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Identification and characterization of plant-specific NAC gene family in canola (*Brassica napus* L.) reveal novel members involved in cell death

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Abstract NAC transcription factors are plant-specific and play important roles in plant development processes, response to biotic and abiotic cues and hormone signaling. However, to date, little is known about the NAC genes in canola (or oilseed rape, *Brassica napus* L.). In this study, a total of 60 NAC genes were identified from canola through a systematical analysis and mining of expressed sequence tags. Among these, the cDNA sequences of 41 NAC genes were successfully cloned. The translated protein sequences of canola NAC genes with the NAC genes from representative species were phylogenetically clustered into three major groups and multiple subgroups. The transcriptional activities of these BnaNAC proteins were assayed in yeast. In addition, by quantitative real-time RT-PCR, we further observed that some of these *BnaNACs* were regulated by different hormone stimuli or abiotic stresses. Interestingly, we successfully identified two novel *BnaNACs*, *BnaNAC19* and *BnaNAC82*, which could elicit hypersensitive response-like cell death when expressed in *Nicotiana benthamiana* leaves, which was mediated by accumulation of reactive oxygen species. Overall, our work has laid a solid foundation for further characterization of this important NAC gene family in canola.

Keywords Abiotic stress · *Brassica napus* · Cell death · NAC · ROS · Transcription factors

Introduction

Abiotic stresses such as high salinity, drought and extreme temperatures are among the major environmental factors that influence the productivity of plants. To survive harsh conditions, plants have developed sophisticated mechanisms to sense environmental cues and transmit these

Boya Wang, Xiaohua Guo, Chen Wang and Jieyu Ma have contributed equally to this work.

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一、我室召开2014年度总结会

1. The Laboratory Successfully Held the 2014 Work Summary Meeting

2014年年度工作总结会
The 2014 annual review meeting

1月21日，我室2014年度工作总结会在校国际交流中心210会议室召开。会议由副主任吉万全教授主持，实验室全体固定研究人员及其团队成员、研究生、实验技术人员参加了会议。

校长助理、科学技术发展研究院常务副院长韦革宏教授出席了会议并讲话，表示科研院将全力支持实验室开展顶层设计、凝练研究内容，并积极协调相关部门支持实验室的建设发展和筹备2016年的评估工作。

总结会上，实验室主任康振生教授汇报了2014年实验室在科学研究与平台建设、人才培养与团队建设、开放交流与国际合作等方面的进展和成绩，剖析了实验室存在的主要问题与面临的挑战，并安排部署了2015年度的各项工作。宋卫宁教授、郁飞教授、康振生教授、马锋旺教授分别代表四个研究方向汇报了本年度的科学研究进展。

On January 21, 2015, the laboratory successfully held the 2014 Work Summary Meeting in Room 210 of the University International Exchange Center. Prof. WanquanJi, Associate Director of the laboratory, presided and all faculty members and their team members, graduate students, and technicians of the laboratory participated the meeting.

Prof. Gehong Wei, Assistant President and Executive Associate Dean of the College of Science and Technology R&D came to the meeting and delivered speech. He expressed the strong support from the college to the laboratory in the top level design, research planning, and actively coordinating related administrative offices to support the laboratory development and preparation for the evaluation in 2016.

At the meeting, Prof. Zhensheng Kang, Director of the laboratory, reported the 2014 progresses and accomplishments of the laboratory in scientific research, platform development, scientist training and team development, opening to public, and international exchange and collaboration. He analyzed the major issues and challenges, and assigned the 2015 tasks to all branches and organizations of the laboratory. Profs.Weining Song, Fei Yu, Zhensheng Kang, and Fengwang Ma, reported the research progresses representing the four scientific fields of the laboratory.

