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# 西北农林科技大学职称任职资格

## 佐 证 材 料

(教师岗位系列)

单 位 农学院

姓 名 刘 杨

现任职资格 副教授

申报资格 教 授

岗 位 类 型 教学科研型教师

## 材料清单

- 一、学历、学位证明
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- 三、优秀毕业论文荣誉证书
- 四、教改论文
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- 七、发表论文
- 八、成果奖
- 九、发明专利
- 十、地方标准



普通高等学校

# 毕业证书



学生 刘杨 性别男， 1984 年 3 月 28 日生，于 2002 年 9 月至 2006 年 6 月在本校农学 专业

4 年制本科学习，修完教学计划规定的全部课程，成绩合格，准予毕业。

校 名：



校 (院) 长：

郑小波

证书编号：103071200605000087

二零零六年 六 月 十五 日





# 学士学位证书

(普通高等教育本科毕业生)



刘杨，男，  
1984年3月生。自2002  
年9月至2006年6月

在农学院

农学

专业

完成了4年制本科学习计划，业已毕业。  
经审核符合《中华人民共和国学位条例》  
的规定，授予农学士学位。

南京农业大学

学位评定委员会主席

郑小波

二零零六年六月十五日

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博士研究生

# 毕业证书



研究生 刘杨 性别 男，一九八四年 三 月二十八 日生，于

二00八年 九 月至二0一一年 六 月在 作物栽培学与耕作学

专业学习，学制 三 年，修完博士研究生培养计划规定的全部课程，成绩合格，  
毕业论文答辩通过，准予毕业。

培养单位：南 京 农 业 大 学

校(院、所)长：

郑小波

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二0一一年 六 月 十五 日





# 博士学位证书

刘杨，男，1984年3月28日生。在南京农业大学

作物栽培学与耕作学

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南京农业大学

校长

郑永波

学位评定委员会主席

证书编号：1030722011000124

二〇一一年六月二十七日



# 证书

刘 杨 同志：

经第六次全国会员代表大会选举，您当选为中国作物  
学会第六届栽培专业委员会委员。

特发此证。

中国作物学会栽培专业委员会

2016 年 12 月 28 日



# 聘 书

兹聘请刘杨为中国农学会  
耕作制度分会第九届理事会理  
事。

中国农学会耕作制度分会

2014年10月20日





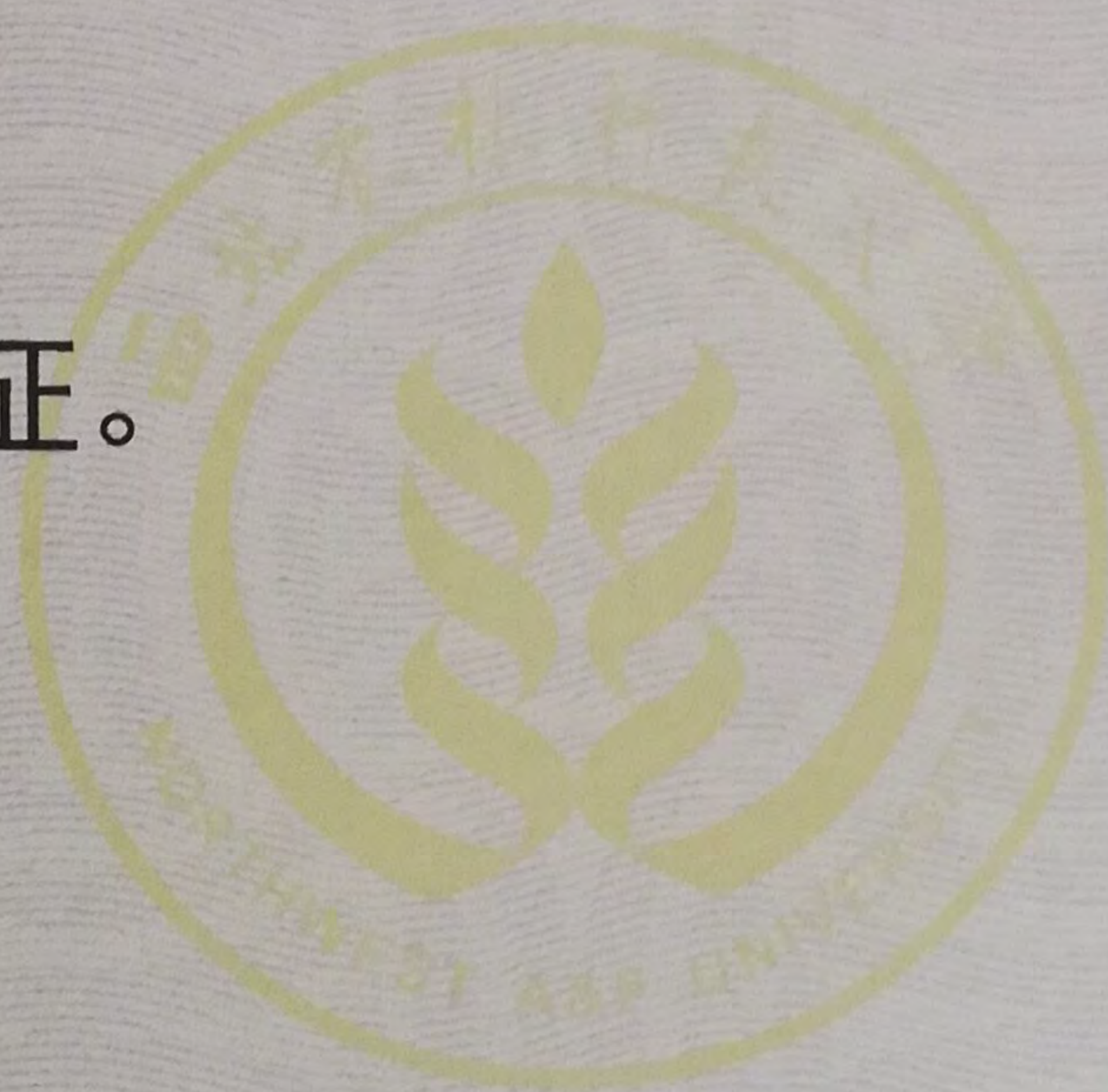


西北农林科技大学  
NORTHWEST A&F UNIVERSITY

## 荣誉证书

梁子蒙同学完成的本科毕业论文《高铵胁迫下细胞分裂素对小麦幼苗生长的影响及其生理机制》被评为 2019 届校级优秀本科毕业论文。

特发此证。



西北农林科技大学

教务处

2019 年 6 月 15 日





## 荣誉证书

李格格 同学：

你的本科毕业论文(设计)《高铵胁迫下外源谷胱甘肽对玉米幼苗生长的影响及其机理》被评为我校2018 届“百篇优秀本科毕业论文(设计)”，特发此证。



西北农林科技大学

教务处

二〇一八年六月



# 人才分类培养背景下农学专业作物栽培学 教学改革与实践

刘 杨 张保军

(西北农林科技大学 农学院 陕西 杨陵 712100)

**摘要** :作物栽培学是高等农业院校农学本科专业的主干专业课程之一。本文针对西北农林科技大学农学专业人才培养模式的变化,探讨了农学专业作物栽培学理论和实验教学如何适应不同的人才分类培养模式,提高课程授课效果。

**关键词** :作物栽培学 ;教学改革 ;人才分类培养

**中图分类号** :G642.0

**文献标志码** :A

**文章编号** :1674-9324(2017)01-0073-02

作物栽培学是研究作物生长发育、产量和品质形成规律及其与环境条件的关系,探索通过栽培管理、生长调控和优化决策等途径,实现作物高产、优质、高效及可持续发展的理论、方法与技术的科学<sup>[1]</sup>。作物栽培学是一门综合性、理论性很强、能直接指导农业生产的应用性科学,是农业科学的重要组成部分,对培养高等农业专业才起到了重要的作用<sup>[2]</sup>。

作物栽培学是高等农业院校农学本科专业的主干专业课程之一。提高作物栽培学课程的教学质量对培养高质量农业现代人才具有重要意义。随着社会的发展、科技的进步、作物生产模式的转型升级,社会对农学等学科人才培养模式及质量的要求发生了很大的变化。为了适应这种变化,培养合格的农学专业人才,各高校从专业课程设置、课程质量标准、教学体系等多个方面进行了探索,取得了一系列的成果,为农学专业人才的培养奠定了良好的基础。

为了适应社会的发展,满足不同行业对农学专业本科生的需求,西北农林科技大学农学院立足“社会对人才需求的多元化”和“学生个人发展的多元化”两大现实需求,以“全面发展、人人成才、个性化教育、系统培养”为指导思想,经过多年的努力探索和积极实践,创新并建立了农学专业多元教学以及学生分类教学模式,有效增强了学生的创新精神和实践能力,促进了学生的学农爱农意识的形成,激发了学生投身现代农业事业热情和信心<sup>[3]</sup>。该模式根据学生兴趣、爱好,结合农学专业特点,在三年级时将学生分流为拔尖创新型和复合应用型两种类型,对学生进行分类培养,提高学生对专业的兴趣、增强学生的学习主动性,对于不同类型人才的培养具有很好的参考价值。作为

农学专业的主干专业课程,作物栽培学教学如何适应这种人才培养模式的变动,对于作物栽培学教学效果以及人才培养效果具有很大的影响,也是一个值得深入探讨的课题。本文基于西北农林科技大学农学院作物栽培教研组在教学上的一些探索,对该问题进行探讨。

## 一、根据不同培养类型,确定理论授课内容

西北农林科技大学农学专业在三年级时将学生分流为拔尖创新型和复合应用型两种类型,对学生进行分类培养。两种类型对学生的培养要求不同,拔尖创新型人才立足于农业科技发展需要,培养学生科技素养,为学生进一步深造、进行科学研究奠定基础,而复合应用型人才立足于农业生产实际需要,培养学生农业实践能力,为学生更好的掌握农业生产实践、直接服务农业生产实践打下良好基础。两种培养类型的不同,对作物栽培学教学的要求也有所不同。按照培养计划,拔尖创新型人才培养模式作物栽培学教学学时为48学时,复合应用型人才作物栽培学教学学时为32学时。作物栽培学教学体系主要包括作物形态发育、作物产量及品质形成、作物对生态环境的响应、作物生产管理三部分。在教学实践中,根据人才培养模式的不同,作物栽培学教研组对教学内容进行了合理优化。针对拔尖创新型人才的培养,在教学中主要着重于作物形态及生长发育、作物产量及品质形成等内容的教学,在教学中更加强调作物栽培学基础理论知识及共性知识的教学,与作物生态学等课程配合,使学生深入掌握作物生长发育的基础知识,为进一步的学习打下基础。而针对复合应用型人才的培养,则在教学中偏向于小麦、玉米、马铃薯、棉花、杂粮等西北

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作者简介 刘杨(1984-),男,河南洛阳人,博士,西北农林科技大学农学院副教授,研究方向:作物栽培学。



旱区广泛种植农作物的生产管理措施,使学生在学的过程中深入了解各种农作物的生产管理措施,为将来学生能够更好的服务于农业生产实践打下良好的基础。

## 二、作物栽培学实验改革

作物栽培学是一门综合性、应用性很强的学科,实验教学在作物栽培学教学体系中起着重要作用。针对不同类型学生培养目标的不同,我们也针对作物栽培学实验教学进行了改革。在坚持理论和实验教学相配合、强化基础、拓宽口径的前提下,将实验教学内容进行了合理分配。针对拔尖创新型人才的培养,在实验教学中主要强调小麦、水稻、玉米、棉花、油菜、大豆等主要农作物形态观察、解剖学特征、器官建成等的观测,使学生更加能够深入了解农作物的生长发育特性,同时增加淀粉含量测定、蛋白含量测定等实验,让学生更加深入的学习作物产量和品质形成的机理。针对复合应用型人才的培养,则强调大田实验的重要

性,引导学生深入田间地头,观测小麦、玉米、马铃薯、棉花、杂粮等西北旱区广泛种植农作物关键生育期的长势,从田间苗情鉴定和分析、田间病虫害观测及防治、产量及产量构成因素测定、品质快速监测等方面使学生对主要农作物的生产进行深入了解,加深对主要农作物生产管理的认识,更好的认知农作物生产管理措施,为将来学生能够更好的服务于农业生产实践打下良好的基础。

通过课程的改革,使得作物栽培学的教学符合不同类型农学专业学生培养的需要,提高学生的学习兴趣,提高授课效果,在教学实践中起到了积极的作用。

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- [2]李雁鸣,李存东,崔彦宏,等.《作物栽培学》课程体系和教学内容改革与建设[J].中国农业教育,2007,(3):31-34.
- [3]海江波,冯炜,廖允成.农学专业多元互补式实践教学模式的创新与构建[J].高等农业教育,2011,(12):50-52.

## Reform and Practice of Crop Cultivation for Agronomy Bachelor Degree Under Personnel Training Mode

LIU Yang,ZHANG Bao-jun

(Agronomy College,Northwest A&F University,Yangling,Shaanxi 712100,China)

Abstract:Crop cultivation is the main professional curriculum for agronomy bachelor degree of agricultural university. In this article,we analyzed the method and approach for the response of the theory and experiment teaching of crop cultivation on the classification training under the change of personnel training mode in Northwest A&F University.

Key words:crop cultivation;reform in education;personnel training mode



高等学校农业经济管理类专业核心课程教材

# 农业概论

主 编 廖允成  
副主编 姜道宏  
杨晓红  
刘 杨

高等教育出版社



#### 内容简介

本书共 11 章,分别是:绪论、农业的起源与发展、农业资源与区划、农业生态系统、农业生产经营、现代农业科技、世界农业、作物学概述、园艺学概述、畜牧学概述、植物保护学概述。

本书可用作农林经济管理、农村区域发展以及农学类各专业的本科生教材,也可用作社会学、管理学和经济学其他相关专业本科生和研究生的选修教材,同时还可作为从事农业经济管理以及农业科技管理工作人员的参考书。

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## 前 言

农业概论是农林经济管理和农村区域发展专业的核心课程之一。随着农林院校专业调整和培养方案的不断修订,课程设置也发生了相应的变化,对课程内容及教学时数有了全新的要求。为了满足教学改革及新形势下人才培养的需要,西北农林科技大学、华中农业大学、西南大学等农业院校联合编写了《农业概论》一书。

农业是国民经济系统的重要组成部分。农业本身也是一个复杂的系统,可以划分为农、林、牧、渔等若干部门,每一个部门可以进一步细分为更小的子系统。全面地了解、认识农业,从全局角度以及系统论的观点看待农业、研究农业知识,是农业院校学生应具备的技能和知识素养。本书内容力求简明、系统,特别强调农业问题的系统性和农业各学科知识的互补性。希望通过课程的学习,学生能够全面地了解农业各学科的基本知识,能够全面地了解农业,为将来更深入地学习、研究农业相关知识打下良好的基础。

本书共 11 章。第一章由廖允成编写,第二章由廖允成、刘杨、吴伟编写,第三章由冯永忠、韩新辉编写,第四章由廖允成、温晓霞、秦晓梁编写,第五章由马红玉、夏显力、韩叙、郭相兴编写,第六章由韩娟、李亚君、秦晓梁编写,第七章由温晓霞、韩娟、李亚君编写,第八章由刘杨编写,第九章由杨晓红编写,第十章由胡建宏编写,第十一章由姜道宏、王满囤、付艳苹、程家森、周爱民编写。全书由廖允成统稿。

由于本书涉及内容较为广泛,缺憾之处在所难免,望广大科教人员及学生批评指正。

编者

2017 年 3 月



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普通高等教育“十二五”规划教材

(second edition)

# SCIENCE OF FARMING SYSTEM

## 农作学 (第二版)

李 军 主编



科学出版社



## 内 容 简 介

农作学是以农作物持续增产、经济高效和生态安全为主要目标,研究建立合理农作制度的基本原理和技术体系的综合性应用学科。本书共分为6章,在分析我国农业资源状况和农业生产现状的基础上,系统介绍了农田种植制度、农田养地制度、农田防护制度、农作经营制度和区域农作制度等农作制度的基本理论和生产技术体系,有助于读者掌握组织农业生产、制定合理农作制度的基本原则和方法,具备研究和解决农业结构调整、农田高效种植、农业可持续发展等方面问题的能力。

本书适合高等农业院校农学、植物科学与技术、种子科学与工程、草业科学等植物生产类本科专业使用,也可供作物栽培学与耕作学专业的研究生及农业管理人员和技术人员参考。

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## 前 言

我国农耕历史源远流长,传统农耕文明较为发达,以农田“精耕细作”技术著称,农耕思想、农业结构、耕作技术、养地手段、种植模式、轮作体系和经营理念等曾一度领先于世界。由于长期采取自给自足的农户家庭经营方式,我国农业土地经营规模较小,土地利用率高,但劳动生产率较低,农产品商品率和经营效益不高。当前,我国农业正处于由传统农作向现代农作的加速转型阶段,正在不断扩大参与经济全球化竞争,农业规模化、机械化和专业化程度较低,农户家庭经营生产成本低而效益差,面临着保障国家粮食安全、增加农民收入、缓解资源环境约束和增强农产品市场竞争力的重大挑战。因此,我国农业发展不仅要持续提供农田生产力和农业系统生产力,而且要积极增加农业经济效益,保护并可持续利用农业资源环境。

农作学是以农作物持续增产、经济高效和生态安全为主要目标,研究建立合理农作制度的基本原理和技术体系的综合性应用学科,是我国高等农业院校农学本科专业的专业主干课程,也是植物科学与技术、种子科学与工程、草业科学等植物生产类本科专业的学科基础课程。通过与作物生产相关学科的交叉融合和兼收并蓄,农作学集成和组装了农学各相关学科的理论和技术,以系统性思想、全局性观点和整体性安排来组织作物生产和经营,形成了以土地用养结合为基础、以高效种植制度为核心、以农作物持续高产为目标的农作制度理论和技术体系,具有宏观和微观相结合、自然科学和社会科学相交叉的学科特点。我国传统农作学教材内容以极尽可能挖掘农田生产潜力、不断提高作物产量为主要发展目标,不够重视经济高效和农民增收问题,对农田节肥、节药和节水等节本增效和保护环境的相关领域研究不足,原有农作学学科内容体系已经难以适应当前我国农业生产和经营的发展需求。

农作学研究对象是我国不同类型农区的农作制度,具有很强的地域特色和综合性特点。由西北农林科技大学和西北地区主要农业院校共同编写、由科学出版社于2003年出版的《农作学》教材,虽然框架体系较为完善,内容也比较丰富,但部分章节内容与其他相关课程交叉重复,部分章节内容较为陈旧,并且由于课程学时压缩、教学时数缩短,原有教材字数偏多、内容不够精练,难以满足当前新形势下农作学课程教学需求。最近15年来,我国农作物生产形势与农作制度发展变化较大。2012年开始,我们再次邀请西部地区16所主要农业院校的30多位主讲教师,修订和编写《农作学》(第二版)教材,以满足西部地区农业院校农作学课程教学需求。根据我国农业生产发展和农作学课程教学需要,本教材遵循“体系完善、内容新颖、文字简练、图文表并茂”的编写原则,注重教材框架重新构建,凝练农作学教材内容体系,补充农作制度最新研究进展,在系统介绍农作学基本原理和我国农作制度主要内容的基础上,适当突出西部地区资源环境和作物生产特色,以符合西部地区农业院校农作学课程教学的需要。

本教材在分析我国农业资源状况和农业生产现状基础上,系统介绍了农田种植制度、农



田养地制度、农田防护制度、农作经营制度和区域农作制度等农作制度的基本理论和生产技术体系,有助于读者掌握组织农业生产、制定合理农作制度的基本原则和方法,具备研究和解决农业结构调整、农田高效种植、农业可持续发展等方面问题的能力。本教材共分为6章。第一章为农作学基本原理,分为3节,介绍了农作学与农作制度概念和内容、农作制度发展演变与类型,简要阐述了农作制度的主要原理和发展战略,分析了中国农业发展面临的问题和农作制度的发展战略。第二章为农田种植制度,分为3节,主要介绍作物布局与结构调整、种植模式与高效用地、作物轮作与连作等农田高产高效用地的理论与技术体系,是我国农作制度的核心内容之一。第三章为农田养地制度,分为3节,主要介绍农田培肥与循环农业、土壤耕作与保护性耕作、农田灌溉与节水农业等农田地力培肥和持续养地的技术原理和方法,也是我国农作制度的核心内容之一。第四章为农田防护制度,分为5节,主要介绍农田防水蚀、防风蚀、防杂草、防污染、防盐碱的技术原理和方法,维护农田生态环境健康发展与地力可持续利用,是西部地区农业生产发展需要着力解决的重要任务。第五章为农作经营制度,分为3节,主要介绍农作经营原理、农场经营制度和农业产业化经营的主要内容和基本途径,积极提高农作经营管理效益和农作制度的经济可持续性,是传统农业向现代农业转变的重要内容。第六章为区域农作制度,分为4节,简要介绍了我国东北地区、黄淮海区、长江中下游区、华南区、黄土高原区、西北绿洲灌区、四川盆地丘陵区 and 云贵高原区8个主要农区的农业资源概况、农作制度特点与主体模式、农作制度发展对策等,展示了我国农区农作制度的地域特色和区域差异。

