laboratory, University of Aberdeen, School of Medical Sciences, Foresterhill, Aberdeen, Scotland, UK.

[2] Scottish Fish Immunology Research Centre, University of Aberdeen, Aberdeen, Scotland, UK.

The fish pathogenic oomycete*Saprolegniaparasitica* causes the disease Saprolegniosis and is responsible for devastating infections of fish with huge economical significance in the aquaculture industry causing every year millions of loss. It is thought that proteins containing an RxLR-amino-acid motif – called RxLR-effector proteins – get secreted by this pathogen and play an important role in the infection. In the present study, a gene encoding a secreted putative RxLR-effector protein – Htp3 – was identified in the *S. parasitica* genome using bioinformatics. Gene expression analyses in the life stages and interaction of *S. parasitica* with the rainbow trout fibroblast cell line RTG-2 indicated that *Htp3* was highly expressed during the later stages of infection. Furthermore, a recombinant version of Htp3 was found to translocate *in vitro* into the RTG-2 cells. These findings suggest that the putative RxLR-effector Htp3 from *S. parasitica* could play an important role in the disease Saprolegniosis.

The *Phytophthora sojae* Effector Avr1d Encodes an RXLR-dEER Protein

Weixiao Yin[1],Suomeng Dong[1],Luchong Zhai[1],Shuyi Duan[1],Dan Yu[1],Xiaobo Zheng[1],Yuanchao Wang[1]

[1] College of Plant Protection, Nanjing Agricultural University, Nanjing, China

Root and stem rot of soybean is caused by the Oomycete *Phytophthora sojae*. The interaction between *P. sojae* and soybean follows the “gene-for-gene” model. Control of the disease based monogenic resistance encoded by resistance to *P. sojae* (*Rps*) genes is the most important and useful strategy. In this study, we identify an Avr effector *Avr1d* from *P. sojae* which can be recognized specifically by *Rps*1d in soybean. Avr1d encodes a predicted protein of 125 amino acids, with an RXLR-dEER protein translocation motif. Transient expression of *Avr1d* in soybean leaves revealed that its gene product could trigger a hypersensitive response (HR) in the presence of *Rps*1d. *Avr1d* was expressed at the germinating cysts stage. Interestingly, the *Avr1d* gene was absent in all tested virulent isolates, while alleles of this gene were amplified from the tested avirulent isolates and showed sequence polymorphisms.

Phosphatidylinositol 3-phosphate produced by *Phytophthora sojae* is important for infection by binding to RXLR effectors

Shan Lu*, Linlin Chen*, Kai Tao, Nannan Sun, Xiaoxue Lu, Yuanchao Wang, Daolong Dou

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

*, equal contribution

Oomycete pathogens, such as *Phytophthora sojae*, encode several hundred RXLR effectors to manipulate host immunity responses inside host cells. It has been shown that phosphatidylinositol-3-phosphate [PI(3)P] binds to host targeting motifs (HTMs) in some effectors to mediate their translocation into host cells, or conversely, binds to the C-terminal regions of effectors to stabilize them. However, the sources of PI(3)P in these interactions are still unclear. Here, we identified two phosphatidylinositol 3-kinase (PI3K) genes (*Ps129311* and *Ps136422*) in *P. sojae*, that encode enzymes that could generate PI(3)P. Both genes are up-regulated at the infection stages and silencing either gene leads to reduced virulence compared with wild-type strains. Transgenic *P. sojae* expressing a PI3P-binding FYVE domain or an *Arabidopsis thaliana* phosphatidylinositol phosphate 5-kinase (AtPIP5K1) also show reduced ability to infect soybean. Alternatively, treatment of *P. sojae* with LY294002, a PI3K-specific inhibitor, depresses its virulence level, though it does not alter resistance when soybean is treated. We also found that secreted FYVE domains fused with GFP accumulated within infection hyphae of *P. parasitica* when the fusion proteins were transiently expressed in *Nicotiana benthamiana*. Furthermore the accumulation level was weakened in the *PI3K*-silenced transformants or in LY294002-treated hyphae. Those results together suggest that *P. sojae* may produce PI(3)P to facilitate its virulence. Therefore, we took advantages of the high levels of PI(3)P on infected hyphae as an *in vivo* PI(3)P- binding assay. As expected, the full length of *P. sojae* RXLR effector Avr1b accumulated on infected hyphae to the same level as the FYVE domains. The deletion of the C-terminal region of Avr1b almost abolished accumulation of the GFP signal on infected hyphae, and mutations of RXLR or/and dEER motifs significantly reduced the levels. Those results indicate that the Avr1b can bind PI(3)P *in planta*, and this process is mediated by both N-terminal and C-terminal regions. Notably, we showed that expression of the secreted PI3P-binding FYVE domain or AtPIP5K1 gene in *N. benthamiana* could substantially increase the resistance of the leaves to *Phytophthora* infection, providing a novel and effective strategy for disease control.

The conserved oomycete RXLR effectors HaRxL96 and PsAvh163 exert virulence functions within the plant cell nucleus

Ryan G. Anderson[1], Megan S. Casady[1], Reed Bryant[1], Devdutta Deb[1], and John M. McDowell[1*]

([1]Department of Plant Pathology, Physiology and Weed Science, Virginia Tech, Blacksburg, VA, 24061, USA)

We are examining the interaction between *Arabidopsis* and its downy mildew pathogen *Hyaloperonospora arabidopsidis (Hpa*) to understand how plant cells are manipulated by oomycete pathogens. Bioinformatic analyses of the *Hpa* genome revealed at least 134 candidate RXLR effector genes. We are focusing on a pair of homologous effectors: *HaRxL96* and *PsAvh163* from *Hpa* and *Phytophthora sojae,* respectively. Transient assays indicate that both effectors suppress diverse elicitors of programmed cell death in soybean, including mammalian Bax and the *P. sojae* elicitor Avr4/6. In addition, transgenic *Arabidopsis* plants expressing either *HaRxL96* or *PsAvh163* partially suppress *RPP4*-mediated resistance to *Hpa* Emoy2 and are more susceptible to *Hpa* Emco5. *HaRxL96* and *PsAvh163* transgenic plants also suppress the callose response to avirulent bacteria, suggesting that both effectors can suppress PAMP-triggered immunity (PTI). PsAvh163 induces an HR-like cell death response in *N. benthamiana* that requires RAR1 and HPS90.3. Effector-YFP fusion proteins exhibit nucleocytoplasmic localization when transiently expressed in *Nicotiana benthamiana*. Genetic mislocalization experiments demonstrate that nuclear localization is required for suppression of PTI and effector triggered immunity (ETI), establishing the relevance of this subcellular address. Contrastingly, recognition of PsAvh163 in *N. benthamiana* is dependent on cytoplasmic localization. These experiments suggest that these homologous effectors from distantly related oomycetes can suppress defense mechanisms from within the host nucleus.

Conserved Effector Proteins from Oomycetes *Hyaloperonospora arabidopsidis* and *Phytophthora sojae* Suppress Immune Responses in Plants

Devdutta Deb[a], Ryan G. Anderson[a], Theresa How-Yew-Kin[a] Kevin Fedkenheuer[a], Brett M. Tyler[b] and John M. McDowell[a]

([a] Department of Plant Pathology, Physiology and Weed Science, Virginia Tech; [b] Center of Genome Research and Biocomputing, Oregon State University)

Effector proteins are exported to the interior of host cells by numerous plant pathogens. Effector proteins have been well characterized in bacteria. Contrastingly, their functions and targets in oomycete pathogenicity are poorly known. Bioinformatic analysis of genome sequences from oomycete pathogens *Phytophthora sojae*, *P. ramorum, P. infestans* and *Hyaloperonospora arabidopsidis (Hpa)* have led to the identification of a large number of candidate effector genes. These effector genes have characteristic motifs (peptide, RxLR and dEER) that target the effectors into plant cells. Although these effector genes are very diverse, certain genes are conserved between *P. sojae* and *H. arabidopsidis*, suggesting that they play important roles in pathogenicity. The goal of my project is to characterize a pair of conserved effector candidates, Ha23 and Ps73 from *Hpa* and *P.sojae* respectively. We hypothesize that these effectors have important conserved roles with regard to infection. The primary objectives of my research center on identifying effector functions and *in planta* targets using both transient assays and stably transformed plants. *Ha23* is expressed early during the course of *Hpa* infection of *Arabidopsis*. Ha23 triggers an ecotype-specific defense response in *Arabidopsis*, suggesting that it is recognized by host surveillance proteins. Ha23 and Ps73 can suppress immunity triggered by pathogen associated molecular patterns (PTI) and by effectors (ETI) *in planta.* Ha23 and Ps73 enhance bacterial virulence in *Arabidopsis* when delivered by the Type III secretion system. Both effectors localize to the nucleus and cytoplasm of plant cells. Experiments with transgenic *Arabidopsis* expressing Ha23 and Ps73 also suggest suppression of immunity triggered by pathogen associated molecular patterns, enhancement of bacterial and oomycete virulence and suppression of defense gene induction. Addition of a Nuclear Localization Signal (NLS) abolishes the ability of both effectors to suppress PTI and ETI, indicating that cytoplasmic localization is essential for functionality. Additionally, we have evidence that suggests similarities between Ha23 and the conserved bacterial effector protein AvrE. Their predicted protein structures show common regions of overlap. Both induce cell death in wild type *Arabidopsis* young plants, suppress PAMP-triggered callose deposition and finally Ha23 and Ps73 can complement the reduced bacterial speck phenotype of the avrE mutant *in planta*.

Fungal effectors contain RxLR variants that are responsible for entry of plant cells

Biao Gu[1, 2], Shiv D. Kale[2], Daolong Dou[3], Brett M. Tyler[2], Weixing Shan[1*]

([1]Northwest A&F University, Yangling, China 712100; [2]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA, USA 24061; [3] Nanjing Agricultural University, Nanjing, China 210095)

The genome-wide prediction of secretome in several plant fungal pathogens revealed that the proportion of secreted proteins reaches ~10% of total encoded proteins (Lowe et al., 2012). These diverse secreted proteins, including known effector proteins, are employed by pathogens, especially obligate biotrophic fungi, to interrupt cell metabolism and disable immunity system of host plant. The predicted intracellular locations of resistant gene products imply that the corresponding effectors should be recognized in the cytoplasm of plant cells. However, the canonical protein transduction motifs, such as RxLR motif in oomycete effectors, were not identified in fungal effectors. The mechanism of entry of fungal effectors into plant cells is not known yet. Based on an extensive investigation of the diversity of oomycete RxLR motif, we defined several RxLR-similar motifs in cloned fungal effectors, such as AvrP123, AvrP4, AvrL567 and AvrM of *Melampsora lini*; AvrPita from *Magnaporthe oryzae* and a secreted protein Ps87 from *Puccinia striformiis* f. sp. *tritici*. A protein transduction assay was used to determine if these effectors' N-terminal motifs fused to Avr1b or GFP could enter the host cells by utilizing the host transduction machinery. All the N-termini of tested fungal effectors were capable of carrying reporter into plant cells. Mutation in RxLR functional variant of AvrL567 blocks entry of recombined protein. Moreover, the N-termini of fungal effectors showed RxLR variant-dependent affinities to phosphatidylinositol-3-phosphate (PI3P) and/or phosphatidic acid (PA). The similarities of function and positioning between oomycete and fungal host-targeting signals suggest that the eukaryotic plant pathogens may share same plant encoding machinery for effector translocation. Whether PA has a role in effector translocation is still under determination.

Polymorphism of the RXLR effector genes *Avr3a*, *Avr4* and *ipiO* in *Phytophthora infestans* isolates from China

Miao Han[a,b] Liyun Guo[a]

([a] Dept. of Plant Pathology, China Agricultural University, Beijing, 100193, China. [b] Lab. of Phytopathology, Wageningen University, the Netherlands. E-mail: miao.han2011@gmail.com. L.Y. Guo, Dept. of Plant Pathology, China Agricultural University, Beijing, 100193, China. E-mail: ppguo@cau.edu.cn, ppguoly@126.com)

Late blight caused by *Phytophthora infestans* is one of the most important diseases of potatoes worldwide. Growing usage of resistant cultivars is a major late blight control measurement. However, late blight resistance is frequently overcome by adaptation of the pathogen. Study of the interaction between *Phytophthora* spp. and its hosts revealed the importance of RXLR effectors in plant infection and colonization. RXLR effectors can act as avirulent factors when recognized by their cognate resistant proteins. In the past decades, a number of RXLR effector genes, such as *Avr3a, Avr4, ipiO,* have been identified in *Phytophthora infestans*. So far, little is known about the distribution of RXLR effector genes in the Chinese *P. infestans* population. In this study, we investigated the distribution of *Avr3a*, *Avr4* and *ipiO* among 52 *P. infestans* isolates collected from major potato producing areas in China. Only 4 of them contain Avr3a (KI) allele which is recognized by R3a, whereas all others contain avr3a (EM), the variant which is no longer detected by R3a. No new alleles of *Avr3a* were detected. *Avr4* was found in all isolates to encode truncated protein. These results indicated that virulent *avr3a* and *avr4* predominately occur in the Chinese *P. infestans* population. Previous studies showed that *P. infestans* isolates lack class I *ipiO* are virulent on *Rpi-blb1* potatoes. Class I variants were present in all the analyzed isolates, except one from Sichuan, which contains only the class III variant *ipiO4*, which cannot trigger *Rpi-blb1* mediated cell death. In addition, several new *ipiO* alleles were identified, functional identification of these new alleles are still in process.

In planta transient expression identifies effector proteins from *Phytophthora parasitica* that induce plant cell death

Yuling Meng[1], Meixiang Zhang[2], Biao Gu[1], Guiyan Huang[2], Qiang Zhang[1], Meng Zhang[1], Junli Quan[1], Weixing Shan[1*]

([1] State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China; [2] State Key Laboratory of Crop Stress Biology in Arid Areas and College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China. * Correspondence to wxshan@nwsuaf.edu.cn)

The genus *Phytophthora* cause enormous economic losses, and *Phytophthora parasitica* is a soilborne pathogen infecting over 60 plant families. *Phytophthora* species secrete large number of effector proteins that facilitate plant infection and colonization. Also plants respond to infection using different immune systems, usually, a hypersensitive cell death response at the infection site. Since most effector proteins of oomycetes fulfill their functions *in planta*, some assays have been developed such as agroinfiltration which allows rapid functional expression of pathogen genes in plants. In this research, we aimed at determination of host specificities between *P. parasitica* and tobacco and identification of effector proteins that induce the plant cell death. Twenty-three strains of *P. parasitica* were tested for virulence on twelve tobacco cultivars by inoculating detached leaves. A high quality cDNA library was constructed using mRNAs derived from tobacco leaves infected with three *P. parasitica* strains which have great difference in virulence spectrum. The library was transformed into *Agrobacterium tumefaciens* AGL1 via triparental mating. We employed a high-throughput *Agrobacterium*-mediated transient expression assay on tobacco leaves to identify effectors. A screen of 10000 cDNAs led to the identification of 12 candidate effectors that have HR-inducing activities.

Identifying essential effectors from the soybean pathogen *Phytophthora sojae*

Hua Wise[1,†], Ryan G. Anderson[1,†], Brett M. Tyler[2], and John M. McDowell[1]
([1] Department of Plant Pathology, Physiology and Weed Science, Virginia Tech; [2] Center for Genome Research and Biocomputing, Department of Botany and Plant Pathology, Oregon State University; † Equal contributors)

Breeding for resistance to plant pathogens is one of the most effective means of disease control. However, the ability of plant pathogens evolve new pathogenicity factors and evade host defense mechanisms drives the continual necessity to identify new resistance genes. We are exploiting genomic technologies in an effector-directed breeding approach that augments traditional breeding efforts against *Phytophthora sojae*, the casual agent of soybean root and seedling rot. This approach is founded on identifying monomorphic *P. sojae* effector genes that are essential for virulence, and using these genes as probes to identify new sources of resistance in soybean and related legumes. Our goal is to identify 10 essential RXLR effector genes. These essential effectors will make excellent candidates for screening for new, durable resistance to *P. sojae*, as these genes cannot be mutated or deleted without a significant fitness penalty. The majority of predicted *P. sojae* RXLR effector genes are polymorphic amongst three sequenced isolates of P. sojae, however, a subset of *P. sojae* RXLR effectors displays little or no allelic diversity. We have established a workflow for transient gene silencing and quantitative virulence assays. To date, we have assessed the virulence contribution of six *PsAvh* genes. Previously, two effectors, PsAvh240 and PsAvh180, have been shown to suppress PAMP-triggered immunity (PTI) and effector triggered immunity (ETI) in *Nicotiana benthamiana* (Wang, et al. 2011). We silenced these genes and observed significantly reduced pathogen growth at early stages of host colonization and reduced disease symptoms at later stages of infection. These results suggest that PsAvh240 and PsAvh180 contribute to overall pathogen fitness, likely through early suppression of host PTI responses. Silencing of PsAvh81 had a weak effect on pathogenicity, while strains in which PsAvh109 was silenced were fully virulent. In sum, our initial screens have yielded two promising candidates for screening, and additional genes are currently being targeted.