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二、2014年度取得的成绩回顾

2. The Accomplishments in 2014

2014年，实验室在科学研究、队伍建设与人才培养、国际合作与学术交流均取得了显著成绩：

In 2014, the laboratory made the following significant accomplishments in scientific research, team development and scientist training, and scientific collaboration and exchange:

◎吉万全教授参加的“小麦种质资源重要育种性状的评价与创新利用”项目获国家科技进步二等奖。

The project “Evaluation, development, and utilization of wheat germplasm for major breeding traits”, participated by Prof. WanquanJi, received the second class award of National Science and Technology Advancement.

◎高翔教授主持的“优质强筋小麦新品种陕627、陕715选育与推广”项目和邹志荣教授主持的“日光温室主动采光蓄热机理与应用技术研究”项目分别获陕西省科学技术一等奖。

The projects “The breeding and extension of high quality wheat cultivars Shaan 627 and Shaan 715” in-charge by Prof. Xiang Gao and “The mechanisms and utilization of automatic light receiving and storage in sunlight greenhouses” in-charge by Prof. Zhirong Zou received the first class award of Shaanxi Provincial Science and Technology.

◎韦革宏教授、单卫星教授入选教育部“长江学者”特聘教授计划。

Prof. Gehong Wei and Weixing Shan were selected as “Changjiang” Special Professors.

◎管清美教授入选国家“青年千人”计划。

Prof. Jingmei Guan was selected as “National Thousand” Program Professor.

◎王晓杰教授和康振生教授分别获批国家自然科学基金优秀青年科学基金和重点项目。

Prof.Xiaojie Wang and Prof. Zhensheng Kang received funding from the Excellent Youth Program and the Key Program of the National Natural Science Foundation, respectively.

◎刘慧泉副研究员博士论文入选全国百篇优秀博士学位论文。

Dr. HuiquanLiu's Ph.D. dissertation was selected as a “National Hundred Excellent Ph.D.” Dissertation.

◎李明军副教授和张宏副研究员分获第十届陕西省青年科技奖，李明军还被授予“陕西青年科技标兵”荣誉称号。

Associate Prof. Mingjun Li and Associate Research Scientist Hong Zhang received the 10th Shaanxi Provincial Youth Science and Technology awards. Mingjun Li was also awarded “Shaanxi Youth Science and Technology Example Scientist”.

◎全年共邀请国内外专家55人次来室学术交流，举办了“逆境生物学”系列学术报告30余场。

The laboratory organized 30 workshops and seminar series on adverse environmental biology, and invited 55 national and international scientists to present seminars or presentations in workshops.

◎实验室与默多克大学生物与食品安全中心共同组建了“中澳生物与非生物逆境治理联合研究中心”。

The laboratory established the 'China-Australia United Research Center on Management of Biotic and Abiotic Stresses” with Murdoch University, Australia.

◎本年度实验室固定研究人员发表署名SCI论文175篇，其中影响因子5以上共18篇；审定农作物新品种9个；获批国家发明专利7项。

The faculty scientists of the laboratory published 175 SCI papers, of which 18 were published in journals with an impact factor 5 or higher; released 9 new crop cultivars; and received approvals for 7 national renovation patents.

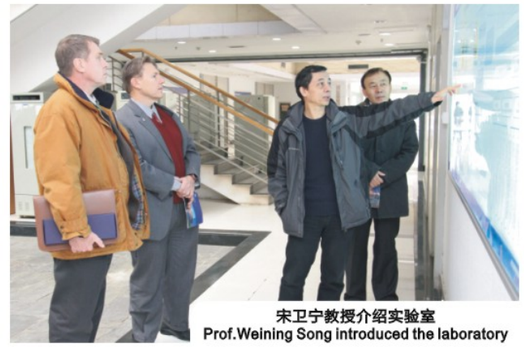
三、学术交流与参观访问

3. Scientific Exchange and Visits

美国阿肯色大学代表团一行来我室交流访问

The Delegation from the University of Arkansas, the United States Visited the Laboratory

