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Melatonin enhances the occurrence of autophagy induced by oxidative stress in *Arabidopsis* seedlings

Abstract: The beneficial effect that melatonin has against mitochondrial dysfunctioning seems to be linked to mitophagy. Roles for melatonin have been demonstrated in promoting health and preventing disease, as well as activating the process of autophagy in general. However, no reports have been made about how the application of melatonin regulates that process when plants are exposed to oxidative stress. We investigated the influence of different concentrations of melatonin (0.0, 0.5, 5.0, 10.0, or 50.0 μM) on autophagy under methyl viologen (MV)-induced oxidative stress. *Arabidopsis* seedlings that were pretreated with 5 or 10 μM melatonin underwent relatively strong induction of autophagy, as evidenced by the number of monodansylcadaverine (MDC)-stained autophagosomes in root samples. Pretreatment with 10 μM melatonin also alleviated MV-induced photo-oxidation damage and significantly reduced the accumulation of oxidized proteins. Those responses might have been due to the strong upregulation of genes that involved in AtATG8-PE conjugation pathway, which enhanced the capacity for autophagy. Histochemical staining revealed that both O_2^- and H_2O_2 were highly accumulated upon MV exposure, although the response did not differ significantly between control and melatonin-pretreated seedlings. By contrast, exogenous melatonin upregulated the expression of two genes for H_2O_2 -scavenging enzymes, that is, *AtAPX1* and *AtCATs*. The activation of autophagy by melatonin without an alteration in ROS production may be part of a survival mechanism that is enhanced by melatonin after cellular damage. Therefore, it represents a second level of defense to remove damaged proteins when antioxidant activities are compromised.

Introduction

Since the first identification and isolation in the bovine pineal gland in 1958 [1], melatonin (N-acetyl-5-methoxytryptamine) has attracted the attention of many researchers seeking to unravel its roles in animals and plants. In animals, this molecule not only acts to regulate sleep-wake cycles and seasonal reproduction [2], but also functions in enhancing immunity [3], protecting against cancer [4, 5], and combating oxidative stress [6–9]. Melatonin has also been discovered in higher plants [10, 11], where it participates as a regulator of multiple developmental processes, such as governing growth of roots, shoots, and explants [12–15], as well as delaying leaf senescence [16]. As a highly effective radical scavenger, melatonin counteracts the effects of various biotic/abiotic stresses, for example, cold, heat, drought, salinity, chemical pollutants, UV irradiation, and fungal pathogens [17–27]. High-throughput sequencing technology is now being used to investigate melatonin-mediated genetic functions, such as upregulation of transcriptional expression by many defense-related genes, including stress receptors, kinases, and transcription factors [28]. Another transcriptome analysis has revealed that melatonin upregulates the expression of genes related

to binding and oxidoreductase activity/processes that are normally inhibited by salt stress, as well as genes involved in cell division, photosynthesis, carbohydrate metabolism, fatty acid biosynthesis, and ascorbate metabolism [29].

Elevated levels of reactive oxygen species (ROS) can lead to oxidative stress, a condition that can cause a loss of cell function through uncontrolled oxidative modifications of macromolecules such as proteins, DNA, and lipids. Because ROS have a dual role in toxicity and as signal molecules, plant cells have developed sophisticated strategies to regulate intracellular ROS concentrations and detoxify excess ROS. Those strategies involve nonenzymatic and enzymatic antioxidants. Protective agents include glutathione, thioredoxin, superoxide dismutases, catalase, glutathione peroxidases, ascorbate peroxidases, and several low-molecular-weight scavengers, such as melatonin, that direct the eradication of free radicals [30, 31]. Along with numerous systems for antioxidative defenses, the strictly regulated removal of oxidized structures is a universal stress response by eukaryotic cells that targets damaged or toxic components for vacuolar or lysosomal degradation.

Autophagy is a ubiquitous process in eukaryotic cells, where part of their cytosolic components are self-digested

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Key words: *Arabidopsis*, *AtATG8s*, autophagy, melatonin, oxidative stress, oxidized protein

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Report

简报

西北农林科技大学与阿尔伯塔大学中加农业与食品创新联合研究中心研讨会合影 Workshop on Agriculture and Food Innovation Joint Research Centre (NWAUFU and U of A)

June 28–30, 2015
Yangling, China



中加农业与食品创新联合研究中心研讨会
The workshop of the China-Canada joint
research center for inventive agriculture and food

一、国际合作

1. International Collaboration

中加农业与食品创新联合研究中心研讨会在我校举办 The China-Canada Joint Research Center for Inventive Agriculture and Food Held a Workshop in Northwest A&F University

为促进我校与加拿大阿尔伯塔大学的学术交流与合作, 由我室承办的西北农林科技大学与阿尔伯塔大学农业与食品创新联合研究中心第一次研讨会, 于6月28–30日在我校举办。

加拿大阿尔伯塔大学农学院院长Stanford Blade教授、副院长Nat Kav教授等一行8人参加研讨交流, 我室固定研究人员及研究生100余人参加了研讨会。副校长罗军教授参加研讨会并致辞。

会议期间, 与会专家分别汇报了研究内容与进展, 双方初步达成围绕小麦、油菜、小杂粮、农田水肥管理和农业经济与政策等领域开展合作交流的意向, 拟定了中加农业与食品创新联合研究中心(西北农林科技大学-阿尔伯塔大学)合作内容方式及运行机制。

Organized by our laboratory, the State Key Laboratory of Crop Stress Biology for Arid Areas (CSBAA), the first workshop of the Northwest A&F University and the University of Alberta Joint Research Center for Inventive Agriculture and Food was held on June 28–30, 2015 at our university. The workshop was to enhance the scientific exchange and collaboration between the two universities.

Prof. Stanford Blade, Dean of the College of Agriculture, Prof. Nat Kav, Associate Dean of the college, and other six members of the University of Alberta delegation participated in the workshop. From the key laboratory, more than 100 faculty and graduate students participated in the workshop. Prof. Jun Luo, Vice President of our university, participated in the workshop and delivered the opening and welcome speech.

During the workshop, scientists from both universities presented research progress. Both delegations agreed to conduct exchanges and collaborations in the research areas of wheat, canola, specialty crops, water and fertilizer management, and agriculture policies. The delegation also decided the type and plans of collaboration and operation for the China-Canada Joint Research Center for Inventive Agriculture and Food (Northwest A&F University and the University of Alberta).

二、学术交流

2. Scientific Exchange

水稻稻瘟菌学术研讨会在我室召开 Rice Blast Pathogen Workshop



许金荣教授主持
Prof. Jinrong Xu presided rice blast pathogen workshop

5月22日，由旱区作物逆境生物学国家重点实验室、西农-普度联合研究中心和植保学院联合举办的“水稻稻瘟菌学术研讨会”在我校召开，来自美国、韩国、中国农科院植保所、浙江大学、中国农业大学、南京农业大学、福建农林大学以及我校的30多名代表参加了此次研讨会。

许金荣教授主持会议，康振生教授参加研讨会并致欢迎词。北卡罗来纳州立大学Ralph Dean教授、首尔国立大学Yong Hwan Lee教授、浙江大学林福呈教授、中国农科院植保所刘文德研究员、中国农业大学杨俊副教授、南京农业大学张海峰副教授以及我校许金荣教授分别从水稻稻瘟菌基因组学、功能基因组学、致病机理以及病原-植物互作等方面作汇报了研究进展。

Organized by CSBAA, the Northwest A&F University and Purdue University Joint Research Center, and the College of Plant Protection, the Rice Blast Pathogen Workshop was held at our university on May 22, 2015. Scientists from the United States, South Korea, and various universities and institutes of China, such as Plant Protection Institute of Chinese Academy of Agricultural Sciences (CAAS), Zhejiang University, China Agricultural University, Nanjing Agricultural University, and Fujian Agriculture and Forestry University, and more than 30 researchers of our university participated in the workshop.