Genomics

Transgenerational Gene Silencing of the *Phytophthora sojae Avr3a* Effector is Associated with the Presence of Discrete small RNA Molecules

Dinah Qutob, B. Patrick Chapman, Mark Gijzen

(Agriculture and Agri-Food Canada, London, ON)

Avirulence (*Avr*) genes of plant pathogens encode effector proteins that trigger immunity in plants that carry appropriate resistance (*R*) genes. The identification of *Avr* genes in plant pathogenic oomycetes has enabled further work on their expression and inheritance. The *Phytophthorasojae Avr3a* gene displays allelic variation in mRNA transcript levels. *P. sojae* strains with detectable *Avr3a* gene transcripts are avirulent on plants carrying the *R*-gene *Rps*3a, whereas strains with loss of transcript (gene silenced) alleles of *Avr3a* escape detection by *Rps*3a and are virulent. However, the inheritance of all known alleles of *Avr3a* has not been tested. We created new crosses between natural isolates of*P. sojae*and discovered that DNA markers for *Avr3a* segregate normally but remarkably we observed non-Mendelianpatterns of inheritance when progeny were tested for the presence of *Avr3a* mRNA transcripts and virulence phenotypes on *Rps*3a soybean plants. We performed deep sequencing of small RNA (sRNA) and identifiedsRNA molecules of 25 nt, matching to the *Avr3a* gene region, that are abundant in gene silenced strains but not in strains with *Avr3a* mRNA. We propose a model whereby sRNA molecules direct epigenetic changes that control the expression state of the *Avr3a* gene in *P. sojae*.

Integrating Oomycete Structural and Functional Genomics Datasets into FungiDB

Sucheta Tripathy[1], Raghuraman Ramamurthy[2], Daniel Borcherding[2], Steve Fischer[4,5], John Brestelli[4,5], Deborah F. Pinney[4,5], Wei Li[4,6], Sufen Hu[4,6], Omar S. Harb[4,6], Brian P. Brunk[4,6], Jessica C. Kissinger[7,8], Christian J. Stoeckert Jr.[4,5], João C. Setubal[1,9], David S. Roos[4,6], Jason E. Stajich[2] and Brett M. Tyler[1,3]

([1] Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA-24061; [2] Department of Plant Pathology & Microbiology, University of California, Riverside, CA 92521, MT; [3] Center for Genome Research and Biocomputing Department of Botany and Plant Pathology Oregon State University Corvallis, OR 97331; [4] Penn Center for Bioinformatics; [5] Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104; [6] Department of Biology, School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA 19104; [7]Center for Tropical & Emerging Global Diseases; [8] Department of Genetics and Institute of Bioinformatics, University of Georgia, Athens, GA 30602, USA and [9] Department of Biochemistry, Institute of Chemistry Universidade de São Paulo, São Paulo, SP 05508-000 Brazil)

Oomycete genomes and functional data are currently being integrated into the fungal database resource, FungiDB www.fungidb.org (Stajich et. al., Nucl. Acids Res. 2012), which uses the NIH-funded EuPathDB platform (www.eupathdb.org). To date 6 oomycete genomes (*Phytophthora sojae*, *Phytophthora ramorum*, *Phytophthora infestans*, *Phytophthora capsici*, *Hyaloperonospora arabidopsidis* and *Pythium ultimum*) have been uploaded into the database. The gene models include computationally assigned annotations as well as existing user annotations (i.e. manually curated effector genes from *P. sojae*, *P. ramorum* and *H. arabidopsidis*). Other user-curated genes from various families such as PLD, PDR for *P. sojae* and *P. ramorum* have also been merged into the list. RNAseq data from *P. sojae*, *P. ramorum*, *H. arabidopsidis* are included in the current release. The powerful graphical user interface of EupathDB (Fischer et. al., Database, 2011), is designed to facilitate the construction of *in silico* experiments (search strategies) based on combining results from a variety of data. The ability to save and share search strategies with others and a plethora of other search options makes the system very useful for the user community. In addition, cross-species genome comparisons among related species can be displayed in the genome browser makes the system uniquely suited for oomycete genomics research. In the future additional available oomycete genomics and functional datasets will be integrated into FungiDB from various sources such as JGI, JCVI, Broad Institute, BGI and individual researchers, making it a comprehensive oomycetes data warehouse.

Whole Genome Gene Discovery in the Pathogenic Oomycete *Pythium insidiosum* by 454 Sequencing Technology

Theerapong Krajaejun[1], Rommanee Khositnithikul[1], Tassanee Lerksuthirat[3], Tassanee Lowhnoo[2], Thidarat Rujirawat[2], Wanta Yingyong[2], Prapat Suriyaphol[4], Sittichoke Tangphatsornruang[5], Gagan Garg[6] and Shoba Ranganathan[6]

([1] Department of Pathology and [2] Research Center, Faculty of Medicine, Ramathibodi Hospital, [3] Molecular Medicine Program, Multidisciplinary Unit, Faculty of Science, [4] Bioinformatics and Data Management for Research, Office for Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok; [5] Genome Sequencing Laboratory, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathumthani, Thailand; [6] Department of Chemistry and Biomolecular Sciences, Macquarie University, Australia)

Oomycetes are unique eukaryotic microorganisms that share a mycelial morphology with fungi. Many oomycetes are pathogenic to plants, but just a few species are pathogenic to animals. *Pythium insidiosum* is the only oomycete that can infect both humans and animals, and causes a can be fatal infectious disease, pythiosis. Most pythiosis patients have life-long handicaps, as results from the inevitable radical excision of infected organs. Many patients die from advanced infection. Better understanding *P. insidiosum* biology and pathogenesis at molecular levels could lead to new forms of prevention and treatment. Genetic and genomic information is lacking for *P. insidiosum*, so we have undertaken a genome-scale expressed sequence tag (EST) study by using the 454-format next generation sequencing technology, and report on 2 genome-scale datasets of (i) 191,311 ESTs (from *Pythium* growth at room/environment temperature [RT]), assembled into 17,174 unigenes (range, 40-9,506 nt; contigs = 10,589 and singletons = 6,585); and (ii) 204,241 ESTs (from *Pythium* growth at body temperature [BT]), assembled into 18,367 unigenes (range, 40-7,239 nt; contigs = 11,938 and singletons = 6,429). Open reading frames (predicted proteins) can be identified in 93% of RT ESTs and 95% of BT ESTs. By BLAST searching through the NCBI database, 12,634 (74%) and 13,432 (73%) of RT and BT ESTs, respectively, had significant hits (E-value < -5). From the combined RT-BT EST dataset, 1,036 and 650 proteins were predicted to be secretory and transmembrane proteins, respectively. DrugBank search showed that 178 proteins can be targets of available drugs, while SPAAN analysis showed that 17 proteins can potentially be vaccine candidates. Simitri genetic comparative analysis revealed, as expected, overall *P. insidiosum* proteins were closely related to other oomycetes than to those proteins of parasitic and fungal pathogens. Approximately 2,000 proteins of *P. insidiosum* significantly matched virulence factors of other pathogens in MvirBD. The EST dataset reported here represents a crucial step in identifying *P. insidiosum* genes that might involve in pathogensis and important biological processes. This genetic information will also facilitate understanding of pathogenic mechanisms of this devastating pathogen, and discovering new targets for more effective treatments of pythiosis.

Computational biology drives the understanding of animal pathogenesis in oomycetes

Rays H.Y. Jiang[1], Brian J. Haas[1], Sean Sykes[1], Broad Genome Annotation Group[1], Sarah Young[1] , Irene De Bruijn[2], Pieter van West[2], Chad Nusbaum[1], Brett Tyler[3] and Carsten Russ[1]

([1] Broad Institute, Cambridge, MA 02142, USA; [2] University of Aberdeen, Aberdeen AB25 2ZD, UK; [3] Virginia Bioinformatics Institute, Blacksburg, VA 24061, USA)

Oomycetes include destructive pathogens of an enormous range of terrestrial plants, as well as a wide variety of aquatic animals such as fish, amphibians, insects and crustaceans. Animal pathogenic oomycetes cause major losses to aquaculture and damage to aquatic ecosystems. We annotated the 53 Mb *Saprolegnia parasitica* genome using ab initio methods and RNA-seq data from multiple life stages. Comparison of the genome of *S. parasitica* to other oomycetes reveals that different host cellular environments have distinctly shaped the evolution of plant and animal pathogen genomes. *S. parasitica* lacks the effector reservoir in plant pathogens but possesses one of largest repertoires of proteases and lectins. We have successfully assembled transcriptomes from 8 species of Saprolegniales by using recently developed algorithms directly from RNA-seq; and resolved several pathogenesis related gene family evolution pattern. We have also shown for the first time that it is feasible to construct a pathogen-host interactome in a genome free fashion. Distinct sets of host and pathogen genes related to infection have been discovered in the interactome.

Bioinformatics analysis of fungi genome

Wenkui Dai, Peixiang Ni

(Beijing Genomics Institute at Shenzhen (BGI-Shenzhen), China. daiwenkui@genomics.cn)

With the breakthrough of the new-generation sequencing technology which results in lower costs, higher throughout and superior accuracy, we can obtain more and better informations about the fungi genomes. BGI has completed and is undertaking some projects about fungi such as *Verticillium dahlia*, *Fusarium oxysporum*, *Metarhizium anisopliae, Metarhizium acridum*, *Puccinia striiformis* etc. Generally, there are two analysis ways for fungi genome: *denovo* analysis and re-sequencing analysis. The main contents are showed as follow, respectively.

***D novo* analysis:**

1. *Denovo* sequencing: We construct libraries with different insert size and adopted the paired-end strategy for fungal genome sequencing.
2. Assembly: An in-home assembler SOAPdenovo is used to assemble the paired-end reads. Consider assembled scaffold size, genome size estimation by reads, k-mer, depth distribution.
3. Annotation: Firstly, we *ab initio* predict the genes by the programme packages GeneMark-ES or Augustus, and we could merge all these genes to obtain the relatively complete gene set. We also predict repeat sequences, and non-coding RNA. Based on those genes, we perform the functional annotation by homologous alignment through blastp to some database such as COG, KEGG, Nr, PHI, CAZyme, TransMemPort, InperproScan.

Re-sequencing analysis

1. Sequencing: Different insert size libraries also are constructed, but we need less data production in comparison with *denovo* process.
2. Sequencing depth distribution and genome coverage calculation.
3. Consensus sequences assembly according to the reference genome.

If related species were given in the *denovo* analysis, both ways could perform the genome comparison to identify structure variation and the homologous relationships with the references genome, and we also do the SNP and Indel calling, segmental duplication, rearrangement with the references, and gene evolution and selection, etc.

Genetic, Genomic, and Transcriptomic Analysis of the Cucumber-Downy Mildew (*Pseudoperonospora cubensis*) Interaction

Brad Day[1,2], Elizabeth Savory[1] and Alyssa Burkhardt[2]

([1]Department of Plant Pathology, Michigan State University, East Lansing, MI 48824 USA;

[2]Program in Cell and Molecular Biology, Michigan State University, East Lansing, MI 48824 USA.)

Cucurbit downy mildew, caused by the oomycete pathogen *Pseudoperonospora cubensis*, is a major limiting factor of cucurbit production throughout the world and is of particular concern to cucumber growers. We have generated a draft (150x coverage) sequence of the ~88.22 Mb genome sequence of *Ps. cubensis* using next-generation sequencing technology, coupled with RNA-Seq to establish transcriptomic resources of both *Ps. cubensis* and *Cucumis sativus* during infection. From these resources we have identified ~270 candidate effector proteins from *Ps. cubensis* as well as additional pathogenicity factors that are up-regulated during infection. Interestingly, the effector complement of *Ps. cubensis* does not seem to be limited to the typical RXLR motif that is found in other sequenced oomycete pathogens. Subsequent analysis of the predicted effector complement of *Ps. cubensis* and comparison to *Phythopthora infestans*revealed limited orthology between the two species, and a number of *Ps. cubensis* effectors with non-effector orthologs in *P. infestans.*Finally, transcriptome analysis of both *Ps. cubensis* and *C. sativus* at multiple time points during infection reveals an interplay of transcriptional gene regulation between pathogen and host. The genome, transcriptome and host-pathogen interaction will be discussed, as well our recent identification of alternative splicing associated with the generation of effector proteins.

Identification and Characterization of tRNA-derived Small RNAs in the Oomycete Pathogen *Phytophthora sojae*

Qinhu Wang[1], Ke Xu[1], Wei Zhang[1], Xiaolong Wang[1,3], Meixiang Zhang[1], Guangjin Fan[1], Weibo Jin[1], Junli Quan[2], Weixing Shan[2*]

([1] State Key Laboratory of Crop Stress Biology in Arid Areas and College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China; [2] State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China; [3] Present address: Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, Freiburg 79104, Germany. * Correspondence to wxshan@nwsuaf.edu.cn)

Small non-coding RNAs are critical for the regulation of post gene transcription of eukaryotes. Oomycetes are fungus-like eukaryotic microorganisms that cause severe losses to agricultural production and damage to ecosystems. However, little is known on the presence and diversity of small RNAs in the oomycete pathogens. In this study, we identified the tRNA-derived small RNAs (tsRNAs) in the soybean pathogen *Phytophthora sojae*, by deep sequencing and classical small RNA cloning. And the presence of oomycete tsRNA is confirmed by Northern. The tsRNAs were selectivity cleaved and conserved within *Phytophthora*. We consistently showed a negative correlation between the accumulation of tsRNAs and their target transcripts by profiling tsRNAs and quantitative analyses of their target transcripts. Functional annotation further revealed that tsRNAs might be involved in the initiation of plant infection and may play an important role in maintaining genome stability in *P. sojae*. The results demonstrated the presence of functional endogenous small noncoding RNAs in oomycetes and further expanded the small RNA families, shedding light on the small RNA biology and epigenetic mechanisms in the eukaryotic pathogens.

Gene duplication and fragment recombination drive functional diversification of a superfamily of cytoplasmic effectors in *Phytophthora sojae*

Danyu Shen, Tingli Liu, Wenwu Ye, Li Liu, Peihan Liu, Yuren Wu, Suomeng Dong, Yuanchao Wang, Daolong Dou*

(Department of Plant Pathology, Nanjing Agricultural University, Nanjing, China)

The genus *Phytophthora*, containing over 100 species, belongs to the oomycetes which share little affinity with true fungi but are instead more closely related to diatoms and brown algae within the stramenopiles or heterokonts. *Phytophthora* and other oomycetes secrete a large number of effector proteins that facilitate infection and colonization. A well-characterized family of cytoplasmic effectors was first discovered in *Phytophthora infestans* as proteins that induced cell death in *Nicotiana benthaminana*, and were termed “crinkling- and necrosis-inducing proteins” (CRN) or “crinklers”. Their functions and mechanisms in pathogenesis are mostly unknown. Here, we investigated the evolution of the CRN gene family in *Phytophthora sojae*, a pathogen that causes soybean root and stem rot, deploying the method of bioinformatics. Our comprehensive analysis of the CRN family reveals three major findings. First, the CRN family was expanded after the divergence of *Phytophthora* species and has evolved in a species-specific manner, via a rapid birth-and-death mechanism. Around two-thirds of the CRN family, with mostly intact genes and higher gene expression values, appear to be most actively involved in the evolution of the family in *P. sojae*. Second, one half of CRN genes were identified to undergo gene duplication, followed by positive selection, which contributed to the expansion of CRN family. Meanwhile, frequent fragment recombination events also occurred, leading to diverse sequence structures. The above two evolution mechanisms appear to account for most of the expansion of the CRN family in *P. sojae*. Third, the CRN effectors, which underwent gene duplication and recombination, resulted in functional diversification. Altogether our data lead us to propose a new model for the evolution of the CRN family in *P. sojae*, which aids in understanding the roles of CRN effectors within each oomycete.

Using Next Generation Sequencing as a diagnostic tool for *Phytophthora ramorum* and *Pythium ultimum*

Andres S. Espindola[1], Jacqueline Fletcher[1], William L. Schneider[2], and Carla Garzon[1]
([1] Oklahoma State University; [2] United States Department of Agriculture)

Molecular biology tools, like immunoassays and nucleic acid based analyses, have permitted a high degree of sensitivity and specificity in diagnostics. Nevertheless, the emergence of new strains of pathogens, principally among those reproducing sexually, may decrease the efficacy of currently available diagnostic tests. This problem emerges from the single target dependence of these methods, meaning that they target either a specific protein motif or a specific gene or locus. Although *Phytophthora ramorum,* the causal agent of Sudden Oak death and Ramorum blight has two different mating types found both in Europe and in the U.S., sexual reproduction may be rare. Nonetheless, its potential to increase genetic diversity may represent an obstacle to sensitive and accurate detection. *P. ramorum* diagnosis and strain characterization is a time demanding multi-step process that can delay crucial trading decisions, with severe economic impact for the Ornamental and Forestry Industries. *Pythium ultimum* is a oomycete soilborne plant pathogen with universal distribution and broad host range. *P. ultimum* has been used as a model system for genetic studies and is the only *Pythium* species whose genome has been characterized to date.