1月9日，美国阿肯色大学农业、食品与生命科学学院院长Michael Vayda教授，植物病理系主任、美国植物病理学会主席Rick A. Bennett教授，大豆育种专家Pengyin Chen教授一行三人来我室交流访问。宋卫宁教授介绍了实验室的研究方向、科研队伍和近期科研工作进展，实验室主任康振生教授及团队成员与来访专家进行了座谈，双方就各自的科研工作进行了深入交流与探讨。



宋卫宁教授介绍实验室
Prof.Weining Song introduced the laboratory

与实验室科研人员座谈交流
Discusstion with the members of the laboratory

On January 9, 2015, the delegation from the University of Arkansas, consisting of Prof. Michael Vayda, Dean of College Agriculture, Food, and Life Sciences, Prof. Rick A. Bennett, Chair of the Department of Plant Pathology and President of American Phytopathological Society, and Prof. Pengyin Chen, Soybean Breeder, visited the laboratory. Prof. Weining Song gave the delegation an introduction on the research directions, scientist team, and recent research progresses of the laboratory. Prof. Zhensheng Kang and his research team members had a meeting with the delegation. They and the guests exchanged research and had deep discussion on mutually interested scientific topics.

美国马萨诸塞大学郭立博士来我室交流访问

Dr. Li Guo from the University of Massachusetts, the United States Visited the laboratory

3月6日，美国马萨诸塞大学郭立博士来实验室交流访问，做了题为“Reconstructing *Fusarium graminearum* gene regulatory networks using a system biology approach”的报告。康振生教授带领郭立博士参观了实验室技术平台，介绍了实验室基本情况。

郭立博士做学术报告
Dr. Li Guo gave a speech

On March 6, 2015,Dr. Li Guo from the University of Massachusetts visited the laboratory. He gave a seminar entitled “ Reconstructing *Fusarium graminearum* gene regulatory networks using a system biology approach”. Prof. Kang showed him the research and technical platforms and gave him a general introduction about the laboratory.

中农发种业集团股份有限公司董事长陈章瑞来我室参观访问

Mr. Zhangrui Chen, CEO of Zhongnongfa Seed Industry Group Co.,Ltd. Visited the Laboratory

1月22日，中农发种业集团股份有限公司董事长陈章瑞来我室参观访问，副主任吉万全教授介绍了实验室研究方向、科研队伍、人才培养以及运行管理等情况并参观了实验室技术平台，双方就农作物育种的科研与推广工作进行了深入交流探讨。

吉万全教授介绍实验室
Prof.Wanquan Ji introduced the laboratory

On January 22, Mr. Zhangrui Chen, CEO of Zhongnongfa Seed Industry Group Co., Ltd. visited the laboratory. Prof. WanquanJi, Associate Director of the laboratory, gave him an introduction on research directions, scientist team, expert training and operation of management of the laboratory. Mr. Chen visited the technical platforms. Prof. Ji and Mr. Chen had deep exchange and discussion on crop breeding and extension.

四、运行与管理

4. Operation and Management

我室开展安全卫生自查工作

The Laboratory Conducted Inspections on Safety and Health

1月12日，为全面排查实验室存在的安全隐患，为广大师生提供一个安全、卫生的实验室条件和科研氛围，我室邀请科研院、保卫处、后勤处等有关单位的负责人组成考察组，对我室各研究平台、公共实验室、人工气候室等科研设施与设备进行安全排查。考察组对实验室有毒有害化学品和易燃易爆物品的存放、使用及处理，以及仪器（设备）的放置与使用，紧急通道通的畅通等各个方面进行了排查，提出了建设性的意见和防护措施，将安全隐患消除在萌芽状态，确保实验室安全高效运转。

安全排查
potential safety hazard checking and controlling

To thorough check for any safety problems and provide everyone a safe and healthy laboratory conditions and research environment, the laboratory invited officers from the Science and Research College, Security Department, and Facility Management Department and other related offices of the university to form an inspection team. The team conducted safety inspections for the facilities and equipment inthe reach platforms, public laboratories, and growth chambers of the laboratory on January 12, 2015. The team especially inspected the storage, utilization, and dispersal of toxic, harmful, and flammable chemicals and those of capable of exploding; setting and use of equipment; and emergency exists. The team made constructive suggestions and provided protection procedures to eliminate safety risks in the beginning stage and ensure the safe and high efficient operation of the laboratory.

