

RESEARCH ARTICLE

# Type VI Secretion System Transports $Zn^{2+}$ to Combat Multiple Stresses and Host Immunity

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## Abstract

Type VI secretion systems (T6SSs) are widespread multi-component machineries that translocate effectors into either eukaryotic or prokaryotic cells, for virulence or for interbacterial competition. Herein, we report that the T6SS-4 from *Yersinia pseudotuberculosis* displays an unexpected function in the transportation of  $Zn^{2+}$  to combat diverse stresses and host immunity. Environmental insults such as oxidative stress induce the expression of T6SS-4 via OxyR, the transcriptional factor that also regulates many oxidative response genes. Zinc transportation is achieved by T6SS-4-mediated translocation of a novel  $Zn^{2+}$ -binding protein substrate YezP (YPK\_3549), which has the capacity to rescue the sensitivity to oxidative stress exhibited by T6SS-4 mutants when added to extracellular milieu. Disruption of the classic zinc transporter ZnuABC together with T6SS-4 or yezP results in mutants that almost completely lost virulence against mice, further highlighting the importance of T6SS-4 in resistance to host immunity. These results assigned an unconventional role to T6SSs, which will lay the foundation for studying novel mechanisms of metal ion uptake by bacteria and the role of this process in their resistance to host immunity and survival in harmful environments.

## Author Summary

One unique feature of type VI secretion system is the presence of multiple distinct systems in certain bacterial species. It is well established that some of these systems function to compete for their living niches among diverse bacterial species, whilst the activity of many such transporters remains unknown. Because metal ions are essential components to virtually all forms of life including bacteria, eukaryotic hosts have evolved complicated strategies to sequester metal ions, which constitute a major branch of their nutritional immunity. Therefore the ability to acquire metal ions is critical for bacterial virulence. This study reveals that the T6SS-4 of *Yersinia pseudotuberculosis* (Yptb) functions to import  $Zn^{2+}$  from the environment to mitigate the detrimental effects such as hydroxyl radicals induced by diverse stresses. Expression of the transporter is activated by multiple



## 第十六届全国植物基因组学大会合影留念



第十六届全国植物基因组学大会  
The 16<sup>th</sup> national plant genome conference

## 一、学术交流

### 1. Scientific Exchange

## 我室举办第十六届全国植物基因组学大会 Hosting the 16<sup>th</sup> National Plant Genome Conference

8月19日–22日, 由我室和生命科学学院共同承办的“第十六届全国植物基因组学大会”在杨凌召开。中国科学院院士张启发、陈晓亚、韩斌以及来自美国加利福尼亚大学、华盛顿大学、北京大学等近百家国内外高校、科研院所以及我校师生近700人参加会议。

The State Key Laboratory and the College of Life Science hosted the 16th National Plant Genome Conference in Yangling on August 19–22, 2015. There were 700 participants at the meeting, including Chinese Academy of Science academicians Qifa Zhang, Xiaoya Chen, Bin Han, and scientists from the University of California, the University of Washington, Beijing University, and other foreign and domestic universities and institutes.

康振生教授主持开幕式, 罗军副校长致辞。研讨会期间, 美国科学院院士、加州大学戴维斯分校 Jorge Dubcovsky 教授、加州大学伯克利分校 Barbara Baker 教授、华盛顿大学 Christine Queitsch 教授等 32 位国内外专家学者围绕 Genome Sequencing and New Technology, Functional Genomics, Proteomics, Metabolomics and Bioinformatics, Transgenic Technology and Genomics-based Breeding, Genome Diversity 以及 Epigenetics and Epigenomics 六个专题展开报告, 并深入讨论。

Prof. Zhensheng Kang, the lab director, presided the opening ceremony, and Vice President Jun Luo made the welcome speech. During the conference, 32 scientists including Dr. Jorge Dubcovsky, American Academy of Science academician and professor of the University of California, Davis; Dr. Barbara Baker, professor of the University of California, Berkeley; and Dr. Christine Queitsch, professor of the University of Washington delivered oral presentations in six special sessions on genome sequencing and new technology; functional genomics; proteomics, metabolomics and bioinformatics; transgenic technology and genomics-based breeding; genome diversity; and epigenetics and epigenomics.

闭幕式由大会执行主席杨维才研究员主持, 大会主席、华中农业大学张启发教授致闭幕辞, 我校孙其信校长和中国科学院上海生命科学研究院副院长韩斌院士共同为优秀研究生报告、优秀墙报颁奖。最后组委会宣布第十七届全国植物基因组学大会由福建农林大学承办并进行了会旗交接。

Dr. Weichai Yang, conference executive chair, presided the close ceremony, and Dr. Qifa Zhang, conference chair, delivered close speech. Dr. Qixin Sun, president of Northwest A&F University, and Dr. Bin Han, vice president of Chinese Academy of Science Shanghai Life Science Institute presented awards to graduate students who gave excellent oral or poster presentations. The conference committee made the announcement of Fujian A&F University to host the 17<sup>th</sup> National Plant Genome Conference, and the conference flag was passed to the next conference host.



## 第六届全国小麦基因组学及分子育种大会召开

The 6<sup>th</sup> National Conference of Wheat Genome and Molecular Breeding Was Held



刘旭 刘旭院士致辞  
Drs. Xu Liu delivered speech



程顺和 程顺和院士致辞  
Drs. Shunhe Cheng delivered speech



贾继增 贾继增研究员致辞  
Prof. Jizeng Jia delivered speech

报告会期间，孙其信教授作了题为“气候变化背景下小麦耐热性遗传改良”的报告，美国加利福尼亚大学Jorge Dubcovsky教授、国家杰青康振生研究员、长江学者康振生教授等26名专家学者围绕小麦的基因组学、新基因发掘、分子育种与种质创新、远缘杂交、遗传多样性及小麦抗条锈病等作了主题报告。大会决定第七届全国小麦基因组学及分子育种大会由河南农业大学承办。

During the conference, Prof. Qixin Sun delivered a presentation titled “Genetic improvement of wheat cultivars for heat tolerance under climate changes”. A total of 26 scientists, including Prof. Jorge Dubcovsky from the University of California, Davis; Dr. Kang Zhong, National Excellent Youth scientist, and Prof. Zhensheng Kang gave presentations in special sessions of wheat genomics, new gene discovery, molecular breeding and germplasm development, hybridization of alien species, genetic diversity, and stripe rust resistance. Based on the conference decision, Henan Agricultural University will host the 7<sup>th</sup> National Wheat Genomics and Molecular Breeding.