我国农业生产区域十分广阔,各地农作制度类型和发展水平千差万别,且正处于农业发展快速转型时期,传统农业中的农田种植制度和养地制度等内容较为成熟和丰富,但现代农业发展中的农作经营制度和区域农作制度等内容尚处于发展形成初级阶段,相关研究成果尚不多见。由于编者的学术水平和现有材料不足,教材内容定有许多疏漏和不足之处,恳请使用本教材的院校、专家和读者予以批评指正,以便今后修编和充实提高。

李 军

2015年9月15日



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(second edition)

# SCIENCE OF FARMING SYSTEM

## 农作学 (第二版)

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# 荣誉证书

刘 杨 同志荣获2017年  
青年教师讲课比赛二等奖。  
特发此证，以资鼓励。

西北农林科技大学  
2017年12月29日



# 荣誉证书

刘杨 老师：

在中国作物学会人才培养与教育专业委员会组织的“2018 年全国农学院作物学科青年教师教学技能大赛（西北片区）”活动中表现突出，荣获

## 一等奖

特发此证，以资鼓励。

中国作物学会作物学人才培养与教育专业委员会（代章）

二〇一八年五月五日



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## 检索报告

根据委托人刘杨委托,通过网络检索,刘杨发表的 9 篇论文被《科学引文索引》扩展版(SCI-Expanded)数据库收录。数据库具体检索结果如下:

1.标题: Effect of polyamines on the grain filling of wheat under drought stress

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小类	PLANT SCIENCES 植物科学	2	-
大类	生物	3	否

2.标题: Effect of polyamine on seed germination of wheat under drought stress is related to changes in hormones and carbohydrates

作者:Liu, Y (Liu Yang)[ 1 ]; Xu, H (Xu Hong)[ 1 ]; Wen, XX (Wen Xiao-xia)[ 1 ]; Liao, YC (Liao Yun-cheng)[ 1 ]

JOURNAL OF INTEGRATIVE AGRICULTURE 卷: 15 期: 12 页: 2759-2774

DOI: 10.1016/S2095-3119(16)61366-7 出版年: 2016 文献类型:Article

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2019 年公布的影响因子: 1.337, 期刊分区:

JCR® 类别	类别中的排序	JCR 分区
AGRICULTURE, MULTIDISCIPLINARY	18/57	Q2

2019 年中科院分区:

	学科名称	分区	Top 期刊
小类	AGRICULTURE, MULTIDISCIPLINARY 农业综合	2	-
大类	农林科学	2	否

3.标题: Effect of non-structural carbohydrate accumulation in the stem pre-anthesis on grain filling of wheat inferior grain

作者:Liang, WX (Liang, Wenxin)[ 1,2 ]; Zhang, ZC (Zhang, Zhichao)[ 1 ]; Wen, XX (Wen, Xiaoxia)[ 1 ]; Liao, YC (Liao, Yuncheng)[ 1 ]; Liu, Y (Liu, Yang)[ 1 ]

FIELD CROPS RESEARCH 卷: 211 页: 66-76

DOI: 10.1016/j.fcr.2017.06.016 出版年: SEP 2017 文献类型:Article

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2019 年公布的影响因子: 3.868, 期刊分区:

JCR® 类别	类别中的排序	JCR 分区
AGRONOMY	7/89	Q1

2019 年中科院分区:

	学科名称	分区	Top 期刊
小类	AGRONOMY 农艺学	2	-
大类	农林科学	1	是

4.标题: Effect of potassium foliage application post-anthesis on grain filling of wheat under



drought stress

作者:Lv, XK (Lv, Xiaokang)[ 1 ] ; Li, T (Li, Tong)[ 1 ] ; Wen, XX (Wen, Xiaoxia)[ 1 ] ; Liao, YC (Liao, Yuncheng)[ 1 ] ; Liu, Y (Liu, Yang)[ 1 ]

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DOI: 10.1016/j.fcr.2017.02.015 出版年: MAY 2017 文献类型:Article

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2019 年公布的影响因子: 3.868, 期刊分区:

JCR® 类别	类别中的排序	JCR 分区
AGRONOMY	7/89	Q1

2019 年中科院分区:

	学科名称	分区	Top 期刊
小类	AGRONOMY 农艺学	2	-
大类	农林科学	1	是

5.标题: Effect of phosphorus and potassium foliage application post-anthesis on grain filling and hormonal changes of wheat

作者:Lv, XK (Lv, Xiaokang)[ 1 ] ; Han, J (Han, Juan)[ 1 ] ; Liao, YC (Liao, Yuncheng)[ 1 ] ; Liu, Y (Liu, Yang)[ 1 ]

FIELD CROPS RESEARCH 卷: 214 页: 83-93

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2019 年公布的影响因子: 3.868, 期刊分区:

JCR® 类别	类别中的排序	JCR 分区
AGRONOMY	7/89	Q1

2019 年中科院分区:



	学科名称	分区	Top 期刊
小类	AGRONOMY 农艺学	2	-
大类	农林科学	1	是

6.标题: Spermidine Increases the Sucrose Content in Inferior Grain of Wheat and Thereby Promotes Its Grain Filling

作者:Luo, J (Luo, Jian)[ 1 ] ; Wei, B (Wei, Bin)[ 1 ] ; Han, J (Han, Juan)[ 1 ] ; Liao, YC (Liao, Yuncheng)[ 1 ] ; Liu, Y (Liu, Yang)[ 1 ]

FRONTIERS IN PLANT SCIENCE 卷: 10

DOI: 10.3389/fpls.2019.01309 出版年: NOV 21 2019 文献类型:Article

通讯作者地址: Han, J; Liu, Y (通讯作者)Northwest A&F Univ, Coll Agron, Yangling, Shaanxi, Peoples R China.

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Web of Science 核心合集中的 "被引频次": 0

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2019 年公布的影响因子: 4.106, 期刊分区:

JCR® 类别	类别中的排序	JCR 分区
PLANT SCIENCES	20/228	Q1

2019 年中科院分区:

	学科名称	分区	Top 期刊
小类	PLANT SCIENCES 植物科学	2	-
大类	生物	2	否

7.标题: Spermidine promotes starch degradation to relieve the inhibitory effect of chilling on wheat seed germination

作者:Gu, XY (Gu, Xiaoyan)[ 1 ] ; Wei, B (Wei, Bin)[ 1 ] ; Liao, YC (Liao, Yuncheng)[ 1 ] ; Wen, XX (Wen, Xiaoxia)[ 1 ] ; Liu, Y (Liu, Yang)[ 1 ]

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DOI: 10.1007/s11738-019-2821-1 出版年: MAR 2019 文献类型:Article

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2019 年公布的影响因子: 1.608, 期刊分区:

JCR® 类别	类别中的排序	JCR 分区
PLANT SCIENCES	104/228	Q2

2019 年中科院分区:

	学科名称	分区	Top 期刊
小类	PLANT SCIENCES 植物科学	4	-
大类	生物	4	否

8.标题: Integrated N management improves nitrogen use efficiency and economics in a winter wheat-summer maize multiple-cropping system

作者:Liang, HY (Liang, Haiyan)[ 1 ]; Zhang, XL (Zhang, Xueling)[ 1 ]; Han, J (Han, Juan)[ 1 ]; Liao, YC (Liao, Yuncheng)[ 1 ]; Liu, Y (Liu, Yang)[ 1 ]; Wen, XX (Wen, Xiaoxia)[ 1 ]

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SOIL SCIENCE	11/35	Q2

2019 年中科院分区:

	学科名称	分区	Top 期刊
小类	SOIL SCIENCE 土壤科学	3	-



大类	农林科学	2	否
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9.标题: Effect of Plastic Film Mulching on the Grain Filling and Hormonal Changes of Maize under Different Irrigation Conditions

作者:Liu, Y (Liu, Yang)[ 1 ] ; Han, J (Han, Juan)[ 1 ] ; Liu, DD (Liu, Didi)[ 1 ] ; Gu, DD (Gu, Dandan)[ 2 ] ; Wang, YP (Wang, Yongping)[ 1 ] ; Liao, YC (Liao, Yuncheng)[ 1 ] ; Wen, XX (Wen, Xiaoxia)[ 1 ]

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2019 年公布的影响因子: 2.776, 期刊分区:

JCR® 类别	类别中的排序	JCR 分区
MULTIDISCIPLINARY SCIENCES	24/69	Q2

2019 年中科院分区:

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小类	MULTIDISCIPLINARY SCIENCES 综合性期刊	3	-
大类	综合性期刊	3	否

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## Research article

## Effect of polyamines on the grain filling of wheat under drought stress

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## ABSTRACT

Drought inhibits wheat grain filling. Polyamines (PAs) are closely associated with plant resistance due to drought and grain filling of cereals. However, little is known about the effect of PAs on the grain filling of wheat under drought stress. This study investigated whether and how PAs are involved in regulating wheat grain filling under drought stress. Two wheat genotypes differing in drought resistance were used, and endogenous PA levels were measured during grain filling under different water treatments. Additionally, external PAs were used, and the variation of hormone levels in grains was measured during grain filling under drought stress. The results indicated that spermidine (Spd) and spermine (Spm) relieve the inhibition caused by drought stress, and putrescine (Put) has the opposite effect. The higher activities of S-adenosylmethionine decarboxylase and Spd synthase in grains promotes the synthetic route from Put to Spd and Spm and notably increases the free Spd and Spm concentrations in grains, which promotes grain filling and drought resistance in wheat. The effect of PA on the grain filling of wheat under drought stress was closely related to the endogenous ethylene (ETH), zeatin (Z) + zeatin riboside (ZR) and abscisic acid (ABA). Spd and Spm significantly increased the Z + ZR and ABA concentrations and decreased the ETH evolution rate in grains, which promoted wheat grain filling under drought. Put significantly increased the ETH evolution rate, which led to excessive ABA accumulation in grains, subsequently aggravating the inhibition of drought on wheat grain filling. This means that the interaction of hormones, rather than the action of a single hormone, was involved in the regulation of wheat grain filling under drought.

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## 1. Introduction

The yield potential of wheat (*Triticum aestivum* L.) is divided into the following three major components: the panicle number per plant, the grain number per panicle, and the grain weight. Grain filling, the final stage of cereal growth, determines the grain weight (Yang and Zhang, 2006). Modern high yield crop production systems require high yield outputs, and for this reason, improved grain filling has become more important than ever (Saini and Westgate, 2000; Zahedi and Jenner, 2003).

Drought is one of the main abiotic stresses limiting crop growth and accounting for considerable grain yield reduction in crops (Jiang and Zhang, 2004). In northern China, one of the most important wheat production regions in China and world, the region's rainy season does not coincide with the growth stage for

wheat. Over 70% of its precipitation falls during the monsoon months from June to September (Li et al., 2000) and, as a result, droughts are a common occurrence during the winter wheat growth stage. In cereals such as wheat, stress caused by drought at the time of grain-filling usually shortens the grain-filling period and reduces the grain-filling rate, which leads to a reduction in grain weight and grain yield (Kobata et al., 1992; Zhang et al., 1998). Thus, how to relieve the inhibition effect of drought stress on the grain filling of wheat is important for wheat production of China.

Polyamines (PA) are organic polycations, which are low molecular weight nitrogen-containing compounds (Igarashi and Kashiwagi, 2000). They have been described as endogenous plant growth regulators or intracellular messengers that regulate plant growth, development, and responses to abiotic stresses (Alcazar et al., 2006; Kusano et al., 2007; Paschalidis and Roubelakis-Angelakis, 2005; Tomosugi et al., 2006). PAs are closely associated with plant resistance to water stress (Groppa and Benavides, 2008). Capell et al. (2004) found that the rice transgenic plant with a high expressing *Datura stramonium adc* gene produced much higher putrescine (Put, one PA) under stress and promoted spermidine

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(Spd, another PA) and spermine (Spm, another PA) synthesis, ultimately protecting the plants from drought. However, the wild-type plants were insufficient in triggering the conversion of Put into Spd and Spm, and they were sensitive for drought stress although the Put increased under drought stress. Yamaguchi et al. (2007) found that an *Arabidopsis* mutant plant, which cannot produce Spm, is hypersensitive to drought, and this phenotype was cured by Spm pretreatment. Yang et al. (2007) suggested that the increased concentrations of free Spd, free Spm, and insoluble-conjugated Put under water stress were significantly correlated with the yield maintenance ratio of rice. These observations indicate that by the manipulation of endogenous PA metabolism, an enhancement in crop drought resistance may occur. Furthermore, Farooq et al. (2009) found that exogenously applied PA increases leaf water status, photosynthesis and membrane properties, thereby improving the drought tolerance of rice. Yamaguchi et al. (2007) found that an *Arabidopsis* mutant plant, which cannot produce Spm, is hypersensitive to drought, and this phenotype was cured by Spm pretreatment. Yang et al. (2007) suggested that the increased concentrations of free Spd, free Spm, and insoluble-conjugated Put under water stress were significantly correlated with the yield maintenance ratio of rice. In addition, PA was thought to be involved in the regulation of grain development. The PA concentration of normal kernels was significantly higher than that of aborting maize kernels (*Zea mays* L.), and the PA concentration was positively correlated with the endosperm nuclei number (Liang and Lur, 2002). Yang et al. (2008) found that higher levels of Spd and Spm promote grain filling and increase the grain weight of rice (*Oryza sativa* L.); however, Put, had the opposite effect. Tan et al. (2009) suggested that low concentrations of Spd and Spm and low Spd/Put and Spm/Put ratios may be important physiological causes of the low grain filling rate and the low grain weight of inferior spikelets in super rice. These studies suggest that the PA is related to drought resistance and grain development in plants. However, the effect of PA on the regulation of wheat grain filling under drought stress and its mechanism remain unclear.

Plant hormones play an important role in regulating grain development. High levels of cytokinins (CTKs) are generally found in the developing grains of cereals, peas, and beans (Dietrich et al., 1995; Michael and Seiler-Kelbitsch, 1972; Morris et al., 1993; Saha et al., 1986; Yang et al., 2000). CTKs are thought to be involved in cell division during seed development (Yang et al., 2000). In rice, superior grains have higher abscisic acid (ABA) concentrations and lower ethylene (ETH) concentrations compared with inferior grains, and the ratio of ABA/ETH is positively and significantly correlated with the grain filling rate (Yang et al., 2006). In addition, the grain filling rate is positively correlated with indole-3-acetic acid (IAA) concentration in rice grains (Xu et al., 2007). High gibberellin 1 (GA<sub>1</sub>) and GA<sub>19</sub> levels were found in the large panicles of rice immediately before and during anthesis (Suzuki et al., 1981).

PA and ETH reportedly share the same S-adenosylmethionine biosynthetic precursor, and increasing PA biosynthesis has a notable effect on ETH synthesis rates (Liang and Lur, 2002). Exogenous PA represses ETH synthesis in oat (*Avena sativa* L.) leaves (Führer et al., 1982) and rice panicles (Yang et al., 2008). In addition, exogenous ABA increased the Put concentration in chickpeas (*Cicer arietinum* L.) (Bueno and Matilla, 1992). This reduced endogenous ABA concentration led to a decrease in the PA levels in maize (Liu et al., 2005). These studies provided clear evidence that there is a close relationship between PA and hormones in the regulation of plant growth. Our previous study suggested that exogenous Spd and Spm significantly increase the endogenous zeatin (Z) + zeatin riboside (ZR), ABA, and IAA concentrations and significantly decrease the ETH concentration in wheat grains, thus affecting wheat grain filling (Liu et al., 2013). However, little is known about

the relationship between PA and hormones in the regulation of wheat grain filling under drought study.

In the present study, two winter wheat cultivars that differ in drought resistance were used. The effects of drought stress on grain filling were monitored, and changes in endogenous Spd, Spm and Put concentrations in grains and leaves were observed. The activities of the enzymes involved in PA biosynthesis, such as ornithine decarboxylase (ODC, EC 4.1.1.17), arginine decarboxylase (ADC, EC 4.1.1.19), S-adenosylmethionine decarboxylase (SAMDC, EC 4.1.1.50) and Spd synthase (EC 2.5.1.16) were measured during wheat grain filling. In addition to these observations, external Spd, Spm and Put were treated under drought stress, and the grain filling characteristics and changes in IAA, Z + ZR, ABA and ETH in the grains were measured during wheat grain filling. The objective of the present study was to investigate the effect of PA on wheat grain filling under drought stress and to determine the relationship of PA and hormones with the regulation of wheat grain filling. We also sought to determine whether the grain filling of wheat can be regulated by manipulating the PA levels under drought stress.

## 2. Materials and methods

### 2.1. Study site description

This study was conducted from 2012 to 2014 at the Crop Specimen Farm in Northwest A&F University, Shaanxi Province, northwestern China. The latitude and longitude of the experimental station are 34°22'N and 108°26'E, respectively. The annual mean precipitation of the experimental station is 550 mm. The annual mean maximum and minimum air temperatures at the site are 42 °C and −19.4 °C, respectively, and the annual mean temperature is 12.9 °C. The total yearly sunshine duration is 2196 h, and the frost-free period is 220 days. The soil at the experiment farm is Eum-Orthrosols (Chinese soil taxonomy), and the mean bulk density of the soil was 1.33 g cm<sup>−3</sup>. The readily available N, P and K quantities were 57.45 mg kg<sup>−1</sup>, 17.33 mg kg<sup>−1</sup>, and 118.57 mg kg<sup>−1</sup>, respectively. The organic matter concentration of the 0–20 cm topsoil was 12.34 g kg<sup>−1</sup>, and the pH was 7.35.

### 2.2. Experimental design and treatments

#### 2.2.1. The first experiment

The experiment was performed in large waterproof sheds. The internal shed dimensions were 32 m (length) × 15 m (width) × 3 m (height). The sheds had a transparent plastic-covered roof and four open sides. Mobile sheds were used to control natural rainfall on rainy days. Each plot area was 3 m × 4 m. The plot was divided by cement barriers.

Two wheat cultivars, Changhan 343 (a drought-resistant cultivar) and Xinong 979 (a drought-susceptible cultivar), were grown. The seeds were sown on Oct 18 for the 2012–2013 growth year and on Oct 20 for 2013–2014. The sowing density was 150 kg ha<sup>−1</sup>, with a row spacing of 0.20 m. Fertilizer at 150 kg ha<sup>−1</sup> urea and 150 kg ha<sup>−1</sup> diammonium orthophosphate was applied at basal levels.

The experiment was a 3 × 2 (three levels of soil moisture and two cultivars) factorial design, with 6 treatment combinations. Each of the treatments contained three plots as replicates in a complete randomized block design. From anthesis until maturity, three levels of soil moisture were imposed on the plants by controlling water application. The well-watered (WW) treatment was maintained soil water potential at −20 ± 5 kPa, the moderately soil-dried (MD) treatment was maintained soil water potential at −40 ± 5 kPa, and the severely soil-dried (SD) treatment was maintained soil water potential at −60 ± 5 kPa. Soil water potential



was monitored at a soil depth of 15- to 20-cm. Five tension meters (SWP-100, Soil Science Research Institute, China Academy of Sciences, Nanjing, China) were installed in each plot. Tension meter readings were recorded at 11:00–12:00 each day. When the readings dropped to a certain value, the appropriate amount of water per plot was added to each of the WW, MD and SD treatments. Before anthesis, the soil water potential was maintained at  $-20 \pm 5$  kPa.

### 2.2.2. The second experiment

The experiment was also performed in large waterproof sheds. The format was the same as in the first experiment. The same two cultivars, Xinong 979 and Changhan 343, were used in external PA application treatments. Each cultivar received five treatments from anthesis until maturity, as follows: (1) CK1: the soil water potential was maintained at  $-20 \pm 5$  kPa; (2) CK2: the soil water potential was maintained at  $-60 \pm 5$  kPa; (3) S1: the soil water potential was maintained at  $-60 \pm 5$  kPa, and Spd was externally sprayed on wheat panicles; (4) S2: the soil water potential was maintained at  $-60 \pm 5$  kPa, and Spm was externally sprayed on wheat panicles; and (5) P1: the soil water potential was maintained at  $-60 \pm 5$  kPa, and Put was externally sprayed on wheat panicles. Before anthesis, the soil water potential was maintained at  $-20 \pm 5$  kPa.

At anthesis, 1 mmol L<sup>-1</sup> Spd (S1), 1 mmol L<sup>-1</sup> Spm (S2), and 2 mmol L<sup>-1</sup> Put (P1) were sprayed on the wheat panicles with a sprayer. The chemicals were applied daily for 6 day at a rate of 5 ml per spike for each application. All of the solutions contained 0.1% (V/V) ethanol and 0.01% (V/V) Tween-20. The same volume of deionized water containing the same concentrations of ethanol and Tween-20 was applied to each of the control plants (CK1 and CK2). Each treatment had three replicates with a completely randomized block design. The Spm, Spd, and Put were purchased from Sigma–Aldrich (USA).

### 2.3. Measurement

Four hundred spikes that flowered on the same day were chosen and tagged in each plot. Twenty tagged spikes from each plot were sampled at 3-d intervals from anthesis to maturity. All grains from each spike were removed. Grains on a spike were divided into superior grains and inferior grains. The most basal grains in the middle spikelets (four to 12 spikelets) from the bottom of a spike were considered superior grains, and the most distal grains in the middle spikelets (four to 12 spikelets) from the bottom of a spike were considered inferior grains (Jiang et al., 2003). Half of the sampled grains were used for measurements of PA, hormones and enzyme activities. The other half of the grains were dried at 70 °C and weighed until a constant weight was observed.