我室召开安全稳定会议

The Laboratory Held a Meeting on Safety and Stability of the New Semester

3月27日，我室新学期安全稳定工作会议召开，实验室全体技术人员，各PI研究室管理员以及相关老师和研究生等二十余人参加了会议。

实验室办公室负责人带领与会人员认真学习了学校有关安全稳定的文件精神，结合学校近期一些实验室发生的事件，重点强调了实验室的安全问题。实验室公共平台负责人黄雪玲高级实验师就实验室公共平台、人工气候室、生物信息室、大型仪器设备等科研条件的运行与管理，预约与使用、安全与卫生、监督与约束等方面进行了全面讲解，同时对实验室公共技术平台仪器设备使用管理进行了说明，提出了不按规范要求使用的约束办法，与会师生进行了充分的沟通交流。

部署新学期安全运行工作
Arranging the safety work of the new semester

On March 27, 2015, the laboratory held a meeting on safety and stability in the new semester. More than 20 people including lab managers of every PI's laboratory and relevant teachers and graduate students participated the meeting.

Lab managers led the study of the university documents on safety and stability, and especially emphasized the safety issue by relating the issue to recent fire incidences in some laboratories within the university. Dr. Xueling Huang, senior technician and manager of the laboratory public platforms provided meeting participants thorough instructions on operation and management of public platforms, growth chambers, bioinformatics laboratory, and major equipment; scheduling and using, safety and health; monitoring; and restrictions; She explained the utilization and management of the equipment in the public platforms, proposed restrictions to avoiding improper use of equipment, and had thorough discussion and exchange with meeting participants.

五、光荣榜：

5. Honorary Recognitions

- 综合办公室获评学校文明办公室。
  The Laboratory Integrated Office was recognized as a “Civilized Office” by the university
- 实验室获2014年度消防安全工作先进集体。
  The laboratory was recognized as a “2014 Excellent Team of Safe Working” by the university
- 网站被评为学校优秀二级网站。
  The web site was awarded outstanding secondary school website
- 办公室主任彭科峰被评为2014年学校先进工作者。
  Kefeng Peng, laboratory manager, was recognized as a “2014 Excellent Worker” by the university
- 简利茹实验师被评为2014年校国有资产管理先进个人。
  Liru Dian, technician, was recognized as an “Excellent Manager of State-own Properties” by the university

2015年1月-3月公开发表的SCI论文
SCI Publications in January-March, 2015

2015年1月至3月，实验室科研人员在SCI收录刊物公开发表署名论文36篇。
From January to March, 2015, scientists of the laboratory published 36 papers in SCI journals.

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Whitefly Parasitoids: Distribution, Life History, Bionomics, and Utilization

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Abstract

Whiteflies are small hemipterans numbering more than 1,550 described species, of which about 50 are agricultural pests. Adults are free-living, whereas late first to fourth instars are sessile on the plant. All known species of whitefly parasitoids belong to Hymenoptera; two genera, *Encarsia* and *Eretmocerus*, occur worldwide, and others are mostly specific to different continents. All parasitoid eggs are laid in—or in *Eretmocerus*, under—the host. They develop within whitefly nymphs and emerge from the fourth instar, and in *Cales*, from either the third or fourth instar. Parasitized hosts are recognized by conspecifics, but super- and hyperparasitism occur. Dispersal flights are influenced by gender and mating status, but no long-range attraction to whitefly presence on leaves is known. Studies on *En. formosa* have laid the foundation for behavioral studies and biological control in general. We review past and ongoing studies of whitefly parasitoids worldwide, updating available information on species diversity, biology, behavior, tritrophic interactions, and utilization in pest management.