我校专家做报告  
Experts in NWSUAF gave speeches

## 康振生教授率团参加第十四届国际锈病白粉病大会并做特邀报告

Prof. Zhensheng Kang led a delegation for the 14<sup>th</sup> International Cereal Rusts and Powdery Mildews Conference and Was Invited to Present Research

7月5日至8日，“第十四届国际锈病白粉病大会”在丹麦赫尔辛格举行。来自美国、英国等20多个国家和地区的180余名研究人员参加。

The 14<sup>th</sup> International Cereal Rusts and Powdery Mildews Conference was held in Helsingør, Denmark from July 5 to 8, 2015. About 180 scientists from 20 countries including the US and UK participated the conference.

康振生教授应邀作了题为“Research progress on the role of barberry in epidemics of wheat stripe rust in China”的大会报告，引起了参会学者的广泛关注，并就小檗在锈菌毒性变异中的作用等各自感兴趣的科学问题同康振生教授交流。会后，与会人员参观了位于Flakkebjerg的全球锈病参考中心（Global Rust Reference Center）及试验田，康振生教授与丹麦、美国等专家就条锈菌有性生殖等问题进行了深入交流。

Prof. Zhensheng Kang delivered a specially invited presentation titled “Research progress on the role of barberry in epidemics of wheat stripe rust in China”, which got great attention from the participants. He had further scientific exchange with conference attendants on barberry's role on the pathogen variation. The meeting delegations visited the Global Rust Reference Center.



康振生教授做学术报告  
Prof. Zhensheng Kang gave a report



汤春蕾博士做学术报告  
Dr. Chunlei Tang gave a report

随后，康振生教授一行访问了丹麦哥本哈根大学植物与环境科学系Hans Thordal Christensen教授的实验室。访问期间，康振生教授和汤春蕾博士分别做了题为“Advance in the study of wheat stripe rust”和“Systematic characterization of the effector repertoire of *Puccinia Striiformis*”的学术报告，双方就小麦条锈菌毒性变异、效应蛋白、非寄主抗性等方面进行了深入的交流。康振生教授还邀请Hans等来我校进行访问交流。

After the conference, Prof. Kang and his delegation visited the laboratory of Hans Thordal Christensen, the Department of Plant and Environmental Sciences, Copenhagen University. He and Dr. Chunlei Tang gave talks entitled “Advances in the study of wheat stripe rust” and “Systematic characterization of the effector repertoire of *Puccinia Striiformis*”, respectively. Guests and hosts had further exchange about virulence variation, effect proteins, and non-host resistance. Prof. Kang invited Dr. Hans to visit our university.



## 郭军教授等参加BGRI Technical Workshop 2015年会 Lab Scientists Attended the 2015 BGRI Technical Workshop

2015年9月15日至23日，郭军教授、韩德俊教授等一行6人参加了在澳大利亚悉尼举办的BGRI Technical Workshop 2015年度会议。会议围绕锈病新抗源的挖掘与利用、锈病原监测、条锈病研究进展、锈菌小种毒性变异，抗锈品种的选育与利用等方面进行了广泛的学术交流。



会议合影  
Participants of the 2015 BGRI technical workshop

Six scientists including Prof. Jun Guo and Prof. Dejun Han from our laboratory attended the 2015 BGRI Technical Workshop held in Sydney, Australia from September 15 to 23, 2015. The workshop had presentations and discussions on discovery and utilization of new sources of resistance, monitoring rust pathogens, stripe rust research progress, virulence variation of rust pathogen races, and breeding for rust resistant wheat cultivars.

### 学术报告 Seminars

7月到9月，实验室邀请国内外6位知名学者来我室访问交流。

1. 美国内布拉斯加大学Steven Harris教授：The stress biology of extremophilefungand the nature of fungal interactions algae.

2. 印度德里大学IndraniDasgupta教授：Use of Rice tungro bacilliform virus to obtain virus resistance and gene silencing in rice.

3. 美国康奈尔大学张胜博士：Orbitrap Fusion Tribrid's technologies and applications for quantitative proteomics and characterization of global protein PTMs.

4. 默多克大学Bernard Dell教授：Drought tolerant research in wheat at Murdoch University

5. 澳大利亚联邦科工组织植物工业研究所王明波博士：DNA demethylases interact with small RNAs to regulate stress response genes in Arabidopsis.

6. 以色列特拉维夫大学HananSela博士：Conservation and utilization of cereal crop wild relatives.

From July to September, 2015, the laboratory invited 6 well-known foreign and domestic scientists for visiting and scientific exchange. The six visitors are the following.

1. Prof. Steven Harris from the University of Nebraska: "the stress biology of extremophile fungi and the nature of fungal interactions algae".

2. Prof. IndraniDasgupta from the University of Dehli: "Use of rice tungro bacilliform virus to obtain virus resistance and gene silencing in rice."

3. Dr. Sheng Zhang from Cornell University: "Orbitrap Fusion Tribrid's technologies and applications for quantitative proteomics and characterization of global protein PTMs."

4. Prof. Bernard Dell from Murdoch University: "Drought tolerant research in wheat at Murdoch University."

5. Dr. Mingbo Wang from CSIRO Institute of Plant Industry: "DNA demethylases interact with small RNAs to regulate stress response genes in Arabidopsis."

6. Dr. HananSela from Tel Aviv University: "Conservation and utilization of cereal crop wild relatives."



应邀专家作报告  
Invited experts gave speeches

## 二、科研进展

### 2. Progress of Scientific Research

沈锡辉教授团队在细菌六型分泌系统抗逆境胁迫机制研究方面取得重要进展

Prof. Xihui Shen's Team Made Important Progress in Type VI Secretory System Related to the Mechanisms of Stress Resistance in Bacteria

沈锡辉教授团队研究发现T6SS-4受到应激反应总调控因子RpoS和饥饿胁迫调控蛋白RovM等多种胁迫相关调控蛋白的调控，其中饥饿诱导的LysR家族转录调节蛋白RovM通过结合在T6SS-4启动子的上游区域激活其表达，与此相反，RovM通过结合在经典的精氨酸依赖抗酸系统AR3启动子-35区而抑制其表达。由此揭示了假结核耶尔森氏菌根据环境营养状况不同而协调表达不同抗酸胁迫系统的机制，该研究结果已在Environmental Microbiology (IF=6.2) 在线发表，博士生宋云洪为论文第一作者，沈锡辉教授为通讯作者。

Prof. Xihui Shen's team discovered that T6SS-4 is elicited under the regulation of inducible protein RovM and other regulatory proteins, among them hungry-induced LysR family transcript regulatory protein ROvM binding at the upstream of promotor T6SS-4. In contrast, RovM can inhibit the expression of T6SS4 through more classic binding at the upstream 35 domain of the AR3 promotor, anargininedependent acid resistant system. The results indicate that bacterium *Yersiniapseudotuberculosis* has different mechanisms for acid resistance under different nutritional conditions. The research has been published online in *Environmental Microbiology* (Impactor factor: 6.2). PhD student Yunhong Song was the first author, and Prof. Shen was the communication author.