#### 2.3.1. Grain-filling process

The grain filling process was simulated using Richards' (1959) growth equation, according to Yang et al. (2006):

$$W = \frac{A}{(1 + Be^{-kt})^{\frac{1}{N}}} \quad (1)$$

The grain-filling rate (G) was calculated as the derivative of Eq. (1):

$$G = \frac{AkBe^{-kt}}{(1 + Be^{-kt})^{\left(\frac{N+1}{N}\right)}} \quad (2)$$

[W, the grain weight (mg); A, the final grain weight (mg); t, time after anthesis (d); B, k and N, coefficients determined by

regression.]

The active grain-filling period was defined as the period when W was between 5% ( $t_1$ ) and 95% ( $t_2$ ) of A. The average grain-filling rate during this period was therefore calculated from  $t_1$  to  $t_2$  (Yang et al., 2006).

#### 2.3.2. PAs

Spd, Spm, and Put were extracted and measured according to Yang et al. (2008) and Liu et al. (2002). Briefly, approximately 0.5 g FW of samples was homogenized in a pre-chilled mortar and pestle in 3–5 ml of 5% (v/v) perchloric acid (PCA). The homogenates were incubated at 5 °C for 1 h and centrifuged at  $25,000 \times g$  for 20 min. After centrifugation, the supernatant and pellet were collected separately. To extract soluble-conjugated PAs, aliquots (2 ml) of the supernatant were mixed with 2 ml 12 N HCl and heated at 110 °C for 18 h in flame-sealed glass ampules. After acid hydrolysis, HCl was evaporated from the tubes by further heating at 80 °C, and the residues were resuspended in 0.5 ml of 5% (v/v) PCA. To extract insoluble-conjugated PAs, the pellet was rinsed four times with 5% PCA to remove any trace of soluble PA. Then, the pellet was dissolved by vigorous vortexing in 2 ml of 1 N NaOH. The mixture was centrifuged at  $25,000 \times g$  for 20 min, and the supernatant was hydrolyzed under the same conditions mentioned above.

PAs in the non-hydrolyzed supernatant, hydrolyzed supernatant and hydrolyzed pellet were derived with benzoyl chloride and quantified by a high-performance liquid chromatography system (Waters 1525 Binary HPLC Pump/2489 UV Detector, Waters, USA). Exactly 20  $\mu$ L of each methanol (60% v/v)-redissolved sample was injected and loaded onto a Waters Symmetry C18 column (7.5 cm  $\times$  4.6 mm, 3.5  $\mu$ m). The column temperature was 30 °C, with a flow rate of 0.7 ml min<sup>-1</sup>. Polyamine peaks were detected at an absorbance of 230 nm.

#### 2.3.3. PA biosynthetic enzyme activity

The activities of ADC, ODC, SAMDC and Spd synthase were measured according to Yang et al. (2008). The sampled grains (approximately 0.5 g FW) were ground to a fine powder and homogenized with 2–3 ml extraction buffer (pH 8.0) containing 25 mM potassium-phosphate, 50  $\mu$ M EDTA, 100  $\mu$ M phenyl-methylsulfonylfluoride, 1 mM 2-mercaptoethanol and 25 mM ascorbic acid. After centrifugation at  $25,000 \times g$  at 4 °C for 20 min, the supernatant was dialyzed overnight against the extraction buffer. The activities of ODC, ADC and SAMDC were determined by measuring CO<sub>2</sub> evolution as described by Lee et al. (1997). Spd synthase activity was assayed according to Kasukabe et al. (2004). An aliquot of the supernatant was incubated at 37 °C for 30 min in a reaction mixture consisting of 0.1 M Tris–HCl (pH 8.0), 30  $\mu$ M Put, 25  $\mu$ M decarboxylated S-adenosylmethionine, and 20  $\mu$ M adenine. The reaction product (5'-deoxy-5'-methyl-thioadenosine) was quantified via an HPLC (Waters 1525 Binary HPLC Pump, Waters, USA) that was equipped with a fluorescence detector (Waters Multi  $\lambda$ ) and a reverse-phase (C18) column (Waters). The proteins in the extract were quantified as described by Bradford (1976). Each independent sample had three measurements.

#### 2.3.4. Hormones

The methods for the extraction and purification of Z + ZR, IAA, and ABA were essentially identical to those described by Yang et al. (2001). A sample of approximately 0.5 g was ground in a mortar (on ice), and 5 ml 80% (v/v) methanol extraction medium containing 1 mmol L<sup>-1</sup> butylated hydroxytoluene (BHT) was used as an antioxidant. The methanolic extracts were incubated at 4 °C for 4 h and centrifuged at  $10,000 \times g$  for 15 min at the same temperature. The supernatants were passed through Chromosep C18 columns (C18 Sep-Park Cartridge, Waters Corp, USA) that had been prewashed



with 10 ml 100% and 5 ml 80% methanol. The hormone fractions were dried with  $N_2$  and dissolved in 1 ml Phosphate Buffer Saline (PBS) containing 0.1% (v/v) Tween 20 and 0.1% (w/v) gelatin (pH 7.5) for analysis by an enzyme-linked immunosorbent assay (ELISA).

The mouse monoclonal antigens and antibodies against Z + ZR, IAA and ABA, and immunoglobulin G-horseradish peroxidase (IgG-HRP) used in the ELISA were produced at the Phytohormones Research Institute, China Agricultural University. The method for quantification of Z + ZR, GAs ( $GA_1 + GA_4$ ), IAA, and ABA by ELISA was described previously (Yang et al., 2001). The recovery rates of IAA, Z + ZR and ABA were  $93.1 \pm 7.3\%$ ,  $88.2 \pm 3.7\%$  and  $89.3 \pm 3.2\%$ , respectively.

Ethylene evolved from grains was measured according to Yang et al. (2006) with modifications. Briefly, sampled grains were placed between two sheets of moist paper for 1 h at 27 °C in darkness to allow wound-induced ethylene production to subside. Each sample contained 80–100 grains. Grains were then transferred to 25-ml glass vials containing moist filter paper, which were immediately sealed with airtight subaseal stoppers and then incubated in the dark for 8 h at 27 °C. A 1-ml gas sample was withdrawn through the subaseal with a gas-tight syringe, and ethylene was assayed using a gas chromatograph (Trace GC Ultra™, Thermo Fisher Scientific, USA) equipped with a Porapak Q column (0.3 cm  $\times$  200 cm, 0.18–0.30 mm) and flame ionization detector (FID). The temperatures of the injection port, column and detector were kept constant at 70, 70 and 150 °C, respectively. Nitrogen was used as a carrier at a flow rate of 40 KPa, and hydrogen and air were used for FID at rates of 35 and 350 ml min<sup>-1</sup>, respectively. The rate of ethylene evolution was expressed as a function of per unit fresh weight (FW).

#### 2.4. Yield and yield components

Plants (except at the border) from a 1-m<sup>2</sup> site from each plot were harvested at maturity for the determination of grain yield. Yield components, i.e., the spikes per square meter, grain number per spike, and grain weight, were determined from plants harvested from a 1-m<sup>2</sup> site (excluding the border plants) randomly sampled from each plot.

#### 2.5. Statistical analysis

The results were analyzed for variance using SPSS 16.0 for Windows. Data from each sampling set were analyzed separately. The means were tested by the least significant difference method at  $P = 0.05$  (LSD 0.05). The PA and hormone levels were presented as the data for 2013–2014.

### 3. Results

#### 3.1. Yield and yield components

The soil moisture significantly affected the grain yield of the wheat, but the response of the grain yield in the two cultivars to soil moisture was different. For Xinong 979, the grain yield of MD was significantly higher than in the WW and SD treatments, and the grain yield of WW was significantly higher than that of SD. For Changhan 343, the grain yields of the MD and WW treatments were all significantly higher than that of the SD treatment, but there was no significant difference for the grain yields of the WW and MD treatments of Changhan 343. Additionally, the yield of Xinong 979 was more sensitive for drought stress compared to Changhan 343. The grain yields of the SD treatment of Xinong 979 was 20.9% and 26.1% lower than that of WW treatment at 2012–2013 and 2013–2014, respectively. However, the grain yield of the SD

treatment of Changhan 343 was only 14.5% and 13.0% lower than that of WW treatment at 2012–2013 and 2013–2014, respectively.

For yield components, the soil moisture significantly affected grain weight, but the soil moisture had no significant effects on the panicles per ha and spikelets per panicle. This result suggested that, in this study, the soil moisture regulates the grain weight in order to regulate the grain yield of wheat.

#### 3.2. Grain filling

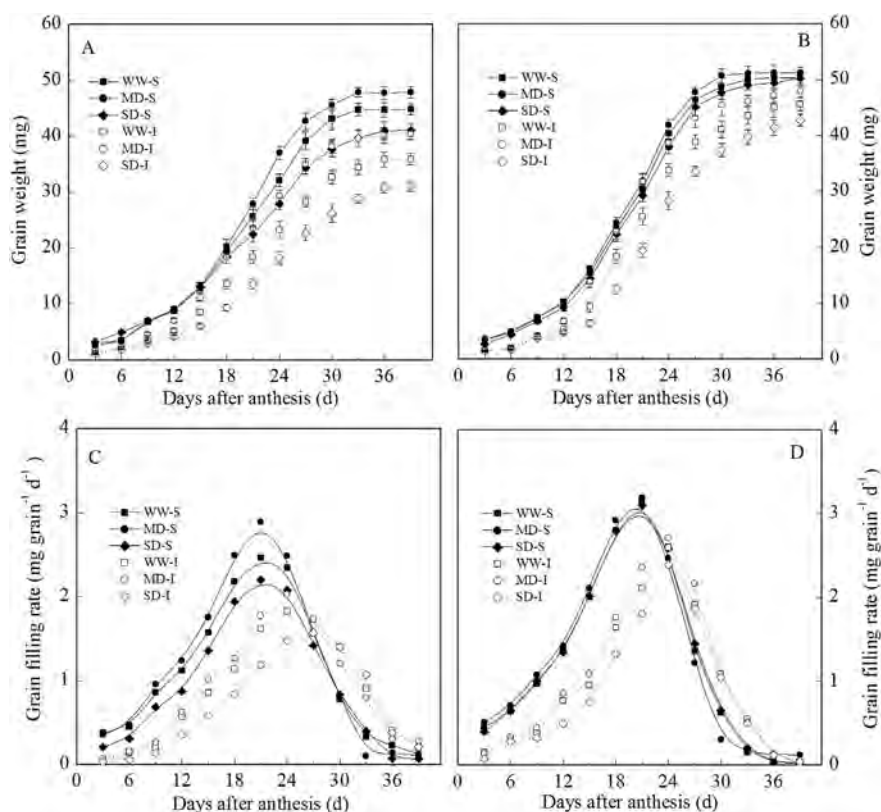
Soil moisture significantly affected the grain filling of wheat. However, there was a significant difference observed for the responses of grain filling of the two cultivars on the soil moisture (Fig. 1, Table 2). Drought stress significantly affected the grain filling of the superior grains and inferior grains of Xinong 979. The MD treatment significantly increased and the SD treatment significantly decreased the maximum grain weights and the maximum and mean grain-filling rates of the superior grains and inferior grains of Xinong 979 compared with WW treatment. Furthermore, drought stress only significantly affected the grain filling of the inferior grain of Changhan 343, and the drought had no significant effect on the grain filling of the superior grains of Changhan 343. The MD treatment significantly increased and the SD treatment significantly decreased the maximum grain weights and the maximum and mean grain-filling rates of the inferior grains of Changhan 343 compared with WW treatment. However, there was no significant difference observed for the maximum grain weights and the maximum and mean grain-filling rates of superior grains among WW, MD and SD treatments of Changhan 343.

#### 3.3. Polyamines in grains

The free Spm and Spd concentrations in the grains transiently increased during early and middle grain filling stages, reaching a maximum at 15 days after anthesis for the superior grains and 18 days after anthesis for the inferior grains, and decreasing thereafter (Fig. 2). The two cultivars showed similar trends. In contrast to these results, the free Put concentration of the grains decreased gradually during the grain filling stage. The free Spd and Spm concentrations in superior grains were significantly higher than that in inferior grains; however, the free Put concentration in superior grains was significantly lower than that in inferior grains. Furthermore, the free Spd and Spm concentrations in grains of Changhan 343 were higher than those of Xinong 979 during the early and middle grain filling stages, but the Put concentration in the grains of Changhan 343 was lower than that of Xinong 979 during the early and middle grain filling stages.

The soil moisture significantly affected the free PA concentrations in grains. The MD treatment significantly increased and the SD treatment significantly decreased the free Spd and Spm concentrations in superior grains and inferior grains of Xinong 979 compared with WW during the early and middle grain filling stages. For Changhan 343, the SD also significantly decreased the free Spd and Spm concentrations in inferior grains and the MD significantly increased these concentrations compared with WW treatment during the early and middle grain filling stages. However, the soil moisture had no significant effect on the free Spd and Spm concentrations in superior grains. In contrast to the effects of Spd and Spm, the drought stress significantly increased the free Put concentration in grains. The free Put concentrations in the superior grains of Xinong 979 and the inferior grains of Changhan 343 and Xinong 979 of WW treatment were significantly higher than those of the MD and SD treatments, and the free Put concentration in the grains of the MD treatment were significantly higher than that of SD treatment.





**Fig. 1.** Effects of drought stress on grain weights (A: Xinong 979; B: Changhan 343) and grain filling rates (C: Xinong 979; D: Changhan 343) of wheat. S: superior grain; I: inferior grain. WW: maintained the soil water potential at  $-20 \pm 5$  kPa after anthesis. MD: maintained the soil water potential  $-40 \pm 5$  kPa after anthesis. SD: maintained the soil water potential  $-60 \pm 5$  kPa after anthesis. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ).

**Table 1**  
Effect of soil moisture on yield and yield component of wheat.

Year	Cultivar	Treatment	No. of panicles ( $\times 10^4 \text{ hm}^{-2}$ )	Spikelets per panicle	Grain weight (mg)	Grain yield ( $\text{t hm}^{-2}$ )
2012–2013	Xinong 979	WW	371.6a	35.62a	41.55b	5.5b
		MD	377.8a	36.73a	44.74a	6.2a
		SD	370.2a	34.71a	33.73c	4.3c
	Changhan 343	WW	397.5a	40.31a	49.12a	7.9a
		MD	390.3a	39.43a	50.03a	7.7a
		SD	386.4a	38.57a	46.16b	6.9b
2013–2014	Xinong 979	WW	401.6a	34.22a	42.36b	5.8b
		MD	401.2a	35.90a	44.92a	6.5a
		SD	400.7a	33.44a	34.38c	4.6c
	Changhan 58	WW	414.3a	42.8a	48.51a	8.7a
		MD	414.8a	42.2a	49.25a	8.6a
		SD	411.5a	41.6a	45.22b	7.7b

Values within a column and for the same year and same cultivar followed by different letters are significantly different at  $P = 0.05$ . WW: maintained the soil water potential at  $-20 \pm 5$  kPa after anthesis. MD: maintained the soil water potential  $-40 \pm 5$  kPa after anthesis. SD: maintained the soil water potential  $-60 \pm 5$  kPa after anthesis.

Different from free polyamine, the soil moisture had no significant effects on the soluble-conjugated and insoluble-conjugated PAs in wheat grain (Figs. 3 and 4). The soluble-conjugated and insoluble-conjugated Spd and Spm concentrations in superior grains were higher than that of inferior grains, and the soluble-conjugated and insoluble-conjugated Put concentrations in superior grains were lower than that of inferior grains. However, no significant difference was observed for the soluble-conjugated and insoluble-conjugated Spd, Spm and Put concentrations among WW, MD and SD treatments.

The regression analysis showed that the maximum grain weight and the maximum and mean grain-filling rate were very significantly and positively correlated with free maximum free Spd and Spm concentrations in grains, and these values were significantly

and negatively correlated with the maximum free Put concentration in grains (Table 3). Unlike free PAs, the correlations of maximum soluble-conjugated and insoluble-conjugated PAs concentrations in grains and the maximum grain weight and the maximum and mean grain-filling rate were insignificant.

### 3.4. Changes in enzymatic activities

To better understand the roles of PAs in wheat grain filling, the activities of four enzymes involved in the biosynthesis of PAs in grains, ADC, ODC, SAMDC, and Spd synthase, were determined. Fig. 5 illustrates the changes in the activities of ADC, ODC, SAMDC, and Spd synthase in grains during grain filling. The changes in the activities of SAMDC and Spd synthase were similar with the



**Table 2**  
Grain-filling characteristics of winter wheat under different soil moisture.

Cultivars	Spikelet category	Treatment	Wmax mg	Gmax mg grain <sup>-1</sup> d <sup>-1</sup>	Gmean mg grain <sup>-1</sup> d <sup>-1</sup>
Xinong 979	S	WW	46.93b	2.46b	1.73b
		MD	48.57a	2.89a	1.83a
		SD	40.52c	2.21c	1.62c
	I	WW	37.30b	1.83b	1.40b
		MD	40.05a	2.02a	1.58a
		SD	27.67c	1.57c	1.20c
Changhan 343	S	WW	51.11a	3.15a	1.92a
		MD	52.00a	3.19a	1.99a
		SD	50.84a	3.10a	1.95a
	I	WW	46.39b	2.60b	1.76b
		MD	47.62a	2.71a	1.84a
		SD	42.12c	2.39c	1.67c

Values within a column and for the same cultivar and same grain type followed by different letters are significantly different at  $P = 0.05$ . WW: maintained the soil water potential at  $-20 \pm 5$  kPa after anthesis. MD: maintained the soil water potential  $-40 \pm 5$  kPa after anthesis. SD: maintained the soil water potential  $-60 \pm 5$  kPa after anthesis. Wmax: the final grain weight; Gmax: maximum grain-filling rates; Gmean: mean grain-filling rates; S: superior grain; I: inferior grain.

changes in concentrations of free Spd and Spm (Fig. 2) and were closely associated with the grain filling rate (Fig. 1). MD increased whereas SD decreased the SAMDC and Spd synthase activities in the inferior grains of the two cultivars and in the superior grains of Xinong 979. However, the MD and SD had no significant effects on the SAMDC and Spd synthase activities in superior grains of Changhan 58. The changing pattern of ADC activity was similar to free Put concentrations in grains, and both MD and SD treatments enhanced ADC activities in inferior grains of the two cultivars and in superior grains of Xinong 979, being more enhanced under SD than under MD. However, the MD and SD had no significant effects on the ADC activities in the superior grains of Changhan 58. Compared with the ADC activity, the ODC activity was much lower and showed no significant difference between WW, MD and SD.

### 3.5. Polyamine in leaves

Free PA concentrations in flag leaves slowly decreased during the grain filling stage and showed no significant differences between the two cultivars when plants were well watered (Fig. 6). The drought stress significantly promoted the free PAs in flag leaves. The concentrations of free PAs in the flag leaves of MD and SD treatments were increased and exhibited one peak for each cultivar. However, the peak values and time of appearance varied greatly according to cultivar. The highest peak value of free Put of Xinong 979, the drought-susceptible cultivar, was shown at 24 d after anthesis. However, the highest peak value of free Put of Changhan 343, the drought-resistant cultivar, was shown at 12 d after anthesis. In addition, the peak value of free Put of Xinong 979 was significantly higher than that of Changhan 343 under the same treatment. During the whole period of withholding water, Changhan 343 had more free Spd and free Spm in the flag leaves compared with Xinong 979. The changes in soluble-conjugated PA and insoluble-conjugated PA concentrations in the flag leaf were similar to those in free PAs (Figs. 7 and 8). The MD and SD treatments significantly increased the soluble-conjugated and insoluble-conjugated PA levels. However, there were no significant differences between the two cultivars in either Put, Spd, or Spm in the soluble-conjugated or the insoluble-conjugated forms when the soil moisture was same.

### 3.6. Effect of external PA on grain filling of wheat under drought stress

The external PAs had a significant effect on the grain filling of wheat under drought stress (Table 4). The external Put, Spd and Spm all had no significant effects on the mean grain-filling rates

and maximum grain weights of superior grains under drought stress. In comparison, the external Spd and Spm significantly promoted the mean grain-filling rates and maximum grain weights of inferior grains under drought stress. However, the external Put significantly decreased the mean grain-filling rates and maximum grain weights of inferior grains under drought stress.

