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Molecular evolution and functional divergence of tubulin superfamily in the fungal tree of life

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Microtubules are essential for various cellular activities and β-tubulins are the target of benzimidazole fungicides. However, the evolution and molecular mechanisms driving functional diversification in fungal tubulins are not clear. In this study, we systematically identified tubulin genes from 59 representative fungi across the fungal kingdom. Phylogenetic analysis showed that α-/β-tubulin genes underwent multiple independent duplications and losses in different fungal lineages and formed distinct paralogous/orthologous clades. The last common ancestor of basidiomycetes and ascomycetes likely possessed two paralogs of α-tubulin (α_1/α_2) and β-tubulin (β_1/β_2) genes but α_2-tubulin genes were lost in basidiomycetes and β_2-tubulin genes were lost in most ascomycetes. Molecular evolutionary analysis indicated that α_1, α_2, and β_2-tubulins have been under strong divergent selection and adaptive positive selection. Many positively selected sites are at or adjacent to important functional sites and likely contribute to functional diversification. We further experimentally confirmed functional divergence of two β-tubulins in *Fusarium* and identified type II variations in FgTub2 responsible for function shifts. In this study, we also identified δ-/ε-/η-tubulins in Chytridiomycetes. Overall, our results illustrated that different evolutionary mechanisms drive functional diversification of α-/β-tubulin genes in different fungal lineages, and residues under positive selection could provide targets for further experimental study.

Tubulins are major components of the microtubules that are involved in many cellular processes, such as cell division, ciliar or flagellar motility, and intracellular transport in eukaryotic organisms. In general, tubulins comprise of the α-, β-, γ-, δ-, ε-, and η-tubulin families[1,2]. The α-, β-, and γ-tubulins are ubiquitous and present in all the eukaryotic organisms. The α- and β-tubulins assemble in a head-to-tail heterodimers to form the basic building block of the microtubule[1]. The γ-tubulins are mainly found in the microtubule organizing center and play essential roles in the initiation of microtubule assembly[1,3]. Interestingly, although they are well conserved in eukaryotes, fungal β-tubulins are the molecular targets of benomyl or MBC fungicides that are effective in controlling many plant diseases caused by ascomycetous fungi[4–6]. Treatments with these fungicides targeted at β-tubulins inhibit microtubule assembly and hyphal growth. Unlike the α-, β-, and γ-tubulin genes, the δ-, ε-, and η-tubulin genes have only been found in animals and some protists[1,2]. To date, their homologs have not been reported in fungi, and are assumed to be lost during fungal evolution[7,8]. However, previous tubulin studies were mainly focused on several model organisms. With fungal species across the Kingdom Fungi being sequenced, it is important to thoroughly examine the distribution and expansion of various tubulin families in different fungal lineages.

Most animals have multiple α- and β-tubulin genes but no more than three γ-tubulin genes[1,2]. For example, the human genome contains at least 15 α-tubulin genes and 21 β-tubulin genes but only 3 γ-tubulin genes[3]. Fungi have much fewer tubulins genes than animals. Many of them contain only a single α-, β-, or γ-tubulin gene. However, some fungi such as *Aspergillus nidulans*[9], *Saccharomyces cerevisiae*[10], *Schizosaccharomyces pombe*[11], and *Fusarium graminearium*[12] are known to have two α- or two β-tubulin genes. In the budding yeast *S. cerevisiae*, either Tub1 or Tub3, two divergent alpha-tubulins, could perform all the functions. However, only Tub1, not Tub3, is essential for normal growth in the haploid strains[13]. Similarly, only one (nda2) of the two α-tubulin genes (nda2 and atb2) is essential for growth in the fission yeast *S. pombe*[11]. In the model filamentous fungus *A.*

第一届学术委员会第四次会议
The 4th Meeting of the First Session of the Academic Committee

一、学术委员会会议
Science and Technology Committee Meeting

召开第一届学术委员会第四次会议
The Laboratory Held the 4th Meeting of the First Science and Technology Committee