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Original Article

Exogenous abscisic acid alleviates zinc uptake and accumulation in *Populus* × *canescens* exposed to excess zinc

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ABSTRACT

A greenhouse experiment was conducted to study whether exogenous abscisic acid (ABA) mediates the responses of poplars to excess zinc (Zn). *Populus* × *canescens* seedlings were treated with either basal or excess Zn levels and either 0 or 10 μM ABA. Excess Zn led to reduced photosynthetic rates, increased Zn accumulation, induced foliar ABA and salicylic acid (SA), decreased foliar gibberellin (GA_3) and auxin (IAA), elevated root H_2O_2 levels, and increased root ratios of glutathione (GSH) to GSSG and foliar ratios of ascorbate (ASC) to dehydroascorbate (DHA) in poplars. While exogenous ABA decreased foliar Zn concentrations with 7 d treatments, it increased levels of endogenous ABA, GA_3 and SA in roots, and resulted in highly increased foliar ASC accumulation and ratios of ASC to DHA. The transcript levels of several genes involved in Zn uptake and detoxification, such as *yellow stripe-like family protein 2* (*YSL2*) and *plant cadmium resistance protein 2* (*PCR2*), were enhanced in poplar roots by excess Zn but repressed by exogenous ABA application. These results suggest that exogenous ABA can decrease Zn concentrations in *P.* × *canescens* under excess Zn for 7 d, likely by modulating the transcript levels of key genes involved in Zn uptake and detoxification.

Key-words: heavy metals; oxidative stress; phytohormones; phytoremediation; transcriptional regulation.

INTRODUCTION

The heavy metal zinc (Zn) rapidly accumulates in soil and water because of anthropogenic activities (e.g. mining, smelting and fertilization with sewage sludge) that lead to Zn contamination (Hassan & Aarts 2011). Zn is an essential element for plants that can be taken up by roots. Therefore, the utilization of certain plants for remediating Zn-contaminated soil has been suggested (Broadley *et al.* 2007). Several herbaceous plants have been identified for Zn hyperaccumulation, including *Arabidopsis halleri*, *Noccaea* (formerly *Thlaspi*) *caerulescens*, and *Sedum alfredii*, which can accumulate more than 10 000 μg Zn g^{-1} dry mass in aboveground tissues (Krämer 2010). However, in these plants, the amount of accumulated Zn is limited by slow growth and low biomass. Thus, fast-growing woody plants, such as *Populus* species, which have a large aboveground biomass, a deep root system and the ability to accumulate intermediate metal concentrations, have been proposed for the phytoremediation of Zn-contaminated soil (Langer *et al.* 2009; Marmiroli *et al.* 2011; Luo *et al.* 2014).

Although Zn is a nutrient element for poplars, excess Zn in the soil can be toxic for these plants (Di Baccio *et al.* 2003, 2005, 2009, 2011). Zn toxicity in plants causes chlorosis, reduced photosynthesis, stunted growth, interference with the uptake of other nutrient elements, outbreak of reactive oxygen species (ROS) and altered antioxidants (Broadley *et al.* 2007). Excess Zn can also lead to the transcriptional regulation of genes involved in Zn accumulation and detoxification in poplars (Di Baccio *et al.* 2011). In plant cells, zinc-regulated transporter/iron-regulated transporter-related proteins (ZIPs) play an essential role in the uptake of extracellular Zn^{2+} (Milner *et al.* 2013). In the genome of *Populus trichocarpa*, the *ZIP* family contains 20 members (Migeon *et al.* 2010). Among these *ZIPs*, *ZIP2* (Potri.009G034600), *ZIP6.2* (Potri.009G074100), *ZIP6.4* (Potri.015G117900) and *ZIP7.2* (Potri.010G134300) act as key components in Zn homeostasis in poplars (Migeon *et al.* 2010; Adams *et al.* 2012). In herbaceous plants, the genes encoding nicotianamine synthase (NAS) and yellow stripe-like family protein (YSL) play critical roles in Zn detoxification and transport, respectively (DiDonato *et al.* 2004; Deinlein *et al.* 2012). Zn^{2+} can bind to nicotianamine (NA), which is a non-proteinogenic amino acid, to form a Zn-NA chelate, which can be further transported across cellular membranes by YSL proteins (DiDonato *et al.* 2004). The transcriptional regulation of *NAS* and *YSL* may represent an important mechanism for controlling Zn homeostasis in plants (Curie *et al.* 2009; Deinlein *et al.* 2012). In addition,