该研究得到了国家自然科学基金，西北农林科技大学杰青培育专项及早区作物逆境生物学国家重点实验室自主研究经费的资助。

These studies were supported by Chinese National Science Foundation, the University Excellent Youth Scientists Training Special Fund, and the Laboratory Self-management Fund.

进一步研究发现T6SS-4可通过分泌一个锌离子结合蛋白YezP而促进细菌对锌离子的转运，进而抑制芬顿反应而降低胞内羟基自由基水平，以提高细菌在多种环境胁迫下的存活率。由此提出一个细菌T6SS转运离子并对抗环境胁迫的机制模型，即在外界胁迫下OxyR感受胁迫信号并上调T6SS-4表达，T6SS-4通过分泌Zn<sup>2+</sup>结合效应蛋白YezP以促进Zn<sup>2+</sup>转运而竞争性抑制芬顿反应，从而抑制羟基自由基的产生，提高细菌对环境胁迫的耐受能力。以上研究结果揭示了细菌VI型分泌系统的一种全新功能，研究结果在PLoS Pathogens (IF=7.5) 发表，博士生王铁涛、司美茹、宋云洪为论文共同第一作者，沈锡辉教授为通讯作者。

The team further found that T6SS-4 can enhance the Zn<sup>2+</sup> transportation through secreting protein YezP that binds Zn<sup>2+</sup> in the bacterium, which inhibits Fenton reaction to reduce the level of hydroxyl free radicals in bacterial cells and increase the survival rate of bacterial cells under environmental stress. Based on the results, they proposed a model for the bacterial T6SS transportation of ions: under external stress, OxyR receives the signal and up-regulates T6SS-4 expression. T6SS-4 enhances Zn<sup>2+</sup> transportation and competitively inhibits the Fenton reaction through secreting Zn<sup>2+</sup>-binding effector protein YezP, Which further inhibits the production of hydroxyl free radicals and increases the tolerance of bacterial cells to environmental stresses. This research reveals a new function of bacterial type VI secretory system and the results are published in *PLoS Pathogens* (Impact factor: 7.5). PhD students Tietao Wang, Meiru Si, and Yunhong Song were co-first authors, and Prof. Shen was the communication author.



## 黄丽丽教授团队在New Phytologist发表研究论文

Prof. Lili Huang' s Team Published Research in *New Phytologist*

黄丽丽教授带领的果树病害研究团队在苹果树腐烂病研究方面取得了突破性进展：研究发现，苹果树腐烂病菌果胶酶、次生代谢物合成、氮转运相关蛋白和酸性外泌蛋白酶等相关基因的扩增和表达可能是该病菌适应性侵染树皮的主要决定因子；次生代谢相关基因（簇）的变异，有可能导致了该病菌的寄主偏好性。研究结果为病害有效防治方法的建立提供了理论依据，以确保我国苹果的安全生产。

Prof. Lili Huang' s team has made breakthrough progress in apple canker disease. They discovered that the pectinase, secondary metabolites, nitrogen-transportation related proteins, and acid secreted proteinase are major pathogenicity factors. The variation in secondary-metabolites related gene families likely determine the host preferences of different isolates of the pathogen. The results provide theoretical basis for developing effective disease management strategies to secure apple production in China.

该成果已发表于New Phytologist (2015年公布影响因子为7.672)；博士生尹志远和刘慧泉副研究员为论文共同第一作者，黄丽丽教授为通讯作者，果树病害研究团队多名师生参与了本项研究工作。该研究得到了国家自然科学基金项目、高等学校博士学科点专项科研基金项目、公益性行业（农业）科研专项和高等学校学科创新引智计划项目的资助。

The research was published in *New Phytologist* (Impact factor: 7.672). PhD student Zhiyuan Yin and associate plant pathologist Huiquan Liu were the co-first authors, and Prof. Huang was the communication author. Several teachers and students in the tree fruit team participated in the research. This study was supported by Chinese National Science Foundation, Special Research Fund for University with PhD Scientific Fields, Publically Beneficial Industry (Agriculture) Science and Technology Special Program, and the “111” University Innovation and Exchange Program.

## 赵惠贤教授团队在小麦控制籽粒大小基因功能研究方面取得新进展

Prof. Huixian Zhao' s Team Made New Progress  
in Functions of Wheat Genes Controlling Kernel Size

日前，作物品质、产量与抗逆分子基础研究团队负责人赵惠贤教授团队在小麦控制籽粒大小基因克隆及功能研究方面中取得新进展：克隆得到位于小麦第7同源群染色体短臂上CYP78A家族的新基因TaCYP78A3；利用BSMV-VIGS技术沉默TaCYP78A3基因在小麦籽粒中的表达，导致小麦种子大小降低11%（ $P<0.01$ ）；通过转基因拟南芥过表达TaCYP78A3基因，导致拟南芥种子增大11%–47%（ $P<0.01$ ）。进一步的细胞学观察和分子水平检测，揭示了小麦TaCYP78A3基因通过促进胚珠和种子表皮细胞增殖来影响种子大小的分子机制。

Recently, Prof. Huixian Zhao' s team has made new progress in cloning and functional analysis of wheat genes controlling kernel size. The team cloned a gene, *TaCYP78A3*, localized on wheat chromosomal 7BS and homologous to *CYP78A*. Through silencing the gene expression in kernels, the BSMV-VIGS approach, they found 11% reduction in kernel size. Over-expression of the gene in Arabidopsis through transformation resulted in seed size increase by 11-47% ( $P<0.01$ ). Through cytological observations and molecular detection, they found that the gene functions increasing seed size through enhancing multiplication of embryo and skin cells.