Prof. Jinrong Xu presided, and Prof. Zhensheng Kang participated in the workshop and delivered the opening and welcome speech. Professors Ralph Dean (North Carolina State University), Yong Hwan Lee (National University of Seoul, South Korea), Fucheng Lin (Zhejiang University), Wende Liu (Plant Protection Institute of CAAS), Prof. Junfu Yang (China Agricultural University), Haifeng Zhang (Nanjing Agricultural University), and Jinrong Xu of our university are among the scientists presenting research progress on genomics, functional genomics, and mechanisms of pathogenicity and interactions with plants for the rice blast fungus.

我室举办禾谷镰刀菌学术研讨会 Workshop on *Fusarium Fungi*

5月29-30日，由旱区作物逆境生物学国家重点实验室和西农-普度联合研究中心共同举办的“禾谷镰刀菌学术研讨会”在我校举行。来自国内外的50多位代表参加了研讨会，实验室主任康振生教授主持会议，副校长钱永华致辞。

美国堪萨斯州立大学John Leslie教授、韩国顺天乡大学Sung-Hwan Yun教授、韩国首尔国立大学Yin-Won Lee教授、福建农林大学王宗华教授、南京农业大学周明国教授、陈长军教授、中国农业科学院刘阳研究员、张昊博士、华中农业大学廖玉才教授、浙江大学马忠华教授、山东农业大学孔令让教授、中科院上海生命科学研究院唐威华教授、江苏省农科院史建荣研究员、张旭研究员、河南农业大学丁胜利教授就小麦赤霉病致病机理、真菌毒素合成调控、抗赤霉病种质资源利用等国内外研究热点和前沿领域汇报了研究进展。



会议合影
Participants of the workshop on *Fusarium fungi*

Organized by CSBAA and the Northwest A&F University and Purdue University Joint Research Center, the Workshop on *Fusarium Fungi* was held at our university on May 29-30, 2015. More than 50 foreign and domestic scientists attended the workshop. Prof. Zhensheng Kang, Director of CSBAA, presided and Vice President Yonghua Qian delivered the opening and welcome speech.

Scientists including professors John Leslie (Kansas State University, USA), Yin-Won Lee (National University of Seoul, South Korea), Zonghua Wang (Fujian Agriculture and Forestry University), Mingguo Zhou and Changjun Chen (Nanjing Agricultural University), Yang Liu and Hao Zhang (CAAS), Yuchai Liao (Huazhong Agricultural University), Zhonghua Ma (Zhejiang Agricultural University), Lingrang Kong (Shandong Agricultural University), Weihua Tang (Shanghai Academy of Life Science of Chinese Academy of Sciences), Jianrong Shi and Xu Zhang (Jiangsu Academy of Agricultural Sciences), and Shengli Ding (Henan Agricultural University) presented their progress on hot topics of advanced research such as pathogenicity mechanisms of the *Fusarium* head blight pathogen, regulation of fungal toxin synthesis, and utilization of plant germplasm for resistance to *Fusarium* pathogens.

植物响应逆境胁迫 学术研讨会召开

Workshop on Plant Responses to Abiotic Stresses

6月19日，由我室承办的“植物响应逆境胁迫学术研讨会”在国际交流中心室举行。来自美国、以色列、韩国、中国台湾的12名专家学者和我校相关领域的科研人员、研究生共200余人参加了研讨会，郁飞教授主持会议。

来自以色列特拉维夫大学的Shaul Yalovsky教授，美国马里兰州的Jianhua Zhu教授、卡内基科学研究所的José R. Dinneny教授和罗格斯大学的Juan Dong教授分别作了精彩学术报告，并和与会人员进行了交流和探讨。

Organized by our laboratory, the Workshop on Plant Responses to Abiotic Stresses was held in the university International Exchange Center on June 19, 2015. More than 200 participants attended the workshop, including 12 scientists from the United States, Israel, South Korea, Taiwan China, and our university. Prof. Fei Yu chaired the workshop.

Prof. Shaul Yalovsky from Tel Aviv University, Israel, Prof. Jianhua Zhu from the University of Maryland, USA, Prof. José R. Dinneny from the Carnegie Institution for Science, USA, and Juan Dong from Rutgers University presented their research and had discussions with workshop participants.



研讨会
Workshop on plant responses to abiotic stresses



与会专家参观重点实验室
Experts visited the key laboratory

学术报告 Seminars

4月-6月，实验室邀请国内外10位知名学者来我室进行访问交流。

1. 英国植物病理学会主席Lesley Torrance 教授：植物与病毒的相互作用；
2. 苏格兰作物研究所Stuart Macfarlane教授：Plant Virology at JHI: Studies of Tobacco rattle virus (TRV) and Raspberry leaf blotch virus (RLBV);
3. 普度大学Daoguo Zhou教授：Mapping high-resolution kinase substrate network in animals and plants;
4. 普度大学Andy Tao教授：Regulation of Salmonella invasion into non-phagocytic cell;
5. 加拿大萨斯喀彻温大学刘国胜教授；
6. 澳大利亚科学院院士Robert McIntosh;
7. 布劳格全球锈病协作组织（Borlaug Global Rust Initiative, BGRI）副主席Ronnie Coffman教授：Rust, Risk, and Biotechnology;
8. 美国犹他州立大学David Hole教授：Genome Wide Association Selection in Wheat and Barley;
9. 加州大学戴维斯分校李国田博士：KitBase: a fully sequence-indexed mutant collection in a model rice variety;
10. 电子科技大学杨足君教授：现代细胞学技术与小麦种质创新。



应邀专家作报告
Invited experts gave speeches

From April to June, our laboratory invited the following scientists for visiting and presented seminars to our faculty and students:

1. Prof. Lesley Torrance, President of UK Plant Pathology Society, presented a seminar on interactions between plants and viruses.
2. Prof. Stuart Macfarlane from Scottish Crop Research Institute presented a seminar entitled “Plant Virology at JHI: Studies of tobacco rattle virus (TRV) and raspberry leaf blotch virus (RLBV)”.
3. Prof. Daoguo Zhou from Purdue University presented a seminar entitled “Mapping high-resolution kinase substrate network in animals and plants”.
4. Prof. Andy Tao from Purdue University presented a seminar entitled “Regulation of Salmonella invasion into non-phagocytic cell”.
5. Prof. Robert McIntosh, Academician of Australian Academy of Sciences, from the University of Sydney.
6. Prof. Guosheng Liu from the University of Saskatchewan, Canada.
7. Prof. Ronnie Coffman, Director of Borlaug Global Rust Initiative (BGRI) presented a seminar entitled “Rust, risk, and biotechnology”.
8. Prof. David Hole from Utah State University presented a seminar entitled “Genome-wide association selection in wheat and barley”.
9. Prof. Guotian Li from the University of California, Davis, USA presented a seminar entitled “KitBase: a fully sequence-indexed mutant collection in a model rice variety”.
10. Prof. Zujun Yang from the University of Electronic Science and Technology of China presented a seminar “Modern cytological technology and development of wheat germplasm”.