12月7日，我室第一届学术委员会第四次会议在我校召开。副校长钱永华主持会议，校长孙其信出席会议并致辞。实验室主任康振生教授汇报了2014年实验室在科学研究、队伍建设、人才培养、开放交流、运行管理等方面的工作及完成情况，汇报了2015年开放课题资助方案，实验室固定研究人员刘同先教授、王中华教授、江元清教授分别汇报了科研工作进展。

学术委员会肯定了实验室一年来的工作成绩，就2015年工作提出三项建议。一是进一步凝练研究方向和内容，充分利用国家和学校的政策，加强小麦和苹果逆境生物学基础研究。二是充分利用学校的政策，吸引和培养青年优秀人才，加强优青、杰青等候选人才培养。三是进一步增加和整合实验室空间资源，加强实验技术队伍建设，为研究人员创造良好的科研条件和学术氛围，并建议实验室提早准备2016年的实验室评估工作。

The first Science and Technology Committee of the State Key Laboratory of Crop Stress Biology for Arid Areas (CSBAA) held its fourth meeting at Northwest A&F University (NWAFU) on December 7, 2014. Vice president Yonghua Qian was presiding the meeting, and President Qixing Sun was attending and delivered the opening speech. Prof. Zhensheng Kang, director of the CSBAA laboratory, delivered the 2014 report on accomplishments and progress in scientific research, team development, expert training, collaboration and scientific exchange, and operation management. He also reported the financial plan for supporting collaborative research projects. Professors Tongxian Liu, Zhonghua Wang, and Yuanqing Jiang reported the research accomplishments and progress in various scientific fields.

The committee made positive comment on the achievement of the laboratory and made the following three suggestions: 1) further fine-turning the research direction and specific objectives, and fully utilizing the national and university policies to strengthen the basic biological research on wheat and apple crops under stress environments; 2) fully utilizing the university policies to attract and train excellent experts, especially strengthen the training of candidates for excellent and outstanding young scientists; and 3) further adding and integrating the space and facilities, strengthening the development of technician team and creating good conditions and favorable environment for scientific research.The committee also proposed early preparation for the laboratory evaluation in 2016.

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二、团队建设
Team Construction

康振生教授荣获2014年宝钢优秀教师特等奖

Prof. Zhensheng Kang Receives the Special Class Award of the 2014 "Baosteel" Excellent Teachers


康振生教授
Professor Zhensheng Kang

日前， 2014年度“宝钢教育奖”评选结果在江苏常熟揭晓，实验室主任康振生教授获得宝钢优秀教师特等奖，这是西北地区唯一获此殊荣的优秀教师，也是宝钢教育奖在我校设立以来第一个获得该项奖励的教师。

The "Baosteel Education Awards" has recently announced its 2014 selections in Changshou, Jiangsu. Prof. Zhensheng Kang, director of the CSBAA laboratory, receives the Special Class award. He has become the first to receive the prestigious award in northwestern China and also the first in NWAFU since Shanghai Baosteel Group Corporation established the awards for teachers who have made excellent achievements.

首届研究生羽毛球团体赛顺利举行

The Laboratory Held the First Graduate Student Badminton Team Competition

10月25日， 2014年研究生羽毛球团体赛在科研楼羽毛球场举行。实验室近50位研究生参加了此次比赛活动。 经过紧张激烈的角逐，作物与有害生物互作机理研究方向代表队获得第一名，作物非生物胁迫应答机理研究方向代表队、作物抗逆种质创新与品种设计研究方向代表队、抗逆种质与基因资源挖掘研究方向代表队分别获得第二、三、四名。通过本次比赛，丰富了我室文化活动，促进了交流与沟通，增强了实验室的凝聚力。

羽毛球赛合影
Group photos of the badminton competition

The first Graduate Student Badminton Team Competition was held in the Badminton Stadium ofthe Scientific Research Building on October 25, 2014. About 50 graduate students representingseveral teams participated in the competition. The matches were very tense and the rankings were very tight. The team of the research group on the interactions between crops and pests received the first prize, and the teams of the research groups on crop responses to abiotic stresses, stress resistant germplasm and cultivar development, and stress resistant germplasm screening and characterization received the awards of the second, third, and fourth places. The competition has enriched the cultural activities, improved the connection and exchange, and enhanced coagulation capacity of thelaboratory.