该成果于2015年7月6日在线发表于The Plant Journal (2015年公布影响因子为5.99)。生命学院博士生马猛为论文第一作者；赵惠贤教授为通讯作者。本研究工作在国家自然科学基金项目：“病毒诱导的基因沉默在小麦穗部的实施及应用（30871578）和“3个小麦种子特异表达的全新microRNA的功能研究(31471482)”等资助下完成。

The research was published in *The Plant Journal* (Impact factor: 5.99) online on July 6, 2015. Meng Ma, a PhD student in the College of Life Science was the first author and Prof. Zhao was the communication author. This research was under the project “Application of virus-induced gene silencing in wheat kernels” (30871578) and “Functional analysis of three new miRNAs expressing specifically in wheat kernels” (30871578) supported by Chinese National Science Foundation. Prof. Zhao is a team leader responsible for basic research of quality, yield, and abiotic stress of crops in the laboratory.

## 三、技术队伍建设

### 3. Development of the Technological Team

## 显微可视平台三名技术人员参加西北五省电镜学术交流及技术研讨会

Technicians Participated in the Northwest  
Five Provinces Electronical Microscope Workshop

2015年7月31日–8月4日，“西北五省第七届电镜学术交流及技术研讨会”在兰州大学举行。会议邀请了国内外的27位专家作会议报告，讲解生物电子显微镜技术的最新发展和生物样品制备和应用方面技术方法。

The Northwest Five Provinces Electronical Microscope Workshop was held in Lanzhou University from July 31 to August 4, 2015. The workshop invited 27 foreign and domestic experts to give presentations on the recent development of electro-microscopic techniques, biological sample preparation, and application techniques.

显微可视技术平台三名实验技术人员参加了此次会议，通过与本领域专家、同行在电镜实验技术和方法经验上的学习、交流，进一步了解了电子显微学的发展前沿，提升了实验技术人员的业务能力，为今后我室电镜工作的更好开展打下了良好基础。

Three technologists from the lab platform of ultrastructural visualization participated in the workshop. Through exchanging techniques and methods, they learned more advanced developments in electron microscopy, enhanced their technical skills laid a good basis for better use of electron microscopy.



## 技术报告交流

### Exchange of Technical Reports

本季度，实验室邀请5位专家进行实验技术交流报告，同时就相关技术进行探讨和实践操作。

1. 张国云实验师：电子显微镜的原理及样品制备；
2. 万智毅：基于NGS的多组学一体化研究思路探讨；
3. 刘三阳：转录组分析与DNA数据挖掘的基本要领与软件使用；
4. 张 峰：高通量测序在动植物育种和发育中的应用；
5. 韦汉福：蛋白质技术在植物研究中的应用。



张国云实验师做技术报告  
Guoyun Zhang was giving talk on techniques

In this quarter, the lab invited five experts for presenting experimental techniques and discussed related techniques and operation.

1. Guoyun Zhang, technician: Theory of electron microscopy and sample preparation.
2. Zhiyi Wan: Study on integration of multi-omics based on next generation sequencing.
3. Sanyang Liu: Transcriptome analysis and the basic skills and software application in DNA data mining.
4. Feng Zhang: High-throughput of DNA sequencing and application in animal and plant breeding and development.
5. Hanfu Wei: Application of protein technology in plant research.

## 四、人才培养

### 4. Training

#### 张勇等三名博士后获批中国博士后科学基金特别资助项目

#### Three Postdoctoral Associates Received Support from China Postdoctoral Science Foundation

近日，中国博士后科学基金会公布了中国博士后科学基金第八批特别资助获资助人员名单，我室共有3名博士后获得本次资助，资助标准为15万元/人。

Chinese Postdoctoral Science Foundation recently announced the 8<sup>th</sup> group special support projects. Three postdoctoral associates in our lab received support of 150,000 yuan:

获资助人员分别是：

- 农业工程流动站博士后张勇（邹志荣教授指导） Dr. Yong Zhang, Agricultural Engineering (Advisor: Prof. Zhirong Zou)  
植物保护流动站博士后汤春蕾（康振生教授指导） Dr. Chunlei Tang, Plant Protection (Advisor: Prof. Zhensheng Kang)  
作物学流动站博士后胡祖庆（张改生教授指导） Dr. Zuqing Hu, Agronomy (Advisor: Prof. Gaisheng Zhang)

#### 汤春蕾等三名博士后获2015年陕西省博士后生活资助

#### Three Postdoctoral Associates Received 2015 Shaanxi Provincial Living Support

近日，陕西省人力资源和社会保障厅公布了2015年陕西省博士后生活资助人员名单，我室共有3名博士后获得本次资助。

Shaanxi Provincial Department of Human Resources and Social Security recently announced awardees of the 2015 Shaanxi Provincial Living Support. Three postdoctoral associates in our lab received the support.

分别是：

- 植物保护流动站博士后汤春蕾（康振生教授指导） Chunlei Tang, Plant Protection, (Advisor: Prof. Zhensheng Kang)  
作物学流动站博士后王冬冬（陈勤教授指导） Dongdong Wang, Agronomy, (Advisor: Prof. Qin Chen)  
园艺学流动站博士后曹贺贺（王晓峰教授指导） Hehe Cao, Horticulture, (advisor: Prof. Xiaofeng Wang)

#### 邹志荣教授和王晓杰教授获陕西省教育系统表彰

#### Two Teachers Received Awards from Shaanxi Education Commission

2015年9月9日上午，中共陕西省委教育工委、省教育厅、省人社厅、省教育工会在西安召开2015年教师节表彰暨师德宣讲启动会，邹志荣教授获“陕西省师德楷模”、小麦条锈病研究室获“陕西教育系统劳模创新工作室”称号。本次表彰大会在全省共邀请52位优秀教师代表和11位劳模代表参加，邹志荣教授、小麦条锈病研究室代表王晓杰教授作为教师及劳模代表应邀参加了此次大会。



陕西省教育系统表彰大会  
Shaanxi education commission

Shaanxi Education Commission, Department of Education, Department of Human Resources, and Provincial Education Workers Committee held a meeting in Xi'an in the morning of September 9, 2015. Prof. Zhirong Zou in the lab received "Model of teachers", and the Wheat Stripe Rust Research Program was recognized as "Innovation work group of Shaanxi education exemplified workers". There were 52 excellent teachers and 11 excellent workers invited to the awarding meeting. Prof. Zhirong Zou and Prof. Xiaojie Wang were among the invited representatives.