三、人才培养

3. Personnel Development

管清美博士入选 国家第十一批“青年千人计划”

Dr. Qingmei Guan Was Selected for the 11th group of the National “Youth Thousand”

教育部公布第十一批“千人计划”入选资格人员名单，我室管清美博士入选“青年千人计划”。管清美博士的入选是重点实验室首次在“青年千人”上的突破，也打破了5年来“青年千人”在我校的沉寂。



管清美博士
Dr. Qingmei Guan

Dr. Qingmei Guan in our laboratory was selected for the 11th group of the National “Youth Thousand” of the Ministry of Education. The selection of Dr. Guan was a breakthrough for our laboratory in the “Youth Thousand”, and broke the recent five-year silence in the program for the whole university.



荣誉证书
The honorary certificate

王晓杰教授获示范区 青年创新创业标兵

Prof. Xiaojie Wang was awarded “Yangling Exemplified Inventive Youth”

五四青年节前夕，示范区团工委开展了创新创业创优评选活动，表彰了一批工作成效突出的创新创业创优先进集体和先进个人。我室王晓杰教授获得“示范区青年创新创业标兵”荣誉称号。

Prior to the 5.4 Youth Day, Yangling Youth Committee carried out evaluation of young people in professional and business invention and development, and awarded a group of teams and individuals for their outstanding work. Prof. Xiaojie Wang received an honorary award of “Yangling Exemplified Inventive Youth”.

四、研究生培养

4. Graduate Student Training

实验室召开第二届研究生学术论坛 The Second Graduate Student Workshop was held



16位研究生做论坛报告
16 students was making presentations

6月13日，以“作物抗逆与种质创新”为主题的旱区作物逆境生物学国家重点实验室第二届研究生学术论坛在国际交流中心104会议室召开。校长助理兼研究生院副院长霍学喜教授出席论坛并致辞。

论坛共收到了论文摘要48篇，论坛组委会从中遴选16篇论文并邀请作者围绕“作物抗逆与种质创新”主题进行汇报交流。来自农学院、植保学院、园艺学院、资环学院、生命学院的12位教授作为评委，听取交流汇报，并从实验设计、数据获取、结论分析等方面进行了点评。

根据论坛的评分标准，经专家组评议，张慧丽同学获得一等奖，张俊祥、王蕊、杨阳三位同学获得二等奖，潘明真、崔立操、李占杰、杨晓菲四位同学获得三等奖，黄淑华、崔振华、高柳、姚文权、孟宪文、焦晨、晁桂梅、陈旺八位同学获得优秀奖。

实验室副主任吉万全对本次论坛进行了点评，并鼓励研究生积极努力投入到科研工作中，取得更大成绩。

与第一届论坛相比，本次论坛首次由校研究生会承办，论坛策划、主持、会务安排等由研究生负责；此次论坛共征集48篇论文，相比上届论坛论文数量大幅增长，而且在质量上有了明显提高，相关研究结果已在*Nature Communications*、*Journal of Experimental Botany*等期刊发表。

The Second Graduate Student Workshop with the theme of “Crop resistance to stresses and germplasm development” was held in the 104 Conference Room of the university International Exchange Center on June 13, 2015. Prof. Xuexi Huo, Assistant to University President and Associate Dean of the Graduate School participated in the workshop and delivered speech.

The workshop received 48 abstracts, from which 16 were selected by the workshop organizing committee for presentations focusing on the theme. As an evaluation committee, 12 professors from the colleges of Agronomy, Plant Protection, Horticulture, Environment and Resources, and Life Science evaluated the reports and made comments on experimental design, data collection and analyses, and conclusions.

Based on workshop evaluation standard and the committee's evaluation, Huili Zhang received the first place; Junxiang Zhang, Xin Wang, and Yang Yang received the second place; Mingzhen Pan, Licao Cui, Zahngjie Li, and Xiaofei Yang received the third place; and Shuhua Huang, Zhenhua Cui, Liu Gao, Wenquan Yao, Xianwen Meng, Chen Jiao, Guimei Chao, and Wang Chen received excellent awards.

Prof. Wanquan Ji summarized the workshop and made comments, encouraging all graduate students actively participating in scientific research and technology development to make more great progress and achievements.

In comparison with the first workshop, this workshop was first organized by the Graduate Student Board. All planning, organizing, and section chairing were done by graduate students. The number of submitted abstracts was greatly increased, and the quality was also significantly improved. Some of the presented research results have been published in *Natural Communication*, *Journal of Experimental Botany*, and other journals.



校长助理兼研究生院副院长霍学喜教授出席开幕式并致辞
Prof. Xuexi Huo participated the workshop and delivered speech



实验室副主任吉万全教授总结
Prof. Wanquan Ji was making the summary speech

五名研究生获学校优秀学位论文奖 Five Graduate Students Received University Excellent Dissertation Awards

我室5名研究生荣获2015年学校优秀学位论文奖，其中3人获博士优秀学位论文奖，2人获硕士优秀学位论文奖。

序号	研究生姓名	指导教师	论文题目
1	毛圆辉 (博士)	陶士珩	翻译过程中mRNA二级结构的功能研究
2	王平 (博士)	马锋旺	外源褪黑素对苹果叶片衰老的调控及相关自噬基因的功能分析
3	成玉林 (博士)	康振生	小麦条锈菌致病相关基因鉴定及其功能研究
4	胡桂林 (硕士)	花保祯	世界蝇蛉科 (长翅目) 昆虫分子系统发育与生物地理学研究
5	魏晓钰 (硕士)	李明军	苹果中糖转运蛋白的鉴定及两个己糖转运蛋白功能的初步研究

Five graduate students from our laboratory received the University Excellent Dissertation Award. They are:

1. Yuanhui Mao, Ph.D. student, major professor: Shiheng Tao, Dissertation: The functions of mRNA secondary structures during translation.
2. Ping Wang, Ph.D. student, major professor: Fengwang Ma, Dissertation: Functional analysis of external melatonins for regulation of apple leaf aging and related autophagy genes.
3. Yulin Cheng, Ph.D. student, major professor: Zhensheng Kang, Dissertation: Identification and function analyses of pathogenicity-related genes in the wheat stripe rust pathogen.
4. Guilin Hu, M.S. student, major professor: Baozhen Hua, Thesis: Phylogenetic and bio-geographic analyses of Panorpidae Mecoptera insects in the world.
5. Xiaoyu Wei, M.S. student, major professor: Mingjun Li, Thesis: Preliminary study on identification of sucrose transporter proteins and functions of two hexose transporter proteins.