单卫星教授、韦革宏教授入选长江学者特聘教授

Prof.Gehong Wei And WeiXing Shan Added "Changjiang Scholar" Specially Appointed Professors

日前，2013、2014年度教育部“长江学者奖励计划”拟聘任人 选名单经“长江学者奖励计划”评审委员会会议审定，我室单卫星教授、韦革宏教授获批特聘教授。

Recently, professors Weixing Shan and Gehong Wei have been approved by the evaluation committee to be specially appointed professors of the "Changjiang Scholar Award Project", adding two more "Changjiang Scholar" professors to the CSBAA laboratory.


单卫星教授
Professor Weixing Shan


韦革宏教授
Professor Gehong Wei

吉万全教授获国家科技进步二等奖

Prof. Ji Wanquan won the second prize of National Science and Technology Progress Award

实验室副主任吉万全教授参与的项目“小麦种质资源重要育种性状的评价与创新利用”获2014年度国家科学技术进步二等奖，吉万全教授为第四完成人。


吉万全教授
Professor WanquanJi

The program "Evaluation and Innovative Use of the Important Breeding Traits of Wheat Germplasm Resources" won the second prize of National Science and Tehnology Progress Award. Prof. Ji Wanquan, the deputy director of State Key Laboratory of Crop Stress Biology for Arid Areas was awarded as the first 4 completed.

李明军副教授、张宏副研究员获陕西省青年科技奖

Associate professors Li Mingjun and Zhang Hong in our lab won the Shaanxi Youth Science and technology awards

日前，第十届陕西青年科技奖评选结果揭晓，我室李明军副教授、张宏副研究员入选。其中，李明军还被授予“陕西青年科技标兵”荣誉称号。

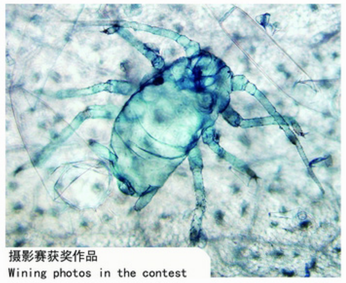
Recently, the tenth session of the Shaanxi Youth Science and technology awards results was announced. In the State Key Laboratory of Crop Stress Biology for Arid Areas, associate professor Li Mingjun and Zhang Hong won the awards. Li Mingjun was also awarded the honorary title of Shaanxi Youth Science and technology pacesetter


李明军副教授
Associate Professor Mingjun Li


张宏副研究员
Associate Professor Hong Zhang

第二届摄影大赛举行

The Laboratory Held the Second Photography Competition


摄影赛获奖作品
Wining photos in the contest

The laboratory successfully completed its second Photography Competition on December 28, 2014. There were 29 pictures mainly showing natural and social sceneries, cultural environments, and scientific exchanges of the laboratory entered for the competition. The pictures were evaluated by a committee consisting of photography experts including the president of Yangling Photography Association. Through evaluation, 1 picture received the first prize, 3 pictures the second prize, 6 the third prize, and 14 the excellent prize. Compared to the first competition, both the number and quality level of the pictures in this completion were significantly increased and improved, fully reflecting the cultural environment and spiritual outlook of the laboratory.

12月28日，实验室第二届摄影比赛落下帷幕。本次比赛共收到29件摄影作品，此次作品以实验室人文景观、文化氛围、学术交流为主。由杨凌示范区摄影协会主席等专家组成的评委对参赛作品进行了评审，评出一等奖1名、二等奖3名、三等奖6名、优秀奖14名。此次比赛相比第一届在数量和水平明显提升并充分展示了实验室的文化氛围和精神面貌。