## 五、调研指导

### 5. Advisory Investigations

#### 孙其信校长来我室检查实验室安全工作

President Qixin Sun and Others Were Checking the Lab Safety



孙其信校长检查实验室安全  
President Qixin Sun checked the lab safety

9月7日上午，孙其信校长及校党委副书记徐养福、吕卫东一行对我室进行了安全检查，副主任吉万全教授汇报了实验室安全运行的有关情况，介绍了公共平台仪器设备安全管理的有关措施和办法。

President Qixin Sun, University Vice Secretaries Yangfu Xu and Weidong Lv, and related officers were checking the lab safety on September 7, 2015. Prof. Wanquan Ji, associate director of the laboratory, reported the information about the lab safety operation and introduced the methods and procedures of the safety management for the equipment and facilities of public platforms.

孙校长一行查看了实验室安全管理规章制度建设、落实，实验仪器设备、实验材料使用和卫生等状况，并与实验室管理人员和实验技术人员进行交流，详细了解实验室仪器运行，有毒有害实验用品的存放、领用、使用、回收、处置等过程管理，实验设施设备安全运行，重点部位自动监控、泄漏检测报警，实验用品仓库通风、防火防爆设施设备维护及运行等情况，对存在的安全隐患及实验室管理问题等提出明确整改要求。他强调，要加大对实验人员的培训管理，规范其操作，确保实验室安全有序运行。

President Sun and others checked the establishment and application of policies and management, discussed with lab managers and technicians, obtained in detail information about the equipment operation; the process for storage, receiving, using, recycling, and discarding of harmful materials; safely operation of equipment and facilities; automatic security control for critical areas; chemical spill check and alarm; air circulation of chemical storage; and maintenance and operation of fire and exploitation-proof facilities. They pointed out potential risks and made clear points for improvement. President Sun stressed training and managing for people conducting experiments, standardizing operation, and making sure safe and efficient operation of the laboratory.

## 六、参观访问

### 6. Visiting

#### 暑期夏令营

#### Summer camps

7月23日，2015年全国青少年高校科学营陕西分营一行100名高中生来我室参观。来自西安、咸阳、宝鸡的优秀高中生在王晓杰教授和黄雪玲博士的带领下参观了重点实验室，两位老师向学生详细介绍了实验室历史沿革、科研队伍、研究方向和近期取得的科研成果，科学营成员实地参观了仪器设备平台并就感兴趣的问题与两位老师进行了交流探讨。

On July 23, 2016, 100 students of the Shaanxi sub-team participating in the 2015 National Youth Scientific Camps were visiting our laboratory under the direction of Prof. Xiaojie Wang and Dr. Xueling Huang. The excellent students were from various high schools in Xi'an, Xianyang, and Baoji. Prof. Wang and Dr. Huang introduced the lab history, research team, and recent progress. Students looked at the equipment and facilities of the lab platforms, and discussed interesting questions.

7月24日，园艺学院2015年“优秀大学生暑期夏令营”一行46名大学生来我室参观。来自华中农业大学、南京农业大学等十余所高校的大学生在黄雪玲博士的带领下参观了重点实验室公共平台。黄博士向学生详细介绍了实验室的研究方向、人员构成、相关仪器设备的操作和在生命科学领域的应用情况。

On July 24, 2016, 46 students participating “the College of Horticulture 2015 Excellent Students Summer Camps” were visiting the laboratory. These undergraduate students were from more than 10 universities including Huazhong Agricultural University and Nanjing Agricultural University. Under Dr. Xueling Huang's direction, they were visiting the public platforms of the key laboratory. Dr. Huang introduced the research directions, teams, equipment operation, and their applications in life science.



基群高处长参观实验室  
Qi Qungao visited the key laboratory



2015全国青少年科学营合影  
Participants of the 2015 national youth scientific camps



黄雪玲博士介绍实验室情况  
Dr. Xueling Huang was introducing the key lab

#### 其他参观访问

#### Other Visitors

本季度以来，贵州大学植物保护系、新疆农业大学调研组，上海农林职业技术学院等代表团陆续到我室参观访问。

During this quarter, delegations from the Department of Plant Protection, Guizhou University, Xinjiang Agricultural University, and Shanghai Agriculture and Forestry College visited the laboratory.



## 七、发表论文

### 7. Publications

2015年7月-9月, 实验室科研人员在SCI收录刊物公开发表署名论文38篇。

From July to September, 2015, scientists of the laboratory published 38 SCI papers.

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# The protein disulfide isomerase 1 of *Phytophthora parasitica* (PpPDI1) is associated with the haustoria-like structures and contributes to plant infection

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Protein disulfide isomerase (PDI) is a ubiquitous and multifunction enzyme belonging to the thioredoxin (TRX) superfamily, which can reduce, oxidize, and catalyze dithiol-disulfide exchange reactions. Other than performing housekeeping functions in helping to maintain proteins in a more stable conformation, there is some evidence to indicate that PDI is involved in pathogen infection processes. In a high-throughput screening for necrosis-inducing factors by *Agrobacterium tumefaciens*-mediated transient expression assay, a typical PDI gene from *Phytophthora parasitica* (PpPDI1) was identified and confirmed to induce strong cell death in *Nicotiana benthamiana* leaves. PpPDI1 is conserved in eukaryotes but predicted to be a secreted protein. Deletion mutant analyses showed that the first CGHC motif in the active domain of PpPDI1 is essential for inducing cell death. Using *P. parasitica* transformation method, the silencing efficiency was found to be very low, suggesting that PpPDI1 is essential for the pathogen. Translational fusion to the enhanced green fluorescent protein (EGFP) in stable *P. parasitica* transformants showed that PpPDI1 is associated with haustoria-like structures during pathogen infection. Furthermore, the PpPDI1-EGFP-expressing transformants increase the number of haustoria-like structures and exhibit enhanced virulence to *N. benthamiana*. These results indicate that PpPDI1 might be a virulence factor of *P. parasitica* and contributes to plant infection.