The advent of new molecular technologies that allow rapid gathering of data is changing the way that molecular diagnostics are applied. Particularly, the high amounts of data generated by next generation sequencing (NGS) requires researchers to devote significant amounts of time to data analysis, using clustered processors and bioinformatics tools. Furthermore, the analyses of raw data can be time consuming and expensive, in terms of software and labor costs. The use of raw sequencing data to detect pathogen sequences would reduce the computational load and provide a rapid and accurate approach to plant disease diagnoses.

The aim of this research was to develop bioinformatics tools along with NGS technology to detect the presence/absence of plant pathogens in a single assay. The methodology is termed Electronic Probe Diagnostic Nucleic acid Analysis (EDNA). Electronic probes (e-probes) were designed for each pathogen with different lengths (20 nt to 140 nt), compared to a near relative, and reverse blasted against raw NGS metagenomic databases from infected and healthy host plants. To estimate the relative efficiency of EDNA, e-probes were blasted against mock sample sequencing databases (MSSDs). Using a bioperl environment in Linux with the Bio::Search module, the amount of hits and matches produced by BLASTn-searches was assessed. *P. ramorum* and *P. ultimum* were detected utilizing EDNA in MSSDs using bioinformatics tools and low computer processing. Simulations of 454 sequencing output data (MSSDs) permitted to set the detection limit to as low as 0.01% of pathogen read abundances for both *P. ramorum* and *P. ultimum*.

Validation of the protocol was conducted with empirical data from *P. ultimum* infected and healthy host plants. Each assay started with total nucleic acid extraction and whole genome/whole transcriptome amplification followed by 454 sequencing. Sample sequencing databases (SSDs) were obtained and analyzed using sets of unique e-probes designed for *P. ultimum.* The total number of e-probes were as high as 270,456 for 20nt long e-probes. E-probes 80bp long were determined as the most informative probe size. Statistical analyses based on e-values and percent identities of blastn-searches of e-probes against the SSDs permitted to corroborate or deny the presence of the target pathogen. *P. ultimum* SSDs produced 61 million bases clustered in 145,922 sequencing reads with an average read length of 423.03 bp. The BLASTn-search produced from 1,070,606 ($1x10^{-3}$ e-value) to 70,250 ($1x10^{-9}$ e-value) hits using all e-probes. To date, the EDNA protocol was able to detect the *P. ultimum* in potato with a detection limit of 0.158% pathogen sequence abundance. Detection specificity ranged from 99.76% to 100% depending on e-probe length. In conclusion, *P. ultimum* can be detected with high sensitivity and specificity using EDNA from infected host plant metagenomic data.

Cloning and Prokaryotic Expression of Pectate Lyase in *Pythium ultimum*

Feifei Fan[1,2], Changsong Li[1], Junshan Qi[1], Lin Li[1], Bo Zhang[1], Yueli Zhang[1],Shihong Zhang[2]

([1] Plant Protection Institute, Shandong Academy of Agricultural Sciences,Jinan, 250100, PR China; [2] College of Plant Science, Jilin University,Changchun, 130062, PR China)

When pathogenic *Pythium* infects the host, it always results in some rotten symptoms on the host ,mainly because a variety of hydrolytic enzymes including pectate lyase with the function of degradating cell wall can be produced in the pathogenic process, and the cell wall will lose its supporting role once hydrolyzed. Due to the effection of pectate lyase, the pectin one of the components of cell wall will be degradated, which will result in losing support from the cellulose structure of the host tissues, and display a soft rot symptom. *Pythium ultimate* has a wide host spectrum, and it will result in basal stem rot when interacting with tomato. In our research, we culture *Pythium ultimate* under the condition of with the present of Inducing pectin and secreted pectate lyase was detected in the medium in order to study the the character of the pectate lyase secreted by *Pythium ultimate*. We obtained a pectate lyase sequences with 800bp from *Pythium ultimate* by PCR cloning, then construct a prokaryotic expression vector *pEASY*-pel carrying the sequence of pectate lyase, then transform it into the competent cell of *E.coli* BL21. We get an experssed target protein with approximately 26 kD induced by IPTG, that demonstrates the pectate lyase gene is successfully expressed in *E.coli*. In the following research, we anticipate that a deletion mutation will be taken, and comband with fluorescence quantitative determination of the expression of pectate lyase from *Pythium ultimate* in the pathogenic process, to determine the function of pectate lyase during the infection process.

In vitro antimicrobial effect of the volatile organic compounds from *Muscodor crispans* against the human pathogenic oomycete *Pythium insidiosum*

Krajaejun T[1], Lowhnoo T[2], Rujirawat T[2], Yingyong W[2], Fucharoen S, Strobel GA

([1] Department of Pathology and [2] Research Center, Faculty of Medicine, Ramathibodi Hospital, [3] Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Bangkok, Thailand; Department of Plant Sciences, Montana State University, Bozeman, MT, USA)

Oomycetes are unique eukaryotic microorganisms that share mycelial morphology with fungi. Many oomycetes are pathogenic to plants, and a limited number are pathogenic to animals. *P. insidiosum* is the only oomycete that is capable of infecting humans, and causes a life-threatening infectious disease, called pythiosis. Conventional antifungal drugs are ineffective against *P. insidiosum* infection. Most of pythiosis patients had life-long handicaps from inevitably radical surgery of infected organs. Many patients die from uncontrolled and aggressive infection. Finding an effective non-invasive way of treatment for pythiosis is urgently needed. In recent years, some organic compounds extracted from entophytic fungi have been reported to have antimicrobial effects against a variety of pathognes. Among them is a mixture of volatile organic compounds (VOCs) from *Muscodor crispans*, a novel endophytic fungus of *Ananas ananassoides* (wild pineapple). The VOCs of *M. crispans* demonstrate a broad antimicrobial effect against human and plant pathogens, including fungi, bacteria, and oomycetes. Here, we investigated *in vitro* effect of the VOCs (at 2.5, 5.0, 10.0, 20.0 or 40.0 µL) against 26 clinical and 4 environmental isolates of *P. insidiosum*, using an agar plate assay. All *P. insidiosum* isolates were completely inhibited with 10.0 µL or more of the VOCs, while the VOCs of at least 20.0 µL killed all isolates. The inhibitory and lethal effects of the *M. crispans* VOCs were dose dependent. In conclusion, The VOCs of *M. crispans* can effectively kill *P. insidiosum*, and it could prove to be useful for treatment of pythiosis.

Loss of heterozygosity and adaptation in *Phytophthora*

Kurt Lamour[1] and Joann Mudge[2]

([1]Department of Entomology and Plant Pathology, The University of Tennessee, Knoxville, Tennessee, USA, [2]National Center for Genome Resources, Santa Fe, New Mexico, USA)

The development of a reference genome and a dense Single Nucleotide Polymorphism (SNP) based linkage map for the vegetable pathogen *Phytophthora capsici*revealed large scale mitotic genetic variation in the form of loss of heterozygosity (LOH). Of the >20K SNPs with normal Mendelian segregation, >11K were impacted by LOH. Contiguous tracts of LOH loci ranged from 300bp to >1Mbp and were associated with a mating type switch (A2 to A1) and loss of pathogenicity. The extent and impact of LOH for field isolates maintained in culture and sexual progeny will be overviewed and the potential functional implications discussed.

Transcriptomic Analysis of a Mid-winter Algal Bloom in Lake Erie Provides Insight on Adaptations to Psychrophilic and Low-light Environments and the Role of Pathogenic Oomycetes

Robyn Edgar Paul Morris, Vipa Phuntumart, George Bullerjahn and Robert Michael McKay
(Department of Biological Sciences Bowling Green state University, Bowling green OH 43403 pmorris@bgsu.edu)

In 2009, samples from a mid-winter algal bloom were collected with bias to filamentous diatoms using a 153µ vertical net tow to a depth of 15m. The algal bloom population was predominately Aulacoseira sp. with notable presence of Stephanodiscus sp. and Fragilaria sp. cDNA was produced from the samples and sequenced using 454. After assembly using MIRA 3.0, the resulting metagenomic library was 11,576 contigs. BLAST analysis against the NCBI non-redundant nucleotide and protein databases, and NCBI's environmental protein and nucleotide databases revealed that ~56% of the assembled contigs had relevant hits (E-value < 1xE-10) and ~60% of those had best hits to algal genomes. The assembled library was also blasted against the *Phytophthora sojae* version 3.0 (predicted proteins and genes from JGI), *Phytophthora infestans (*proteins from the BROAD), AphanoDB, *Saprolegnia parasitica* (proteins from the BROAD), and *Pythium ultimum (*proteins). This analysis identified an additional ~300 sequences with stronger homology to oomycetes than anything in NCBI's databases and ~10% of these hits were secreted proteins. The surprising abundance of organisms that are known to be major pathogens in terrestrial ecosystems suggests that they may be playing a similar role in the annual decline of this bloom.

Differentially expressed microRNAs in soybean during *Phytophthora sojae* infection

James T. Wong and Wenbo Ma

(Department of Plant Pathology and Microbiology,University of California, Riverside, CA 92521 USA)

MicroRNAs (miRNAs) have essential regulatory roles in many plant processes including development, metabolism and responses to abiotic and biotic stresses. Studies have shown that specific miRNA expression changes are induced in plant hosts upon interaction with pathogens. Some miRNAs are involved in plant innate immunity against pathogen infections and potentially contribute to disease resistance. Viral and bacterial pathogens have evolved effector proteins to manipulate host microRNA pathways in order to suppress defense and facilitate disease development. To date, the functions of these small RNA in plant interactions with oomycete pathogens remains largely unknown. To fill this gap in our knowledge, we analyzed small RNA populations in soybean upon infection with *Phytophthora sojae*. Our central hypothesis is that *P. sojae* infection will trigger expression changes in specific small RNA species, which may play a role in plant immunity by regulating their respective target genes.

To examine miRNA changes in soybean, total RNA are isolated from *P. sojae* infected and mock-infected soybean root tissue. Following high throughput Illumina sequencing and bioinformatic analysis, we have identified thirteen known soybean miRNAs and approximately twelve potentially novel soybean miRNAs that are differential expressed in the *P. sojae*-infected soybean roots. Of these miRNAs, eleven were experimentally verified to differentially express when infected with *P. sojae*. These miRNAs are likely to have potential roles in soybean defense response. Our findings suggest that some miRNAs, including miR393, may have a conserved function in protecting plants from infection of both bacterial and fungal/oomycete infection. Current progress is underway to elucidate the molecular mechanism underlying the functions of selected miRNAs during soybean interaction with *P. sojae*.

Cell Biology and Population Genetics

Cell wall biochemistry to illuminate mechanisms of pathogenicity in *Phytophthora infestans*

Laura J. Grenville-Briggs, Annie Inman, Stefan Klinter, Francesco Vilaplana, Osei Ampomah and Vincent Bulone

(Division of Glycoscience, School of Biotechnology, Royal Institute of Technology (KTH), Stockholm, Sweden)

The cell wall is a dynamic extracellular compartment protecting the cell, providing rigidity, and playing an essential role in the uptake of molecules and signalling. In pathogenic organisms, the cell wall is at the forefront of disease, providing contact between the pathogen and host. Using a multidisciplinary approach, my research programme seeks to understand the role of the cell wall in oomycete disease, both as a communication centre with the host organism and as a compartment that is continually reshaped and strengthened throughout the lifecycle, to penetrate and colonise the host. Understanding these mechanisms in more detail will pave the way for better control of oomycete diseases. We are combining novel chemical genomics approaches with state-of-the-art biochemistry and biophysics to study the cell wall and to develop new anti-oomycete drugs. Neutral sugar determination, combined with linkage analysis of cell wall carbohydrates throughout the *P. infestans* lifecycle, highlights differences in cell wall structure and composition that can be exploited to develop novel control mechanisms for oomycete diseases, whilst furthering our fundamental understanding of oomycete pathogenicity. We have previously establishing an essential role for a cellulosic cell wall in appressorium production and infection of potato by *P. infestans* (Grenville-Briggs et al 2008). We are now working to elucidate the precise functions of individual cellulose synthases and a putative chitin synthase in the *P. infestans* cell wall and in pathogenicity.

Molecular mechanisms of zoosporogenesis, motility, chemotaxis and differentiation of Peronosporomycete zoospores

Md. Tofazzal Islam[1], Andreas von Tiedemann[2] and Harold Meijer[3]
([1] Department of Biotechnology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh. [2] Division of Plant Pathology and Plant Protection, University of Göttingen, Grisebachstr. 6, D 37077 Göttingen, Germany. [3] Tel. +88-0171-4001414, E-mail: tofazzalislam@yahoo.com)

The zoosporogenesis, motility and differentiation of zoospores are critical in the disease cycles of Peronosporomycetes that cause devastating diseases in plants, fishes, vertebrates, and microbes. The biflagellate motile zoospores released from sporangia of these phytopathogens are believed to locate their host plants by chemotaxis, after which they undergo a series of morphological changes before penetrating the host tissues to establish the diseases. We conducted series to experiments to understand underlying molecular mechanisms of zoosporogenesis, motility, chemotaxis and differentiation of two important peronosporomycetes *Aphanomyces cochlioides* and *Plasmopara viticola*. Bioassay-guided chromatographic investigations using *A. cochlioides* zoospores identified a host-specific plant signal cochliophilin A (5-hydroxy 6,7-methylenedioxyflavone) in root exudates which are not only responsible for chemotaxis but also trigger developmental transitions (encystment and germination) of zoospores on host surface to initiate infection. Interestingly, the morphological changes of zoospores induced by host-specific signaling compounds appeared to be linked to polymerization/depolymerization of the filamentous actin in their cells. In contrast, nonhost plants possess diverse secondary metabolites (chemical weapons) such as nicotinamide (motility inhibitor), polyflavonoid tannins (lytic factors) to ward-off phytopathogenic zoospores.

We also found that exogenous applications of G protein activator matoparan, primary alcohols (*n*-BuOH) and pure phospholipase C enzymes triggered differentiation of both *A. cochlioides* and *P. viticola* zoospores. Milimolar level of Ca^{2+} triggered germination of mechanically-induced cystospores that blocked by both Ca^{2+} channel blockers or calmodulin antagonists. We observed that both differentiations of zoospores into cystospores and germination of cystospores to haphal germ tubes by pharmacological effectors and Ca^{2+}, respectively were associated with induction of ^{32}P-phosphatidic acid (PA) accumulation in the treated cells. Interestingly, an antagonist of PA generation, lisofylline suppressed encystment of zoospores by mastoparan and Ca^{2+}-induced germination of cystospores and remarkably decreased ^{32}P-PA accumulation in the respective cells. Our results suggest that both PLD and PLC pathways are likely to be involved with *P. viticola* zoospore encystment and PA might act as a second messenger during germination of cystospores by Ca^{2+}.

To understand how motility of zoospores is maintained, we searched inhibitory compounds from *Streptomyces* species. We isolated a broad-spectrum kinase inhibitor, staurosporine from a *Streptomyces* sp. strain B 5136, which impaired motility of zoospores without causing any lysis. Among the 22 known kinase inhibitors tested, the PKC inhibitor chelerythrine was the most potent to arrest the motility of zoospores. Inhibitors that targeted kinase pathways other than PKC pathways did not practically show any activity in impairing zoospore motility. Interestingly, both staurosporine (5 nM) and chelerythrine (10 nM) also inhibited the release of zoospores from the *P. viticola* sporangia. In addition, staurosporine completely suppressed downy mildew disease in grapevine leaves at 2 µM, suggesting the potential of small-molecule PKC inhibitors for the control of peronosporomycete phytopathogens. Taken together, these results suggest that PKC is likely to be a key signaling mediator associated with zoosporogenesis and the maintenance of flagellar motility in peronosporomycete zoospores.

Live-cell Image of Compatible and Incompatible Interaction between *Phytophthora sojae* and Host

Kai Tao, Maofeng Jing, Xiaobo Zheng, Yuanchao Wang

(Lab of Oomycetes and Fungal Molecular Biology, College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, P.R.China.)