五、科研进展

5. Progress of Scientific Research

国家公益性行业(农业)科研 专项现场考察会在洛川举行

On-site Evaluation Meeting for
the National Non-profit Scientific Research
(Agriculture) Special Project was Held in Luochuan

4月10日,陕西省农业厅组织专家在洛川对黄丽丽教授主持的国家公益性行业(农业)科研专项-苹果树腐烂病防控示范基地进行了现场考察。经过考察讨论,专家组一致认为通过夏季涂干等关键技术的实施,示范园苹果树腐烂病新发病株率远低于常规管理园,同时老病疤治愈率高于常规管理果园,示范园区苹果树腐烂病防控效果明显。专家组建议将该技术进一步扩大示范面积,加大推广力度,使更多的果农受益。



会议合影
Participants of on-site evaluation meeting

The on-site evaluation meeting for the National Non-profit Scientific Research (Agriculture) Special Project - A demonstration base for management of Valsa canker of apple, led by Prof. Lili Huang, was held in Luochuan, Shaanxi on April 10, 2015. The evaluation was conducted by a committee of experts assembled by the Department of Agriculture, Shaanxi Province. Through on-site observations and discussion, the evaluation committee concluded that significant control was achieved by implementing the key techniques including pasting diseased stems of apples trees with effective fungicide solutions in summer. The orchards managed with the new control techniques had much lower recently infected trees and much higher healed cankers than orchards managed with the conventional practices. The committee made suggestions for enlarging areas of demonstration and putting greater effort in extending the technique to let more apple growers receive the benefits.

谢长根副教授研究 团队取得重要进展

Associate Prof. Changgen Xie's Research Team Made Important Progress

谢长根副教授研究团队在植物ABA信号转导研究工作中取得新进展:植物3型蔗糖非发酵型蛋白激酶(Sucrose Non-Fermenting 1-Related Protein Kinase Subgroup 3, SnRK3)家族成员-*PKS5* (SOS2-Like Protein Kinase5, 也被称为SnRK3.22或CIPK11)通过磷酸化bZIP型转录因子ABI5 (ABA Insensitive 5)第42位丝氨酸残基参与正向调节植物ABA反应。

该研究结果发表在美国植物生物学会(ASPB)期刊《Plant Physiology》上(2015年公布影响因子为6.841)。周晓娜,郝红梅,张玉国为本研究论文共同第一作者,谢长根副教授为通讯作者。

Associate Prof. Changgen Xie's research team in our laboratory has made important progress. They identified SOS2-LIKE PROTEIN KINASE5 (PKS5), an SNF1-RELATED PROTEIN KINASE3-type protein kinase, is important for abscisic acid responses in Arabidopsis through phosphorylation of ABSCISIC ACID-INSENSITIVE5 (ABI5). Their data demonstrate that PKS5-mediated phosphorylation of ABI5 at Ser-42 is critical for the ABA regulation of seed germination and gene expression in Arabidopsis.

The study was published in *Plant Physiology*, the official journal of American Society of Plant Biology, which has an impact factor of 6.841 based on the 2015 announcement. Xiaona Zhou, Hongmei Hao, and Yuguo Zhang were co-first authors, and Prof. Xie was the corresponding author.

康振生教授团队在小麦条锈菌致病机理研究工作中取得新进展

Prof. Zhensheng Kang's Team Made New Progress in Research on Mechanisms of Pathogenicity in the Wheat Stripe Rust Pathogen

康振生教授团队相关研究结果《*PsANT*, the adenine nucleotide translocase of *Puccinia striiformis*, promotes cell death and fungal growth》发表在《Scientific Reports》上(2015年公布影响因子为5.578)。

论文中明确了条锈菌腺苷酸转位酶(adenine translocase, *PsANT*)参与能量供给,调控条锈菌初期生长发育,从而影响病菌致病性,并且探明*PsANT*是条锈菌细胞凋亡调控途径的重要成员。汤春蕾博士为论文第一作者,康振生教授为通讯作者。

The study of Prof. Zhensheng Kang's team on mechanisms of pathogenicity in the wheat stripe rust pathogen was published in *Scientific Reports*. The journal has an impact factor of 5.578 based on the 2015 announcement.

In this study, they discovered that *PsANT*, the adenine nucleotide translocase of the wheat stripe rust pathogen is involved in energy supply, regulation of early growth, and thus affecting the pathogenicity of the fungal pathogen. Their data showed that *PsANT* is an important member of cell death regulators of the pathogen. Dr. Chunlei Tang was the first author, and Prof. Kang was the corresponding author.

六、技术队伍建设

6. Technology Team Development

裴国亮等参加全国生物医学农林电镜 技术研讨会暨生物电镜前沿技术培训班

Guoliang Pei and Juanni Yao Participated in the Workshop of Nationwide Electro-Microscopy Technology in Biology, Medical, Agriculture and Forestry and the Training Class of Advanced Electro-Microscopy Technology in Biology

5月29日至6月2日,我室裴国亮、姚娟妮参加了2015全国生物医学农林电镜技术研讨会暨生物电镜前沿技术培训班,并取得结业证书。本次会议在浙江大学举行,会议邀请了国内外知名专家教授和电镜工作者讲授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,并安排学员参加实验操作及演示。

Guoliang Pei and Juanni Yao from our laboratory participated in the Workshop of Nationwide Electro-Microscopy (EM) Technology in Biology, Medical, Agriculture and Forestry and the Training Class of Advanced Electro-Microscopy Technology in Biology from May 29 to June 2, 2015, and received training certificates. The workshop and training class were held at Zhejiang University. Several well-known foreign and domestic experts were invited to teach the recent development in biological EM technology and methods in biological sample preparation and utilization. Participants were given demonstrations and took part in operations.



培训证书
The training certificates

张国云获两项实用新型专利 Guoyun Zhang Got Two New and Applicable Patents



专利证书
The patent certificates

张国云实验师申报的《一种染色液量可调节的超薄切片的染色辅助装置》、《一种超薄切片的染色辅助装置》获国家实用新型专利授权。

Two applications by Guoyun Zhang, an experimentalist in our laboratory, have been granted China new and applicable patents. The first application was entitled "A staining supplementary device for ultra-thin cutter that is able to adjust the amount of stain solution" and the second application was entitled "A staining supplementary device for ultra-thin cutting".

技术交流 Technology Exchange

本季度，实验室邀请6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. 周晓娜实验师：蛋白质层析的基本原理及AKTA系统在蛋白质层析中的应用与操作；
2. 裴国亮实验师：激光共聚焦显微镜在生命科学研究中的应用与实践；
3. 陈伟博士：Raindrop - the most sensitive nucleic acid detection system today;
4. 韩伟博士：FEI扫描电子显微镜在生物领域的应用及探索；
5. 张姣：实时荧光定量PCR原理及应用；
6. 钱俊博士：高通量技术在农业方面的应用案例分享。

During the second quarter, the laboratory invited six experts to give talks on techniques, discuss, and give demonstrations.

1. Xiaona Zhou, experimentalist, presented "The basic concepts of protein chromatography and the utilization and operation of the AKTA system in protein chromatography".
2. Guoliang Pei, experimentalist, presented "Applications of the laser confocal microscope in life science research".
3. Dr. Wei Chen presented "Raindrop - the most sensitive nucleic acid detection system today".
4. Dr. Wei Han presented "Exploration and utilization of FEI scanning electro-microscope in life science research".
5. Jiao Zhang presented "The concept and utilization of real-time fluorescent quantitative PCR".
6. Dr. Jun Qian presented "Case studies on utilization of high throughput technology in agriculture".