**Keywords:** *Phytophthora parasitica*, protein disulfide isomerase, cell death, haustoria, plant infection, virulence factor

## Introduction

Disulfide bonds, which are covalent linkages formed between the side chains of cysteine residues, play important roles for the stability of correct folding state of many proteins (Hatahet and Ruddock, 2009). The formation of disulfide bond is a critical step in the folding of nascent peptides in the endoplasmic reticulum (ER). In this process, increasing evidence supports the catalytic role of protein disulfide isomerase (PDI) family (Ellgaard and Ruddock, 2005; Appenzeller-Herzog and Ellgaard, 2008). The PDI family includes PDI and PDI-like proteins with thioredoxin domains,

# The MADS-box transcription factor FgMcm1 regulates cell identity and fungal development in *Fusarium graminearum*

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## Summary

In eukaryotic cells, MADS-box genes are known to play major regulatory roles in various biological processes by combinatorial interactions with other transcription factors. In this study, we functionally characterized the *FgMCM1* MADS-box gene in *Fusarium graminearum*, the causal agent of wheat and barley head blight. Deletion of *FgMCM1* resulted in the loss of perithecia production and phialide formation. The *Fgmcm1* mutant was significantly reduced in virulence, deoxynivalenol biosynthesis and conidiation. In yeast two-hybrid assays, FgMcm1 interacted with Mat1-1-1 and Fst12, two transcription factors important for sexual reproduction. Whereas *Fgmcm1* mutants were unstable and produced stunted subcultures, *Fgmcm1 mat1-1-1* but not *Fgmcm1 fst12* double mutants were stable. Furthermore, spontaneous suppressor mutations occurred frequently in stunted subcultures to recover growth rate. Ribonucleic acid sequencing analysis indicated that a number of sexual reproduction-related genes were upregulated in stunted subcultures compared with the *Fgmcm1* mutant, which was downregulated in the expression of genes involved in pathogenesis, secondary metabolism and conidiation. We also showed that culture instability was not observed in

the *Fvmcm1* mutants of the heterothallic *Fusarium verticillioides*. Overall, our data indicate that FgMcm1 plays a critical role in the regulation of cell identity, sexual and asexual reproduction, secondary metabolism and pathogenesis in *F. graminearum*.

## Introduction

*Fusarium graminearum* (Teleomorph *Gibberella zeae*) is one of the major causal agents of Fusarium head blight (FHB) or scab. It also infects barley, maize and other small grains (Bai and Shaner, 2004; Goswami and Kistler, 2004). In addition to yield losses caused by head blight, infested cereals often are contaminated with mycotoxins, such as deoxynivalenol (DON) and zearalenone (Desjardins, 2003; Walter *et al.*, 2010). *Fusarium graminearum* overwinters in plant debris and produces fruiting bodies in the spring. Plant infection is initiated when airborne ascospores forcibly released from perithecia are landed on flowering spikelets as the primary inoculum (Trail *et al.*, 2002; Schmale *et al.*, 2005). The pathogen then spreads from the infected floret to the rachis and causes severe damage under favourable environmental conditions. Therefore, sexual reproduction or ascospore production plays a critical role in FHB. *Fusarium graminearum* also produces macroconidia on infected plants, which is important for colonization of vegetative tissues and disease spreading in the field. Under laboratory conditions, macroconidia are as virulent as ascospores and are often used in infection assays with flowering wheat heads. In the past decade, genes of various biochemical activities or physiological functions have been shown to be important for sexual and asexual reproduction in *F. graminearum*, including key components of conserved signal transduction pathways (Jenczmionka *et al.*, 2003; Urban *et al.*, 2003; Bluhm *et al.*, 2007; Yu *et al.*, 2008; Nguyen *et al.*, 2012; Hu *et al.*, 2014) and transcription factors with different deoxyribonucleic acid (DNA)-binding domains (Son *et al.*, 2011; Wang *et al.*, 2011a; Min *et al.*, 2012).

As a homothallic fungus, sexual reproduction is controlled by two linked mating idiomorphic alleles *MAT1-1* and *MAT1-2* in *F. graminearum*. Like in many other filamentous ascomycetes, the mating type (*MAT*) locus

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# Expression of *TaCYP78A3*, a gene encoding cytochrome P450 CYP78A3 protein in wheat (*Triticum aestivum* L.), affects seed size

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## SUMMARY

Several studies have described quantitative trait loci (QTL) for seed size in wheat, but the relevant genes and molecular mechanisms remain largely unknown. Here we report the functional characterization of the wheat *TaCYP78A3* gene and its effect on seed size. *TaCYP78A3* encoded wheat cytochrome P450 CYP78A3, and was specifically expressed in wheat reproductive organs. *TaCYP78A3* activity was positively correlated with the final seed size. Its silencing caused a reduction of cell number in the seed coat, resulting in an 11% decrease in wheat seed size, whereas *TaCYP78A3* over-expression induced production of more cells in the seed coat, leading to an 11–48% increase in Arabidopsis seed size. In addition, the cell number in the final seed coat was determined by the *TaCYP78A3* expression level, which affected the extent of integument cell proliferation in the developing ovule and seed. Unfortunately, *TaCYP78A3* over-expression in Arabidopsis caused a reduced seed set due to an ovule developmental defect. Moreover, *TaCYP78A3* over-expression affected embryo development by promoting embryo integument cell proliferation during seed development, which also ultimately affected the final seed size in Arabidopsis. In summary, our results indicated that *TaCYP78A3* plays critical roles in influencing seed size by affecting the extent of integument cell proliferation. The present study provides direct evidence that *TaCYP78A3* affects seed size in wheat, and contributes to an understanding of the cellular basis of the gene influencing seed development.

**Keywords:** cytochrome P450, *CYP78A*, *Triticum aestivum*, *TaCYP78A3*, seed size, BSMV-VIGS.

## INTRODUCTION

Seed size is an important agronomic trait that occupies a pivotal position in crop yield. There is a striking diversity of seed size among the plant species of the world, even within the same community (Harper *et al.*, 1970; Venable, 1992). Seed size in a crop is usually reported as the 1000-seed weight, and is an important trait that has been selected during domestication and modern crop breeding (Shomura *et al.*, 2008).