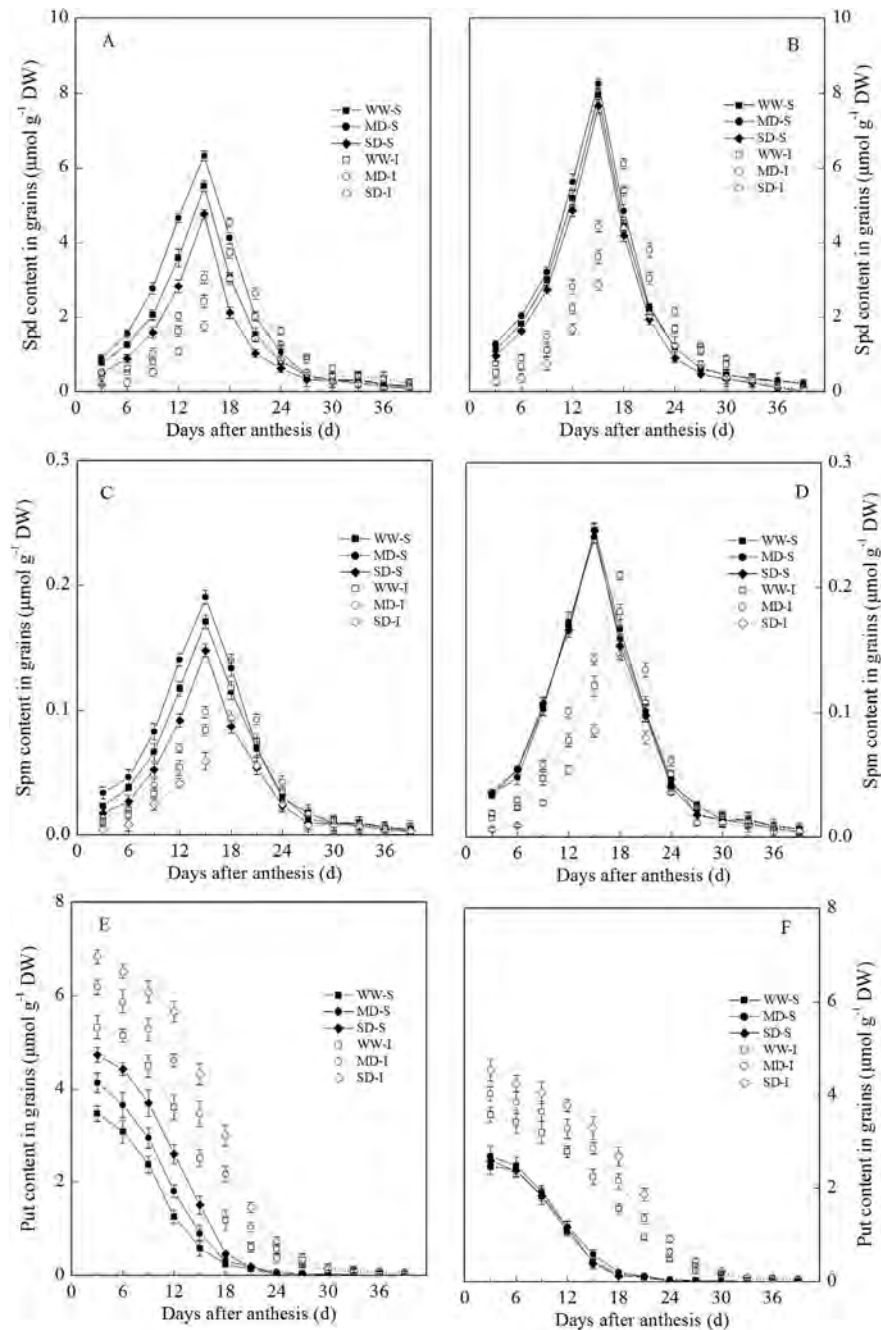
### 3.7. Effect of PA on endogenous hormones in grains under drought stress

The IAA and Z + ZR concentrations in grains showed similar patterns during grain filling. The IAA and Z + ZR concentrations in the grains transiently increased at the early grain filling stage and reached a maximum at 12 days after anthesis for superior grains and 18 days after anthesis for inferior grains. The concentrations decreased thereafter (Fig. 9). The IAA and Z + ZR concentrations in superior grains were significantly higher than that in inferior grains during the early and middle grain filling stages.

The drought stress significantly decreased the IAA and Z + ZR concentrations in the inferior grains of Xinong 979 and Changhan 343, and it significantly decreased the IAA and Z + ZR concentrations in the superior grains of Xinong 979. Under drought stress, the external applications of Spm, Spd, and Put all significantly increased the IAA concentration in the grains during the early and middle grain filling stages. The IAA concentrations in the superior and inferior grains of S1, S2 and P1 treatments were all significantly higher than those of the CK2 treatment at 6–18 days post-anthesis. In contrast to the IAA, the different PAs had different effects on the grain Z + ZR concentrations in grains under drought stress. The external Spd and Spm significantly increased the Z + ZR concentrations in the grains during the early and middle grain filling stages. Under drought stress, the Z + ZR concentrations in the grains treated with S1 and S2 were significantly higher than those of the CK2 from 6 to 18 d post-anthesis. However, there was no significant difference in the Z + ZR concentrations of grains between the P1 and CK2 treatments during the grain filling stage.

Similar to the IAA and Z + ZR concentrations, the ABA grain concentration transiently increased at the early grain filling stage and then decreased (Fig. 10). However, the ABA concentration in the grains reached a maximum at 18 days and 24 days after anthesis for superior grains and inferior grains, respectively. External applications of Spd, Spm, and Put under drought stress all significantly increased the ABA concentration in the wheat grains. The ABA concentrations in the grains from the S1, S2, and P1 treatments were all significantly higher than that of the CK2 treatment from 6 to 24 days after anthesis. In addition, the ABA concentration of the grains from the P1 treatment was significantly higher than those of





**Fig. 2.** Effect of drought stress on the free PA content in wheat grains (A, C and E: Xinong 979; B, D and F: Changhan 343). S: superior grain; I: inferior grain. WW: maintained the soil water potential at  $-20 \pm 5$  kPa after anthesis. MD: maintained the soil water potential  $-40 \pm 5$  kPa after anthesis. SD: maintained the soil water potential  $-60 \pm 5$  kPa after anthesis. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ).

the S1 and S2 treatments; compared with Spd and Spm, the externally applied Put had a more notable effect on the ABA concentration in the grains.

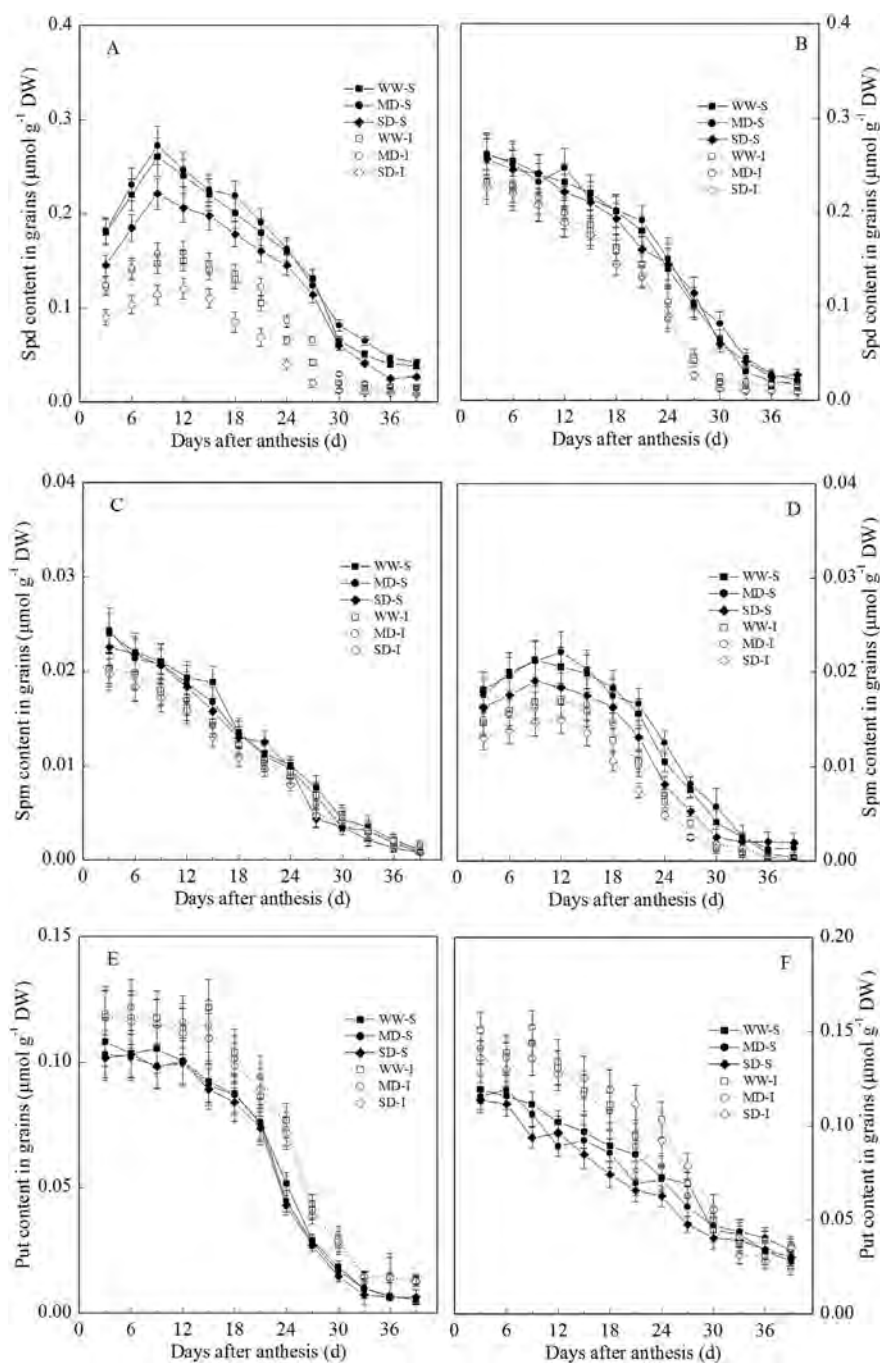
During the grain filling stage, the ETH evolution rate in the grains gradually decreased (Fig. 10). The external PAs all significantly affected the ETH evolution rate in the grains under drought stress. However, the different PAs had different effects on ETH evolution rate in grains under drought stress. Under drought stress, the external Spd and Spm significantly decreased the ETH evolution rate in the wheat grains, whereas the external Put significantly increased the ETH evolution rate in the grains during the grain filling stage.

## 4. Discussion

### 4.1. Effects of PAs on the grain filling of wheat under drought stress

Grain filling determines the grain weight and represents an important agronomic trait for wheat production. Drought significantly affected the grain filling of crops such as wheat and rice (Yang and Zhang, 2006). PA was thought to be involved in the regulation of grain filling of cereals. During the early and middle grain filling stages of rice, the Spd and Spm concentrations in superior grains were significantly higher than that in inferior grains; however, the Put concentration of the superior grains was lower



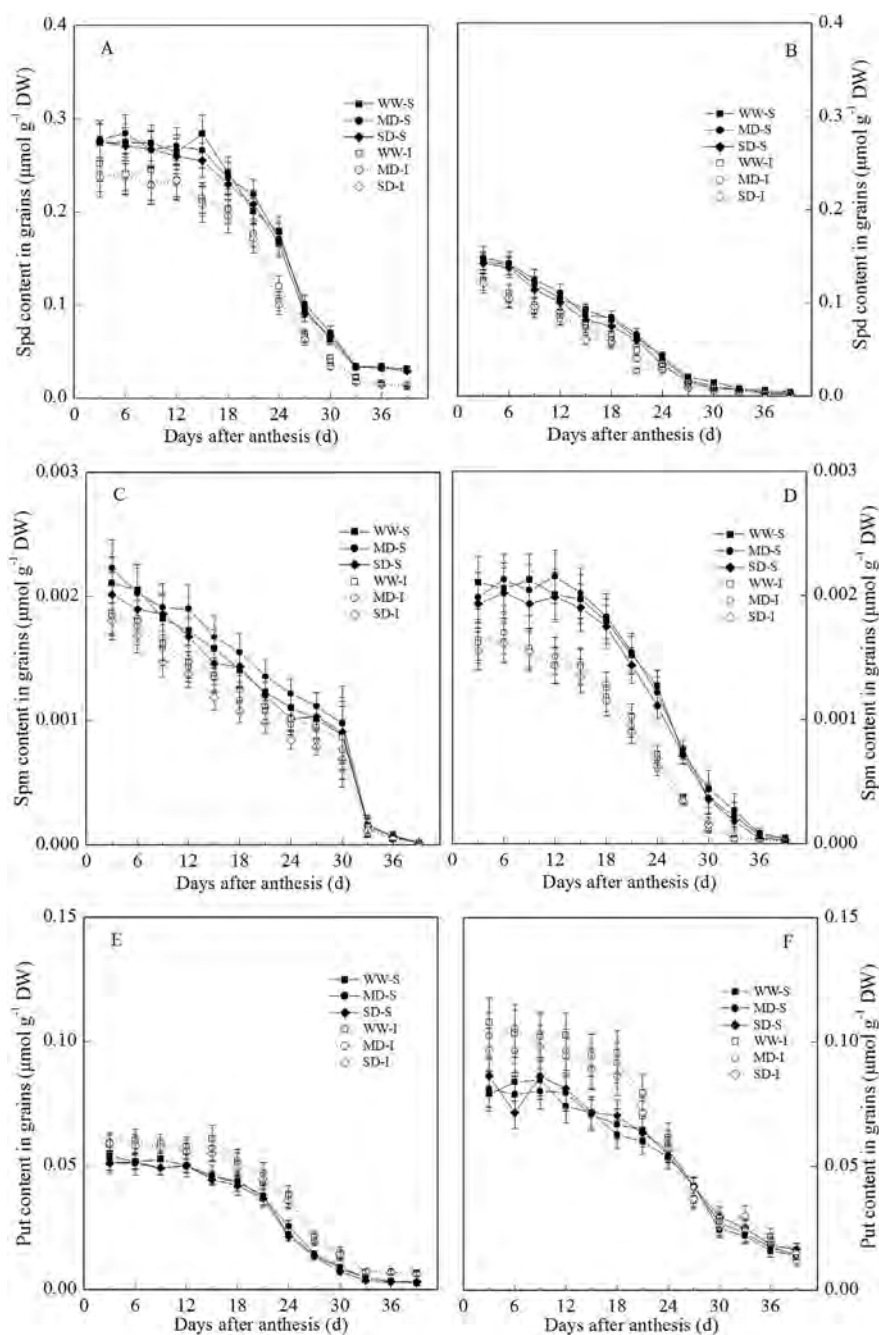


**Fig. 3.** Effect of drought stress on the soluble-conjugated PA content in wheat grains (A, C and E: Xinong 979; B, D and F: Changhan 343). S: superior grain; I: inferior grain. WW: maintained the soil water potential at  $-20 \pm 5$  kPa after anthesis. MD: maintained the soil water potential  $-40 \pm 5$  kPa after anthesis. SD: maintained the soil water potential  $-60 \pm 5$  kPa after anthesis. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ).

than that of the inferior grains (Yang et al., 2008). The grain filling rate and grain weight of rice were positively and significantly correlated with the Spd and Spm concentrations and the Spd/Put and Spm/Put ratios in the grains, but the grain filling rate and weight were negatively correlated with the Put concentration of the grains (Tan et al., 2009). In addition, PA was involved in the drought resistance of plants and the manipulation of PA metabolism may enhance crop drought resistance (Capell et al., 2004). Exogenously applied PA improved the drought tolerance of rice (Farooq et al., 2009). In the present study, the free Spd and Spm concentrations in grains and flag leaves of the drought-resistant

cultivar, Changhan 343, were significantly higher than those of Xiong 979, the drought-susceptible cultivar. However, the free Put concentrations in the grains and flag leaves of Changhan 343 were significantly lower than in those of Xinong 979. The free maximum Spd and Spm concentrations in grains were significantly and positively correlated with the maximum grain weight and the maximum and mean grain-filling rate of wheat, and the free maximum Put concentration in grains was significantly and negatively correlated with the maximum grain weight and the maximum and mean grain-filling rate of wheat. Additionally, the external application of Spd and Spm significantly increased the grain filling





**Fig. 4.** Effect of drought stress on the insoluble-conjugated PA content in wheat grains (A, C and E: Xinong 979; B, D and F: Changhan 343). S: superior grain; I: inferior grain. WW: maintained the soil water potential at  $-20 \pm 5$  kPa after anthesis. MD: maintained the soil water potential  $-40 \pm 5$  kPa after anthesis. SD: maintained the soil water potential  $-60 \pm 5$  kPa after anthesis. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ).

rate and weight of wheat under drought stress. However, the external application of Put significantly decreased the grain filling rate and weight of wheat under drought stress (Fig. 1, Table 1). These results demonstrated that free Put, Spm and Spd play an important role in the response of grain filling on drought stress of wheat. Free Spd and Spm can relieve the inhibition of drought stress on wheat grain filling. However, free Put aggravates the inhibition.

In contrast to free PA, the soil moisture had no significant effects on the soluble-conjugated PAs and insoluble-conjugated PAs in wheat grains (Figs. 3 and 4). However, the soil moisture significantly increased the soluble-conjugated and insoluble-conjugated

PA levels in flag leaves. Soil moisture had no significant effect on the soluble-conjugated and insoluble-conjugated PA levels in grains, and there were no significant differences between the two cultivars in terms of Put, Spd, or Spm in the soluble-conjugated and insoluble-conjugated form in grains and leaves when the soil moisture levels were the same. These results suggest that the soluble-conjugated PAs and the insoluble-conjugated PAs could play a minor role in responding to soil drying and in regulating the grain filling of wheat.

In higher plants, Put can be directly synthesized from ornithine via ODC or indirectly from arginine via ADC (Gemplová et al., 2006). Spd and Spm are synthesized via Spd synthase and Spm



**Table 3**

Correlation coefficients of peak PAs contents in wheat grain with the maximum grain filling rate (Gmax), mean grain filling rate (Gmean), and maximum grain weight (Wmax) of wheat.

	Wmax	Gmax	Gmean
Free Spd	0.918**	0.966**	0.935**
Free Spm	0.934**	0.974**	0.953**
Free Put	−0.926**	−0.924**	−0.905**
Soluble-conjugated Spd	0.395	0.341	0.327
Soluble-conjugated Spm	0.160	0.128	0.098
Soluble-conjugated Put	0.512	0.573	0.567
Insoluble-conjugated Spd	−0.439	−0.514	−0.503
Insoluble-conjugated Spm	0.413	0.440	0.375
Insoluble-conjugated Put	0.495	0.501	0.545

\*\*Significant at the 0.01 probability level ( $n = 6$ ). Wmax: the final grain weight (mg); Gmax: maximum grain-filling rates; Gmean: mean grain-filling rates.

synthase, respectively, by the sequential addition of aminopropyl groups to Put. The aminopropyl groups are generated from S-adenosyl-L-methionine (SAM) by SAMDC (Maiale et al., 2004). The present results show that increasing the free Put concentrations in grains is closely associated with increasing the ADC activities under both MD and SD treatments (Fig. 3A and Fig. 5A, E). The soil moisture treatments exhibited no significant effect on ODC activities (Fig. 5B, F). The results suggested that, similarly to rice (Yang et al., 2008), the Put synthesis in wheat grains occurs primarily via ADC as opposed to ODC. Additionally, the activities of SAMDC and Spd synthase are closely associated with the changes of free Spd and Spm in grains, and the soil moisture significantly affected the activities of these enzymes. The MD increased whereas SD decreased the SAMDC and Spd synthase activities in the inferior grains of the two cultivars and in the superior grains of Xinong 979. The activities of SAMDC and Spd synthase in the grains of Changhan 343 were significantly higher than in those of Xinong 979. The activities of SAMDC and Spd synthase in grains were significantly and positively correlated with maximum free Spd and Spm concentrations in grains and the maximum grain weight and the maximum and mean grain-filling rate. However, there was no significant difference between the activities of ADC in grains of the two cultivars and the correlations of ADC activity in grains and the maximum grain weight, and the maximum and mean grain-filling rates were insignificant. Capell et al. (2004) suggested that the modulation of the PA biosynthetic pathway in transgenic rice promoted the Spd concentration in plants and confers tolerance of drought stress. Based on these results, we suggest that the higher activities of SAMDC and Spd synthase in the grains of Changhan 343 promote the synthetic route from Put to Spd and Spm and increase the concentrations of free Spd and Spm in grains, thereby promoting grain filling and drought resistance compared with Xinong 979.