裴国亮实验师做技术报告
Guoliang Pei was giving talk on techniques

周晓娜实验师做技术报告
Xiaona Zhou was giving talk on techniques

七、指导调研 7. Officers' Visits



吉万全教授介绍实验室情况
Prof. Wanquan Ji was introducing the key laboratory



王晓杰教授介绍小麦条锈菌研究进展
Prof. Xiaojie Wang was presenting research progresses on wheat rust

陕西省委副书记胡和平来我室调研指导 Mr. Heping Hu, Vice Secretary of Shaanxi CPC, Visited the Laboratory

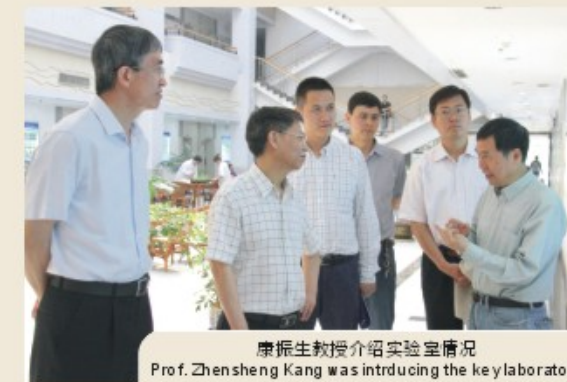
6月8日，陕西省委副书记胡和平来我室调研指导，校长孙其信、副书记徐养福、杨凌示范区党工委书记郭社荣等陪同。

实验室副主任吉万全教授介绍了实验室定位、研究方向与内容、人才培养与队伍建设、科研成果与社会服务等；王晓杰教授汇报了真菌病害科研团队围绕小麦锈病和赤霉病重大科学问题开展的研究及取得的重要进展。

Mr. Heping Hu, Vice Secretary of Shaanxi Communist Party Committee (CPC), visited our laboratory on June 8, 2015. Dr. Qixin Sun, University President, Mr. Yangfu Xu, University Vice Secretary, and Mr. Sherong Guo, Secretary of CPC Yangling accompanied his visit.

Prof. Wanquan Ji, Vice Director of CSBAA gave the introduction on the direction, research areas, scientist training and team development, achievements in science and technology, and social serves of the laboratory. Prof. Xiaojie Wang reported major progress of the Fungal Disease Research Team on wheat stripe rust and Fusarium head blight.

科技部农村司司长马连芳来我室调研 Lianfang Ma, Director of Countryside Bureau of the Ministry of Science and Technology, visited the laboratory



康振生教授介绍实验室情况
Prof. Zhensheng Kang was introducing the key laboratory

5月4日，科技部农村司司长马连芳来我室调研，副校长钱永华、科研院副院长韦革宏等陪同。

实验室主任康振生教授汇报了实验室研究方向与内容、人才培养与队伍建设、科研任务与研究进展、开放共享与运行管理等。

Lianfang Ma, Director of Countryside Bureau of the Ministry of Science and Technology, visited the laboratory on May 4, 2015. Mr. Yonghua Qian, University Vice President and Prof. Gehong Wei, Associate Dean of the University College of Scientific Research accompanied the visit.

Prof. Zhensheng Kang, Director of CSBAA, gave the introduction on the direction, research areas, scientist training and team development, tasks and achievements in science and technology, facility sharing, operation, and management of the laboratory.



合影
Participants

八、参观访问

8. Public Visits

我室举行开放日活动

Laboratory Open Day



钱永华副校长致开幕词
Vice President Yonghua Qian
was delivering the opening speech.



康振生教授作专题报告
Prof. Zhensheng Kang gave a special report

为进一步加强实验室开放共享,充分发挥实验室科技资源在科学研究中的作用,4月16日,我室举行开放日活动,副校长钱永华出席活动并致辞,学校各单位主管科研领导、2015年杰青(优青)培养科研专项资助对象、学校中青年科教人员、实验技术团队、大型仪器设备管理和操作人员、研究生、高年级本科生等近百人参加此次活动。

活动期间,实验室主任康振生作了专题报告,详细回答了提问,并和与会人员就实验室开放共享、研究生培养、科研协作等进行了交流探讨,随后康振生教授带领大家参观了实验室。同时周晓娜实验师和裴国亮实验师做了题为“蛋白质层析的基本原理及AKTA系统在蛋白质层析中的应用与操作”与“激光共聚焦显微镜在生命科学研究中的应用与实践”的技术讲座。

To strengthen the role of the laboratory in facility and equipment sharing and maximizing the use of scientific resources in research, the laboratory held an Open Day on April 16, 2015. Mr. Yonghua Qian, University Vice President, participated and delivered the opening speech. More than 100 people including administrators in charge of scientific research from various offices of the university, scientists who have received 2015 Outstanding (Excellent) Young Scientist Supporting Program grants, young teachers, research technician team, managers and operators of large equipment, graduate students, and senior undergraduate students participated in the Open Day activities.

At the Open Day, Prof. Zhensheng Kang, Director of the laboratory, gave a special topic report, answered questions, and discussed about the laboratory open and sharing, training graduate student, and collaboration with participants. He directed participants to visit the facilities and equipment of the laboratory. Drs. Xiaona Zhou and Guoliang Pei gave technical lectures entitled “The basic concepts of protein chromatography and the utilization and operation of the AKTA system in protein chromatography” and “Applications of laser-confocal microscopy in life science research”, Respectively.

陕西中医药大学学生来我室参观学习

Students from Shaanxi Chinese Medicine University Visited the Laboratory

公众开放活动期间,陕西中医药大学2012级生物技术专业48名学生来我室参观学习。黄雪玲博士详细介绍了实验室的研究方向、人员构成、研究进展、仪器设备共享和社会服务情况。随后,我室汤春蕾博士为师生做了题为“小麦-条锈菌”的学术讲座。



陕西中医药大学参观
Students from Shaanxi Chinese Medicine
University Visited the key Laboratory

During the period of open to public, 48 students of the 2012 class of Biotechnology from the Shaanxi Chinese Medicine University visited the laboratory. Dr. Xueling Huang directed their visit and introduced to the guests about the research areas, personnel structure, research progress and sharing facilities and equipment. Dr. Chunlei Tang presented a seminar entitled “Wheat - the stripe rust pathogen interactions to the guests”.

陕西科技大学校长姚书志一行来我室参观访问

Shuzhi Yao, President of Shaanxi University of Science and Technology, Visited the Laboratory



姚书志校长参观实验室
President Shuzhi Yao visited the key laboratory

6月3日,陕西科技大学校长姚书志一行来我室参观访问。王晓杰教授介绍了实验室研究方向、科研队伍、平台建设、运行管理以及组建以来取得的科研成果等情况。姚书志一行实地参观了实验室技术平台仪器设备,双方就实验室运行管理、科研平台开放共享等进行了深入交流。

Mr. Shuzhi Yao, President of Shaanxi University of Science and Technology leading his university delegation, visited the laboratory on June 3, 2015. Prof. Xiaojie Wang gave the guests an introduction on research areas, teams, platform construction, operation and management, and research achievements since the establishment of the laboratory. The delegation observed the technical platform facilities and equipment. The hosts and guests exchanged information on laboratory operation, management and research platform sharing.