Seed development involves complex processes, including three growth programs – those of the diploid embryo, the triploid endosperm and the maternal integument – which coordinate to affect the final seed size (Ohto *et al.*, 2005; Sundaresan, 2005; Berger *et al.*, 2006). Functional loss of *HAIKU2* (*IKU2*) and *MINISEED3* (*MINI3*), encoding a receptor-like kinase and a WRKY transcription factor,

respectively, resulted in small seeds with precocious endosperm cellularization and reduced embryo growth (Garcia *et al.*, 2003; Luo *et al.*, 2005). *IKU1*, *IKU2* and *MINI3* were found to act in a genetic pathway regulated by *SHORT HYPOCOTYL UNDER BLUE1* (*SHB1*); gain-of-function mutants of *IKU1*, *IKU2* and *MINI3* produced larger seeds due to enhanced endosperm proliferation and delayed endosperm cellularization (Garcia *et al.*, 2003; Luo *et al.*, 2005; Zhou *et al.*, 2009; Wang *et al.*, 2010). Expression of *TRANSPARENT TESTA GLABRA 2* (*TTG2*) and *APETALA 2* (*AP2*) has been reported to promote seed growth by increasing cell expansion in the integuments; mutants of this gene have defective seed integuments and reduced seed size (Johnson, 2002; Garcia *et al.*, 2005; Jofuku *et al.*, 2005). By controlling the proliferation of seed integument

# Activation of the signalling mucin MoMsb2 and its functional relationship with Cbp1 in *Magnaporthe oryzae*

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## Summary

Various surface signals are recognized by *Magnaporthe oryzae* to activate the Pmk1 MAP kinase that is essential for appressorium formation and invasive growth. One of upstream sensors of the Pmk1 pathway is the MoMsb2 signalling mucin. However, the activation of MoMsb2 and its relationship with other sensors is not clear. In this study, we showed that the cleavage and transmembrane domains are essential for MoMsb2 functions. Cleavage of MoMsb2 was further confirmed by western blot analysis, and five putative cleavage sites were functionally characterized. Expression of the extracellular region alone partially rescued the defects of *Momsb2* in appressorium formation and virulence. The cytoplasmic region of MoMsb2, although dispensable for appressorium formation, was more important for penetration and invasive growth. Interestingly, the *Momsb2 cbp1* double mutant deleted of both mucin genes was blocked in Pmk1 activation. It failed to form appressoria on artificial surfaces and was non-pathogenic. In addition, we showed that MoMsb2 interacts with Ras2 but not with MoCdc42 in co-immunoprecipitation assays. Overall, results from this study indicated that the extracellular and cytoplasmic regions of MoMsb2 have distinct functions in appressorium formation, penetration and invasive growth, and MoMsb2 has overlapping functions with Cbp1 in recognizing environmental signals for Pmk1 activation.

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## Introduction

For foliar pathogens, the penetration of plant cuticle and cell wall is a critical step to initiate infectious growth. Many of them, including the rice blast fungus *Magnaporthe oryzae*, form a specialized infection structure known as appressorium for plant penetration (Tucker and Talbot, 2001; Hamel *et al.*, 2012; Li *et al.*, 2012). As a model for studying fungal–plant interactions, *M. oryzae* has been extensively studied for molecular mechanisms regulating plant infection processes. Several well-conserved signal transduction pathways are shown to regulate surface recognition, appressorium formation, turgor generation, and differentiation of penetration pegs and invasive hyphae (Ebbole, 2007; Zhao *et al.*, 2007; Wilson and Talbot, 2009; Li *et al.*, 2012). Germ tubes of *M. oryzae* are known to recognize surface hydrophobicity and hardness for appressorium formation. However, appressorium formation on hydrophilic surfaces can be induced with cAMP, cutin monomers and components of plant epicuticular waxes (Lee and Dean, 1993; Gilbert *et al.*, 1996; Liu *et al.*, 2011). Recognition of chemical and physical cues of leaf surfaces also have been reported to regulate appressorium formation in other pathogenic fungi, including *Ustilago maydis* and *Colletotrichum* species (Münch *et al.*, 2008; Vollmeier *et al.*, 2012).

In *M. oryzae*, the Pmk1 MAP kinase (MAPK) homologous to Fus3 and Kss1 of the budding yeast *Saccharomyces cerevisiae* is essential for appressorium formation and pathogenesis (Xu and Hamer, 1996). The *pmk1* deletion mutant is blocked in appressorium formation and non-pathogenic. Deletion of several upstream components of the Pmk1 pathway, including *MST50*, *MST11* and *MST7*, resulted in the same defects in appressorium development and plant infection (Zhao *et al.*, 2005; Park *et al.*, 2006; Zhou, 2014). Orthologues of the Pmk1 MAPK have been shown to regulate various plant infection processes in a number of phytopathogenic fungi, including *Colletotrichum lagenarium*, *Cochliobolus heterostrophus*, *Botrytis cinerea*, *Verticillium dahliae*, *U. maydis* and *Fusarium oxysporum* (Takano *et al.*, 2000; Di Pietro *et al.*, 2001; Brefort *et al.*, 2009; Hamel *et al.*, 2012; Li *et al.*, 2012). In *M. oryzae*, the MoMsb2 signalling mucin is likely one of the receptors that are involved in the



## Characterization of protein kinase *PsSRPKL*, a novel pathogenicity factor in the wheat stripe rust fungus

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### Summary

As in other eukaryotes, protein kinases (PKs) are generally evolutionarily conserved and play major regulatory roles in plant pathogenic fungi. Many PKs have been proven to be important for pathogenesis in model fungal plant pathogens, but little is currently known about their roles in the pathogenesis of cereal rust fungi, devastating pathogens in agriculture worldwide. Here, we report on an *in planta* highly induced PK gene *PsSRPKL* from the wheat stripe rust fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*), one of the most important cereal rust fungi. *PsSRPKL* belongs to a group of PKs that are evolutionarily specific to cereal rust fungi. It shows a high level of intraspecific polymorphism in the kinase domains and directed green fluorescent protein chimeras to plant nuclei. Overexpression of *PsSRPKL* in fission yeast induces aberrant cell morphology and a decreased resistance to environmental stresses. Most importantly, *PsSRPKL* is proven to be an important pathogenicity factor responsible for fungal growth and responses to environmental stresses, therefore contributing significantly to *Pst* virulence in wheat. We hypothesize that cereal rust fungi have developed specific PKs as pathogenicity factors for adaptation to their host species during evolution. Thus, our findings provide significant insights into pathogenicity and virulence evolution in cereal rust fungi.