As ultrastructural details of *Phytophthora sojae* infection processes and defense response from host have been known for years, advances in fluorescent protein and imaging technology provide us a noval way to trace this interaction in vivo. Here we applied the live cell-imaging to examine the compatible and incompatible interactions between *P. sojae* P6497 GFP-tagging isolate and two soybean cultivars, one of which is susceptible and the other has *Rps3b* resistant gene. The characterization of infection processes contains the spatial extension, from epidermis to xylem, and temporal development, from 30 min to 36h post-inoculation, of invasive hypha in two combinations. In compatible interactions, as having penetrated epidermis, invasive hypha can extend to xylem the center part of the host almost at 12 h post-inoculation, while invasive hypha are still delayed in cortical cells layer almost near the penetration site in incompatible interaction. In both compatible and incompatible interactions, spherical or mastoid-like haustoria developed from invasive hypha are apparently present, some of which are surrounded by callose; and approximately by 15hpi, it appears to develop kinds of intumescent structures intercellularly and intracellularly in epidermal cell layer of host, such as knot-like, rhizomorph-like, broom-like and lobe-like. Main differences are defense response of host cell emanating strong autofluorescence regarded as results of phenolic compounds, which is significant in incompatible interactions and weak in compatible interactions; meanwhile those differentiated structures including haustoria are covered by unknown materials with autofluorescence. Illustrations of theses processes will extend details of the interaction between *P. sojae* and host, and initiates researches on effector's secretion, translocation and localization.

The Phenotypic and Genotypic Characterization of *Phytophthora capsici* in Gansu China

Jian Hu[1], Zhili Pang [1], Yang Bi[1] and Xili Liu[1*]

([1] Department of Plant Pathology, China Agricultural University, Beijing, China P. R., 100193. * Corresponding author: seedling@cau.edu.cn; author email: jaffyhu@gmail.com)

Phytophthora capsici is a destructive soilborne pathogen that causes great losses to pepper production in China. Our main objective was to characterize the phenotypic and genotypic diversity of *P. capsici* and its spread mechanism in Gansu province. 279 isolates were collected from 6 big regions (38 field sites in total) in Gansu from 2007 to 2011. Both A1 and A2 mating type isolates exit in the same region even in the same site. The number of metalaxyl sensitive, intermediate sensitive and insensitive isolates were 155, 88 and 36 respectively. Only two field sites had high proportion of insensitive isolates. 29 of the isolates were tested for physiological races, most of which were race 3, 5 of which were race2 and only one was race 1. SSR analysis showed that isolates were high genetic diversity in Gansu province. Cluster analysis separated the isolates into 6 genetic groups, and isolates from different regions showed different genetic diversity. Our results indicate that sexual recombination and host pressure probably caused the genetic variation of *P. capsici* in Gansu province.

Phenotypic and Genotypic Characterization of Potato Late Blight Pathogen *Phytophthora infestans* in Northern Shaanxi of China

Yuee Tian, Jieping Sun, Yunfang Ma, Dandan Liu, Junli Quan, Weixing Shan*

(State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China. * Correspondence to wxshan@nwsuaf.edu.cn)

Potato late blight caused by *P. infestans* is the most serious disease in Northern Shaanxi, an emerging potato production region in China. In this study, we aimed at determination of genetic diversity of *P. infestans* population in the region. A total of 125 single-lesion isolates were purified from samples collected in 2009 and were characterized for mating types, pathotypes, mitochondrial DNA (mtDNA) haplotypes and SSR (simple sequence repeats) genotypes. Mating type assay showed that both mating types（A1 and A2）occurred in the region, and self-fertile isolates were also detected but at very low frequency. Virulence determination on a set of differential near-isogenic lines containing R1 to R11, respectively, showed the presence of two pathotypes, in which the majority of isolates was of the pathotype 3.4.10. Super-virulent pathotype that virulent to all tested resistance genes was detected but at very low frequency. Analysis of mtDNA haplotypes showed that all of the isolates examined were IIa. A total of 25 multilocus genotypes were distinguished as determined with microsatellite markers, at seven polymorphic loci, and the genotype MLG10 was the dominant one with a frequency of 30.4% and presented in all of the four sampled regions. Multiple shared genotypes were detected among regions. The phenotypic and genotypic structure of the *P. infestans* populations in Northern Shaanxi suggests low level of genetic diversity and that migration of seed potato tubers may serve as a main source of initial inoculum. The occurrence of low frequency A2 isolates and supervirulent isolates suggested potential threat of late blight to potato production in Northern Shaanxi.

Identification of flagellar mastigoneme proteins from *Phytophthora*

Blackman L.M.[1], Hee, W. [1], Arikawa M.[2, 3], Yamada S.[2], Suzaki T.[2], and Hardham A.R.[1]

([1] Plant Science Division, Research School of Biology, Australian National University, Canberra, ACT 2601, Australia; [2] Department of Biology, Graduate School of Sciences, Kobe University, Nada-ku, Kobe 657-8501, Japan; [3] Department of Cardiovascular Control, Kochi Medical School, Nankoku, Kochi 783-8505, Japan)

Motile, flagellate zoospores of *Phytophthora* and *Pythium* species play a key role in pathogen dissemination and the initiation of infection of host plants. The diseases these pathogens cause are highly destructive and result in extensive losses in agriculture and natural ecosystems worldwide. Tripartite tubular hairs called mastigonemes on the anterior flagellum of *Phytophthora* and *Pythium* and other protists in the Stramenopile taxon are responsible for reversing the thrust of flagellar beat and for cell motility. Immunoprecipitation experiments using antibodies directed towards mastigonemes on the flagella of zoospores of *Phytophthora nicotianae* have facilitated the identification of three similar proteins. A gene for one of these proteins has been cloned and encodes a mastigoneme shaft protein. Expression of the gene, designated *PnMas2*, is up-regulated during asexual sporulation, a period during which many zoospore components are synthesized. Analysis of the sequence of the PnMas2 protein has revealed that, like other Stramenopile mastigoneme proteins, PnMas2 has an N-terminal secretion signal and contains four cysteine-rich epidermal growth factor (EGF)-like domains. Evidence from non-denaturing gels indicates that PnMas2 forms large oligomeric complexes, most likely through disulphide bridging. Bioinformatic analysis has revealed that *Phytophthora* species typically have three or four putative mastigoneme proteins containing four EGF-like domains. These proteins are similar in sequence to mastigoneme proteins in other Stramenopile protists including the algae *Ochromonas danica, Aureococcus anophagefferens* and *Scytosiphon lomentaria* and the diatoms *Thalassiosira pseudonana* and *T. weissflogii*.

The resistance evolution of *Phytophthora sojae* to flumorph via a gradually accumulated process

Meng Cai, Xili Liu*

(Department of Plant Pathology, China Agriculture University, Beijing Key laboratory of quarantine and prevention control on Seed-borne diseases, Beijing, 100193. The work was funded by Ph. D. Programs Foundation of Ministry of Education of China, No. 200800190025. *Correspondent Author. Email: liuxili2009@gmail.com)

Phytophthora sojae is the causal agent of soybean root rot, which has an impact on the soybean cultivation. When fungicides are introduced for disease control, the pathogens are capable of adjusting to the selection pressure of fungicides, and the resistance evolution presumably emerges in this situation. In this study, one wild type isolate of *P. sojae* was exposed to flumorph at 2.5 μg/ml. After several transfers, two mutants (RF-2 and RF-4) were obtained with a frequency of approximately 10^{-4}, which were low resistant to flumorph (the resistance factor < 10). Subsequently, another four mutants (RF-2-1, RF-4-1, RF-2-16 and RF-4-4) with a higher resistance level were derived from RF-2 and RF-4 with a frequency of nearly 10^{-3} by exposure to flumorph at 5 μg/ml. The potential target cellulose synthase 3 (CesA3) of flumorph was also investigated. The result showed that RF-2 and RF-4 carried a single mutation (Ile to Val at codon 1027) on CesA3, whilst RF-2-1, RF-4-1, RF-2-16 and RF-4-4 also carried mutation at codon 1027, but with an additional mutation at codon of 1020 (Gly (GGC) to Ser (AGC) or Val (GTC)) on CesA3. It was supposed that the mutation at codon 1027 on CesA3 in *P. sojae* conferred a low level of resistance. Following the mutation, an amino acid substitution at codon 1020 was considered to be associated with the presence of a higher resistance. The results implied that there was a micro-evolutionary progression towards the higher resistance to flumorph in *P. sojae*.

Fitness of metalaxyl-resistnace mutants of *Phytophthora boehmeriae* Saw.

Fangxin Chen, Yongxia Qi and Zhimou Gao *

(College of Plant Protection, Anhui Agricultural University, Hefei 230036, China)

Phytophthora boehmeriae Sawada is an important pathogenic oomycete, which causes ramie blight, cotton blight and several other plant blights. Some biological characteristics and their inheritance of the metalaxyl-resistance mutants (Mt^r) of *Phytophthora boehmeriae*, acquired by metalaxyl-induction on lima bean agar (LBA) media, were studied i*n vitro*. The results showed that there were no obvious difference between the Mt^r mutants and their wide-type parents in temperature for mycelial growth, zoosporangium production, sensitivity to malachite green and the pathogenicity to cotton seedlings. However, the oospore productions of the mutants were much lower than that of their parents. The growth rate and the colony morphology of Mt^r mutants and their parents all displayed obvious variation or separation and were inherited unsteadily in their single zoospore and oospore progenies. At the same time the homothallic character of Mt^r mutants could inherit steadily as well as that of the wide-type isolates did in asexual and sexual reproduction. It could reveal that the Mt^r mutants of *P. boehmeriae* were strong in fitness to nature, and would develop the metalaxyl-resistance population easily in a short time as long as the Mt^r mutants formed.

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*Corresponding author: Z. M. Gao; e-mail: gaozhimou@126.com

Genetic diversity of *Photophthora capsici* isolates in Anhui based on ISSR-PCR markers

Ping Li, Zhimou Gao*, Shun Cao, Yuli Dai, Xiuli Li, Dafeng Xu, Min Guo and Yuemin Pan

(College of plant protection, Anhui Agricultural University, Hefei Anhui 230036, China)

In order to probe into the genetic relationships among isolates of *Photophthora capsici* from three large regions of Anhui Province in eastern China, inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) was conducted to investigate the genetic diversity of *P. capsici* isolates. 13 random primers were screened and with them the 51 individuals were amplified. As a result, a total of 158 reproducible ISSR fragments,were obtained, of which 89.9% (142) were polymorphic, revealing high polymorphism among the isolates. Genetic similarity coefficients among all of the isolates ranged from 0.56 to 0.94 with the average of 0.84 based on the ISSR data, indicating high level of genetic variation in *P. capsici* isolates from Anhui Province of China. Cluster analysis using the unweighted pair-group method with arithmetic averages (UPGMA) indicated that the Anhui isolates were divided into seven groups according to the DNA fingerprints, and that no correlation between ISSR group and geographic origin. Isolates from the same location showed no clustering based on the year of sampling. Analysis of molecular variance partitioned variability among (13.6%) and within populations (86.4%). The value of gene flow among populations differed from 2.804 to 4.937, with average value of 3.545，indicating highly frequent gene exchange. Genetic distances and genetic differentiation were negatively correlated with geographic distances.

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*Corresponding author: Z. M. Gao; e-mail: gaozhimou@126.com

Resistance Genetic Mechanisms of *Phytophthora capsici* to Iprovalicarb and Mandipropamid

Zhili Pang[1], QianWang[1], Xili Liu[1]*

([1] Department of Plant Pathology, China Agricultural University, Beijing, China P. R., 100193. * Corresponding author: XiLi Liu; E-mail address: seedling@cau.edu.cn)

Phytophthora capsici is an important soil-borne oomycete pathogen which can infect a variety of hosts and pose great risk to agricultural production. CAA fungicides, which belong to a novel class of chemical agent, were widely applied to control the oomycete diseases nowadays. Iprovalicarb and mandipropamid are two representative ones of CAA fungicides. The objective of this study was to investigate the resistance inheritance mechanism of *P. capsici* to iprovalicarb and mandipropamid. The results are as follows:

1. The concentrations of sensitivity for *P. capsici* to iprovalicarb and mandipropamid were determined as 1 μg/mL and 0.1 μg/mL respectively. Then the sensitivity to iprovalicarb and mandipropamid of 500 *P. capsici* isolates were tested.

2. Thirty-two resistant isolates and five sensitive isolates were determined about the mating types, and conducted the selfed-crossing to determine the genetic background to be homozygous or heterozygous. As a result, the sensitive parent 22 and the resistant parent 8[83] were obtained, and crossed to obtain 138 sexual progeny.

3. According to cDNA sequence of 3314 and 3325 mutation, which are SNPs of parents in cellulose synthase (*CesA3*) of *P. capsici*, selfing progeny from either one parent or sexual progeny from both parents could be differentiated. Among 138 isolates tested, 49 isolates were sexual progeny from both parents (F1), and 89 were selfed isolates. Phenotype of F1 progeny were resistant to both fungicides tested. However, the resistance level of F1 was between sensitive parent and resistant parent. Therefore, we speculated that the resistance genotype was semi-dominance. In order to determine the number of resistant gene, several isolates in F1 population were randomly selected to mate to get sib-mating or selfing. Thirty progeny were obtained from isolates 17 and 27, and the sensitivity assay showed that the segregation of sensitivity to both two fungicides was about 3:1 (resistant:sensitive) verified by the chi-square test. The results indicated that the gene controlling the fungicide resistance could be a single nuclear gene. In all, we speculated that the resistance of *P. capsici* to iprovalicarb and mandipropamid was controlled by a single semi-dominance nuclear gene.

Characterization of *Phytophthora* species infecting ornamental crops

Jaimin S. Patel, David Norman, Mary Brennan and Gul Shad Ali*

(Department of Plant Pathology/Mid-Florida REC, University of Florida/IFAS, 2725 S Binion Rd, Apopka, FL-32703)

Various *Phytophthora* spp. infect a variety of foliar and woody ornamental plants in Florida. Based on morphological characteristics and previous pathological history of a particular crop, several isolates were tentatively identified to a species level. In this project, we have characterized close to 40 isolates cultured from 17 genera of ornamental crops with molecular phylogenetic analyses using the internal transcribed spacer 1 and 2 (ITS) regions of ribosomal RNA genes. A vast majority of these strains belong to *P. nicotianae*, followed by *P. tropicalis*, *P. palmivora* and *P. cinnamomi*. For managing fungicide-resistance, it is important to determine prevalence of *Phytophthora* isolates that are insensitive to Mefenoxam, a commonly used fungicide for controlling *Phytophthora* diseases. In mefenoxam sensitivity assays, we found moderate to complete insensitivity in several isolates. These studies suggest that several *Phytophthora* spp infect ornamental crops in Florida and that the build up of fungicide-insensitive *Phytophthora* populations might become problematic for disease management in ornamentals. Mitigating this potential problem will require prudent fungicide resistance management strategies including rotation of chemicals with different modes of actions.

Screening fungicides to control metalaxyl-resistant *Phytophthora* blight of pepper

Qi Rende[1,2], Wangtao[1], Zhao Wei[1], Li Ping[1], Gao Zhimou[2]

([1] Institute of Plant Protection, Anhui Academy of Agricultural Sciences, Hefei 230031; [2] College of Plant Protection, Anhui Agricultural University, Hefei 230036. Special Fund for Agro-scientific Research in the Public Interest, 201003004. Correspondent author. E-mail: gaozhimou@126.com)

Phytophthora capsici is one of the ubiquitous root pathogens which causes a devastating disease (called phytophthora blight) on many crops, including cucurbits, eggplants, and tomatoes et al. Phytophthora blight occurred in many pepper growing areas in the world, and it could cause damping off of seedlings, root rot, and blights that could lead to significant yield and economic losses, sometimes the losses is up to 100% in pepper fields. Disease management of phytophthora blight mainly relies on cultural practices and application of few fungicides. The intensive uses of phenylalamide fungicide (e.g. metalaxyl, mefenoxam) have led to the metalaxyl-resistant (MR) isolates of *P. capsici* rapidly extend in worldwide. In order to screen the fungicide to control metalaxyl-resistant phytophthora blight of pepper, the EC_{50} values of ten fungicides (metalaxyl, mandipropamid, infinito, dimehomorph, flumorph, **pyrimorph,** trifloxystrobin, azoxystrobin, cymoxanil, and propamocarb) were determined in inhibiting mycelia growth and sporangia germination against metalaxyl-sensitive (MS) and MR isolates of P. capsici, and the efficacies of these fungicides in controlling phytophthora blight of pepper were tested in pots experiments. The results showed that mandipropamid, infinito, dimehomorph, flumorph, **pyrimorph,** trifloxystrobin (+**salicylhydroxamic acid,** SHAM), azoxystrobin (+SHAM) showed excellent inhibitory activity in mycelia growth and sporangia germination of both MS and MR P. capsici isolates, the EC_{50} values for mycelia growth and sporangia germination were 0.0678-4.7064 μg/mL and 0.0028-3.5780μg/mL, respectively. The efficacies of mandipropamid and other 8 fungicides in controlling *phytophthora* blight of pepper were over 70%. The results also showed that the other 9 fungicides did not exhibited cross-resistance with metalaxyl, and thus, they could be used as alternative fungicides in managing metalaxyl resistance in controlling *phytophthora* blight of pepper.