三、学术报告
Workshops and Seminars

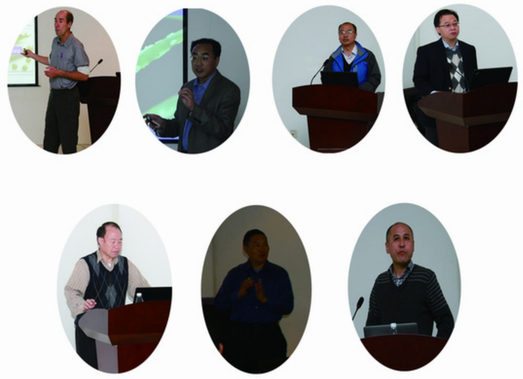
12月7日，我室举办了“小麦遗传育种理论与实践”学术报告。中国工程院刘旭院士和程顺和院士应邀做了题为“中国小麦资源和育种的回顾与几个问题的探讨”，“基因组学技术在小麦遗传改良中的应用问题探讨”学术报告，并与研究生进行了讨论交流。


程顺和院士做报告
Academician Shunhe Cheng gave a speech

The laboratory held the workshop of “Concepts and Practices of Wheat Genetics and Breeding” on December 7, 2014. Professors Xu Liu and Shunfa Cheng, academicians of Chinese Academy of Engineering were invited to present “The retrospect of wheat germplasm and breeding and discussion of issues in China” and “Applications of the genomics technology on wheat genetics and improvement”, respectively. They also exchanged ideas with graduate students.


刘旭院士做报告
Academician Xu Liu gave a speech

本季度，受实验室邀请加拿大萨斯卡切温大学Yangdou Wei教授，日本国立农业资源研究所资深研究员Takao Komatsuda博士、美国农业部麦类作物条锈病防控岗位科学家Xianming Chen教授、中玉金标记（北京）生物技术股份有限公司CEO兼首席科学家卢洪博士、农业部全国农业技术推广服务中心病虫害防治处处长杨普云博士、加拿大不列颠哥伦比亚大学植物系Reinhard Jetter教授、美国加州大学戴维斯分校植物科学系Mingcheng Luo教授等7位国内外著名学者来我室开展学术交流并做学术报告。


应邀专家做报告
Invited experts gave speeches

In this quarter, the laboratory invited the following seven scientists to visit and present seminars: Dr. Takao Komatsuda,senior scientist from the National Institute of Agricultural Resources, Japan; Dr. Xianming Chen, Research Plant Pathologist, US Department of Agriculture, Agricultural Research Service, USA; Dr. Hong Lu, CEO and senior scientist of China Yujin Marker Biotechnology LTD, Beijing; Dr. Puyun Yang, director of Pest Management Division, National Center of Agricultural Technology Extension and Service, China Ministry of Agriculture; Dr. Yangdou Wei, professor of the Department of Biology, University of Saskatchewan, Canada; Dr. Reinhard Jetter, Prof. of the Department of Botany, University of British Columbia, Canada; and Dr. Mingcheng Luo, professor of the Department of Plant Sciences, University of California, Davis, USA.

11月16日，我室举办了“现代植物分子病理学研究进展”学术报告会。来自南京农业大学周明国教授和王源超教授、北京大学李毅教授、上海交通大学陈功友教授、福建农林大学魏太云教授、中科院微生物所郭惠珊研究员和钱韦研究员、中科院遗传发育所周俭民研究员和沈前华研究员分别作了学术报告，并与我室师生进行了热烈讨论。

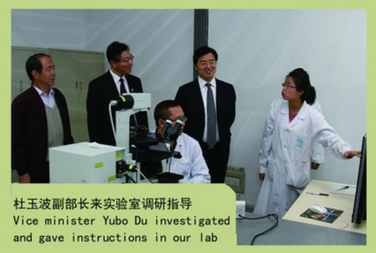
On November 16, 2014, the laboratory held a seminar series on “Progress of modern molecular plant pathology research”.