#### 4.2. Relationship of hormone and PA in regulation of wheat grain filling

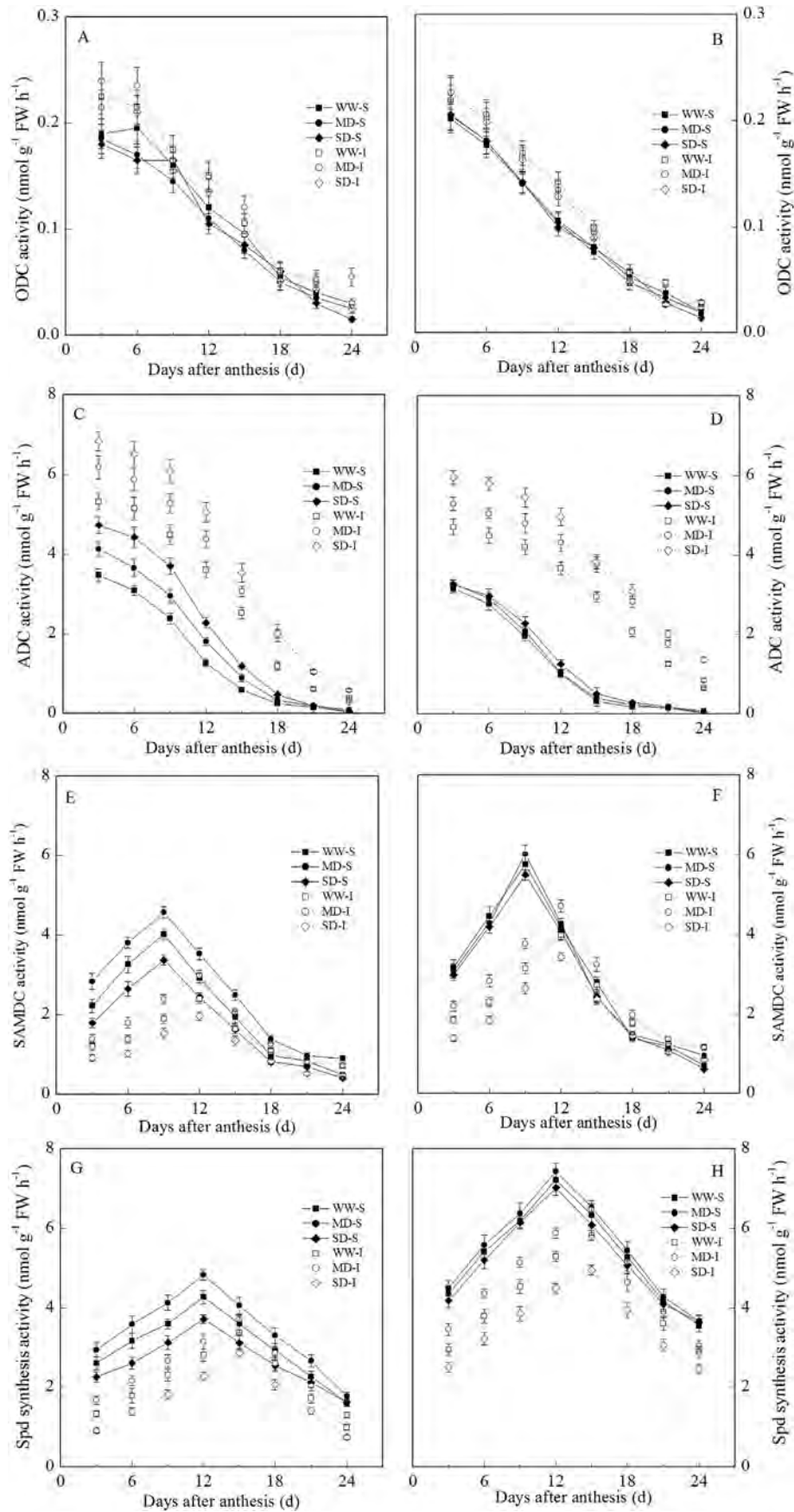
It has been reported that PA, by interacting in some way with hormones, regulates the growth and development of plants (Kusano et al., 2007; Smith et al., 1985). PA and ETH share the same biosynthetic precursor S-adenosylmethionine, and PA biosynthesis was notably affected by the ETH synthesis rates (Liang and Lur, 2002; Walden et al., 1997). Fuhrer et al. (1982) reported that exogenous PAs could repress ethylene synthesis in oat leaves. Yang et al. (2008) indicated that the external application of Spd or Spm decreases the ETH level in rice panicles. The previous studies suggested that ETH inhibits grain filling of rice and wheat because it

can aggravate the premature senility of plants (Liu et al., 2013; Yang et al., 2007). In the present study, drought stress significantly promoted the ETH evolution rate of wheat grain, and the external Spd and Spm significantly decreased the ETH evolution rate in grains under drought stress. This result implies that higher Spd, Spm and ETH exhibit an antagonistic relationship. Similar observations were also reported in *Arabidopsis thaliana* (Hu et al., 2006), maize (Feng et al., 2011), tomato (*Solanum lycopersicum*) (Nambeesan et al., 2012) and rice (Chen et al., 2013). It is therefore speculated that a potential metabolic interaction or competition between free Spd and Spm and ETH biosynthesis may mediate the effects of soil drying on the grain filling of wheat. Spd and Spm may relieve the premature senility of plants under drought stress by inhibiting ETH biosynthesis and increasing the grain filling rate and grain weight under drought stress. In contrast to Spd and Spm, the external Put significantly increased the ETH evolution rate in grains under drought stress. This suggests that Put promotes the ETH synthesis, and this may be the reason why Put notably decreases the maximum grain weight and the maximum and mean grain-filling rate under drought stress.

Previous studies suggested that ABA was involved in regulating the grain filling of rice and wheat and the resistance of plant to drought stress (Yang et al., 2006). External PA increased the endogenous ABA levels (Steiner et al., 2007). In the present study, the external Put, Spd and Spm all significantly increased the ABA concentration in grains under drought stress; however, the ABA concentration in the grains of Put treatment was significantly higher than those of Spd and Spm treatments under drought stress. ABA accumulation in plants can promote the drought resistance of plants. However, the excessive accumulation of ABA in plants may work against the drought resistance of plants (Yang et al., 2006). Yang and Zhang (2006) found that an increase in ABA concentration in grains under moderately dry soil conditions increased the grain-filling rate of rice, whereas much higher ABA concentrations under severely soil-dried condition reduced the grain-filling rate of rice. This previous study suggested that ABA promotes the transport of carbohydrates from the stem to the grains, but the excessive transport of carbohydrates may lead to deficiencies of carbohydrates in vegetative organs such as leaves and roots and may aggravate the premature senility of plants. Based on these results, we suggest that the effect of Spd and Spm on grain filling of wheat under drought stress may be related to the promotion of ABA accumulation in grains, which increases the transport of carbohydrates from the stem to the grains. However, Put treatment led to excessive ABA accumulation in grains, which exacerbated the premature senility of plants and decreased the drought resistance of grain filling of wheat.

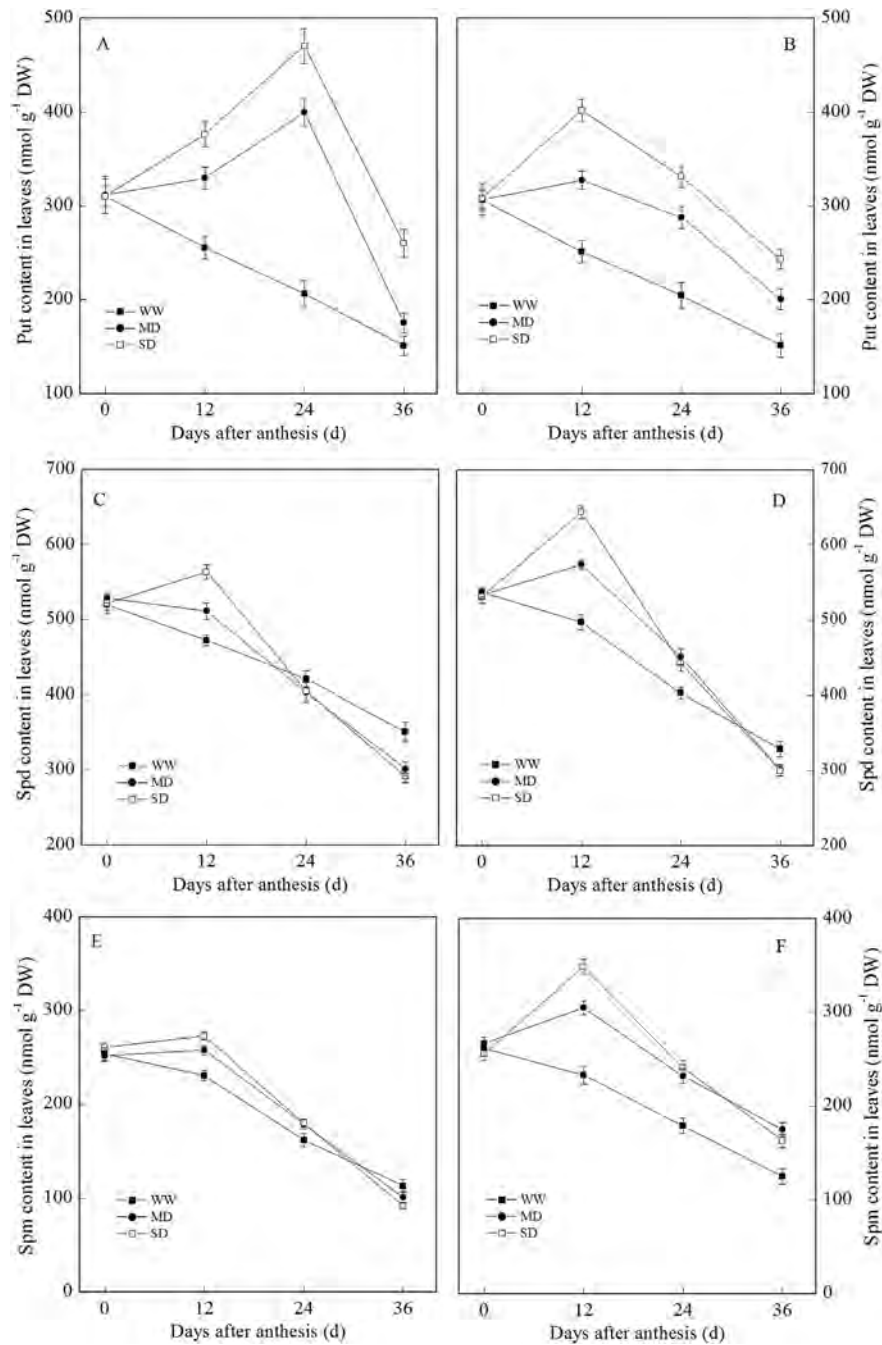
In addition to ABA and ETH, CTK plays an important role in regulating the grain filling of cereal (Yang et al., 2002; Zhang et al., 2009a). In rice, wheat, maize, and barley (*Hordeum vulgare* L.), a higher CTK concentration was generally observed in the endosperm of the grains, and CTKs were thought to be involved in cell division during the early phase of seed development (Dietrich et al., 1995; Michael and Seiler-Kelbitsch, 1972; Morris et al., 1993; Saha et al., 1986; Xu et al., 2007; Yang et al., 2000). Yang et al. (2008) indicated that external Spd and Spm significantly increased the Z + ZR levels in rice inferior grains, whereas methylglyoxal-bis(guanyldrazone) (MGBG), which inhibits the biosynthesis of Spd and Spm, significantly reduced Z + ZR levels in rice inferior grains. In the present study, external Spd and Spm significantly increased the Z + ZR concentrations in the inferior grains of Xinong 979 and Changhan 343 and the superior grains of Xinong 979 under drought stress. However, the external Put had no significant effect on the Z + ZR concentrations in the grains of two wheat cultivars. The regression analysis demonstrated that the maximum grain





**Fig. 5.** Effect of drought stress on the PA biosynthetic enzyme activity in wheat grains (A, C, E and G: Xinong 979; B, D, F and H: Changhan 343). S: superior grain; I: inferior grain. WW: maintained the soil water potential at  $-20 \pm 5$  kPa after anthesis. MD: maintained the soil water potential  $-40 \pm 5$  kPa after anthesis. SD: maintained the soil water potential  $-60 \pm 5$  kPa after anthesis. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ).



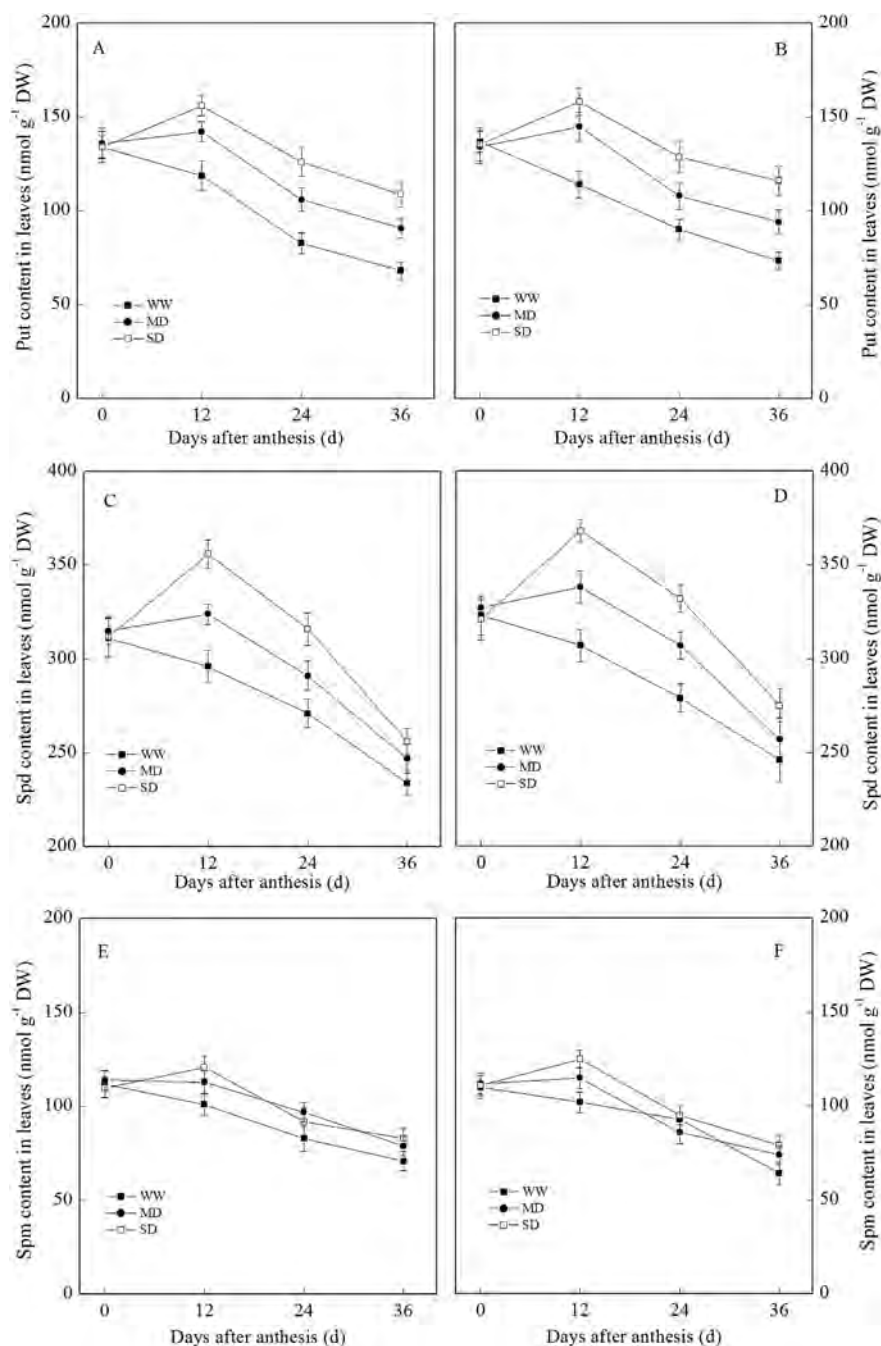


**Fig. 6.** Effect of drought stress on the free PA content in flag leaves (A, C and E: Xinong 979; B, D and F: Changhan 343). WW: maintained the soil water potential at  $-20 \pm 5$  kPa after anthesis. MD: maintained the soil water potential  $-40 \pm 5$  kPa after anthesis. SD: maintained the soil water potential  $-60 \pm 5$  kPa after anthesis. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ).

weight and mean grain-filling rate were significantly and positively correlated with maximum Z + ZR concentrations in grains ( $R = 0.928^{**}$  and  $0.934^{**}$ , respectively). The results were similar to those of Yang et al. (2008). This means that the effect of PA on the grain filling of wheat under drought stress was closely related to the endogenous CTK in grains. In addition to CTKs, IAA is involved in the regulation of the grain filling process (Xu et al., 2007; Yang and Zhang, 2006; Zhang et al., 2009b). IAA notably regulates the endosperm cell division in developing grains (Davies, 1987). High IAA concentrations in the grains could create an “attractive power” leading to an increase in CTK concentrations in grains (Seth and

Waering, 1967; Singh and Gerung, 1982). In the present study, external Spd, Spm and Put all significantly increased the IAA concentrations in grains under drought stress. However, external Spd, Spm and Put had different effects on the grain filling of wheat under drought stress. The regression analysis demonstrated that the correlations of maximum IAA concentration in grains, and the maximum grain weight and mean grain-filling rate were insignificant ( $R = 0.452$  and  $0.503$ , respectively). Based on these results, we suggest that IAA may be an important factor for regulating wheat grain filling. However, the effect of PA on the grain filling of wheat under drought stress showed no close relationship with





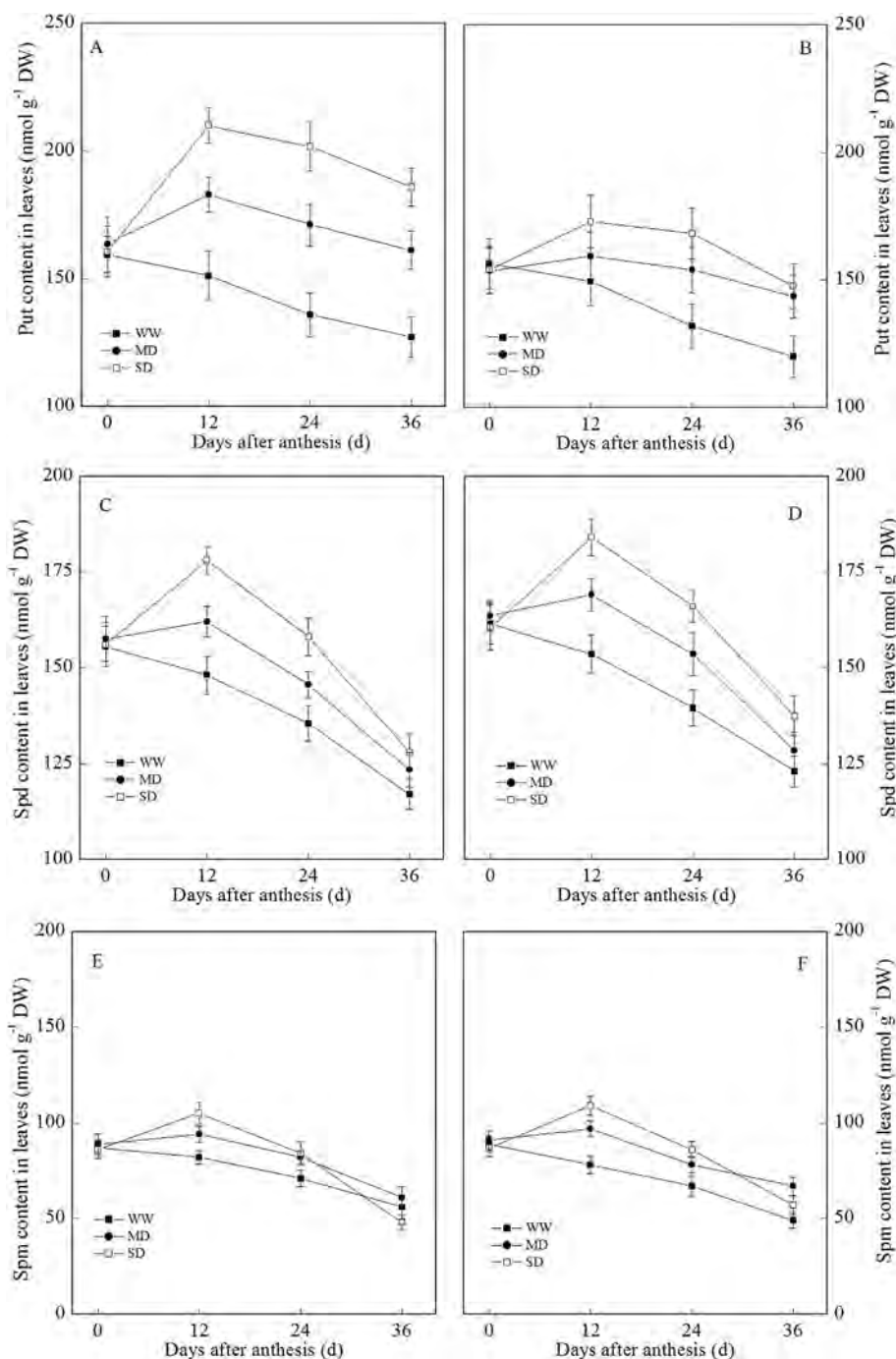
**Fig. 7.** Effect of drought stress on the soluble-conjugated PA content in flag leaves (A, C and E: Xinong 979; B, D and F: Changhan 343). WW: maintained the soil water potential at  $-20 \pm 5$  kPa after anthesis. MD: maintained the soil water potential  $-40 \pm 5$  kPa after anthesis. SD: maintained the soil water potential  $-60 \pm 5$  kPa after anthesis. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ).

endogenous IAA.