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### Introduction

Reversible protein phosphorylation by protein kinases (PKs) and phosphatases is a ubiquitous signalling mechanism in all eukaryotic cells (Miranda-Saavedra and Barton, 2007). Genes that encode PKs tend to evolve slowly over time among different organisms and rarely undergo accelerated evolution (Kong *et al.*, 2010). Conventional PKs can be classified into eight groups (AGC, CAMK, CK1, CMGC, RGC, STE, TK and TKL) based on similarities in amino acid sequence, domain structure and regulation (Hanks and Hunter, 1995; Miranda-Saavedra and Barton, 2007). Genome sequences are now available for a number of model fungal plant pathogens, including *Magnaporthe oryzae*, *Ustilago maydis* and *Fusarium graminearum*, in which about 1% of predicted genes encode PKs (Cuomo *et al.*, 2007; Ma *et al.*, 2010; Wang *et al.*, 2011). Classical genetic studies show that a number of these PK genes are important for pathogenesis (Lee *et al.*, 2003; Zhao *et al.*, 2007; Nguyen *et al.*, 2008; Wang *et al.*, 2011). Especially, the well-studied subgroup of MAP kinases (MAPKs) (CMGC group) has been proven to be well-conserved in eukaryotes and play critical roles in regulating fungal growth and plant infection (Brachmann *et al.*, 2003; Kahmann and Kamper, 2004; Zhao *et al.*, 2007). However, there are only few reports about the role of PKs in the pathogenesis of obligate biotrophic pathogens because of the fact that they only grow *in planta* and the lack of an efficient and reliable transformation system. Some MAPK homologues from obligate biotrophic rust fungi have also been proven to be important for fungal growth and plant infection, using heterologous expression assays or barley stripe mosaic virus (BSMV) induced RNAi (Hu *et al.*, 2007; Guo *et al.*, 2011; Panwar *et al.*, 2013). These results indicate that PKs may also play an important role in rust pathogenicity.

Serine-arginine (SR) PKs (SRPKs) also belong to the CMGC group but are a relatively novel PK subgroup. They phosphorylate SR splicing factors (also named SR proteins) with remarkable specificity and efficiency (Giannakourou *et al.*, 2011). SRPKs were originally thought to be devoted to constitutive and alternative mRNA splicing. However, they are now known to expand their influence to additional steps in mRNA maturation and other cellular activities, such as chromatin

## Genome sequence of *Valsa* canker pathogens uncovers a potential adaptation of colonization of woody bark

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**Key words:** *Cytospora* spp., fungal genomes, necrotrophic fungi, pectinase, secondary metabolism, tree disease.

### Summary

- Canker caused by ascomycetous *Valsa* species are among the most destructive diseases of woody plants worldwide. These pathogens are distinct from other pathogens because they only effectively attack tree bark in the field. To unravel the potential adaptation mechanism of bark colonization, we examined the genomes of *Valsa mali* and *Valsa pyri* that preferentially infect apple and pear, respectively.
- We reported the 44.7 and 35.7 Mb genomes of *V. mali* and *V. pyri*, respectively. We also identified the potential genomic determinants of wood colonization by comparing them with related cereal pathogens.
- Both genomes encode a plethora of pathogenicity-related genes involved in plant cell wall degradation and secondary metabolite biosynthesis. In order to adapt to the nutrient limitation and low pH environment in bark, they seem to employ membrane transporters associated with nitrogen uptake and secrete proteases predominantly with acidic pH optima. Remarkably, both *Valsa* genomes are especially suited for pectin decomposition, but are limited in lignocellulose and cutin degradation. Besides many similarities, the two genomes show distinct variations in many secondary metabolism gene clusters.
- Our results show a potential adaptation of *Valsa* canker pathogens to colonize woody bark. Secondary metabolism gene clusters are probably responsible for this host specificity.

### Introduction

For woody plants, canker diseases are among the most destructive and difficult to manage problems worldwide. *Valsa* canker, caused by pathogenic ascomycetes of the Valsaceae family (Sordariomycetes, Diaporthales), is one of the most widespread canker diseases. It affects > 70 species of trees worldwide, including fruit trees, hardwood trees, shade trees, and shrubs (Spielman, 1985; Agrios, 2005). These fungi cause extensive damage and even death to trees by forming cankers on the bark and girdling branches or the main stem (Fig. 1a–d). *Valsa* canker of apple is predominantly caused by *Valsa mali* (anamorph *Cytospora sacculus*) (Tanaka, 1919; Wang *et al.*, 2011). It is one of the most destructive diseases of apple trees in eastern Asia and is often associated with severe yield losses (Lee *et al.*, 2006; Li *et al.*, 2013).

The genus *Valsa* comprises > 500 species that infect diverse host plants. The most common species is *Valsa fabianae* (anamorph *Cytospora eucalypticola*) which causes canker and death of *Eucalyptus* trees throughout the world (Adams *et al.*, 2005). Infected bark tissue often becomes reddish-brown, water-soaked, slightly intumescent, softened, alcohol smelling, and ultimately shrivels and separates from the underlying wood (Fig. 1b). Since

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the pathogen penetrates extensively into the host phloem and xylem (Ke *et al.*, 2013) (Fig. 1c), chemical treatment cannot effectively cure or control *Valsa* canker (Abe *et al.*, 2007). However, infectious hyphae of *Valsa* spp. can colonize but not effectively degrade the xylem vessels (Fig. 1d). Late in the developing stage, the pathogen produces pycnidia on the cankers (Ke *et al.*, 2013). *Valsa* spp. mainly infect host bark by conidia (Wang *et al.*, 2014) (Fig. 1e). Conidia can germinate on both wounded or intact bark, but successful infection only occurs on wounded plants (Ke *et al.*, 2013) (Fig. 1f,g). After infection of wounded tissue, infection hyphae develop inter- and intracellularly and colonize all bark tissue, resulting in severe tissue maceration and necrosis (Fig. 1h–j). Therefore, better understanding of their pathogenicity mechanisms is crucial for developing more effective disease management strategies. Genomics has greatly accelerated the pace of discovery in the study of plant pathogens. In the past decade, over 100 plant pathogenic fungi have been sequenced, and genomic studies have significantly advanced our understanding of different pathogens.

In this study, we sequenced the genomes of the *Valsa* canker pathogens *V. mali* and *V. pyri*, which preferentially infect apple and pear, respectively. Comparative analysis revealed that their genomes encode a diverse set of genes that are potentially involved in the infection and colonization of the host bark. In particular, the gene families of pectinases, glutamic proteases,

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