Prof. Mingguo Zhou from Nanjing Agricultural University;
Prof. Yuanchao Wang from Nanjing Agricultural University;
Prof. Yi Li from Beijing University;
Prof. Gongyou Chen from Shanghai Jiao Tong University;
Prof. Taiyun Wei from Fujiang Agriculture and Forest University;.
Prof. Huishan Guo from the Institute of Microbiology, Chinese Academy of Sciences;
Prof. Wei Qian from the Institute of Microbiology, Chinese Academy of Sciences;
Prof. Jianmin Zhou from the Institute of Genetics and Development, Chinese Academy of Sciences;
Prof. Qianhua Shen from the Institute of Genetics and Development, Chinese Academy of Sciences

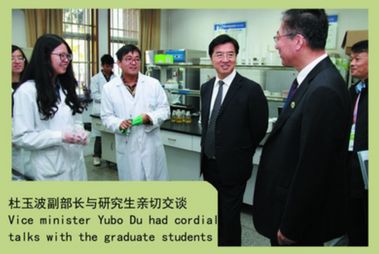
四、调研指导
Leaders’ Visits and Direction

教育部副部长杜玉波来我室考察指导工作
Vice Minister Yubo Du from China Ministry of Education Visited the Laboratory

11月5日，教育部党组副书记、副部长杜玉波一行来我室考察指导工作，校长孙其信教授陪同考察。实验室副主任吉万全教授汇报了实验室的定位，研究方向、科研团队、条件平台以及近年取得的科研成果。杜玉波副部长实地考察了我室大型仪器设备平台和专业实验室，并与正在做实验的研究生进行了亲切交谈。


杜玉波副部长来实验室调研指导
Vice minister Yubo Du investigated and gave instructions in our lab

On November 5, 2014, Mr. Yubo Du, vice minister, leading a delegation of China Ministry of Education, was visiting the laboratory, accompanied by President Qixing Sun. Prof. Wanquan Ji, vice director of the laboratory, reported the localization, research direction, faculty, facilities and equipment, and recent achievements of the laboratory to the delegation. Mr. Du and other delegation members visited the major facilities and equipment platforms and specific laboratories, and talked with faculty and graduate students.


杜玉波副部长与研究生亲切交谈
Vice minister Yubo Du had cordial talks with the graduate students

钱永华副校长来我室调研指导工作

Vice President Yonghua Qian Came to the Laboratory for Investigation and Direction

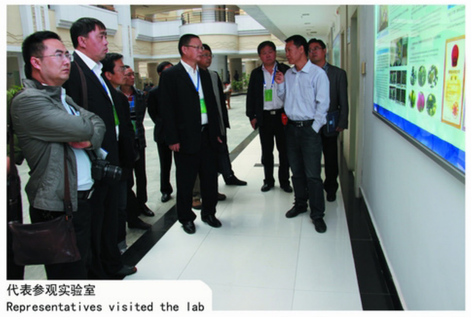
10月15日，钱永华副校长来我室调研指导工作，科研院常务副院长韦革宏教授陪同调研。实验室主任康振生教授从实验室定位与研究方向的凝练、人才培养与队伍建设、承担任务与主要成果、平台建设与开放交流、实验室运行与管理以及面临的挑战与存在问题等方面进行了汇报。

On October 15, 2014, vice president Yonghua Qian came to the laboratory for work investigation and direction, accompanied by Prof. Gehong Wei, executive vice dean, and others from the university Scientific Research School. Prof. Zhensheng Kang, director of the laboratory, reported the laboratory's work from every aspect including achievements, progress, challenges and issues.

钱永华副校长来实验室调研
Vice president Yonghua Qian investigated in the lab

五、参观考察

Public and Other Visits



代表参观实验室
Representatives visited the lab

百余名中学负责人来我室考察

High School Administrators Visited the Laboratory

10月10日，来我校参加招生工作研讨会的全国15个省67所中学及地方教育部门的百余名代表来实验室参观考察，胡银岗教授介绍了实验室的战略定位、研究内容、研究成果、社会服务和科技贡献，并就现代生物技术等进行了讨论。

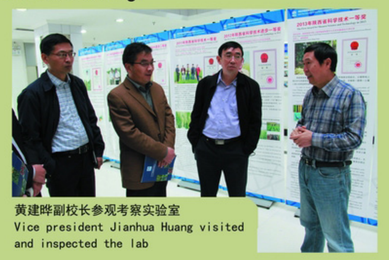
On October 10, 2014, more than 100 administrators from 67 high schools and regional education offices in 15 provinces, who were attending the University Admission Forum, were visiting the laboratory. Prof. Yingang Hu gave the gusts an introduction about the laboratory, including the strategic position, research directions and achievements, social services and contributions to science and technology, and discussed