These results suggest that multiple hormones are involved in the regulation of grain filling of wheat. The previous study suggested that the interaction of hormones significantly affects the grain filling of cereal (Yang et al., 2001). The ratio of ABA/ETH was significantly and positively correlated with the grain filling of wheat (Yang et al., 2006). In the present study, we found that the correlations of the maximum ABA concentration in grains with the maximum grain weight and mean grain-filling rate were insignificant ( $R = 0.013$  and  $0.059$ , respectively). However, the ratio of ABA/ETH in grains was significantly and positively

correlated with maximum grain weight and mean grain-filling rate ( $R = 0.688^{**}$  and  $0.713^{**}$ , respectively). Additionally, the maximum Z + ZR concentrations in grains were significantly and positively correlated with maximum grain weight and mean grain-filling rate, and the ETH evolution rate in grains was significantly and negatively correlated with maximum grain weight and mean grain-filling rate. These observations indicate that the effect of PA on grain filling of wheat is significantly related to other hormones. The interactions of hormones or plant growth regulators, not just a single type of hormone, were involved in the regulation of wheat grain filling. The accumulation and transport





**Fig. 8.** Effect of drought stress on the insoluble-conjugated PA content in flag leaves (A, C and E: Xinong 979; B, D and F: Changhan 343). WW: maintained the soil water potential at  $-20 \pm 5$  kPa after anthesis. MD: maintained the soil water potential  $-40 \pm 5$  kPa after anthesis. SD: maintained the soil water potential  $-60 \pm 5$  kPa after anthesis. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ).

of photosynthate determined the grain weight of cereal (Yang et al., 2006). Our previous study suggested that external PA promotes photosynthesis in wheat flag leaves (Liu et al., 2013). In addition, ABA, Z + ZR, and ETH all affect the accumulation or transport of photosynthate (Yang et al., 2006). Therefore, we suggest that PA and hormones may be involved in the grain filling of wheat, which may be related to the accumulation and transport of photosynthate. Further studies on the specific effects of the individual hormones and their interactions with each other and their effects on the grain filling in wheat are necessary.

## 5. Conclusion

The results indicate that moderate drought significantly promotes grain filling and severe drought significantly inhibits grain filling in wheat. Endogenous free PAs play an important role in the response of grain filling during drought stress in wheat. Free Spd and Spm can notably relieve the inhibition of drought stress on wheat grain filling, and free Put aggravates the inhibition of drought stress on wheat grain filling. However, endogenous soluble-conjugated PAs and insoluble-conjugated PAs play a minor

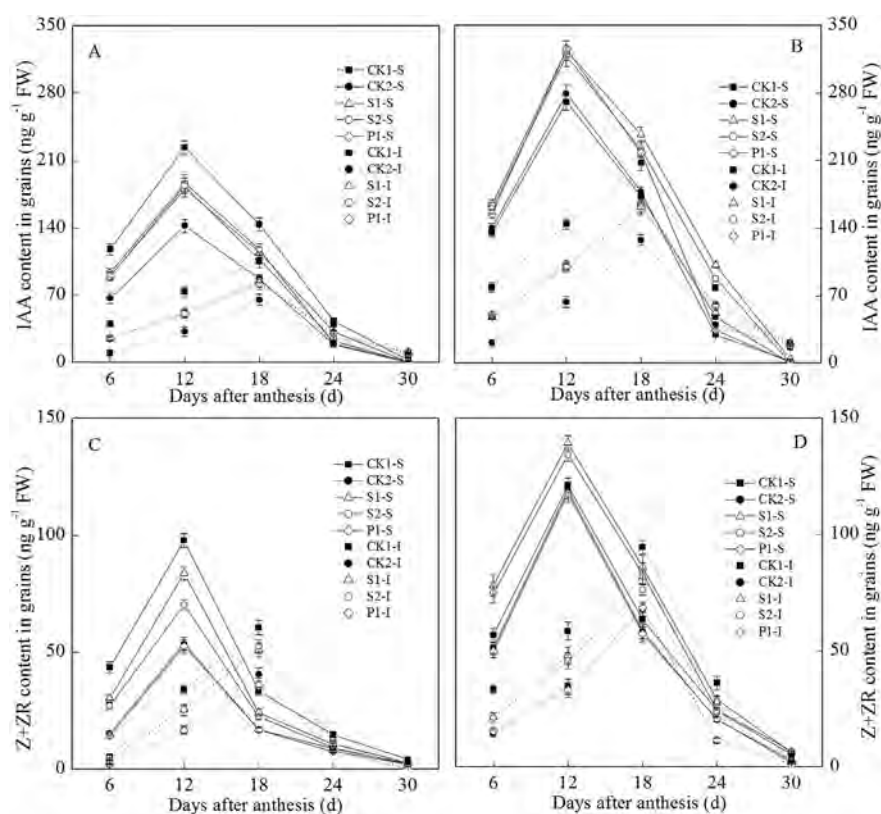


**Table 4**

Effect of external PAs on grain filling characters under drought stress.

Cultivars	Spikelet category	Treatment	Wmax mg	Gmean mg grain <sup>-1</sup> d <sup>-1</sup>	D d
Xinong 979	S	CK1	47.14a	1.73a	27.25a
		CK2	40.49b	1.61b	25.15b
		S1	41.31b	1.64b	25.19b
		S2	41.35b	1.63b	25.37b
		P1	39.97b	1.59b	25.14b
	I	CK1	38.02a	1.43a	26.59a
		CK2	27.90c	1.21c	23.06c
		S1	34.12b	1.36b	25.09b
		S2	33.49b	1.34b	24.99bc
		P1	24.68d	1.07d	23.07c
Changhan 343	S	CK1	51.88a	1.99a	26.07a
		CK2	50.39a	1.95a	25.84a
		S1	50.69a	1.97a	25.73a
		S2	51.56a	1.99a	25.91a
		P1	49.12a	1.93a	25.45a
	I	CK1	47.13a	1.78a	26.48a
		CK2	41.34c	1.62c	25.52b
		S1	43.40b	1.72b	25.23b
		S2	43.18b	1.71b	25.25b
		P1	38.79d	1.52d	25.19b

Values within a column and for the same cultivar and same grain type followed by different letters are significantly different at  $P = 0.05$ . CK1: soil water potential was maintained at  $-20 \pm 5$  kPa. CK2: soil water potential was maintained at  $-60 \pm 5$  kPa. S1: soil water potential was maintained at  $-60 \pm 5$  kPa and external sprayed Spd on wheat panicles. S2: soil water potential was maintained at  $-60 \pm 5$  kPa and external sprayed Spm on wheat panicles. P1: soil water potential was maintained at  $-60 \pm 5$  kPa and external sprayed Put on wheat panicles. Gmean: mean grain-filling rates; D: active grain-filling period; S: superior grain; I: inferior grain.

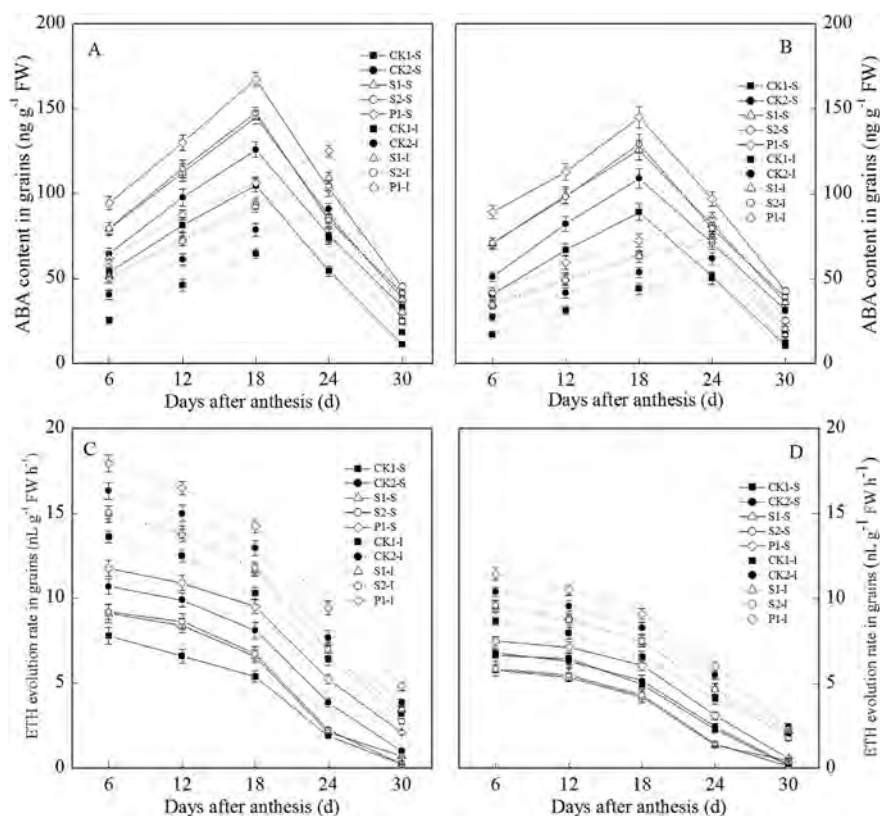


**Fig. 9.** Effect of external PA on the IAA and Z + ZR contents in wheat grains under drought stress (A and C: Xinong 979; B and D: Changhan 343). S: superior grain; I: inferior grain. CK1: soil water potential was maintained at  $-20 \pm 5$  kPa. CK2: soil water potential was maintained at  $-60 \pm 5$  kPa. S1: soil water potential was maintained at  $-60 \pm 5$  kPa and external sprayed Spd on wheat panicles. S2: soil water potential was maintained at  $-60 \pm 5$  kPa and external sprayed Spm on wheat panicles. P1: soil water potential was maintained at  $-60 \pm 5$  kPa and external sprayed Put on wheat panicles. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ).

role in responding to soil drying and in regulating the grain filling of wheat. The higher activities of SAMDC and Spd synthase in grains can promote the synthetic route from Put to Spd and Spm and notably increase the free Spd and Spm concentrations in grains,

thus promoting grain filling and drought resistance in wheat. The Spd and Spm significantly increased the Z + ZR and ABA concentration and decreased the ETH evolution rate in grains, which promoted wheat grain filling under drought. Put significantly





**Fig. 10.** Effect of external PA on the ABA content and ETH evolution rate in wheat grains under drought stress (A and C: Xinong 979; B and D: Changhan 343). S: superior grain; I: inferior grain. CK1: soil water potential was maintained at  $-20 \pm 5$  kPa. CK2: soil water potential was maintained at  $-60 \pm 5$  kPa. S1: soil water potential was maintained at  $-60 \pm 5$  kPa and external sprayed Spd on wheat panicles. S2: soil water potential was maintained at  $-60 \pm 5$  kPa and external sprayed Spm on wheat panicles. P1: soil water potential was maintained at  $-60 \pm 5$  kPa and external sprayed Put on wheat panicles. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ).

increased the ETH evolution rate and led to excessive ABA accumulation in grains, which aggravated the inhibition of drought on wheat grain filling. This means that the interaction of hormones, rather than the action of a single hormone, was involved in the regulation of wheat grain filling under drought.

## Contribution

Conceived and designed the experiments: Yang Liu, Yuncheng Liao.

Performed the experiments: Yang Liu, Haiyan Liang, Xiaokang Lv, Didi Liu.

Analyzed the data: Yang Liu, Xiaoxia Wen.

Wrote the paper: Yang Liu, Yuncheng Liao.

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RESEARCH ARTICLE

## Effect of polyamine on seed germination of wheat under drought stress is related to changes in hormones and carbohydrates



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### Abstract

Drought is a multifaceted stress condition that inhibits crop growth. Seed germination is one of the critical and sensitive stages of plants, and its process is inhibited or even entirely prevented by drought. Polyamines (PAs) are closely associated with plant resistance to drought stress and seed germination. However, little is known about the effect of PA on the seed germination of wheat under drought stress. This study investigated the involvement of PAs in regulating wheat seed germination under drought stress. Six wheat genotypes differing in drought resistance were used, and endogenous PA levels were measured during seed germination under different water treatments. In addition, external PA was used for seed soaking and the variation of hormones, total soluble sugar and starch were measured during the seed germination under different water treatments. These results indicated that the free spermidine (Spd) accumulation in seeds during the seed germination period favored wheat seed germination under drought stress; however, the free putrescine (Put) accumulation in seeds during the seed germination period may work against wheat seed germination under drought stress. In addition, seed soaking in Spd and spermine (Spm) significantly relieved the inhibition of seed germination by drought stress; however, soaking seeds in Put had no significant effect on seed germination under drought. External Spd and Spm significantly increased the endogenous indole-3-acetic acid (IAA), zeatin (Z)+zeatin riboside (ZR), abscisic acid (ABA), and gibberellins (GA) contents in seeds and accelerated the seed starch degradation and increased the concentration of soluble sugars in seeds during seed germination. This may promote wheat seed germination under drought stress. In conclusion, free Spd and Put are key factors for regulating wheat seed germination under drought stress and the effects of Spd and Put on seed germination under drought notably related to hormones and starch metabolism.

**Keywords:** polyamine, drought, seed germination, wheat, abscisic acid, starch

## 1. Introduction

Abiotic and biotic stresses occur frequently during a plant's life cycle (Munne and Muller 2013). Drought is one of the main abiotic stresses that can limit crop growth and accounts for considerable grain yield reduction in crops. Winter wheat (*Triticum aestivum* L.) is one of the most important food crops in China and the world. However, the rainy season in the main wheat-producing region of China does not coincide with the growth stages of winter wheat (Li *et al.* 2000). As

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a result, drought is a common occurrence during the winter wheat growth stage.

Seed germination is usually the most crucial phase during seedling establishment (Hubbard *et al.* 2012; Shi *et al.* 2014). However, this process is inhibited or even entirely prevented by drought (Hubbard *et al.* 2012). In the northern arid and semi-arid regions, one of the most important wheat producing regions in China, drought is one of the main factors that limits seed germination and the grain production of wheat. Hence, methods that can relieve the inhibition of the seed germination of wheat by drought are important for the wheat production of China and the world.

Polyamines (PAs) are endogenous plant growth regulators that mediate many plant physiological processes and the response to environmental stressors. The three main PAs in plants are spermidine (Spd), spermine (Spm) and putrescine (Put) (Paschalidis and Roubelakis-Angelakis 2005; Alcazar *et al.* 2006; Tomosugi *et al.* 2006; Kusano *et al.* 2007; Yang *et al.* 2008). Polyamine is involved in the seed germination of plants. The PA content increases during the first 15 d of *Ocotea catharinensis* seed germination and then decreases and stabilizes between 30 and 60 d of germination (Dias *et al.* 2009). Exogenous PAs improve the seed germination of the hot pepper (Khan *et al.* 2012). Inhibition of PA biosynthesis retards the pea germination process (Villanueva and Huang 1993). The PA levels increase during the seed development of soybeans and rice (Sen *et al.* 1981; Lin *et al.* 1984). In addition, PAs are closely associated with plant resistance to drought stress (Groppa and Benavides 2008). The manipulation of PA metabolism may enhance crop drought resistance (Capell *et al.* 2004). Farooq *et al.* (2009) found that exogenously applied PAs increase leaf water status, photosynthesis and membrane properties, which improves the drought tolerance of rice. Yamaguchi *et al.* (2007) found that an *Arabidopsis* mutant plant, which cannot produce Spm, is hypersensitive to drought and that this phenotype was cured by Spm pretreatment. Yang *et al.* (2007) suggested that the increasing of free Spd, free Spm and insoluble-conjugated Put during water stress significantly correlated with the yield maintenance ratio of rice. These studies suggested that PAs were notably related to the drought resistance and seed germination of plants.

Plant hormones play important roles in regulating seed germination. Absciscic acid (ABA) inhibited the seed germination of plants such as *Arabidopsis thaliana* (Kucera *et al.* 2005; Muller *et al.* 2006). Graeber *et al.* (2010) suggested that ABA delayed the radicle expansion, which inhibited seed germination. Gibberellins (GA) antagonized the effect of ABA on seed germination (Miransari and Smith 2014), which stimulated the synthesis and production of hydrolases, especially  $\alpha$ -amylase, resulting in the germination of seeds

(Yamaguchi 2008). Auxins and cytokinins (CTK) are also involved in the regulation of seed germination. CTKs are active during all stages of seed germination (Chiwocha *et al.* 2005; Nikolic *et al.* 2006; Riefler *et al.* 2006). MicroRNA60 inhibits auxin response factor 10 during *Arabidopsis thaliana* seed germination, which allows seed germination (Liu *et al.* 2007).

There was a significant relationship between PAs and hormones on the regulation of plant growth. PAs and ethylene share the biosynthetic precursor S-adenosyl-L-methionine (SAM), and increases in Spd and Spm biosynthesis are likely to affect the rate of ethylene synthesis (Walden *et al.* 1997; Liang and Lur 2002). Exogenous Spd and Spm significantly increased the zeatin (Z)+zeatin riboside (ZR) content in rice grains (Yang *et al.* 2008). Both PA and ABA are involved in the response of grape rootstocks to salinity (Upreti and Murti 2010).

These studies provide clear evidence that PAs significantly affect the seed germination and the drought resistance of plants. However, little is known about the effect of PAs on the seed germination of wheat under drought stress. Pieruzzi *et al.* (2011) suggested that the effect of PA on the seed germination of *Araucaria angustifolia* (Gymnosperm) and *Ocotea odorifera* (Angiosperm) was significantly related to indole-3-acetic acid (IAA) and ABA. However, it is not known whether the effect of PA on seed germination of wheat is related to these hormones.

In our previous study, we found that the seed germination of different wheat cultivars was significantly different under drought stress (Xu *et al.* 2014). In the present study, two experiments were conducted: (1) six wheat cultivars, the seed germination of which varied in drought resistance, were used, and we measured the variation of Spm, Spd and Put in seed during seed germination induced by different water treatments; and (2) external Spd, Spm and Put were used for seed soaking, and the variation of hormones, total soluble sugar and starch in seed were measured during the seed germination under different water treatments. The purpose of the present study was to investigate the effect of PA on wheat seed germination under drought stress and the relationship of PA, hormones and starch during wheat seed germination induced by drought stress.

## 2. Materials and methods

### 2.1. The first experiment

Six wheat cultivars, the seed germination of which was different in drought resistance, were used. Changhan 58, Lunxuan 988 and Xiza 5 are the drought-resistant cultivars, and Wanmai 52, Luomai 18 and Zhengmai 7698 are the non-resistant cultivars (Xu *et al.* 2014). Uniform seeds



of each wheat cultivar were selected and soaked in 2.5% sodium hypochlorite for 10 min for surface sterilization, and washed with distilled water following the protocol of Li *et al.* (2013). Two pieces of filter paper, with a 14-cm diameter, were placed in each petri dish. Polyethylene glycol (MW 6000, PEG-6000, 15% w/v) was used to stimulate the water stress. An equal volume of distilled water (CK) or 15% PEG-6000 (DT) was added to soak the paper. Then, 50 seeds were placed in each dish.

The growth temperatures were 22°C/18°C (day/night), the day and night times were 12 h/12 h, and the illumination intensity was 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The experiment used a completely randomized block design with three replicates for each treatment. Samples were harvested at 0, 1, 2, 3, 4, and 5 d after sowing for endogenous PA measurements. At 7 d post sowing the samples were harvested to determine the seed germination rate and the length and weight of the coleoptile and radicle.

## 2.2. The second experiment

Two wheat cultivars, Changhan 58 (drought-resistant at seed germination stage) and Luomai 18 (drought-susceptible at seed germination stage), were used. Seed surface sterilization was the same as in the first experiment. Before germination, three treatments were set by presoaking the seeds in 0.1 mmol L<sup>-1</sup> Spm, Spd and Put for 6 h. The Spm, Spd and Put concentrations used here were proven to be optimal in preliminary experiments (Du *et al.* 2010). Seeds pre-soaked with distilled water were used as the control.

Two pieces of filter paper, with a diameter of 14 cm, were placed in each petri dish. Polyethylene glycol (MW 6000, PEG-6000) was used to stimulate the water stress. An equal volume of distilled water (T1), 15% PEG-6000 (T2) or 20% PEG-6000 (T3) was added to soak the paper. Then, 50 seeds were placed in each dish. The growth environment was same as the first experiment. The experiment used a completely randomized block design, with three replicates for each treatment. Samples were harvested at 2 and 4 d post sowing, and the total soluble sugar, starch and hormone were measured. At 7 d post sowing, the samples were harvested to determine the seed germination rate and the length of the coleoptile and radicle.

## 2.3. Germination rate (GR) and germination index (GI)

At 7 d after sowing, GR was measured and defined as the percentage of germinated seeds in each Petri dish.  $GI = \sum(G_i/T_i)$ , where  $G_i$  is the germination percentage at the  $i$ th d and  $T_i$  is the day of the germination test (Wang *et al.* 2004; Li *et al.* 2013). If the length of the radical and coleoptile reached the full and half length of the seed, respectively, the seed

was defined as germinated.

## 2.4. Radical and coleoptile growth of germinating seeds

The length and dry weight of the radical and coleoptile were measured at 7 d post sowing. Coleoptile, radicle and the seed residues of germinating seeds were isolated and killed at 105°C for 2 h and then dried at 70°C until they reached a constant weight.

## 2.5. Drought resistance index

The drought-tolerant coefficient (DC) of each trait measured was calculated as:

$$DC = \frac{X_{ijDT}}{X_{ijCK}}$$

The drought resistance indexes (DI) of each trait measured were calculated as:

$$DI = DC \times \frac{X_{ijDT}}{X_{ijCK}}$$

$X_{ijDT}$  and  $X_{ijCK}$  were the values of trait ( $j$ ) for the genotype ( $i$ ) evaluated under DT (15% PEG-6000 was used for seed germination) and CK (the distilled water was used for seed germination) treatments, respectively (Xu *et al.* 2014).