其他参观访问

Other Visitors

4月-6月,先后有康奈尔大学生物技术研究院院长Jocelyn Rose教授、俄克拉荷马州立大学副校长Thomas Coon、加拿大阿尔伯塔大学农学院院长Stanford Blade教授、中国农业科学院植物保护研究所科研处处长郑传临、山东农业大学植保学院院长李向东、中国农科院作物科学研究所肖世和研究员等代表团来我室参观访问。

From April to June, Prof. Jocelyn Rose, Dean of the College of Bioscience and Technology of Cornell University; Mr. Thomas Coon, Vice President of Oklahoma State University; Prof. Stanford Blade, Dean of the College of Agriculture, University of Alberta; Mr. Chuanlin Zheng, Director of Scientific Research of Plant Protection Institute, Chinese Academy of Agricultural Sciences (CAAS); Mr. Xiangdong Li, Dean of Plant Protection, Shandong Agricultural University; Prof. Shihe Xiao, Crop science Institute, CAAS led their delegation visiting the laboratory.

九、发表论文

9. Publications

2015年4月-6月, 实验室科研人员在SCI收录刊物公开发表署名论文35篇。

From April to June, 2015, scientists of our laboratory published 35 papers in SCI journals.

1. Cheng, YF; Cui, JM; Li, ZJ; Hu, ZX; Xing, ZN; Wang, J; Zhao, HX; Hu, SW. The sulphonylurea herbicide monosulphuron ester sodium as a special male gametocide in *Brassica napus* L. CZECH JOURNAL OF GENETICS AND PLANT BREEDING 2015, 51: 16-22.
2. Dai, LM; Zhou, Q; Li, RM; Du, YJ; He, J; Wang, D; Cheng, SY; Zhang, JX; Wang, YJ. Establishment of a picloram-induced somatic embryogenesis system in *Vitis vinifera* cv. chardonnay and genetic transformation of a stilbene synthase gene from wild-growing *Vitis* species. PLANT CELL TISSUE AND ORGAN CULTURE. 2015, 121: 397-412.
3. Guo, JK; Ding, YZ; Feng, RW; Wang, RG; Xu, YM; Chen, C; Wei, XL; Chen, WM. *Burkholderia metalliresistens* sp nov., a multiple metal-resistant and phosphate-solubilising species isolated from heavy metal-polluted soil in Southeast China. ANTONIE VAN LEEUWENHOEK INTERNATIONAL JOURNAL OF GENERAL AND MOLECULAR MICROBIOLOGY. 2015, 107: 1591-1598.
4. Guo, RR; Zhao, J; Wang, XH; Guo, CL; Li, Z; Wang, YJ; Wang, XP. Constitutive expression of a grape aspartic protease gene in transgenic *Arabidopsis* confers osmotic stress tolerance. PLANT CELL TISSUE AND ORGAN CULTURE. 2015, 121: 275-287.
5. Hou, L; Chen, XM; Wang, MN; See, DR; Chao, SM; Bulli, P; Jing, JX. Mapping a large number of QTL for durable resistance to stripe rust in winter wheat Druchamp using SSR and SNP markers. PLOS ONE. 2015, 10: DOI: 10.1371.
6. Hu, GL; Yan, G; Xu, H; Hua, BZ. Molecular phylogeny of *Panorpidae* (Insecta: Mecoptera) based on mitochondrial and nuclear genes. MOLECULAR PHYLOGENETICS AND EVOLUTION. 2015, 85: 22-31.
7. Hu, XH; Xu, ZR; Xu, WN; Li, JM; Zhao, N; Zhou, Y. Application of gamma-aminobutyric acid demonstrates a protective role of polyamine and GABA metabolism in muskmelon seedlings under $\text{Ca}(\text{NO}_3)_2$ stress. PLANT PHYSIOLOGY AND BIOCHEMISTRY. 2015, 92: 1-10.
8. Jiang, L; Gao, QH; Hua, BZ. Larval morphology of the hanging-fly *Bittacus trapezoideus* Huang & Hua (Insecta: Mecoptera: Bittacidae). ZOOTAXA. 2015, 3954: 324-333.
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10. Li, C; Sun, XK; Chang, C; Jia, DF; Wei, ZW; Li, CY; Ma, FW. Dopamine alleviates salt-induced stress in *Malus hupehensis*. PHYSIOLOGIA PLANTARUM. 2015, 153: 584-602.
11. Li, P; Chen, QZ; Liu, TX. Effects of a juvenile hormone analog, pyriproxyfen, on *Serangium japonicum* (Coleoptera: Coccinellidae), a predator of *Bemisia tabaci* (Hemiptera: Aleyrodidae). BIOLOGICAL CONTROL. 2015, 86: 7-13.
12. Li, YL; Tan, YX; Shao, Y; Li, MJ; Ma, FW. Comprehensive genomic analysis and expression profiling of diacylglycerol kinase gene family in *Malus prunifolia* (Willd.) Borkh. GENE. 2015, 561: 225-234.
13. Li, YX; Liu, TX. Oviposition preference, larval performance and adaptation of *Trichoplusia ni* on cabbage and cotton. INSECT SCIENCE. 2015, 22: 273-282.
14. Lin, XL; Pan, QJ; Tian, HG; Douglas, AE; Liu, TX. Bacteria abundance and diversity of different life stages of *Plutella xylostella* (Lepidoptera: Plutellidae), revealed by bacteria culture-dependent and PCR-DGGE methods. INSECT SCIENCE. 2015, 22: 375-385.
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16. Ma, P; Bai, TH; Ma, FW. Effects of progressive drought on photosynthesis and partitioning of absorbed light in apple trees. JOURNAL OF INTEGRATIVE AGRICULTURE. 2015, 14: 681-690.
17. Mandal, MS; Fu, Y; Zhang, S; Ji, WQ. Erratum to: proteomic analysis of the defense response of wheat to the powdery mildew fungus, *Blumeria graminis* f. sp. *tritici*. THE PROTEIN JOURNAL. 2015, 34: 236.
18. Meng, XW; Wang, C; Rahman, SU; Wang, YX; Wang, AL; Tao, SH. Genome-wide identification and evolution of HECT genes in soybean. INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES. 2015, 16: 8517-8535.
19. Shu, DT; He, YL; Yue, H; Wang, QY. Microbial structures and community functions of anaerobic sludge in six full-scale wastewater treatment plants as revealed by 454 high-throughput pyrosequencing. BIORESOURCE TECHNOLOGY. 2015, 186: 163-172.
20. Si, MR; Zhang, L; Chaudhry, MT; Ding, W; Xu, YX; Chen, C; Akbar, A; Shen, XH; Liu, SJ. *Corynebacterium glutamicum* methionine sulfoxide reductase uses both mycoerdoxin and thioredoxin for regeneration and oxidative stress resistance. APPLIED AND ENVIRONMENTAL MICROBIOLOGY. 2015, 81: 2781-2796.
21. Sun, PP; Zhao, XB; Shangguan, NN; Chang, DW; Ma, Q. The roles of inoculants' carbon source use in the biocontrol of potato scab disease. CANADIAN JOURNAL OF MICROBIOLOGY. 2015, 61: 257-262.
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26. Wang, P; Sun, X; Wang, N; Tan, DX; Ma, FW. Melatonin enhances the occurrence of autophagy induced by oxidative stress in *Arabidopsis* seedlings. JOURNAL OF PINEAL RESEARCH. 2015, 58: 479-489.
27. Wei, SP; Ji, ZQ. Novel N-Acylated Benzimidazolone Derivatives: Synthesis, 2D-QSAR and targets prediction. JOURNAL OF THE BRAZILIAN CHEMICAL SOCIETY. 2015, 26: 633-641.
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Molecular phylogeny of Panorpidae (Insecta: Mecoptera) based on mitochondrial and nuclear genes