扬州大学副校长黄建晔一行来我室考察

Vice President Jianye Huang from Yangzhou University Visited the Laboratory

10月22日，扬州大学副校长黄建晔一行来我室参观考察，我校副校长冷畅俭、科研院有关负责人陪同考察。实验室主任康振生教授介绍了实验室情况，就实验室运行管理、国际合作、开放共享等进行了交流探讨。

On October 22, 2014, Prof. Jianye Huang, vice president from Yangzhou University, leading a delegation was visiting the laboratory, accompanied by vice president Changjian Leng and officers of the School of Scientific Research of NWAFU. Prof. Zhengsheng Kang gave the guests an introduction about the laboratory and discussed with the guest about laboratory operation and management, international collaboration, and public opening and resources sharing.



黄建晔副校长参观考察实验室
Vice president Jianhua Huang visited and inspected the lab

加拿大农业与食品部副部长来我室参观访问

Vice Minister of the Department of Agriculture and Agri-Food Canada Visited the Laboratory

11月5日，加拿大农业与农业食品部副部长吉勒斯尚登一行来我室参观访问。实验室副主任吉万全教授介绍了实验室情况，大型仪器设备平台运行和管理，并就小麦重大病害研究工作进行了沟通交流。

Gilles Schanden参观访问实验室
Gilles Schanden visited the lab

On November 5, 2014, Mr. Gilles Schanden, vice minister, leading a delegation of the Department of Agriculture and Agri-Food Canad, was visiting the laboratory. Prof. Wanquan Ji, vice director of the laboratory, presented the guests an introduction of the laboratory including major facilities and equipment and lab operation and management, and exchanged research and control of major wheat diseases.

Jairo A Palta参观访问实验室
Jairo A Palta visited the lab

澳大利亚联邦科学与工业研究组织来我室参观访问

Australia Commonwealth Scientific and Industrial Research Organization (CSIRO) Scientist Visited the Laboratory

11月21日，澳大利亚联邦科学与工业研究组织（CSIRO）环境与生命科学研究中心资深研究科学家Jairo A Palta来我室参观访问，王晓杰副研究员介绍了实验室情况，双方就感兴趣的科学研究问题进行了交流探讨。

On November 21, 2014, Dr. Jairo A. Palta, senior scientist of the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia, was visiting the laboratory. Dr. Xiaojie Wang gave the guest an introduction about the laboratory, and discussed mutually interested research topics.

Valley Murray参观访问实验室
Valley Murray visited the labb

加拿大驻华大使馆农业参赞谷默雷一行来我室参观访问

Canadian Embassy in China Agricultural counselor Valley Murray line to visit the Laboratory

12月16日，加拿大驻华大使馆农业参赞谷默雷先生等一行4人来我室参观访问。胡银岗教授介绍了实验室情况，双方就学术交流与国际合作等进行了交流。

On December 16, 2014, Mr. Valley Murray, Agricultural Counselor of Canadian Embassy and his delegation were visiting the laboratory. Prof. Yingang Hu introduced the guests about the laboratory and discussed about scientific exchange and international collaboration.

六、实验平台建设

Construction of Research Platforms

日立公司应邀对我室透射电镜进行技术维护

Hitachi Electronics Engineer Conducted TEM Technical Maintenance

12月23日至24日，日立电子公司高级工程师金相会应邀来我室对透射电子显微镜进行技术回访和设备维护，并进行技术培训和指导。

技术指导
Technology instruction

From December 23 to 24, 2014, Mr. Xianghui Jin, senior Engineer, Hitachi Electronics Co. LTD was conducting technical returning investigation and maintenance for our transmission electron microscopes. He also provided technical training and instruction.

10月—12月公开发表的SCI论文

SCI Publications from October to December, 2014

2014年10月至12月，实验室科研人员在SCI收录刊物公开发表署名学术论文34篇。

From October to December, 2014, scientists of the laboratory published 34 SCI papers.