## 2.6. Extraction and quantification of PAs

Spd, Spm and Put were extracted and measured according to Yang *et al.* (2008) and Liu *et al.* (2002). Briefly, approximately 0.5 g fresh weight (FW) of samples was homogenized in 5 mL of 5% (v/v) perchloric acid (PCA), and the homogenates were incubated at 5°C for 1 h. Then, they were centrifuged at 25 000g for 20 min. The supernatant and pellet were collected separately. The supernatant was used to extract the free PAs and soluble-conjugated PAs. The pellet was used for insoluble-conjugated PA extraction (dissolved by 1 mol L<sup>-1</sup> NaOH). To extract soluble-conjugated and insoluble-conjugated PAs, 2 mL of 5% PCA supernatant and NaOH supernatant were acid hydrolyzed by 2 mL of 12 mol L<sup>-1</sup> HCl and resuspended in 0.5 mL of 5% (v/v) PCA, respectively.

PAs in the non-hydrolyzed supernatant, hydrolyzed supernatant and hydrolyzed pellet were derived with benzoyl chloride and quantified with a High-Performance Liquid Chromatography System (Waters 1525 Binary HPLC Pump/2489 UV Detector, Waters, USA). Exactly 20  $\mu\text{L}$  of each sample was injected and loaded onto a waters Symmetry C18 column (7.5 cm×4.6 mm, 3.5  $\mu\text{m}$ ). The column temperature was 30°C, with a flow rate of 0.7 mL min<sup>-1</sup>. Polyamine peaks were detected at an absorbance of 230 nm.



## 2.7. Extraction and quantification of hormones

Approximately 0.5 g FW of sample was taken, and the endogenous Z+ZR, GAs ( $GA_1+GA_4$ ), IAA, and ABA were extracted according to the previous studies (Yang *et al.* 2001; Liu *et al.* 2011). The samples were homogenized with 5 mL of 80% (v/v) methanol, which contained 1 mmol  $L^{-1}$  butylated hydroxytoluene (BHT). The extracting solution was passed through Chromosep C18 columns (C18 Sep-Park Cartridge, Waters Corp., Millford, MA, USA), and the fractions were vacuum dried at 40°C and dissolved in 1 mL of phosphate-buffered saline (PBS) containing 0.1% (v/v) Tween 20 and 0.1% (w/v) gelatin (pH 7.5) for enzyme-linked immunosorbent assay (ELISA). The ELISA Kits were manufactured by the Phytohormones Research Institute, China Agricultural University. The quantification of Z+ZR, GAs ( $GA_1+GA_4$ ), IAA, and ABA was performed by ELISA as previously described (Yang *et al.* 2001; Liu *et al.* 2011). The recovery rates for IAA, Z+ZR, ABA, and GAs were (85.4±4.7), (93.1±6.2), (89.5±3.2), and (78.2±5.4)%, respectively.

## 2.8. Concentrations of starch and soluble sugars in germinating seed

To extract sugars, 0.1 g powdered dried germinating seed sample was extracted twice with 80% (v/v) ethanol at 80°C and centrifugation at 3 000×g. After centrifugation, the supernatant and pellet were collected separately. The supernatant was collected for the measurement of soluble sugar concentration measurement. To extract starch, the

pellet was dissolved by vigorous vortexing in 36 and 18 mol  $L^{-1}$  perchloric acid and centrifugation at 3 000×g. The perchloric acid supernatants were combined for starch concentration measurement. The soluble sugar and starch concentration measurements were performed using the anthrone method, 0.1 mL of the supernatant was boiled in 5 mL of anthrone- $H_2SO_4$  solution (0.15 g anthrone in 100 mL of 70%  $H_2SO_4$ ) for 20 min at 80°C, and then the absorption at 620 nm was measured (Fales 1951; Liu *et al.* 2011).

## 2.9. Statistical analysis

The results were analyzed using SPSS 16.0. The data from each sampling set were analyzed separately. The means were tested by the least significant difference method at  $P=0.05$  (LSD 0.05).

## 3. Results

### 3.1. Effect of drought on the seed germination of different wheat cultivars

Drought significantly inhibited the seed germination of wheat. At 7 d after germination, the seed germination rate, seed germination index, coleoptile and radicle length of seed, coleoptile and radicle weight of seed of drought treatment were all significantly lower than that of the control for the all six cultivars (Table 1).

However, there was a significant difference observed for the drought resistance of seed germination among the six cultivars. The DI of the seed GR and GI and the length and

**Table 1** Effects of drought on the seed germination of different wheat cultivars<sup>1)</sup>

Cultivars	Treatments <sup>2)</sup>	GR (%)	GI (%)	CL (cm)	RL (cm)	CW (mg seed <sup>-1</sup> )	RW (mg seed <sup>-1</sup> )
Luomai 18	CK	96.67 a	70.06 a	2.88 a	3.90 a	2.95 a	3.60 a
	DT	13.33 b	9.50 b	0.09 b	0.35 d	0.24 b	0.61 b
Wanmai 52	CK	92.22 a	89.65 a	2.58 a	4.33 a	3.13 a	2.36 a
	DT	15.56 b	0.23 b	0.10 b	0.22 d	0.17 b	0.36 b
Zhengmai 7698	CK	91.11 a	73.50 a	2.27 a	4.11 a	3.00 a	2.39 a
	DT	11.11 b	9.37 b	0.09 b	0.20 d	0.09 b	0.19 b
Lunxuan 988	CK	100.00 a	73.19 a	1.64 a	2.78 bc	3.41 a	3.77 a
	DT	57.78 b	18.50 b	0.83 b	2.06 c	1.02 b	2.31 b
Changhan 58	CK	97.78 a	79.61 a	3.08 a	4.14 a	3.63 a	2.93 a
	DT	72.22 b	50.39 b	1.57 b	3.37 ab	2.14 b	1.71 b
Xiza 5	CK	97.78 a	76.72 a	2.99 a	3.92 a	3.41 a	3.95 a
	DT	73.33 b	45.85 b	1.21 b	2.89 bc	1.67 b	1.90 b
Source of variation (F-value)	Genotype (G)	11.74**	57.47**	28.39**	10.55**	14.07**	12.52**
	Water (W)	186.49**	16.75**	671.91**	174.85**	372.45**	11.34**
	G×W	11.43**	498.24**	15.33**	14.68**	4.31**	12.13**

<sup>1)</sup> GR, germination rate; GI, germination index; CL, coleoptile length; RL, radical length; CW, radical weight; RW, radical weight. The same as below.

<sup>2)</sup> CK, the distilled water was used for seed germination; DT, 15% PEG-6000 was used for seed germination.

\*\*, significantly different at the 0.01 probability level. Values within a column and for the same cultivar followed by different letters are significantly different at  $P=0.05$ .



dry weight of seed coleoptile and radicle of Lunxuan 988, Changhan 58 and Xiza 5 were all significantly higher than those of Luomai 18, Wanmai 52 and Zhengmai 7698 (Table 2). This result indicates that the drought resistance of seed germination of Lunxuan 988, Changhan 58 and Xiza 5 was significantly higher than that of Luomai 18, Wanmai 52 and Zhengmai 7698.

### 3.2. Effect of drought on the changes of PA contents at germination period

The free Spd and Put contents in the germinating seeds showed increasing trends during seed germination (Fig. 1). The drought significantly affected the free Spd and Put contents in the germinating seeds. However, the response of free Spd and Put contents to drought had a notable genotype difference.

The drought-susceptible cultivars, Luomai 18, Zhengmai 7698 and Wanmai 52, had significantly lower free Spd content in seeds of the DT treatment than CK treatment at the 5th d after treatment. In contrast, the free Spd content in seeds of the DT treatment was significantly higher than that of the CK treatment at the 2nd d after treatment for the drought-resistant cultivars, Changhan 58, Xiza 5 and Lunxuan 988. In addition, the free Spd content in seeds of drought-resistant cultivars was significantly higher than that of drought-susceptible cultivars during the 2nd to 5th d after treatment.

The change in free Put of seeds induced by drought stress was different from that in the free Spd. For the drought-resistant cultivars, the free Put content in seeds of DT treatment was lower than that of CK at the 5th d after treatment. In contrast, the free Put content in seeds of the DT treatment was significantly higher than that of the CK treatment at the 2nd d after treatment for the drought-susceptible cultivars. In addition, the free Put content in the seeds of drought-resistant cultivars was significantly lower than that of drought-susceptible cultivars during the 2nd to 5th d after treatment.

During seed germination, the soluble- and insoluble-conjugated Spd and Put contents in the germinating seeds showed increasing trends (Figs. 2 and 3). Drought stress significantly promoted the soluble- and insoluble-conjugated

Spd and Put contents in seeds. The soluble- and insoluble-conjugated Spd contents in seeds of the DT treatment were significantly higher than those of the CK treatment during the 2nd to 4th d after treatment, and the soluble- and insoluble-conjugated Put contents in seeds of the DT treatment were significantly higher than those of the CK treatment on the 2nd and 3rd d after treatment.

### 3.3. Effect of external PA on the seed germination of wheat under drought stress

Drought stress significantly inhibited the seed germination of wheat. A notable difference was observed in seed germination between the two wheat varieties under drought stress (Fig. 4). Under normal conditions, there was no significant difference in seed germination between Changhan 58 and Luomai 18. However, at 15% PEG-6000 and 20% PEG-6000 conditions, the germination rate, coleoptile and radicle length of Changhan 58 were significantly higher than that of Luomai 18.

External PA also significantly affects wheat seed germination, and there was a notable difference in the effects of Spd, Spm and Put on the seed germination under different water treatments. The T1 treatment (no water stress), the external Spd, Spm and Put had no significant effects on the seed germination rate of wheat. At T2 (15% PEG-6000) and T3 (20% PEG-6000) treatments, external Spd and Spm significantly promoted the seed germination rate compared to the control but external Put had no significant effect on the seed germination rate of wheat. In addition, external Spd and Spm significantly promoted the coleoptile and radicle growth of wheat seeds. The coleoptile and radicle length of the Spd and Spm treatments were significantly higher than those of the control treatment at all water conditions. However, no significant difference was observed for the coleoptile or radicle length between Put and the control treatment in all water conditions.

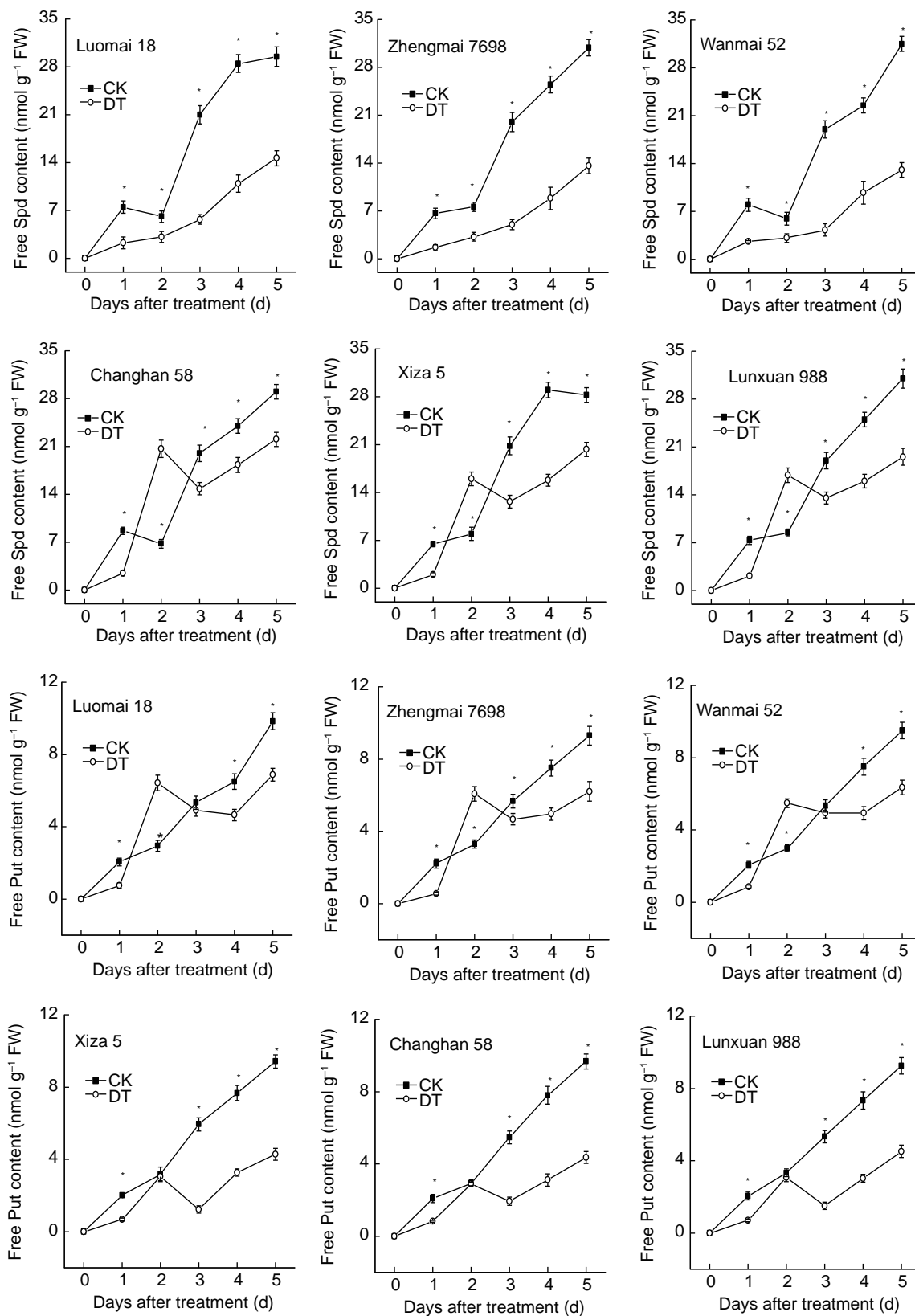
### 3.4. Effect of external PA on the hormonal changes during seed germination under drought stress

With increasing PEG-6000 concentration, the GAs, IAA and Z+ZR contents in seeds decreased (Figs. 5–7). However,

**Table 2** Drought resistance index (DI) of the different wheat cultivars at germination period

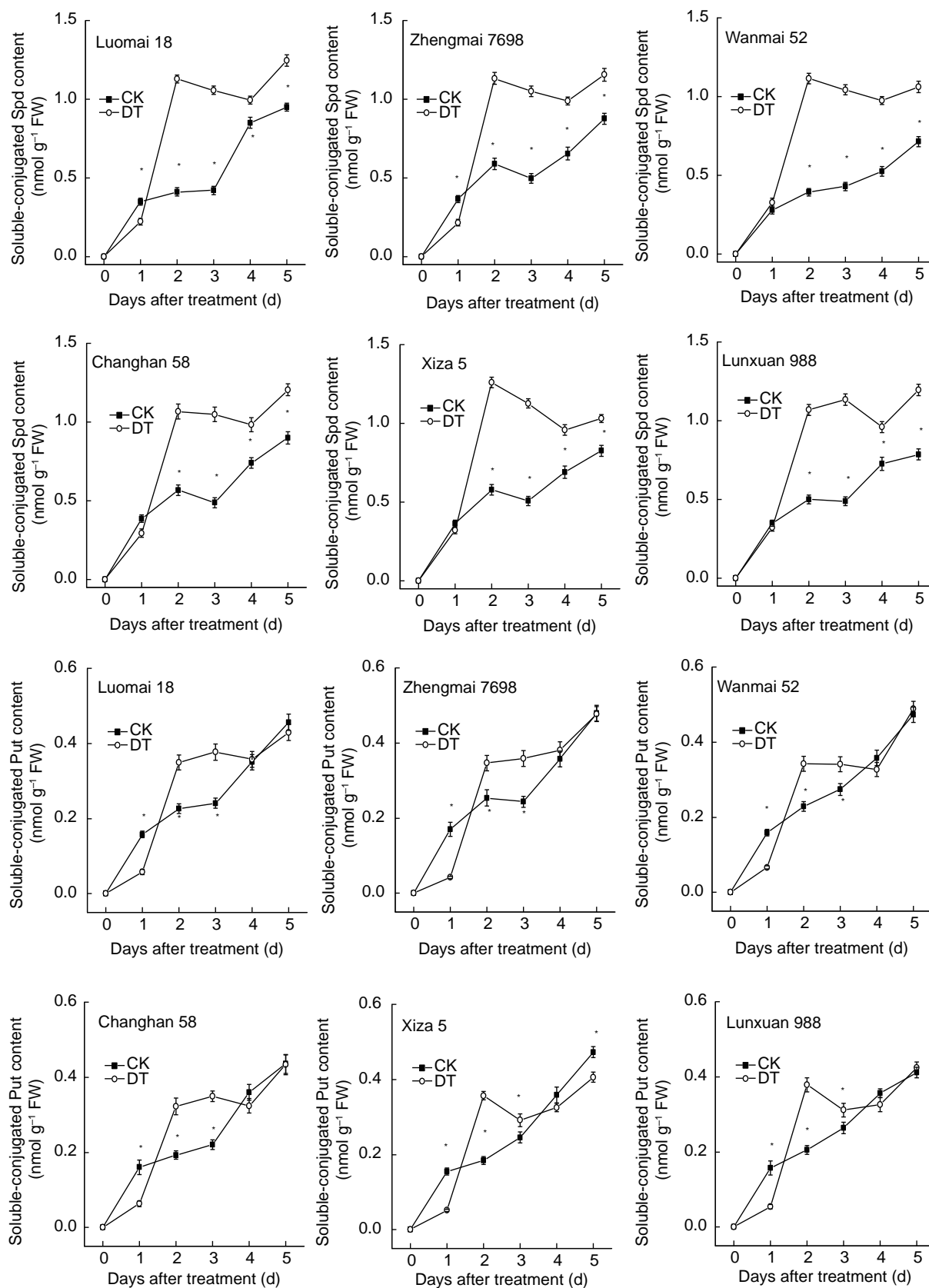
Cultivar	GR	GI	CL	RL	CW	RW
Luomai 18	0.019	0.015	0.001	0.008	0.006	0.033
Wanmai 52	0.027	0.014	0.002	0.003	0.003	0.018
Zhengmai 7698	0.014	0.009	0.001	0.003	0.001	0.005
Lunxuan 988	0.348	0.255	0.163	0.395	0.255	0.448
Changhan 58	0.556	0.413	0.311	0.71	0.389	0.315
Xiza 5	0.573	0.364	0.190	0.552	0.252	0.288





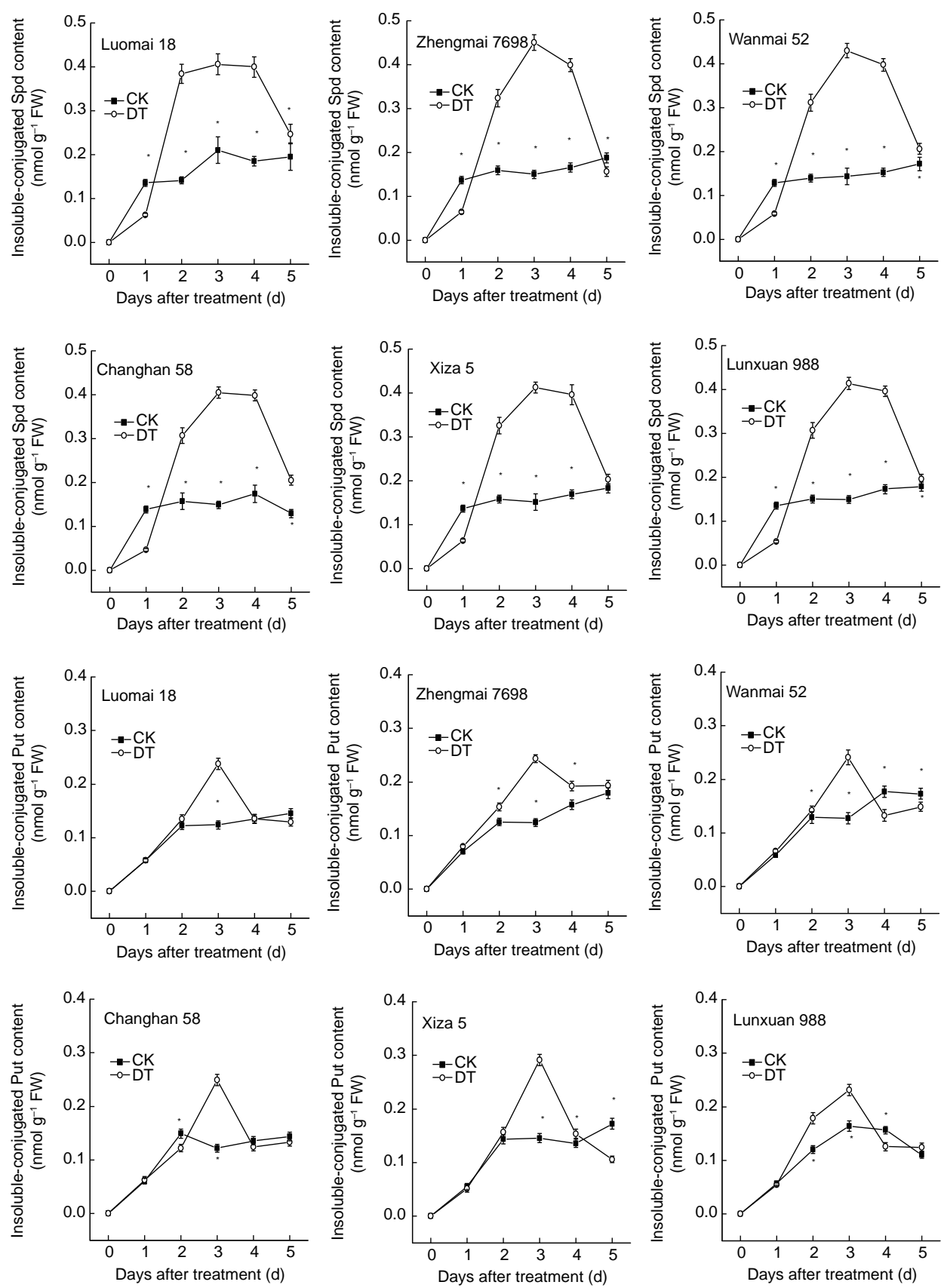
**Fig. 1** Changes in free spermidine (Spd) and putrescine (Put) contents in wheat seeds during seed germination under drought stress. CK, distilled water was used for seed germination; DT, 15% PEG-6000 was used for seed germination. Vertical bars represent the standard error of the mean ( $n=3$ ). \*, values for the same days were significantly different at  $P=0.05$ . The same as below.