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ABSTRACT

Panorpidae are the largest family in Mecoptera, covering approximately 70% species of the order. However, the phylogenetic relationship within Panorpidae has not been adequately explored. Here we analyzed the phylogenetic relationships among 70 species of five genera in Panorpidae using maximum likelihood and Bayesian inference based on two mitochondrial (*cox1* and *cox2*) and one nuclear (28S rRNA) gene fragments with *Panorpodes kuandianensis* and *Brachypanorpa carolinensis* in Panorpidae as outgroups. The results show that the genera *Neopanorpa*, *Sinopanorpa* and *Dicerapanorpa* are monophyletic, while the widespread genus *Panorpa* is reconfirmed to be a paraphyletic group. The *P. centralis* group is monophyletic and may merit a generic status, while the *P. davidi* and *P. amurensis* groups are paraphyletic. The divergence time estimated from BEAST analysis indicates that the Panorpidae may originate in the period from early Paleogene (63.6 mya) to middle Eocene (41.2 mya), and most diversification within Panorpidae occurred in the Cenozoic. The phylogeny and biogeography of Panorpidae are briefly discussed.

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1. Introduction

Panorpidae is the most speciose family in the order Mecoptera and comprises approximately 400 described species (Byers and Thornhill, 1983; Kaltenbach, 1978; Penny and Byers, 1979). They are commonly called scorpionflies because their male genital bulb is enlarged and recurved upward, resembling the tail of scorpions. The species of Panorpidae are currently assigned to six genera, *Panorpa* Linnaeus, 1758; *Leptopanorpa* MacLachlan, 1875; *Neopanorpa* Weele, 1909; *Sinopanorpa* Cai & Hua, 2008; *Furcatopanorpa* Ma & Hua, 2011, and *Dicerapanorpa* Zhong & Hua, 2013. The monotypic *Furcatopanorpa* is distributed in central China (Ma and Hua, 2011). *Sinopanorpa* (3 species) and *Dicerapanorpa* (8 species) are also endemic to China (Cai et al., 2008; Zhong and Hua, 2013a). *Leptopanorpa* (13 species) is claimed to be exclusively distributed in Java, Indonesia (Chau and Byers, 1978). *Neopanorpa* (129 species) is found from India, Southeast Asia, Indo-China and southern China (Byers and Thornhill, 1983). The dominant speciose genus *Panorpa* (246 species) is distributed in the whole Holarctic region and northern Oriental region (Zhong and Hua, 2013a).

Panorpa and *Neopanorpa* are the most species-rich genera in Panorpidae and consist of more than 90% species of the family. *Neopanorpa* differs from *Panorpa* by vein 1A joining the hind margin of forewing before the origin of Rs, usually with one cross vein between 1A and 2A, tergum III of males with a developed notal organ, and the main plate short and simple, with the axis undeveloped and generally not extending beyond the main plate (Cai et al., 2008; Cheng, 1957; Ma and Hua, 2011; Ma et al., 2012; Zhong and Hua, 2013a). *Panorpa* is the widespread species-rich genus in Panorpidae (Penny and Byers, 1979), and is such a diverse taxon that its component species are categorized into different species groups for regional faunas based on morphological criteria (Byers, 1993; Carpenter, 1931, 1938; Cheng, 1957; Esben-Petersen, 1921; Issiki, 1933; Ward, 1983; Willmann, 1977).

Previous phylogenetic studies of Panorpidae are mainly based on morphological data (Issiki, 1933; Ma et al., 2012; Willmann, 1977, 1989). *Panorpa* was considered paraphyletic with *Neopanorpa* and *Leptopanorpa* based on wing venation (Willmann, 1989) and female genital plates and other characters (Ma et al., 2012). Based on molecular data, Misof et al. (2000) and Whiting (2002) also concluded that *Panorpa* is paraphyletic with *Neopanorpa*. The limited studies on the phylogenetic information of Panorpidae are essentially restricted to a small number of European, American and Asian species (Ma et al., 2012; Misof et al., 2000; Whiting, 2002; Willmann, 1977, 1989). Recent studies

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OPEN PsANT, the adenine nucleotide translocase of *Puccinia striiformis*, promotes cell death and fungal growth

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Adenine nucleotide translocase (ANT) is a constitutive mitochondrial component that is involved in ADP/ATP exchange and mitochondrion-mediated apoptosis in yeast and mammals. However, little is known about the function of ANT in pathogenic fungi. In this study, we identified an ANT gene of *Puccinia striiformis* f. sp. *tritici* (*Pst*), designated *PsANT*. The *PsANT* protein contains three typical conserved mitochondrion-carrier-protein (mito-carr) domains and shares more than 70% identity with its orthologs from other fungi, suggesting that ANT is conserved in fungi. Immuno-cytochemical localization confirmed the mitochondrial localization of *PsANT* in normal *Pst* hyphal cells or collapsed cells. Over-expression of *PsANT* indicated that *PsANT* promotes cell death in tobacco, wheat and fission yeast cells. Further study showed that the three mito-carr domains are all needed to induce cell death. qRT-PCR analyses revealed an in-plant induced expression of *PsANT* during infection. Knockdown of *PsANT* using a host-induced gene silencing system (HIGS) attenuated the growth and development of virulent *Pst* at the early infection stage but not enough to alter its pathogenicity. These results provide new insight into the function of *PsANT* in fungal cell death and growth and might be useful in the search for and design of novel disease control strategies.

Apoptosis is a form of cell death that plays key roles in development, tissue homeostasis and disease¹. The misregulation of apoptosis might lead to degenerative diseases, such as the Alzheimer disease in humans². Apoptosis is governed in cells by a sophisticated machinery with an elaborate array of checks and balances³. Mitochondria contribute to apoptosis induction by changing mitochondrial membrane permeability (MMP), and an *in vitro* system for apoptosis induction requires the presence of mitochondria⁴. The lethal change in MMP results from a primary mitochondrial outer membrane permeabilization (MOMP) and the mitochondrial permeability transition (MPT) in the mitochondrial inner membrane⁵. MPT activation compromises the normal integrity of the mitochondrial inner membrane, which makes the inner membrane freely permeable and allows the free redistribution of solutes and water across the inner membrane. This finally results in matrix expansion and mechanical rupture of the outer membrane⁶. MPT is regulated by the opening of the permeability transition pore complex (PTPC), a supra-molecular complex that is assembled at the contact sites between the mitochondrial outer and inner membranes through the dynamic interaction of multiple proteins, including voltage-dependent anion channels (VADC), adenine nucleotide translocase (ANT) and cyclophilin D⁷. ANT is a constitutive mitochondrial inner membrane ADP-ATP antiporter that imports ADP into the mitochondria and exports ATP to the cytosol. The ANT dimer exists in two conformations that are referred to as the matrix (m)

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FgSKN7 and *FgATF1* have overlapping functions in ascospore germination, pathogenesis and stress responses in *Fusarium graminearum*