Molecular characterization of the chitin synthase genes from the fish pathogen *Saprolegnia parasitica*

Elzbieta Rzeszutek, Sara Diaz, Hugo Mélida and Vincent Bulone

(Division of Glycoscience, School of Biotechnology, Royal Institute of Technology (KTH), Stockholm, Sweden)

Saprolegnia parasitica is one of the most severe fish pathogens causing significant economical losses in aquaculture. The analysis of the cell wall composition of the micro-organism and the characterization of key enzymes involved in cell wall biosynthesis may facilitate the identification of target proteins for disease control. The cell wall of *S. parasitica* consists mainly of cellulose, β-(1→3)- and β-(1→6) glucans. It also contains minute amounts of chitin. The main objective of this work was to test the effect of nikkomycin Z, a competitive inhibitor of chitin synthase, on the growth of *S. parasitica.* Genome mining allowed the identification of six different putative chitin synthase genes. Their expression in the mycelium was analyzed using Real-Time PCR. Four of the six genes were expressed at a significantly high level while the two others exhibited either a much lower level of expression or no expression in the mycelium. Nikkomycin Z did not influence the expression level of any of the six genes. *In vitro* synthesis of chitin was performed using an optimized specific radiometric assay and cell-free extracts as a source of enzymes. It was possible to detect chitin synthase activity in both intact cell membranes as well as in detergent-extracts of membranes, which confirmed the capacity of the mycelium to synthesize chitin. The mycelium was highly sensitive to the presence of nikkomycin Z in the culture medium and isolated chitin synthases were strongly inhibited *in vitro*. Although the amount of chitin in the cell wall of *S. parasitica* does not exceed a few percent, our data indicate a key role of chitin in hyphal growth and demonstrate that chitin synthases represent promising targets of anti-oomycete drugs.

Effects of soil environments on oospore germination of *Phytophthora sojae*

Wen Jingzhi, Cui Renfang

(Department of Plant Protection, College of Agriculture, Northeast Agricultural University, Harbin 150030, China)

Corresponding author: Wen Jingzhi, E-mail: jzhwen2000@yahoo.com.cn

Phytophthora root and stem rot, caused by *Phytophthora sojae*, is an extremely destructive soil-borne disease that causes significant yield losses of soybean production. *P. sojae* can survive in soil for a long time in the form of a dormant structure, oospore，that germinates and infects soybean under favorable conditions. Therefore, knowledge of the survival of oospores in soil is essential for understanding epidemics of this disease.

In the present study, a fluorescence microscopy detection method that can distinguish oospores in different survival state was developed based on the results of MTT staining and observation of enhanced green fluorescent protein (EGFP) - labeled oospores of *P. sojae*. This method allows real time observation of oospore survival states of *P. sojae.*

Effects of soil temperatures and water contents on survival of oospores of *P. sojae* were investigated in the present study. The results showed that lower soil temperatures (-15°C, -5°C, 4°C and 8°C) were favorable for dormancy of oospores; while higher temperature, 25°C, was suitable for oospore entering the active phase which is the preparation stage before germination. At 25°C, the water content from 30% to 60% was suitable for oospore germination, and the best water content for oospore germination was 30%.

At the conditions of 25°C and 30 % soil water content, the effects of soil types and crop rotations on survival of oospores of *P. sojae,* and correlation between oospores germination rates and organic content, pH, and Ca^{2+} in soil were investigated. The results showed that oospore germination was negatively correlated with organic content and soil pH, but positively correlated with Ca^{2+} content. Black soil was most suitable for activation of oospores, followed by meadow soil, while albic soil, sandy soil, and alkaline soil were not suitable for the activation of oospores. The results suggested that intermediate organic content, pH and Ca^{2+} content in black soil provide favorable conditions for oospore germination, while high pH of alkaline soil, high organic content and low Ca^{2+} content in albic soil, and Low Ca^{2+} content in sandy oil were not suitable for activation of oospores.

In the present study, inhibition of soil microorganisms from different types of crop rotations against mycelia growth of *P. sojae* was evaluated, and correlation between activation rate of oospores and the number of bacteria, fungi, actinomycetes, sum total of microbes, antagonistic bacteria and their inhibition effect was investigated. The results showed that the activation rate of oospores was negatively correlated with the number of bacteria, number of antagonistic bacteria, and sum total of microbes, but positively correlated with the number of actinomycetes and number of fungi. Soybean continuous cropping and corn continuous cropping were suitable for activation of oospores because there were larger amount of actinomycetes and fungi, but no antagonistic bacteria in soybean continuous cropping soil, while fewer bacteria and sum total of microbes in corn continuous cropping soil. More antagonistic bacteria and sum total of microbes could be the reason why wheat continuous cropping was suitable for activation of oospores. Therefore, for controlling Phytophthora root and stem rot, soybean should be planted in the fields that had wheat or corn as previous crops. The soil that planted wheat in previous year can inhibit the activation of oospores. The soil with corn as previous crop make oospores activate, but germinated oospores cannot infect corn. The results of the present study suggested that crop rotation practice may annually reduce the number of oospores in soil, and effectively control the disease.

Enhanced green fluorescent protein (EGFP) as a report gene for studying the infection of *Phytophthora sojae*

Wen Jingzhi, Li Lijun

(Department of Plant Protection, College of Agriculture, Northeast Agricultural University, Harbin 150030, China)

Corresponding author: Wen Jingzhi, E-mail：jzhwen2000@yahoo.com.cn

Phytophthora root rot, caused by *Phytophthora sojae*, is a widely distributed, extremely harmful soil-borne disease. Environmental conditions are conducive to the occurrence of this disease even lead to have no harvests. The present study on *P. sojae* has great limitations due to its soil-borne characteristics. In order to research the infection of *P. sojae*, transgenic *P. sojae* trains that produce enhanced green fluorescent protein (EGFP) were obtained after stable DNA integration using the $CaCl_2$-PEG-mediated transformation method. The expression of EGFP in hypha, zoosporangia and oospores of *P. sojae* was observed using a fluorescent microscope. The EGFP gene in the transformants was detected using the PCR examination and the vegetative and reproductive characters of transformants were evaluated in the media. The track of an EGFP-marked zoospore of *P. sojae* swimming to different plant rootlet and the situation of germination and penetration after quiescence were observed using a fluorescent microscope. The results showed that EGFP gene can be stably expressed in hyphae, zoosporangia and oospores of *P. sojae*. There were no significant difference between the transformants and the wild type in mycelial growth, while remarkable or extremely remarkable difference was detected in the vegetative and reproductive characters between some transformants and the wild type, the pathotype of one transformant has changed. The track of an EGFP-marked zoospore of *P. sojae* swimming to a rootlet of compatible soybean plant was a similar sine curve with radicle vertical range 0-60 μm scopes from the rootlet, the one of non-compatible soybean plant was a similar sine curve with radicle vertical range 0-100 μm scopes from the rootlet, and the one of non-host soybean plant was a similar sine curve with radicle vertical range 60-150 μm scopes from the rootlet. When the EGFP-marked zoospore of *P. sojae* swam to a rootlet of host plant, after some exploratory contacts, the zoospore adhered to the rootlet and became into a cyst, the cyst germinated a germ tube in 1 h, the top of the germ tube adhered to the rootlet, the cyst was pushed away from the rootlet. When the zoospore infected the compatible soybean plant, the length of germ tube and the distance from the cyst to the rootlet were shorter than it infected the non-compatible soybean plant. When the EGFP-marked zoospore of *P. sojae* swam to a rootlet of host plant, after only one exploratory contact, the zoospore did not adhere to the rootlet, it germinated to a germ tube in the water when the zoospore interacted with the non-host plant, the growth direction of the germ tube was random. It shows that there is some attraction of the host plant root exudation to the zoospore of *P. sojae* and there is no attraction of the non-host plant root exudation to the zoospore of *P. sojae*. So we can presume that the selection of *P. sojae* to the host is in connection with root exudation.

Infection Mechanisms of Fish Pathogenic Oomycetes

Ariane Willems, Herbert van den Berg, Kirsty L. Minor, Chris J. Secombes, Pieter van West
(Aberdeen Oomycete Laboratory, University of Aberdeen, College of Life Sciences and Medicine, Institute of Medical Sciences, Foresterhill, Aberdeen, AB25 2ZD, Scotland UK)

The infection mechanisms of plant pathogenic oomycetes have been well characterized, however for animal pathogenic oomycetes these data are still scarce. Due to the tremendous economic and environmental impact of these infections, a better understanding of the infection mechanisms of animal pathogenic oomycetes is mandatory. New insights into these mechanisms could lead to novel means for better control of this disease in aquaculture and natural ecosystems.

In the aquaculture industry the major pathogenic oomycetes are *Saprolegnia parasitica* and *Saprolegnia diclina* –mainly infecting fish and eggs, respectively. Recently, it was reported by van West *et al.* (2010) and Wawra *et al*. (2012) that *S. parasitica* translocates effector proteins into trout cells. This process could be involved in the modulation of host defenses against the emerging infection, as was previously described in plant pathogenic oomycetes. However unlike these plant pathogenic oomycetes, *S. parasitica* does not seem to form haustoria-like structures. The exchange of nutrients and translocation of effector proteins seems to occur via specialized flattened “feeding”-structures connecting invaded cells with *Saprolegnia* hyphae. *S. diclina* does seem to form structures similar to appressoria that penetrate fish eggs, however the molecular and cellular mechanisms facilitating the infection process remain unidentified.

In the fish-farming industry differences in susceptibility of eggs originating from different hens were noted. Since the immune system in the eggs is not developed yet, these differences could either be explained by passive immunity, the physical aspects of the eggs (aspects influenced by the hen) or both. One of the first physical barriers to overcome by *S. diclina* is the chorion. Therefore we hypothesized that the chorion thickness in infection-resistant eggs is greater than the chorion thickness of infection-prone eggs. To test this hypothesis we collected a large number of uninfected eggs from hens producing infection-resistant and from hens producing infection-prone eggs. Chorion thickness will primarily be analyzed with H&E staining. Additional analysis to differentiate between distinct layers in the chorion and surface characteristics of the chorion will be performed by transmission and scanning electron microscopy respectively.

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Characterization of regulated protein secretion in *Phytophthora* zoospores

Weiwei Zhang, Leila M. Blackman and Adrienne R. Hardham

(Plant Science Division, Research School of Biology, College of Medicine, Biology and Environment, The Australian National University, Canberra, ACT 0200, Australia)

In many species of *Phytophthora* and other oomycetes, motile biflagellate zoospores initiate plant infection. Within the zoospore cytoplasm, organelles, including three types of cortical vesicles, are distributed with a distinct polarity. Rapid exocytosis or migration of these vesicles during zoospore encystment indicates that the cortical vesicles may play important roles during early infection. During encystment at the host surface, *Phytophthora* zoospores become immobile and firmly attached to the plant epidermis. Within the first 2 minutes of this process, the contents of ventral and dorsal cortical vesicles are secreted, delivering adhesives and a putative protective coating onto the surface of the cysts. By contrast, the third category of cortical vesicles, the large peripheral vesicles, move away from the plasma membrane, become randomly distributed within the cyst cytoplasm and ultimately degraded. Unexpectedly, we found that PnCcp, a 12 kDa protein component of the large peripheral vesicles is somehow selectively secreted during encystment. Double immunolabelling studies have shown that in sporangia and zoospores, PnCcp colocalises with PnLpv, a high molecular weight glycoprotein also resident in the large peripheral vesicles. However, in hyphae, the large peripheral vesicles sometimes contain only PnLpv and quantitative analysis suggests that during vesicle development, PnCcp is added to large peripheral vesicles after PnLpv. Quantitative, real-time RT-PCR shows that expression of *PnLpv* precedes that of *PnCcp* and that *PnCcp* but not *PnLpv* is expressed in zoospores. PnCcp and PnLpv were also found to be differentially compartmentalised within the vesicles. The differential synthesis and secretion of large peripheral vesicle proteins in *Phytophthora* zoospores provides a novel system in which to study selective protein secretion in eukaryotes.

Comparative Study on Resistance Molecular Mechanism of *Peronophythora litchii* to Novel QoI Fungicides

Yuxin Zhou,[1] Botao Li[1] and Xili Liu[1*]

(Department of Plant Pathology, China Agricultural University, Beijing, China P. R., 100193. * Corresponding author, seedling@cau.edu.cn, author email: rita1720@126.com)

Litchi downy blight caused by *Peronophythora litchii* is a destructive disease suffered by litchi in China. The control of the disease mainly depends on the application of chemical fungicides. In this study, the main objective was to investigate the resistance molecular mechanism of *P. litchii* to 4 novel QoI fungicides which were Enestroburin, SYP-1620, SYP-2815, and ZJ-0712. One ZJ-0712-resistant mutant (BU) and two SYP-2815-resistant mutants (SU1 and SU2) were obtained by UV-radiation induction. One SYP-2815-resistant mutant (S38), two SYP-1620-resistant mutants (A11-4 and A0902), one ZJ-0712-resistant mutant (B0908) and one Azoxystrobin-resistant mutant (M11-4) were obtained by fungicide adaption. All the mutants showed high and stable resistance level (>100) compared to sensitive isolates. The *cytochrome b* of *P. litchii*, which has a full length of 382 amino acids, was cloned from both sensitive and resistant isolates. The result showed that single-mutation G142A, G142S, Y131C and F128S were found in M11-4 & S38, BU & B0908, SU2 & SU2 and A11-4 & A0902, respectively. G142A/S and Y131C showed high resistant level to 4 QoI fungicides, while F128S showed different resistant level. The model of QoI fungicides docking into the pocket and the related mutant pocket were built for SYP-2815, SYP1620, ZJ-0712 and Enestrobilurin, which well explained the correlations between mutant site and fungicide resistance.

Functional Genomics

Tyrosinase is required for melanin production in zoosporangia of the fish pathogenic oomycete *Saprolegnia parasitica*

Saraiva, M[1]; de Bruijn, I[1]; Grenville-Briggs, L[1,2]; McLaggan, D[1]; Bulone, V[2]; van West, P[1*]

[1] Aberdeen Oomycete laboratory, University of Aberdeen, Foresterhill, AB25 2ZD, Aberdeen, UK

[2]KTH – Royal Institute of Technology, School of Biotechnology AlbaNova University Center SE-106 91 Stockholm Sweden

* Corresponding author – p.vanwest@abdn.ac.uk

Melanin plays an important role in pathogenicity of filamentous fungi and is often considered as an indirect virulence factor by acting as a protective agent against several threats and hazards that these organisms may encounter such as oxidants, killing by macrophages, UV and also antimicrobial compounds. Tyrosinase is the first enzyme involved in the melanin biosynthetic pathway. It is a mono-oxygenase that catalyses the *O*-hydroxylation of monophenols and subsequent oxidation of *O*-diphenols to quinines. Tyrosinases are widely distributed in nature, where they have been found in prokaryotes, eukaryotic microorganisms, invertebrates, plants and mammals.

Here we describe the functional characterisation of a tyrosinase (*SpTyr*) from the fish pathogenic oomycete *Saprolegnia parasitica*. This aquatic water mould is able to infect a wide range of fish, amphibians and crustaceans that are relevant to the aquaculture industry and aquatic ecosystems. Previously we found that *Sptyr* is highly expressed in sporulating mycelium. After developing and employing a transient gene silencing method, based on the delivery of *in vitro* synthesized dsRNA into protoplasts of this water mould, we obtained *SpTyr*-silenced lines with a significantly decreased tyrosinase activity of 40-60% compared to control lines. The tyrosinase activity correlated directly with the level of *SpTyr*-silencing in the transient lines, which ranged from 68.7-37.5%. Furthermore, the melanin content was measured spectrophotometrically in the *SpTyr*-silenced lines and found to be significantly reduced to 2-70%. Microscopic observations of *SpTyr*-silenced lines resulted in aberrant zoosporangium formation, with less pigment and abnormal morphology.

Moreover, our results demonstrate that transient gene silencing can be successfully used to functionally characterise genes in *S. parasitica* and could provide a high-throughput tool for *S. parasitica* functional genomics.

Functional analysis of novel membrane receptors in *Phytophthora*

Hua C.[1], Meijer H.J.G.[1], Zhao W.[2], Yang X.[2], Wang Y.[2] and Govers F[1].

([1]Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; [2]Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China)

Phytophthora development and spore behaviour are influenced by many environmental components including temperature, humidity and plant secreted compounds. Extracellular signals are mainly transduced into cells via membrane receptors that activate downstream signalling pathways. Mining and comparative genomics of whole genome sequences revealed that *Phytophthora* harbours many novel enzymes with aberrant combinations of catalytic and regulatory domains. One group, that is well conserved among oomycetes, comprises GPCR-PIPKs, transmembrane proteins in which features of two super families are combined: G-protein coupled receptors (GPCRs) and phosphatidylinositol-phosphate-kinases (PIPKs). This group of proteins potentially activates G protein and/or phospholipid mediated signal transduction pathways which might be involved in developmental or infection processes. We studied the expression of *GPCR-PIPK* genes and functionally analysed GPCR-PIPKs by gene-silencing or overexpression in *Phytophthora*. These membrane localized receptors are present in various cellular compartments rather than in the plasma membrane, as anticipated. Furthermore, this research revealed a vital role for GPCR-PIPKs in various stages of the *Phytophthora* life cycle. As such they are excellent and promising candidates for novel drug-targets.