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Journal of Pineal Research

Melatonin regulates proteomic changes during leaf senescence in *Malus hupehensis*

Abstract: Despite the relationship between melatonin and aging, the overall changes and regulation of proteome profiling by long-term melatonin exposure during leaf senescence is not well understood. In this study, leaf senescence in *Malus hupehensis* plants was delayed when exogenous melatonin was regularly applied to the roots for 2 months compared with natural leaf senescence. Proteins of samples 0 and 50 day for both treatments were extracted and labeled with TMT regents before being examined via NanoLC-MS/MS. The proteomics data showed that 622 and 309 proteins were altered by senescence and melatonin, respectively. Our GO analysis by Blast2GO revealed that most of the altered proteins that are involved in major metabolic processes exhibited hydrolase activity and were mainly located in the plastids. These proteins were classified into several senescence-related functional categories, including degradation of macromolecules, redox and stress responses, transport, photosynthesis, development, and other regulatory proteins. We found that melatonin treatment led to the downregulation of proteins that are normally upregulated during senescence. The melatonin-related delay in senescence might have occurred due to the altering of proteins involved in processes associated with senescence. And as well, there are many unknown regulatory proteins possibly being involved in the melatonin's function. This study is the first to demonstrate changes at the proteome level in response to exogenous melatonin in plants. Our findings provide a set of informative and fundamental data about the role of melatonin in apple leaf senescence.

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Key words: GO, leaf senescence, *Malus*, melatonin, NanoLC-MS/MS, proteome, tandem mass tag

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Introduction

Leaf senescence is an inevitable and complex developmental process in which essential nutrients are recycled. Cells in those leaves undergo an orderly disintegration of their organelles and dramatic changes in metabolism. These changes include a decrease in photosynthetic capacity and massive hydrolysis of macromolecules, for example, proteins, lipids, and nucleic acids [1, 2]. Although critical and evolutionarily acquired for plant fitness, leaf senescence may limit yields and biomass accumulations due to the reduction in photosynthesis. Perennial fruit trees such as apple (*Malus* sp.) often encounter various environmental stresses that can lead to early leaf senescence and strongly inhibit the accumulation of carbohydrate in leaves, thus delay fruit development and influencing the production of desirable flavors [3]. Therefore, an improved understanding of leaf senescence is of great practical significance and new strategies, perhaps utilizing melatonin, must be explored for preventing or delaying this process under certain circumstances, to enhance ecological and safety crops, and to protect plants in environment.

Melatonin is an indolamine derived from the essential amino acid tryptophan, and it is a conserved molecule widespread in plant and animal kingdoms. In animals and humans, its functions are related to regulating circadian rhythms and photoperiods [4, 5], promoting immunomodulation, and conferring anti-aging and anti-inflammatory properties [6, 7]. In plants, its role as a powerful antioxidant and a growth promoter is most supported by experimental evidences. For instance, melatonin helped to protect red cabbage seedlings and a macroalga *Ulva* sp. against high concentrations of heavy metals [8, 9]; Melatonin enhanced the resistance to UV radiation in Mediterranean plants [10]; Melatonin also had positive protection against cold-induced apoptosis in a suspension culture of carrot (*Daucus carota* L.) [11] and recently Bajwa et al. [12] reported that melatonin enhanced cold tolerance in *Arabidopsis*, and Shi and Chan [13] found one Cysteine2/Histidine2-type zinc finger transcription factor 6 (*ZAT6*)-activated *C-REPEAT-BINDING FACTOR* (*CBF*) pathway was involved in melatonin-mediated freezing stress response in *Arabidopsis*; Melatonin as well increased the tolerance of water-deficient stress in cucumber seedlings [14] and grape cuttings [15]. In addition to these, our previous work of *Malus* provided a series of evidence on its effective delay in dark-induced [16], drought-induced [3] and natural leaf senescence [17]. In plant growth and

FgKin1 kinase localizes to the septal pore and plays a role in hyphal growth, ascospore germination, pathogenesis, and localization of Tub1 beta-tubulins in *Fusarium graminearum*

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Key words: ascospore germination, ascospore release, beta-tubulins, cytokinesis, pathogenesis, septation.