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Functional characterization of a vanillin dehydrogenase in *Corynebacterium glutamicum*

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Vanillin dehydrogenase (VDH) is a crucial enzyme involved in the degradation of lignin-derived aromatic compounds. Herein, the VDH from *Corynebacterium glutamicum* was characterized. The relative molecular mass (Mr) determined by SDS-PAGE was ~51 kDa, whereas the apparent native Mr values revealed by gel filtration chromatography were 49.5, 92.3, 159.0 and 199.2 kDa, indicating the presence of dimeric, trimeric and tetrameric forms. Moreover, the enzyme showed its highest level of activity toward vanillin at pH 7.0 and 30°C, and interestingly, it could utilize NAD^+ and $NADP^+$ as coenzymes with similar efficiency and showed no obvious difference toward NAD^+ and $NADP^+$. In addition to vanillin, this enzyme exhibited catalytic activity toward a broad range of substrates, including *p*-hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde, *o*-phthaldialdehyde, cinnamaldehyde, syringaldehyde and benzaldehyde. Conserved catalytic residues or putative cofactor interactive sites were identified based on sequence alignment and comparison with previous studies, and the function of selected residues were verified by site-directed mutagenesis analysis. Finally, the *vdh* deletion mutant partially lost its ability to grow on vanillin, indicating the presence of alternative VDH(s) in *Corynebacterium glutamicum*. Taken together, this study contributes to understanding the VDH diversity from bacteria and the aromatic metabolism pathways in *C. glutamicum*.

Corynebacterium glutamicum, a fast growing Gram-positive soil bacterium, is one of the most important microorganisms in industrial biotechnology. Since its discovery, *C. glutamicum* has been widely used for industrial production of amino acids, vitamins, nucleotides and various other bio-based chemicals[1]. As a soil bacterium, recent studies have demonstrated that *C. glutamicum* is able to utilize a large variety of lignin derived aromatic compounds (e.g. vanillin, ferulate, *p*-coumarate, cinnamate, etc.) for growth[2,3]. The outstanding capability of *C. glutamicum* in assimilation of aromatic compounds provides it with a distinct advantage in using lignocellulosic hydrolysates as sustainable and inexpensive feedstocks in industrial fermentation, thanks to its capability to detoxify and assimilate great amounts of lignin derived aromatic inhibitors in lignocellulosic hydrolysates as an alternative source to sugars for carbon and energy.

The main lignin-derived aromatic inhibitors in lignocellulosic hydrolysates are ferulate, vanillin, *p*-coumarate, 4-hydroxybenzoic acid (4-HBA), and vanillic acid, and most of which can be assimilated into TCA cycle intermediates by *C. glutamicum*[3–5]. Catabolism of ferulate follows a CoA-dependent non-β-oxidative pathway that contains feruloyl-CoA synthetase (Fcs) and enoyl-CoA hydratase/aldolase (Ech), yielding vanillin[6]. Vanillin is further converted into protocatechuate catalyzed by an aldehyde dehydrogenase (Vdh) and a demethylase (VanAB)[7,8]. Although some peripheral pathways converting various phenylpropanoids (such as vanillin, vanillate, caffeate, *p*-coumarate, and cinnamate) to protocatechuate have been suggested in *C. glutamicum*, and the genes *van*AB encoding vanillate demethylase that catalyzes the conversion of vanillate to protocatechuate have been functionally identified[3,6], the upstream vanillin dehydrogenase gene (*vdh*) has not been experimentally investigated.