A Yeast One-Hybrid Assay Captures a Novel DNA-Binding Domain in *Phytophthora sojae*

Brian D. M. Rutter & Vipaporn Phuntumart
(Bowling Green State University)

Automated and manual genome annotation approaches have identified more than 1000 different transcription factors belonging to approximately 60 families in the genomes of six oomycete species: *Phytophthora sojae, P. ramorum, P. infestans, Pythium ultimum, H. arabidopsidis* and *Saprolegnia parasitica*. A large body of research has delved into how *P. sojae* grows and infects its host, as well as the anatomy and functions of its effector proteins. Little, however, is known about the gene regulation coordinating the oomycete's growth, development and virulence. In order to amend this lack of information, a yeast one-hybrid assay was performed with the intent of identifying known and novel transcription factors in *P. sojae*. A "GCCGCC" motif was identified as a significant sequence in the upstream regions of *P. sojae* genes. This motif, which was found on multiple scaffolds of the *P. sojae* genome, is a common binding domain for transcription factors that regulate growth, development and stress responses in plants. The "GCCGCC" motif was used as bait in a yeast-one hybrid assay designed to screen the total cDNA extracted from *P. sojae* mycelium and zoospores. The assay captured heretofore-uncharacterized gene sequences that encode a potentially novel DNA-binding domain.

Identification of target proteins associated with *Phytophthora sojae* G-protein alpha subunit PsGPA1 involved in chemotaxis to soybean isoflavones

Xin Zhang, Yujuan Hao, Chenlei Hua, Suomeng Dong, Yuanchao Wang,
(Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China)

Phytophthora sojae has a narrow host range and is one of the most important pathogens which causes the root and stem rot of soybean. Zoospore is a key stage in the life cycle of *P. sojae*. It is believed that chemotaxis of zoospores to isoflavones is critical for host recognition and initiating infection. To investigate the role of G-protein in chemotaxis, we analyzed the expression of several genes known to be involved in these pathways. The G-protein alpha subunit *PsGPA1* was identified and characterized, which was expressed specifically in sporangia and zoospores while not in mycelium. *PsGPA1*-deficient stable transformants were obtained by gene silencing strategies. Our results showed that zoospore behavior including chemotaxis to the soybean isoflavone daidzein was severely impaired in the silenced transformants. *PsGPA1* silencing also affected zoospore encystment and cyst germination and led to the infectious inability of the *PsGPA1*-silenced mutants to soybean. To better understand the G-protein signaling in chemotaxis, we used yeast two-hybrid screening to identify proteins related with PsGPA1. We found that PsGPA1 physically interacted with the receptor for activated C-kinase 1 homologue PsRACK1. *PsRACK1* silencing distinctly reduced the yeild of oospores but did not impair chemotaxis of zoospores, which suggested that PsRACK1 may not be the downstream target of PsGPA1.

Screening and functional analysis of pathogenicity related genes from SNARE family in protein secretion pathways from *Phytophthora sojae*

Zhijian Zhao [1,2], Delong Li [1], Jinggan Li [1], Zhaojun Lu [1], Meng Yao [1], Chuhua Zhai [1], Yidan Huang [1], Suomeng Dong [1], Xiaobo Zheng [1], Yuanchao Wang [1*]

([1], The Key Laboratory of Monitoring and Management of Plant Diseases and Insects of the Ministry of Agriculture, College of Plant Protection, Nanjing Agricultural University, Nanjing 210095，China)

([2], Agricultural Enviroments & Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming, 650205, China)

[*]Corresponding author: Yuanchao Wang, Email: wangyc@njua.edu.cn

Oomycete plant pathogens, a fungal-like diverse group in the stramenopile lineage are destructive to a vast variety of plants important to agriculture, forestry, and natural ecosystems. *Phytophthora sojae* causes root and stem rot of soybean and leads to annual $1-2 billion losses worldwide. To successfully colonize their hosts, a diversity of plant pathogens secrete an arsenal of effector proteins and other molecules to disturb host defense response and enable parasitic infection. In eukaryotic cells, vesicle trafficking of extracellular proteins molecules through the secretory pathway relies on packaging and delivery of membrane vesicles. The membrane fusion process is highly conserved in all eukaryotes and the central components SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) driving the membrane fusion events are involved in vesicle delivery to target membranes. Until recently, only a few new clues were obtained that Oomycete RXLR effector proteins were delivered inside host cells through PI3P-mediated mechanisms. However, we have no idea how these effector proteins were transported and secreted outside from Oomycete pathogens. In order to address this question, we analyzed seven sequenced Oomycete genomes and mined total 34 SNARE genes from *P. sojae* genome based on BLAST of the homologues reported in yeast, *Arabidopsis* and human, which were divided into Qa, Qb, Qc and R SNAREs according to the conserved SNARE motifs. We clustered their expression levels in ten different developmental and infectious stages using the RNA sequecing data of *P. sojae* and picked up twelve candidate SNARE genes that were highly expressed in zoospore, cyst germination or infection stage. Both transient and stable -silenced transformation based on RNA interference strategy were utilized to screen these twelve candidate SNARE genes. Our results showed that nine of them were related to pathogenicity of *P. sojae* and all of silenced mutants showed severely attenuated on soybean infection. We also used yeast two-hybrid to validate the interaction among different SNARE proteins and primary results indicated several SNARE proteins interacted each other. Now stable-silenced transformants from five of them were obtained and their phenotypic and functional characterizations are being investigated.

Conserved R-SNARE family gene *PsVAMP714* is involved in effector secretion, sexual reproduction and soybean infection by *Phytophthora sojae*

Zhijian Zhao [1,2], Jinggan Li [1], Delong Li [1], Zhaojun Lu [1], Meng Yao [1], Yidan Huang [1], Xiaobo Zheng [1], Yuanchao Wang [1*]

([1], The Key Laboratory of Monitoring and Management of Plant Diseases and Insects of the Ministry of Agriculture, College of Plant Protection, Nanjing Agricultural University, Nanjing 210095，China)

([2], Agricultural Enviroments & Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming, 650205, China)

[*]Corresponding author: Yuanchao Wang, Email: wangyc@njua.edu.cn

Phytophthora sojae, a homothallic species of Oomycetes, is responsible for 'damping off' of seedlings and root and stem rot of soybean and is one of the most important pathogens of soybean, causing losses of $1-2 billion per year worldwide. To successfully colonize their hosts, a diversity of plant pathogens secrete an arsenal of effector proteins and other molecules to modulate host innate immunity and enable parasitic infection. These proteins were delivered by vesicle-mediated transport within pathogen cells and finally secreted to outside by exocytosis. SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) are now generally accepted to be the major players in the final stage of fusion of diverse vesicle-mediated transport events. The SNARE-mediated process is conserved evolutionally in eukaryotic cells. Here, we reported a conserved longin domain-containing R-SNARE family gene *PsVAMP714* of *P. sojae*, which was predicted to encode a 224-amino acid protein and displayed up-regulated expression in soybean late infection stages by real-time PCR analysis. *PsVAMP714* fused with mCherry RFP was localized on the organelles in cytoplasm. Four part-silenced transformants of *PsVAMP714* were obtained by stable genetic transformation based on RNA interference strategy and the phenotypic and functional analysis was characterized. Our results showed that germinated cysts of *PsVAMP714*-silenced mutants were abnormal and the pathogenesis was severely impaired to soybean infection. *PsVAMP714* silencing altered cell wall integrity and affected chitin and β-glucan distribution of hyphal tip.To further analysze the reasons of attenuated pathogenesis, we investigated host defense response, including callose deposition, ROS accumulation and PR gene expression, and tolerance variations to osmotic or salt stresses. We performed the infiltration assay on leaves of *Nicotiana benthamiana* using culture filtrate of *P. sojae*, secreted protein analysis on SDS-PAGE electrophoresis and extracellular enzymes assay. The results suggested that *PsVAMP714* may be involved in secretary transport of elicitors and some extracellular enzymes. We also found that *PsVAMP714* influenced sexual reproduction of *P. sojae*, because oospore formation was significantly reduced in silenced mutants compared with that of donor wild type.

A *Phytophthora sojae* Heat Shock Transcription Factor PsHSF1 is Required for Oxidative Stress Tolerance and Suppression of Plant Immunity

Yuting Sheng[1], Yonglin Wang[1], Suomeng Dong[1], Kai Tao[1],Chenlei Hua, Harold Meijier[2], Xiaobo Zheng[1], Francine Govers[2] , Yuanchao Wang[1*]

[1]Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China; [2]Laboratory of Phytopathology, Plant Sciences Group, Wageningen University, Binnenhaven

Although much is known about that reactive oxygen species (ROS) play central roles in plant defense system, the mechanisms how phytopathogens adapt and counteract the plant-driven ROS remain little understood. Genome-wide analysis of ROS tolerance-related transcription factors in *Phytophthora* species uncovered an extremely huge gene family of heat shock transcription factors (HSFs), 22 HSF genes in *P. sojae*, 24 HSF genes in *P. infestans* and 18 HSF genes in *P. ramorum*, while only 1-4 HSFs in true fungi, such as *Magnaporthe* or yeast. A highly up-regulated *P. sojae* HSF under oxidative stress condition, PsHSF1, was given a detailed study, which was also strongly up-regulated under heat shock stress and at cyst germination stage. *PsHSF1* silencing reduced the rate of cyst germination. Hyphal growth of *PsHSF1*- silenced transformants was severely affected under the stress of hydrogen peroxide or heat shock compared with wide-type (WT). The soybean infection of *PsHSF1*-silenced transformants was significantly delayed. Cytological staining showed that the mutants compromised to suppress hydrogen peroxide accumulation and innate immunity of soybean cell around the infection area. The results demonstrate that PsHSF1 is required for the adaptation and response to stress mediated with ROS in *P. sojae*, and suggest that PsHSF1 played an important role in counteracting ROS-mediated plant innate immunity.

PnPMA1, an atypical plasma membrane H^+-ATPase, is involved in zoospore development in *Phytophthora parasitica*

Meixiang Zhang[1], Yuling Meng[2] , Qinhu Wang[1], Dandan Liu[2], Junli Quan[2], Adrienne R. Hardham[3], Weixing Shan[2*]

([1] State Key Laboratory of Crop Stress Biology in Arid Areas and College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China; [2] State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China; [3] Plant Science Division, Research School of Biology, College of Medicine, Biology and Environment, The Australian National University, Canberra, ACT 2601, Australia. * Correspondence to wxshan@nwsuaf.edu.cn)

Species in the genus *Phytophthora* are destructive plant pathogens that can infect many agriculturally and ornamentally important plants. Biflagellate zoospores can be used by *Phytophthora* pathogens for dispersal and plant infection. Once released from sporangia, zoospores swim and actively target host tissues by various mechenisms including chemotaxis, autotaxis, and electrotaxis. However, the molecular mechanisms that control zoospore development and behavior are still largely unknown. *PnPMA1* gene was identified in our previous studies and has been shown to be highly expressed in zoospores and germinated cysts of *Phytophthora parasitica*, and it encodes an atypical plasma membrane H^+-ATPase containing an insertion of ~155 amino acid residues at the C terminus. Using the lacZ fusion-based topology determination approach, we show that the C-terminal insertion loop in the PnPMA1 protein is located in the extracellular space. To elucidate the biological function of PnPMA1, *PnPMA1*-silenced transformants were generated by introduction of hairpin and antisense constructs respectively, and were confirmed by quantitative PCR of *PnPMA1* transcripts and detection of associated small interfering RNAs (siRNAs). High levels of *PnPMA1* silencing in *P. parasitica* led to production of non-flagellate and large aberrant zoospores, rapid transition from zoospores to cysts, and a decreased germination rate of cysts. These results indicate that PnPMA1 plays essential roles in zoospore development. We postulate that PnPMA1 controls biological processes by regulation of ion homeostasis and cell wall-plasma membrane interaction.

Functional Analysis of a Novel Diaminopimelate decarboxylase from the Oomycete *Saprolegnia parasitica*

Josie Hug[1], Lingxiao Ge[1], Stan Oome[2] and Paul Morris[1]
[1]Bowling Green State University, Ohio, USA, [2]Utrecht University, Netherlands

Oomycetes are members of the kingdom Stamenopiles and include many important plant pathogens, including *P. sojae*, pathogen of soybeans and *P. infestans,* pathogen of potatoes and tomatoes. Genomic sequences for five oomycetes have now been published and the sequence for a sixth genome from the fish pathogen *Saprolegnia parasitica* has just been released. Analyses of these genomes have revealed that oomycetes genomes contain several novel biosynthetic strategies for the synthesis of primary metabolites. One such example is lysine biosynthesis. In nature, there are at least seven independent strategies for the synthesis of lysine. Plants and bacteria use an epimerase to convert L, L-diaminopimelate to meso-diaminopimelate. This substrate meso-diaminopimelate is then converted to lysine by a diaminopimelate decarboxylase. Oomycetes are also predicted to synthesize lysine from L, L-diaminopimelate. However, the epimerase is only present in *S. parasitica* but absent in *P. sojae*, *P. infestans*, *P. ramorum*, *P. ultimum*, and *H. arabidopsidis*. Additionally, the similarity of diaminopimelate decarboxylase from *S. parasitica*, *P. sojae*, *P. infestans*, *P. ramorum*, *P. ultimum*, and *H. arabidopsidis* indicates that the enzyme from different organisms should function in the same way. Thus, we postulate that an epimerase is not needed and the diaminopimelate decarboxylase of oomycetes is able to convert L, L-diaminopimelate directly to lysine.

To confirm the catalytic activity of diaminopimelate decarboxylase, its nucleotide sequence from *S. parasitica* was synthesized and amplified. The gene will be cloned in an Escherichia-Pseudomonas shuttle vector pHERD20T via cold fusion cloning. The constructed plasmid will then be transformed and expressed in *E. coli* and *P. aeruginosa* mutants that are deficient in diaminopimelate decarboxylase in order to test complementation of the *S. parasitica* homologue. *S. parasitica* diaminopimelate decarboxylase will also be purified and its catalytic function will be investigated with L, L-diaminopimelate in vitro. Diaminopimelate decarboxylase should be able to convert L, L-diaminopimelate to lysine directly. Substrate specificity of diaminopimelate and production of lysine will be tested by HPLC analysis.

Lysine is an essential amino acid used as an important supplement for animal feed. Lysine production for animal feed is an important industry worldwide which makes an annual market value of several billion. Diaminopimelate decarboxylase may represent a novel amino acid biosynthetic strategy for lysine synthesis, so it could be applied to increase the efficiency of lysine production. Additionally, a finding that direct synthesis is possible with conversion of L, L-diaminopimelate to meso-diaminopimelate also indicates that the role of epimerase is to make an intermediate which is likely to be a component of Saprolegnia cell wall. The presence of epimerase in *S. parasitica* and the absence of it in other oomycetes suggest that meso-diaminopimelate is utilized as the cell wall component as in bacteria. So in addition to confirming a novel pathway, this provides potential insight into evolutionary changes in the cell wall of oomycetes that have taken.

PsVPS1, a dynamin-related protein, is involved in zoospore germination and soybean infection

Delong Li, Zhijian Zhao, Jinggan Li, Zhaojun Lu, Meng Yao, Chuhua Zhai, Yidan Huang, Suomeng Dong, Xiaobo Zheng, Yuanchao Wang*

(The Key Laboratory of Monitoring and Management of Plant Diseases and Insects of the Ministry of Agriculture, College of Plant Protection, Nanjing Agricultural University, Nanjing 210095，China)

*Corresponding author: Yuanchao Wang, Email: wangyc@njua.edu.cn

Plant pathogenic oomycetes have unique physiological characteristics and devastating effects on crops and natural ecosystems all over the world. Recent studies of proteins secreted by plant pathogenic oomycetes have been implicated in pathogenesis. Secreted proteins plays critical role for the pathogens. However, little is known about the pre-vacuolar secretory pathway in these pathogens. To investigate if the pre-vacuolar is crucial for fungal pathogenicity, here we identified a gene, *Psvps1*, a dynamin-related protein, in the genome sequence of *Phytophthora. sojae*. Moreover *Psvps1* is homologous to the *Saccharomyces cerevisiae* vacuolar protein sorting gene vps1 that mediates budding of clathrin-coated vesicles from the late Golgi that are diverted from the general secretory pathway to the vacuole. Three part-silenced transformants were generated by the PEG-mediated protoplast stable transformation. Our results showed silenced mutant was impaired in invasion in susceptible soybean plant. To further analysze the reasons of attenuated pathogenesis, we investigated the expansion of infection hyphae of silenced mutant in host epidermal cell. Cyst germination of *Psvps1* silenced mutants was abnormal and aberrant in chitin and polysaccharides distribution of hyphal tip. Silenced mutants shows reduced vegetative growth, but reduced vegetative growth can't affected pathogenicity. The hyphae of silenced mutants contains highly fragmented vacuoles. By infiltration assay on leaves of *N. benthamiana* using filtrate of *P. sojae*, the results suggested that *Psvps1* affected the transport of elicitors and some extracellular enzymes.