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Summary

Fusarium head blight caused by *Fusarium graminearum* is one of the most destructive diseases of wheat and barley. Deoxynivalenol (DON) produced by the pathogen is an important mycotoxin and virulence factor. Because oxidative burst is a common defense response and reactive oxygen species (ROS) induces DON production, in this study, we characterized functional relationships of three stress-related transcription factor genes *FgAP1*, *FgATF1* and *FgSKN7*. Although all of them played a role in tolerance to oxidative stress, deletion of *FgAP1* or *FgATF1* had no significant effect on DON production. In contrast, *Fgskn7* mutants were reduced in DON production and defective in H₂O₂-induced *TRI* gene expression. The *Fgap1* mutant had no detectable phenotype other than increased sensitivity to H₂O₂ and *Fgap1 Fgatif1* and *Fgap1 Fgskn7* mutants lacked additional or more severe phenotypes than the single mutants. The *Fgatif1*, but not *Fgskn7*, mutant was significantly reduced in virulence and delayed in ascospore release. The *Fgskn7 Fgatif1* double mutant had more severe defects in growth, conidiation and virulence than the *Fgatif1* or *Fgskn7* mutant. Instead of producing four-celled ascospores, it formed eight small, single-celled ascospores in each ascus. Therefore, *FgSKN7* and *FgATF1* must have overlapping functions in intracellular ROS signalling for growth, development and pathogenesis in *F. graminearum*.

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Introduction

Fusarium head blight (FHB) or scab is a destructive disease of wheat and barley (Bai and Shaner, 2004; Goswami and Kistler, 2004). One major causal agent is *Fusarium graminearum*, which also infects other small grains. The spikes of host plants are most susceptible to *F. graminearum* infection at anthesis. Under favorable environmental conditions, FHB can cause severe yield losses and reduce grain quality. In addition, *F. graminearum* produces toxic secondary metabolites, including deoxynivalenol (DON) that is a potent inhibitor of protein synthesis in eukaryotic organisms (Van de Walle *et al.*, 2010). The accumulation of harmful mycotoxins such as DON in infested grains is a great threat to human and animal health. DON is synthesized by the trichothecene biosynthesis (*TRI*) genes (Brown *et al.*, 2004; Alexander *et al.*, 2009). Except the *TRI1*, *TRI16* and *TRI101* genes, all other 11 *TRI* genes, including *TRI5*, *TRI6* and *TRI10*, are in the main *TRI* gene cluster. *TRI5* encodes the enzyme responsible for trichodiene synthesis, which is the first and key step for DON biosynthesis. *TRI6* and *TRI10* are two transcription factor genes that regulate *TRI* gene expression (Seong *et al.*, 2009; Nasmith *et al.*, 2011).

In *F. graminearum*, several environmental factors have been implicated in the regulation of DON production, including pH and reactive oxygen species (ROS) stress. Acidic pH is conducive to *TRI* gene expression and DON production. At alkaline pH conditions, expression of *TRI* genes and DON production are suppressed (Gardiner *et al.*, 2009b; Merhej *et al.*, 2011). Several studies also showed that ROS is involved in regulating DON synthesis in *F. graminearum* (Ochiai *et al.*, 2007; Audenaert *et al.*, 2010). Low concentrations of H₂O₂ or sublethal levels of prothioconazole fungicides stimulated DON production. Interestingly, expression of *TRI* genes could be detected at early infection stages (Guldener *et al.*, 2006; Ilgen *et al.*, 2009). Because oxidative burst is a common plant defense response, *F. graminearum* may use ROS generated by the host as a trigger to upregulate DON biosynthesis. DON also is phytotoxic and DON production is an important virulence factor in FHB (Proctor *et al.*, 1997; Desjardins *et al.*, 2000).

SOS2-LIKE PROTEIN KINASE5, an SNF1-RELATED PROTEIN KINASE3-Type Protein Kinase, Is Important for Abscisic Acid Responses in Arabidopsis through Phosphorylation of ABSCISIC ACID-INSENSITIVE5^{1[OPEN]}

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Abscisic acid (ABA) plays an essential role in seed germination. In this study, we demonstrate that one SNF1-RELATED PROTEIN KINASE3-type protein kinase, SOS2-LIKE PROTEIN KINASE5 (PKS5), is involved in ABA signal transduction via the phosphorylation of an interacting protein, ABSCISIC ACID-INSENSITIVE5 (ABI5). We found that *pks5-3* and *pks5-4*, two previously identified PKS5 superactive kinase mutants with point mutations in the PKS5 FISL/NAF (a conserved peptide that is necessary for interaction with SOS3 or SOS3-LIKE CALCIUM BINDING PROTEINS) motif and the kinase domain, respectively, are hypersensitive to ABA during seed germination. PKS5 was found to interact with ABI5 in yeast (*Saccharomyces cerevisiae*), and this interaction was further confirmed in planta using bimolecular fluorescence complementation. Genetic studies revealed that ABI5 is epistatic to PKS5. PKS5 phosphorylates a serine (Ser) residue at position 42 in ABI5 and regulates ABA-responsive gene expression. This phosphorylation was induced by ABA in vivo and transactivated ABI5. Expression of ABI5, in which Ser-42 was mutated to alanine, could not fully rescue the ABA-insensitive phenotypes of the *abi5-8* and *pks5-4abi5-8* mutants. In contrast, mutating Ser-42 to aspartate rescued the ABA insensitivity of these mutants. These data demonstrate that PKS5-mediated phosphorylation of ABI5 at Ser-42 is critical for the ABA regulation of seed germination and gene expression in Arabidopsis (*Arabidopsis thaliana*).

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X.Z. and C.G.X. designed the research; X.Z., C.G.X., H.H., Y.Z., Y.B., W.Z., F.Y., and M.W. performed the research; X.Z., C.G.X., H.H., Y.Z., Y.B., W.Z., Y.Q., F.Z., J.H., H.X., A.G., H.Z., Y.Z., C.C., and Y.Y. contributed to data analysis; C.G.X., X.Z., Y.G., and K.S.S. wrote the article.

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The phytohormone abscisic acid (ABA) plays important roles in plant growth and developmental processes (Finkelstein *et al.*, 2002; Cutler *et al.*, 2010). Recently, a phosphorylation cascade has been shown to play a fundamental role in early ABA signaling (Fujita *et al.*, 2009; Cutler *et al.*, 2010). When bound to ABA, PYRABACIN RESISTANCE1/PYRABACIN RESISTANCE1-LIKE/REGULATORY COMPONENTS OF ABSCISIC ACID RECEPTOR-type ABA receptors (hereafter referred to as PYLs) interact with and inhibit clade A PROTEIN PHOSPHATASE2C (PP2C). This leads to the release of SNF1-RELATED PROTEIN KINASE2 (SnRK2)-type protein kinases from PP2C-SnRK2 complexes, allowing SnRK2s to phosphorylate and activate downstream effectors of ABA responses, such as ABSCISIC ACID-INSENSITIVE5 (ABI5) and other ABSCISIC ACID-RESPONSIVE ELEMENT-BINDING FACTORS (ABFs; Fujita *et al.*, 2009; Cutler *et al.*, 2010).

The phosphorylation of ABI5 and ABFs by ABA-activated kinases is required for ABA-responsive gene expression (Lopez-Molina *et al.*, 2001, 2003; Furihata *et al.*, 2006; Rodrigues *et al.*, 2013). ABI5 and ABFs

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