The vanillin dehydrogenase is a critical enzyme for the degradation of lignin derived phenylpropanoids (such as vanillin, vanillate, caffeate, *p*-coumarate, and cinnamate) and studies on vanillin dehydrogenase gene (*vdh*) in Gram-negtive bacteria have been well documented. For instance, *vdh* has been characterized in *Pseudomonas fluorescens*[9], *Pseudomonas putida*[10,11], *Pseudomonas* sp. strain HR199[12], and *Sphingomonas paucimobilis* SYK-

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Fgk3 glycogen synthase kinase is important for development, pathogenesis, and stress responses in *Fusarium graminearum*

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Wheat scab caused by *Fusarium graminearum* is an important disease. In a previous study, the *FGK3* glycogen synthase kinase gene orthologous to mammalian GSK3 was identified as an important virulence factor. Although GSK3 orthologs are well-conserved, none of them have been functionally characterized in fungal pathogens. In this study, we further characterized the roles of *FGK3* gene. The Δ*fgk3* mutant had pleiotropic defects in growth rate, conidium morphology, germination, and perithecium formation. It was non-pathogenic in infection assays and blocked in DON production. Glycogen accumulation was increased in the Δ*fgk3* mutant, confirming the inhibitory role of Fgk3 on glycogen synthase. In *FGK3*-GFP transformants, GFP signals mainly localized to the cytoplasm in conidia but to the cytoplasm and nucleus in hyphae. Moreover, the expression level of *FGK3* increased in response to cold, H_2O_2, and SDS stresses. In the Δ*fgk3* mutant, cold, heat, and salt stresses failed to induce the expression of the stress response-related genes *FgGRE2, FgGPD1, FgCTT1*, and *FgMSN2*. In the presence of 80 mM LiCl, a GSK3 kinase inhibitor, the wild type displayed similar defects to the Δ*fgk3* mutant. Overall, our results indicate that *FGK3* is important for growth, conidiogenesis, DON production, pathogenicity, and stress responses in *F. graminearum*.

Fusarium graminearum is the predominant species that causes Fusarium head blight (WHB) or scab of wheat and barley worldwide[1,2]. Under favorable conditions, it can cause severe yield losses, and often contaminates infected grains with harmful mycotoxins. One of the mycotoxins produced by *F. graminearum* is trichothecene mycotoxin deoxynivalenol (DON), which is a potent inhibitor of eukaryotic protein synthesis and an important virulence factor[3]. The trichothecene biosynthetic gene clusters and biosynthesis pathways in *F. graminearum* and related species have been extensively studied in the past decade[4,5].

To better understand fungal pathogenesis, we systematically characterized protein kinase genes in a previous study[6]. In total, 42 of them were found to be important for pathogenicity or virulence in infection assays with flowering wheat heads and corn stalks. In addition to components of the well-conserved cAMP signaling and MAP kinase pathways, we found 31 protein kinase genes that had not been previously characterized as important pathogenicity factors. One of them, FGSG_07329, is orthologous to the mammalian GSK3β glycogen synthase kinase gene[7].

GSK3 orthologs from different eukaryotic organisms, including mammals, insects, fungi, nematodes, and protozoa, have similar structures and well-conserved ATP binding sites[8]. Although GSK3 was first characterized as a Ser/Thr protein kinase responsible for the phosphorylation and inactivation of glycogen synthase, later studies have shown that GSK3 functions in multiple cellular processes[7]. In addition to enzymes involved in metabolism, its substrates include structural and signaling proteins, as well as transcription factors. In mammalian cells, GSK3 plays roles in many signaling pathways that are involved in cell proliferation and differentiation, microtubule dynamics, development, and oncogenesis[9,10]. Alzheimer's disease is one of several human diseases that are known to be related to the hyperactivity of GSK3[11]. In plants, GSK3 kinases function in hormonal signaling networks that involve brassinosteroids, abscisic acid, and auxin during growth and development[12]. They also play roles in floral organ development and cell expansion, as well as in responses to biotic and abiotic stresses[13].