Two G protein-coupled receptors with a phosphatidylinositol phosphate kinase domain have a role in asexual and sexual developments in *Phytophthora sojae*

Xinyu Yang[a], Wei Zhao[a], Chenlei Hua[b], Wenwu Ye[a], Harold J.G. Mejer[b], Francine Govers[bc], Xiaobo Zheng[a], Yuanchao Wang[a,*]

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

[b], Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, NL-6708 PB Wageningen, The Netherlands

[c], Centre for BioSystems Genomics, P.O. Box 98, NL-6700 AB Wageningen, The Netherlands

Xinyu Yang and Wei Zhao contributed equally to this study

G protein-coupled receptors (GPCRs) are cellular key components that mediate mostly extracellular signals into intracellular responses. They share an architecture consisting of seven transmembrane domains with a, at the cytosolic side located, C-terminal tail that initiates downstream signaling. A novel class of GPCRs exist in oomycetes that have a phosphatidylinositol phosphate kinase (PIPK) domain fused their C-terminus. *Phytophthora* species contain twelve GPCR-PIPKs (GK1-GK12). The present study focusses on two *P. sojae* GKs. Expression analysis revealed that *PsGK4* and *PsGK5* are differentially expressed during some development stages. The expression levels of *PsGK4* and *PsGK5* are peaking at the cyst stage or during cyst germination and during late infection stages. Functional analysis of *PsGK4* and *PsGK5* was accomplished by obtaining silenced transformants. Whereas The *PsGK4* silenced transformants exhibited no difference in hyphae morphology, growth rate and oospore production in comparison to wild type and control transformants, they showed a higher level of encystment and a reduced cyst germination rate. In addition, zoospores lost the chemotaxis response to soybean root tips. The *PsGK5* silenced transformants exhibited no differences in asexual development. Instead, the oospore production of silenced transformants was severely impaired. Both *PsGK4* and *PsGK5*-silenced transformants showed reduced pathogenicity. RFP fusions of GK5 revealed the absence of the protein from the transmembrane but its presence in small vesicles of which a significant fraction moved rapidly. The obtained results point to involvement of GKs during various life-stages regulating zoospore behavior and oospore development. We propose that each of the GKs heads a pathway involved in development.

Mitogen-Activated Protein Kinase Cascades in *Phytophthora sojae*

Meng Zhang, Jing Lu, Aining Li, Kai Tao, Suomeng Dong, Xiaobo Zheng, Yuanchao Wang

College of Plant Protection, Key Laboratory of Integrated Management of Crop Diseases and Pests (Ministry of Education), Nanjing Agricultural University, Nanjing 210095, China

Mitogen-activated protein kinase (MAPK) cascades are universal signal transduction modules in yeasts, animals and plants. These protein phosphorylation cascades link extracellular stimuli to a wide range of cellular responses. Completion of the *Phytophthora sojae* genome-sequencing project has revealed the existence of 14 MAPKs, including 4 MAPK kinases and 3 MAPK kinase kinases. *PsSAK1* gene, a mitogen-activated protein (MAP) kinase from *P. sojae*, was identified in our previous studies and has been shown to play an important role in host infection and zoospore viability. In this study, the 3'-tag digital gene expression (DGE) profiling was applied for the global transcriptional sequence of *PsSAK1*-silenced mutants at the cyst and 1.5 h after inoculation stages. A set of 1,193 genes were down-regulated during the two stages compared with the recipient *P. sojae* strain. Among them, higher rate were detected in Myb family that was related to *PsSAK1* silencing, including a R2R3-type Myb transcription factor, *PsMYB1*. qRT-PCR indicated that the transcriptional level of *PsMYB1* decreased due to *PsSAK1* silencing. The transcript of *PsMYB1* was up-regulated at sporulation, germinated cysts, and early infection stages. Silencing of *PsMYB1* results in the following phenotypes different from wild-type: a) no cleavage of the cytoplasm into uninucleate zoospores or release of normal zoospores, b) direct germination of sporangia, and c) reduced virulence consistent with the phenotype of *PsSAK1*-silenced strains. Thus, the data indicated that the transcription factor PsMYB1 functions downstream of MAP kinase PsSAK1 and is required for zoospore development and the virulence of *P. sojae*. Another mitogen-activated protein kinase, PsMPK1, is involved in asexual developmental process, cell wall integrity and pathogenicity. Loss of *PsMPK1*, *P. sojae* strains showed hypersensitivity to cell wall degrading enzyme, produced less zoospores significantly, and grew in a severely retarded way compared with wild-type. TEM observations showed that the innermost layer and the inner cell membrane of *P. sojae* contained rugae and invaginations. Necrosis was observed aroud the inoculation site of etiolated soybeans, and the hyphae of the mutants could not extend among the cells of host. Transcript profiling revealed that PsMPK1 regulated the transcript of some elicitor proteins and Avh proteins. Therefore we hypothesized that *PsMPK1* is related with various signals of infection processes of *P. sojae*.

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Participants

Aining, Li
lail025@yahoo.com.cn
Chinese Academy of Science
52 Sanlihe Rd.
100101 Beijing
China

Ali, Shad, Gul
gsali@ufl.edu
Mid-Florida Research & Education Center
Institute of Food and Agricultural Sciences
University of Florida,
2725 Binion Road,
32703 Apopka
USA

Anderson, Gabriel, Ryan
andersrg@vt.edu
McDowell Laboratory, Virginia Tech
536 Latham Hall
24060 Blacksburg, VA
USA

Birch, Paul
Paul.birch@hutton.ac.uk
Division of Plant Sciences
University of Dundee (at JHI)
Invergowrie
Dundee
DD2 5DA Dundee
UK

Blackman, Leila
leila.blackman@anu.edu.au
Research School of Biology
Australian National University
0200 Canberra, ACT
Australia

Bottin, Arnaud
bottin@lrsv.ups-tlse.fr
Universit de Toulouse
Laboratoire de Recherche en Sciences Végétales
UPS, UMR 5546;BP 42617
F-31326 Tolosan ,Castanet
France

Breen, Susan
Susan.breen@hutton.ac.uk
James Hutton Institute
University of Dundee
Errol Road Invergowrie
DD2 5DA Dundee
UK

Brunner, Frédéric
Frederic.brunner@zmbp.uni-tuebingen.de
ZMBP - Center for Plant Molecular Biology
Plant Biochemistry
University of Tübingen
Auf der Morgenstelle 5
D-72076 Tübingen
Germany

Cai, Meng
cm122587@163.com
Department of Plant Pathology
China Agricultural University
2 Yuanmingyuan West Road, Haidian District
100193 Beijing
China

Cao, Hua
Cao7448hua@nwsuaf.edu.cn
Northwest A&F University
No.3 Taicheng Road
712100 Yangling, Shaanxi
China

Chai, Chunyue
ccyuer1019@126.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Chen, Lei
chenleicau@gmail.com
College of Agronomy and Biotechnology
China Agricultural University
2 Yuanmingyuan West Road,Haidian District
100193 Beijing
China

Chen, Linlin
2009102012@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Chen, Qinghe
chenqh@faas.cn
Fujian Academy of Agricultural Science
247 Wusi Road Fuzhou
350003 Fuzhou
China

Chen, Xiaoren
Xrchen_hn@hotmail.com
Yangzhou University
88 South University Ave.
225009 Yangzhou, Jiangsu
China

Choi, HyunJin
choi4@vbi.vt.edu
Virginia Tech
536 Latham Hall
24060 Blacksburg, VA
USA

Dai, Tingting
2010202021@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Dai, Wenkui
daiwenkui@genomics.cn
BGI-Shenzhen
Beishan Industrial Zone, Yantian District
518083 Shenzhen
China

Day, Brad
bday@msu.edu
Michigan State University
Board of Trustees
48820 East Lansing, Michigan
USA

Deb, Devdutta
ddeb1@vt.edu
Plant Pathology, Physiology and Weed Science
Virginia Tech
536 Latham Hall
24061 Blacksburg VA
USA

Dong, Suomeng
smdong@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Dou, Daolong
ddou@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Du, Juan
juan2.du@wur.nl
Laboratory of Plant Breeding
Plant Sciences Group
Wageningen University and Research Centre
6708 PB Wageningen
The Netherlands

Duan, Shuyi
2011202021@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Engelhardt, Stefan
Stefan.engelhardt@hutton.ac.uk
Division of Plant Sciences
University of Dundee
JHI, Errol Road, Invergowrie
DD2 5DA Dundee
UK

Espindola, Sebastian, Andres
espindo@ostatemail.okstate.edu
National Institute for Microbial Forensics & Food and Agricultural Biosecurity
Department of Entomology & Plant Pathology
127 Noble Research Center
Oklahoma State University
74078 Stillwater, Oklahoma
USA

Fan , Sujie
Jay19861224@sina.com
Soybean Research Institute
Northeast Agricultural University
No.59 Mucai Street Xiangfang District
150030 Harbin
China

Fang, Yufeng
yufengf1@vt.edu
Center for Genome Research and Biocomputing
Oregon State University
97331 Corvallis, Oregon
USA

Fedkenheuer, E, Kevin
fedkenke@vt.edu
Department of Plant Pathology, Physiology, and Weed Science
Virginia Tech
536 Latham Hall
24060 Blacksburg, VA
USA

Flores, Cristina
Cristina.flores@email.ucr.edu
CMDB Graduate Program
University of California, Riverside
92507 Riverside, California
USA

Gao, Jian
2010202022@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Gao, Zhimou
gaozhimou@126.com
College of Plant Protection
Anhui Agricultural University
130 West Changjiang Rd.
230036 Hefei, Anhui
China

Ge, Lingxiao
glingx@bgsu.edu
Lab 532 Department of Biological Sciences
Bowling Green State University
43403 Bowling Green, Ohio
USA

Geng, Lihua
Lihuag316@yahoo.com.cn
Beijing Vegetable Research Center
Beijing Academy of Agriculture and Forestry Sciences
100097 Beijing
China

Gijzen, Mark
mark.gijzen@agr.gc.ca
Agriculture and Agri-Food Canada
1341 Baseline Road
N5V 4T3 London
Canada

Gil, Juliana, Rosa
giljuliana@gmail.com
University of California – Davis
One Shields Avenue Davis, CA
95616 Davis, CA
USA

Gil, Juliana, Rosa
giljuliana@gmail.com
University of California – Davis
One Shields Avenue Davis, CA
95616 Davis, CA
USA

Gilroy, Marjorie, Eleanor
eleanor.gilroy@hutton.ac.uk
Office D111/D107 lab
The James Hutton Institute
DD2 5DA Dundee
UK

Govers, Francine
francine.govers@wur.nl
Droevendaalsesteeg 1
Laboratory of Phytopathology
Wageningen University
6708 PB Wageningen
The Netherlands

Gu, Biao
bgu@nwsuaf.edu.cn
State Key Laboratory of Crop Stress Biology for Arid Areas
Northwest A&F University
3 Taicheng Road
712100 Yangling, Shaanxi
China

Guo, Liyun
ppguo@cau.edu.cn
China Agricultural University
2 Yuanmingyuan West Road, Haidian District
100191 Beijing
China

Guo, Mei
guo_plum@yahoo.com
Virus-free Seedling Research Institute
Heilongjiang Academy of Agricultural Sciences
368 Xuefu Road,Nangang District
150086 Harbin
China

Guo, Na
2008201011@njau.edu.cn
College of Agriculture
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Haldar, Kasturi
khaldar@nd.edu
Massachusetts Institute of Technology
University of Notre Dame
46556 Notre Dame, IN
USA

He, Qin
heqin1024@126.com
Key Laboratory of Horticultural Plant Biology
Huazhong Agricultural University
No.1,shizishan Street,Hongshan District
430070 Wuhan
China

Hein, Ingo
Ingo.Hein@hutton.ac.uk
The James Hutton Institute
Invergowrie–Dundee
DD2 5DA Scotland
UK

Heyman, Fredrik
fredrik.heyman@slu.se
Department of Forest Mycology and Pathology
Inst för skoglig mykologi och patologi
Swedish University of Agricultural Sciences
Box 7026 Ulls v 26A
750 07 Uppsala
Sweden

Hu, Jian
jaffyhu@gmail.com
University of Tennessee & China Agricultural University
37919 Knoxville, Tennessee
USA

Hua, Chenlei
chenlei.hua@wur.nl
Wageningen University & Research centre
6708 PB Wageningen
The Netherlands

Islam, M Tofazzal
tofazzalislam@yahoo.com
Department of Biotechnology
Bangabandhu Sheikh Mujibur Rahman Agricultural University
1706 Gazipur
Bangladesh

Jiang, H.Y., Rays
rjiang@broad.mit.edu
The Broad Institute of MIT and Harvard
02142 MA Cambridge MA
USA

Jiang, Hua
2009101002@njau.edu.cn
College of Agriculture
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Jiang, jizhi
jiangjizhi@yahoo.com
Biochemistry and Molecular Biology
College of Life Sciences, Hebei University
Road-East Wusi 180, Baoding
071002 Hebei
China

Jiang, Liangyu
Liangyu0113@163.com
Soybean Research Institute
Northeast Agricultural University
No.59 Mucai Street Xiangfang District
150030 Harbin
China

Jiang, Zide
zdjiang@scau.edu.cn
South China Agricultural University
Wushan RD.,Tianhe District
510642 Guangzhou
China

Jing, Maofeng
jingmfeng@163.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Kamoun, Sophien
sophien.kamoun@tsl.ac.uk
The Sainsbury Laboratory
Norwich Research Park
NR4 7UH Norwich
United Kingdom

Krajaejun, Theerapong
mr_en@hotmail.com
Department of Pathology
Ramathibodi Hospital
Mahidol University
10400 Bangkok
Thailand

Lamour, Haas, Kurt
klamour@utk.edu
University of Tennessee
37923 Knoxville, Tennessee
USA

Laura J. Grenville-Briggs
lauragb@kth.se
Division of Glycoscience
School of Biotechnology
Royal Institute of Technology (KTH)
106 91 Stockholm
Sweden

Li, Changsong
Lics1011@sina.com
Plant Protection Institute
Shandong Academy of Agricultural Science
250100 Jinan, Shandong
China

Li, Delong
2009202012@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Li, Honghao
leehh071@yahoo.com.cn
Institute of Plant Protection
Sichuan Academy of Agricultural Sciences
610066 Chengdu
China

Li, Jinggan
2009102059@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Li, Hongjuan
woheaiyouyue921@163.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Li, Qi
liqihenzhao@126.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Liang, Chen
Liangchen@qau.edu.cn
Qingdao Agriculture University
266109 Qingdao, Shandong
China

Lin, Yanling
1148122@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Liu, Hui-Rong
Lhz17@yahoo.com.cn
College of Life Sciences
Inner Mongolia Agricultural University
10018 Hohhot
China

Liu, Tingli
2007202019@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Liu, Xili
seedling@cau.edu.cn
China Agricultural University
2 Yuanmingyuan West Road, Haidian District
100193 Beijing
China

Liu, Li
1216108@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Liu, Peihan
lphgood@126.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Lobach, Lars
lars.loebach@abdn.ac.uk
Institute of Medical Sciences
University of Aberdeen
AB25 2ZD Aberdeen
UK

Lu, Chenchen
sango64@126.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Lu, Jing
2009102050@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Lu, Shan
2009202004@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Lu,Xiaoxue
1217105@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Lv, Rongfei
walkinglv@126.com
Shandong Agricultural University
Daizong Road No.61
271000 Taian,Shandong
China

Ma, Huifei
huifei211204@sohu.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Ma, Wenbo
wenbo.ma@ucr.edu
University of California,Riverside
900 University Ave.
92521 Riverside, CA
USA

Ma, Zhenchuan
masen8866@163.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

McDowell, Matthew, John
johnmcd@vt.edu
Virginia Tech
536 Latham Hall
24060 Blacksburg, VA
USA

Meng, Yuling
yuling.meng1985@gmail.com
Northwest A&F University
No.3 Taicheng Road
712100 Yangling, Shaanxi
China

Min, Fanxiang
minfanxiang@126.com
Virus-free Seedling Research Institute of
Heilongjiang Academy of Agriculture Scicences
150086 harbin
China

Morris, Paul Francis
pmorris@bgsu.edu
Bowling Green State University
1001 East Wooster Street
43403 Bowling Green, OH
USA

Nasir
nasirrajput81@gmail.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Peng, Youliang
pengyl@cau.edu.cn
Chinese Agricultural University
2 Yuanmingyuan West Road, Haidian District
100193 Beijing
China

Qi, Rende
rende7@126.com
Institute of Plant Protection
Anhui Academy of Agricultural Sciences
No.40 Nongke South Road
30031 Hefei
China

Qiao, Jiachen
qiao.jiachen@163.com
Inner Mongolia Potato E&T Research Center
No.235 University West Road
10021 Hohhot
China