Summary

- The Kin1/Par-1/MARK kinases regulate various cellular processes in eukaryotic organisms. Kin1 orthologs are well conserved in fungal pathogens but none of them have been functionally characterized. Here, we show that *KIN1* is important for pathogenesis and growth in two phytopathogenic fungi and that FgKin1 regulates ascospore germination and the localization of Tub1 β-tubulins in *Fusarium graminearum*.
- The *Fgkin1* mutant and putative *FgKIN1*^{S172A} kinase dead (nonactivatable) transformants were characterized for defects in plant infection, sexual and asexual reproduction, and stress responses. The localization of FgKin1 and two β-tubulins were examined in the wild-type and mutant backgrounds.
- Deletion of *FgKIN1* resulted in reduced virulence and defects in ascospore germination and release. FgKin1 localized to the center of septal pores. *FgKIN1* deletion had no effect on Tub2 microtubules but disrupted Tub1 localization. In the mutant, Tub1 appeared to be enriched in the nucleolus. In *Magnaporthe oryzae*, MoKin1 has similar functions in growth and infection and it also localizes to septal pores. The S172A mutation had no effect on the localization and function of *FgKIN1* during sexual reproduction.
- These results indicate that *FgKIN1* has kinase-dependent and independent functions and it specifically regulates Tub1 β-tubulins. FgKin1 plays a critical role in ascospore discharge, germination, and plant infection.

Introduction

The filamentous ascomycete *Fusarium graminearum* is one of the causal agents of Fusarium head blight (FHB) of wheat and barley (Bai & Shaner, 2004; Goswami & Kistler, 2004). It is also one of the pathogens causing stalk and ear rots of maize. Unlike many other plant pathogenic fungi, *F. graminearum* uses ascospores as the primary inoculum to infect wheat or barley heads. The pathogen overwinters in infected plant tissues and produces perithecia on plant debris. Ascospores are forcibly discharged from mature perithecia (Trail *et al.*, 2002; Trail, 2007) to infect wheat and barley heads that are susceptible from anthesis to the dough stage (Bai & Shaner, 2004). Asexual spores produced by this pathogen on diseased plants are primarily for disease spreading. Under favorable environmental conditions, *F. graminearum* can cause severe yield losses and it produces harmful mycotoxins, such as deoxynivalenol (DON) and zearalenone, in infected plant tissues. As an inhibitor of protein synthesis in eukaryotic organisms, DON is also an important virulence factor during plant infection (Proctor *et al.*, 1995; Bai *et al.*, 2002). Mutants defective in DON production are defective in spreading from the initial infection site via the rachis to the rest of wheat or barley heads.

Because ascospores are the primary inoculum, sexual reproduction plays a critical role in the infection cycle of *F. graminearum*, which is a homothallic fungus that contains two linked mating type idiomorphs and four pheromone and pheromone receptor genes (Kim *et al.*, 2008; Lee *et al.*, 2008; Zheng *et al.*, 2013). In addition to these well-conserved mating-related genes, a number of genes with various biological functions are known to be important for sexual production in *F. graminearum*, including components of three well conserved mitogen-activated protein kinase pathways and a number of transcription factor genes (Hou *et al.*, 2002; Jenczmionka *et al.*, 2003; Urban *et al.*, 2003; Son *et al.*, 2011; C. Wang *et al.*, 2011; Nguyen *et al.*, 2012). Whereas mutants like the *mgv1* and *Gpmk1* deletion mutants were female sterile and failed to form perithecia, some mutants, such as the *FgstuA*, *roa*, *zif1* and *Gzrum1* mutants, were defective in ascospore formation or produced ascospores with abnormal morphology (Min *et al.*, 2010; Kim *et al.*, 2011; Lysoe *et al.*, 2011; Y. Wang *et al.*, 2011). In addition, several genes, such as *GEA1*, *MID1*, and *CCH1*, are known to be important for forcible

*These authors contributed equally to this work.