Qiao, Yongli
yongli.qiao@ucr.edu
University of California, Riverside
900 University Ave.
92521 Riverside, CA
USA

Quan, Junli
junliquan@gmail.com
College of Plant Protection
Northwest A&F University
No.3 Taicheng Road
712100 Yangling, Shaanxi
China

Rutter, Douglas, Brian
rutterb@bgsu.edu
Bowling Green State University
1001 East Wooster Street
43403 Bowling Green, OH
USA

Rzeszutek, Elzbieta
elrz@kth.se
Division of Glycoscience,
School of Biotechnology
Royal Institute of Technology (KTH)
Roslagstullsbacken21
10691 Stockholm
Sweden

Saraiva, Raquel Maia, Marcia
m.saraiva@abdn.ac.uk
Institute of Medical Sciences
University of Aberdeen
AB25 2ZD Aberdeen
UK

Shan, Weixing
wxshan@nwsuaf.edu.cn
College of Plant Protection
Northwest A&F University
No.3 Taicheng Road
712100 Yangling, Shaanxi
China

Shen, ChongYao
cyshen@cau.edu.cn
Dept. of Plant Pathology
China Agricultural University
2 Yuanmingyuan West Road, Haidian District
100193 Beijing
China

Shen, Danyu
2009102014@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Sheng, Yuting
2009202013@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Song, Tianqiao
2009102015@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Song, Weiwei
weiweisdau@126.com
Shandong Agricultural University
Daizong Road No.61
271000 Taian,Shandong
China

Stam, Remco
remco.stam@hutton.ac.uk
James Hutton Institute
University of Dundee
DD2 5DA Dundee, Scotland
UK

Su,liming
suliming1@126.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Sui, Yangyang
Syy341@163.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Sun,nannan
snn0327@163.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Tang, Junli
tangjunli2@163.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Tao, Kai
Kaitao.china@gmail.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Tian, Yue-e
tianyuee1985@163.com
Northwest A&F University
No.3 Taicheng Road
712100 Yangling, Shaanxi
China

Tian, Zhendong
Tianzhd@mail.hzau.edu.cn
Key Laboratory of Horticultural Plant Biology
Huazhong Agricultural University
No.1,shizishan Street,Hongshan District
430070 Wuhan
China

Tripathy, Sucheta
tsucheta@gmail.com
Virginia Tech
536 Latham Hall
76002 Blacksburg, VA
USA

Tyler, Brett
Brett.tyler@oregonstate.edu
Center for Genome Research and Biocomputing,
Oregon State University
97331 Corvallis, OR
USA

Vossen ,Jack
jack.vossen@wur.nl
Wageningen UR Plant Breeding
Wageningen University and Research Center
Droevendaalsesteeg 1
6708PB Wageningen
The Netherlands

Wang , Xin
693338830@qq.com
Soybean Research Institute
Northeast Agricultural University
No.59 Mucai Street Xiangfang District
150030 Harbin
China

Wang ,Yonglin
ylwang@bjfu.edu.cn
Beijing Forestry University
35 qinghua east Road, Haidian District
100083 Beijing
China

Wang, Huizheng
huizhengwang@yahoo.cn
Shandong Agricultural University
Daizong Road No.61
271000 Taian,Shandong
China

Wang, Qinhu
qinhu.wang@gmail.com
Northwest A&F University
No.3 Taicheng Road
271000 Yangling, Shaanxi
China

Wang, Qunqing
qunqing.wang@oregonstate.edu
Center for Genome Research and Biocomputing
Oregon State University
97331 Corvallis, OR
USA

Wang, Xiaowen
xiaowenwang@cau.edu.cn
China Agricultural University
2 Yuanmingyuan West Road, Haidian District
100191 Beijing
China

Wang, Yan
yan.wang@wur.nl
Lab of Phytopathology
Wageningen University
6708PB Wageningen
The Netherlands

Wang,Rongbo
wrb16128@163.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Wei,Qiwei
weiqiwei2008@126.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Wang,Yuanchao
wangyc@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Wen, Jingzhi
Jzhwen2000@yahoo.com.cn
Northeast Agricultural University
No.59 Mucai Street Xiangfang District
150030 Harbin
China

Willems, Ariane
Ariane.willems@abdn.ac.uk
Aberdeen Oomycete Laboratory
College of Life Sciences and Medicine
Institute of Medical Sciences
University of Aberdeen
AB25 2ZD Aberdeen
UK

Wise, Zhang, Hua
huawise@vt.edu
Center for Genome Research and Biocomputing
Oregon State University
97331 Corvallis, OR
USA

Wong, Tac, James
Jwong032@ucr.edu
University of California, Riverside
900 University Ave.
92521 Riverside, CA
USA

Wong, W, Joan
jnwong@ucdavis.edu
University of California – Davis
One Shields Avenue
95616 Davis, CA
USA

Wu,Yuren
1217407@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Xiong, Qin
Xiongqin1226@hotmail.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Xu, Jing
1218213@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

XuanYuan, Guochao
xuanyuanguochao@163.com
Inner Mongolia Potato E&T Research Center
010021 Huhhot
China

Yan, Qiang
2008101141@njau.edu.cn
College of Agriculture
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Yang, Shuai
2009102049@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Yang, Shuai
yangshuai20000@163.com
Northeast Agricultural University
No.59 Mucai Street Xiangfang District
150030 Harbin
China

Yang, Xinyu
2010202017@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Ye, Wenwu
wenwuye@gmail.com
Center for Genome Research and Biocomputing
Oregon State University
97331 Corvallis, OR
USA

Yin, Weixiao
2011202022@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Yu, Dan
2008202013@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Yu, Xiaoli
yuxiaoli92jn@gmai.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Zhang, Meixiang
zhiwuyazhe@gmail.com
Northwest A&F University
No.3 Taicheng Road
712100 Yangling, Shaanxi
China

Zhang, Meng
Zhangmeng.1204@163.com
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Zhang, Mengyan
Zmy791756937@163.com
Shandong Agricultural University
Daizong Road No.61
271000 Taian,Shandong
China

Zhang, Shuzhen
dnzhshzh@163.com
Soybean Research Institute
Northeast Agricultural University
No.59 Mucai Street Xiangfang District
150030 Harbin
China

Zhang, Weiwei
weiwei.zhang@anu.edu.au
The Australian National University
0200 Canberra, ACT
Australia

Zhang, Xin
2009202014@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

Zhang, Xiuguo
zhxg@sdau.edu.cn
Shandong Agricultural University
Daizong Road No.61
271000 Taian,Shandong
China

Zhao, Li
mushanshuizhiji@163.com
Shandong Agricultural University
Daizong Road No.61
271000 Taian,Shandong
China

Zhao, Zhijian
zhijianzhao@hotmail.com
Agricultural Environments & Resources Institute
Yunnan Academy of Agricultural Science
Taoyuan Village, Longtou Street
650205 Kunming
China

Zheng, Xiangzi
xiangzi.zheng@student.uni-tuebingen.de
Center of Plant Molecular Biology (ZMBP)
Tübingen university
72076 Tübingen
German

Zheng,Xiaobo
xb.zheng@njau.edu.cn
Plant Pathology Department
Nanjing Agricultural University
No.1 Weigang Road
210095 Nanjing
China

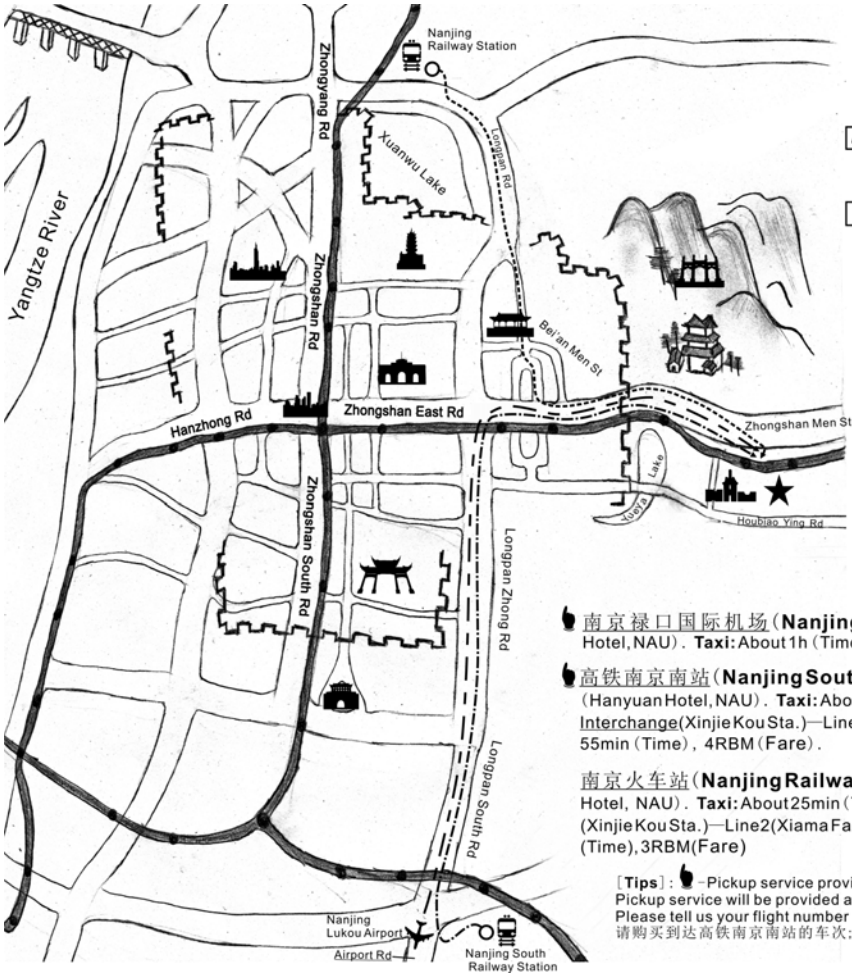
Zhili,Pang
pzl19870611@126.com
China Agricultural University
2 Yuanmingyuan West Road, Haidian District
100193 Beijing
China

Zhou, Jianmin
zhoujianmin@nibs.ac.cn
Institute of Genetics and Development Biology
China Agricultural University
West Lincui Road, Chaoyang District
100101 Beijing
China

Zhou, Yuxin
Rita1720@126.com
Department of Plant Pathology
China Agricultural University
2 Yuanmingyuan West Road, Haidian District
100193 Beijing
China

Zhu, jie
Zhujie1216@hotmail.com
Shandong Agricultural University
Daizong Road No.61
271000 Taian,Shandong
China

Zhu, Xiaoqiong
mycolozhu@cau.edu.cn
China Agricultural University
2 Yuanmingyuan West Road, Haidian District
100191 Beijing
China



Location Map

For OMGN May 25-28, 2012

Draw by Xu,Wu Annotated by Wu, Yuren

Meeting Address:

南京农业大学 (NAU)
卫岗1号 (No.1 Weigang Rd)

翰苑大厦 (Hanyuan Hotel)
童卫路20号 (No.20 Tongwei St)

Tour Spot:

- Confucius Temple
- Gujiming Temple
- Minggugong
- Dr. Sun Yat-Sen's Mausoleum
- Presidential Palace
- Gate of China
- Xinjiekou
- Zifeng Mansion

Note:

- Subway
- Ming Dynasty City Wall

Important Rd. and St. with Chinese Name:

机场高速(Airport Rd)；
龙蟠南路(Longpan South Rd)；
龙蟠中路(Longpan Zhong Rd)；
中山东路(Zhongshan East Rd)；
中山门大街(Zhongshan Men St)；
龙蟠路(Longpan Rd)；
北安门街(Bei' an Men St)；
后标营路（Houbiao Ying Rd）

到达路线方式(Route)

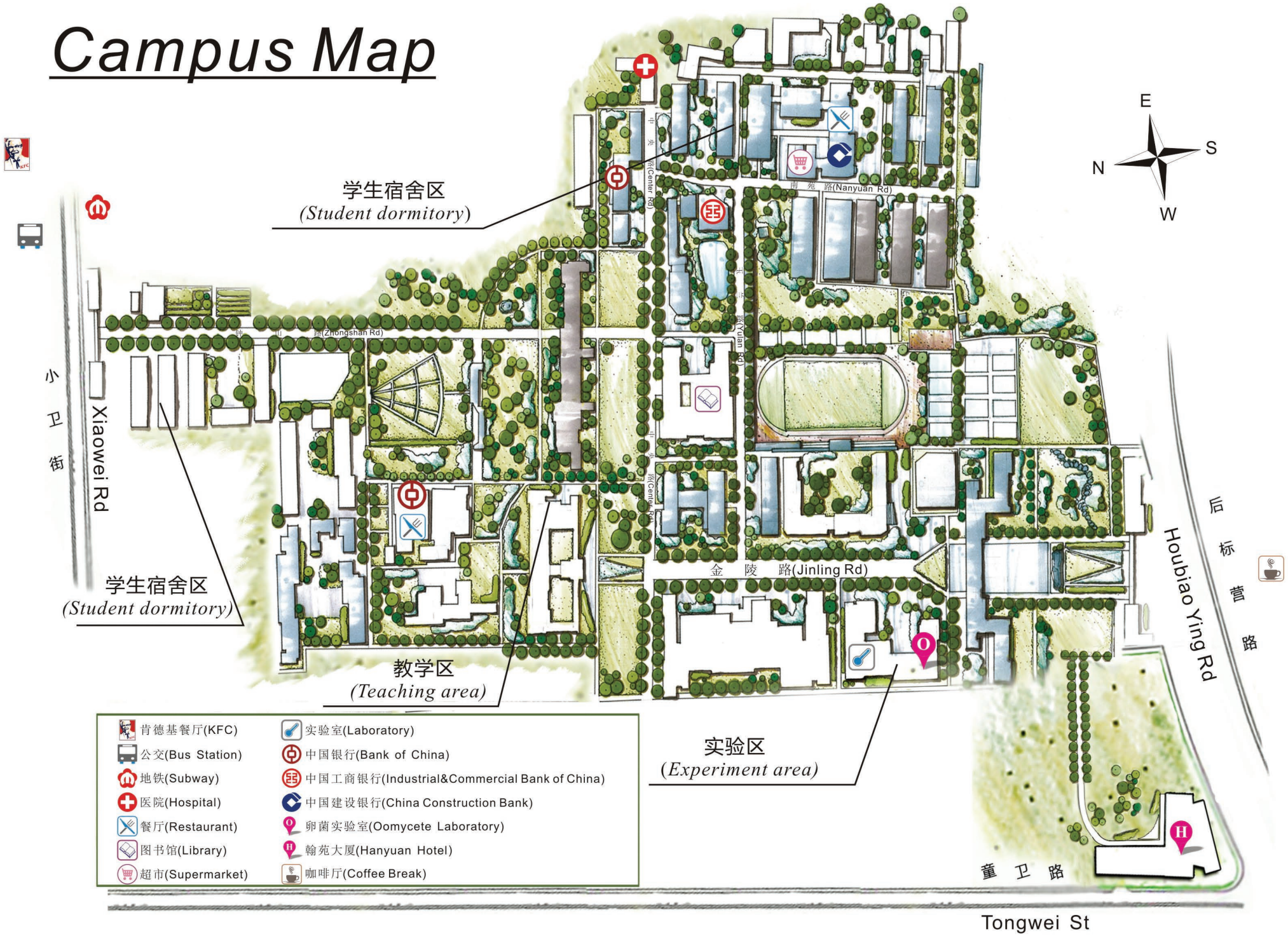
南京禄口国际机场(**Nanjing Lukou Airport**)——→南京农业大学.翰苑大厦(Hanyuan Hotel,NAU). **Taxi:** About 1h (Time),150RBM

高铁南京南站(**Nanjing South Railway Station,NSRS**)-·-·→南京农业大学.翰苑大厦(Hanyuan Hotel,NAU). **Taxi:** About 40min (Time), 50RBM (Fare); **Subway:** Line1(NSRS)—Interchange(Xinjie Kou Sta.)—Line2(Xiama Fang Sta.)—10minutes' walk to Hanyuan Hotel, about 55min (Time), 4RBM (Fare).

南京火车站(**Nanjing Railway Station, NRS**)·····→南京农业大学.翰苑大厦(Hanyuan Hotel, NAU). **Taxi:** About 25min (Time), 25RBM (Fare); **Subway:** Line1(NRS)—Interchange (Xinjie Kou Sta.)—Line2(Xiama Fang Sta.)—10minutes' walk to Hanyuan Hotel, about 40min (Time), 3RBM(Fare)

[**Tips**]: -Pickup service provided at this site.
Pickup service will be provided at **Nanjing Lukou Airport** and **Nanjing South Railway Station**. Please tell us your flight number as soon as possible so that you can get this service conveniently.
请购买到达高铁南京南站的车次；为便于及时接机，请尽快告知我们您的航班信息。

Campus Map



Draw by Luo,Chang Colored by Song,Jiawei Annotated by Wu,Yuren