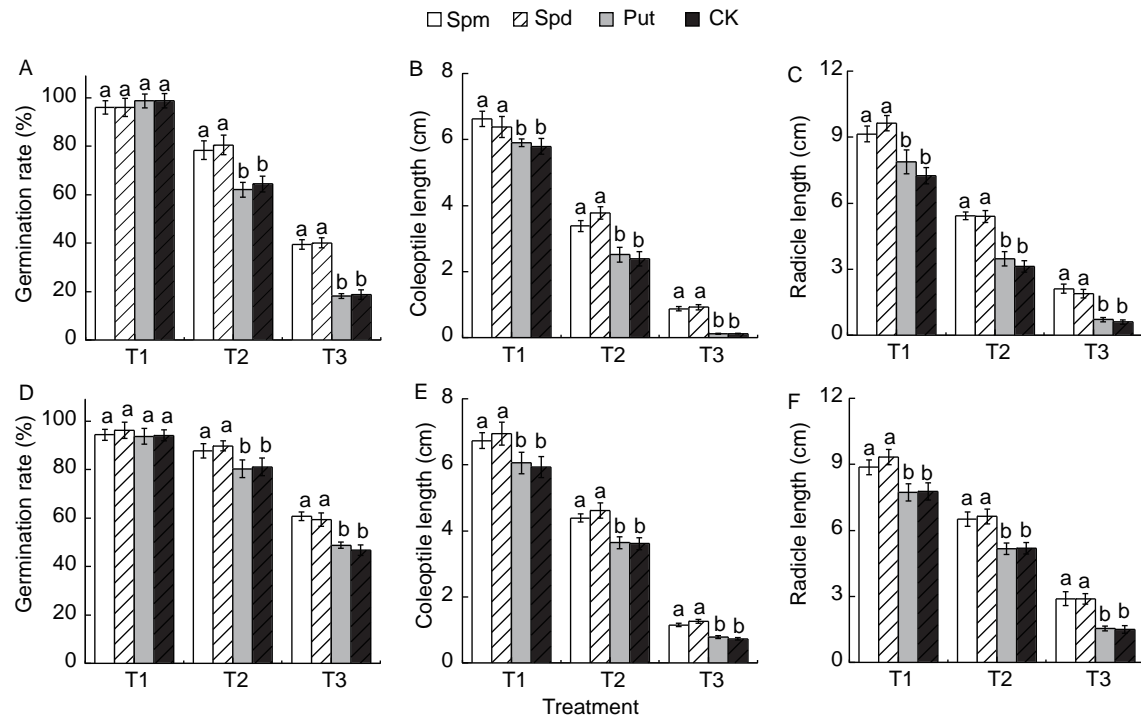
**Fig. 2** Changes in soluble-conjugated Spd and Put contents in wheat seeds during seed germination under drought stress.



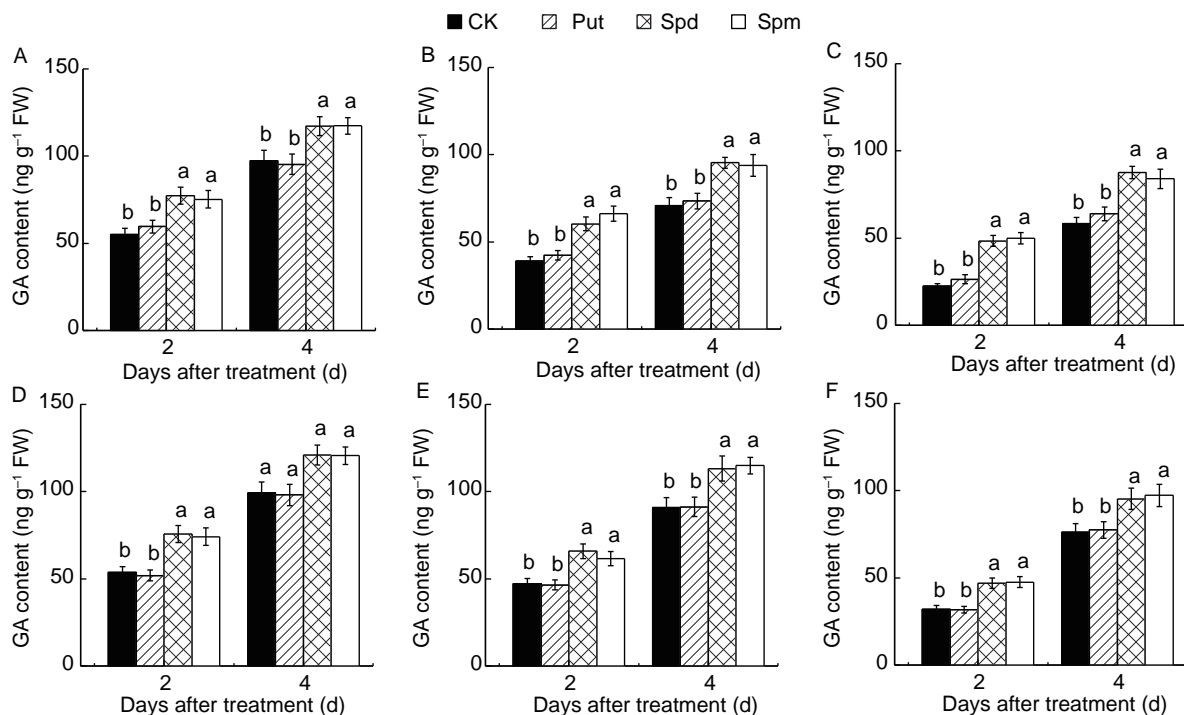


**Fig. 3** Changes in insoluble-conjugated Spd and Put contents in wheat seeds during seed germination under drought stress.



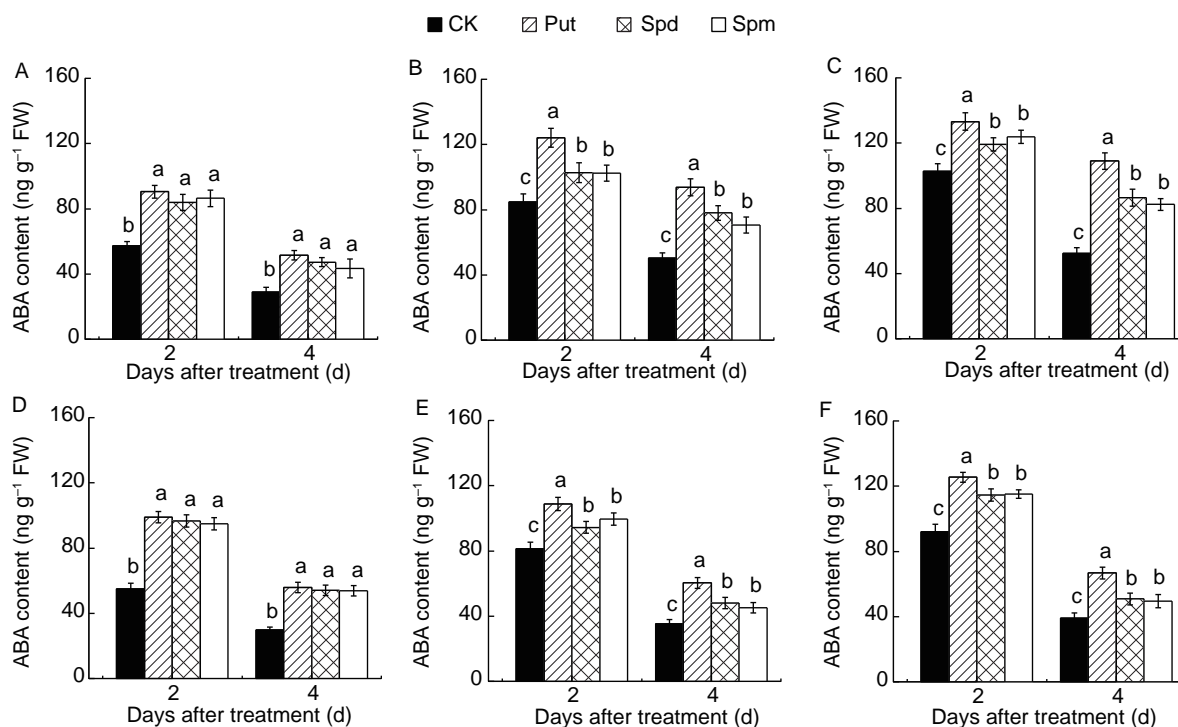


**Fig. 4** Effect of polyamine (PA) soaking on the seed germination rate, coleoptile and radicle length of different wheat cultivars (A, B and C, Luomai 18; D, E and F, Changhan 58) under drought stress. T1, distilled water was used for seed germination; T2, 15% PEG-6000 was used for seed germination; T3, 20% PEG-6000 was used for seed germination. Spm, Spd and Put: presoaking the seeds in 0.1 mmol L<sup>-1</sup> Spm, Spd and Put for 6 h. Values for the same water treatment followed by different letters are significantly different at  $P=0.05$ . The same as below.

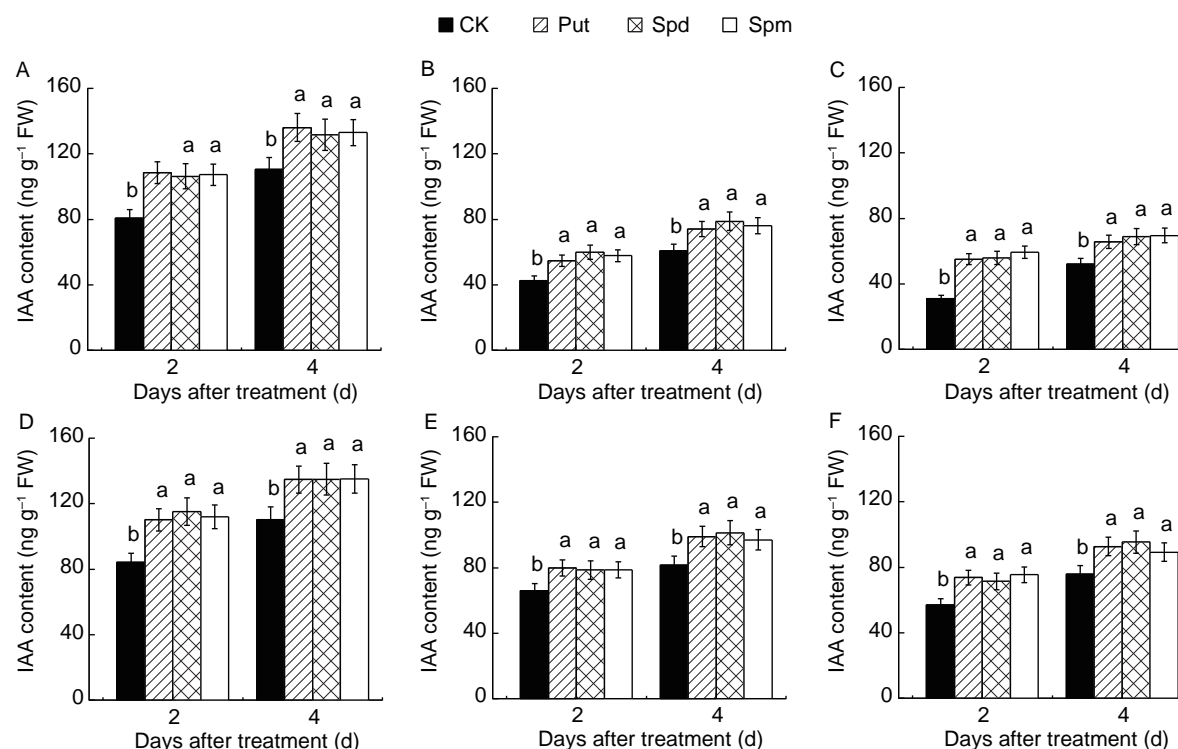


**Fig. 5** Effect of PA soaking on the change in gibberellins (GA) contents in wheat seeds during the seed germination of different wheat cultivars (A, B and C, Luomai 18; D, E and F, Changhan 58) under drought stress. A and D, distilled water was used for seed germination. B and E, 15% PEG-6000 was used for seed germination. C and F, 20% PEG-6000 was used for seed germination.





**Fig. 6** Effect of PA soaking on the change of abscisic acid (ABA) contents in wheat seeds during seed germination of different wheat cultivars (A, B and C, Luomai 18; D, E and F, Changhan 58) under drought stress. A and D, distilled water was used for seed germination. B and E, 15% PEG-6000 was used for seed germination. C and F, 20% PEG-6000 was used for seed germination.



**Fig. 7** Effect of PA soaking on the change of indole-3-acetic acid (IAA) contents in wheat seeds during seed germination of different wheat cultivars (A, B and C, Luomai 18; D, E and F, Changhan 58) under drought stress. A and D, distilled water was used for seed germination. B and E, 15% PEG-6000 was used for seed germination. C and F, 20% PEG-6000 was used for seed germination.



the ABA content in seeds increased with increasing PEG-6000 concentration (Fig. 8). There was a notable difference observed for the hormone contents in seeds during seed germination between the two wheat varieties under drought stress. There was no significant difference for the GAs, IAA, ABA, or Z+ZR contents in seeds during seed germination between any water stress levels in the Changhan 58 and Luomai 18 cultivars. However, the GAs, IAA and Z+ZR contents in seeds of Changhan 58 were higher than those of Luomai 18 at 15 and 20% PEG-6000 conditions.

External PA significantly affected the hormone content in seeds during seed germination. External Spd and Spm significantly increased the GAs, IAA and Z+ZR contents in seeds at the 2nd and 4th d after treatment. External Put had no significant effects on the GA or Z+ZR contents in seeds, but it significantly increased the IAA content in seeds at the 2nd and 4th d after treatment. In contrast to these hormones, external Spd, Spm and Put all significantly increased the ABA content in seeds; however, the ABA contents in seeds of Spd and Spm treatments were significantly lower than those of Put treatment. The ABA content in seeds of Put treatment was significantly higher than that of Spd, Spm and control treatments at the 2nd and 4th d after treatment, and there was no significant

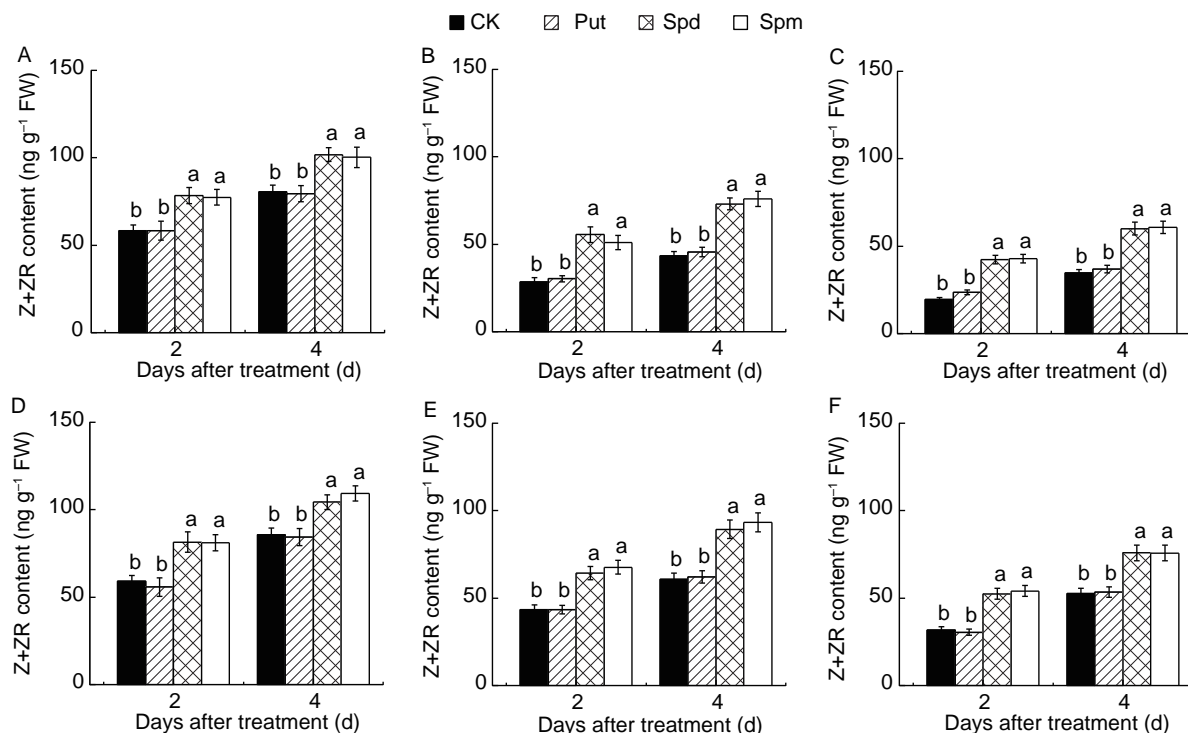
difference observed for the ABA content in seeds of Spd, Spm or control treatments.

### 3.5. Effects of pre-soaking with PA on starch and total soluble sugar in seed

The total soluble sugar concentration in seeds had an increasing trend, and the starch concentration in seeds had a decreasing trend during seed germination (Figs. 9 and 10). Drought stress decreased the total soluble sugar concentration in seeds and increased the starch concentration in seeds. External Spd and Spm significantly increased the total soluble sugar concentration in seeds and significantly decreased the starch concentration in seeds during seed germination. However, external Put had no significant effect on the total soluble sugar or starch concentration in seeds during germination.

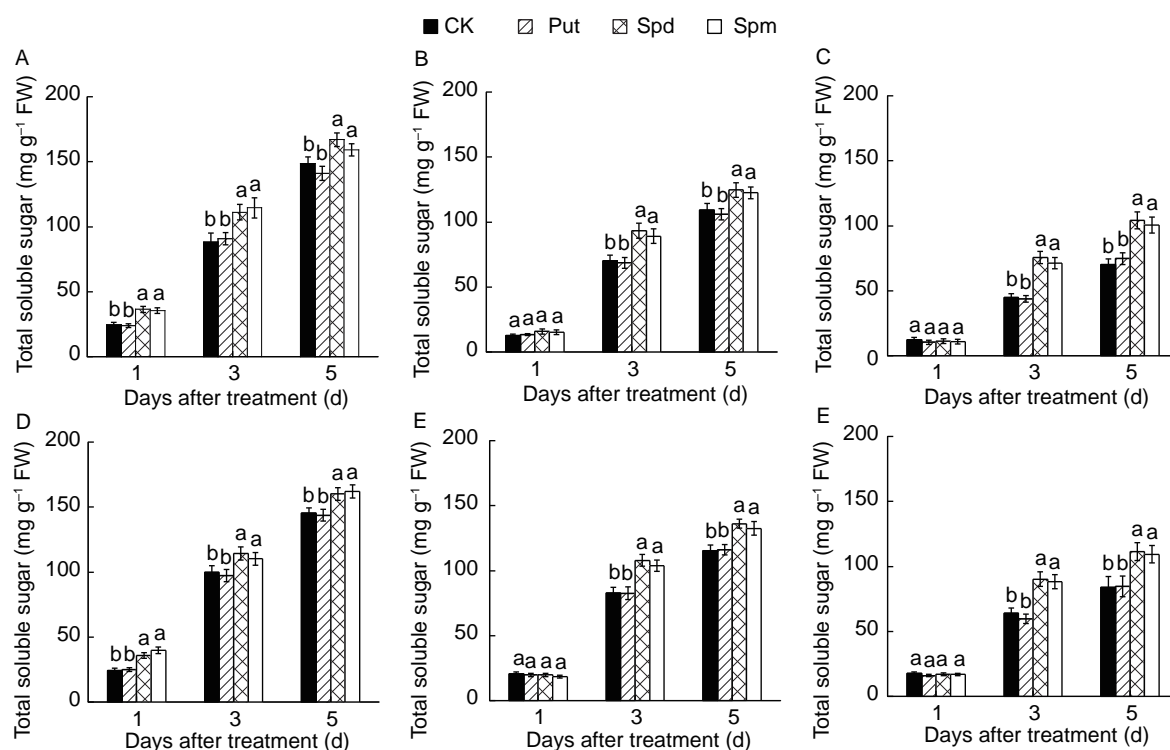
## 4. Discussion

Drought is a multifaceted stress condition that inhibits crop growth and causes serious crop yield limitations (Muscolo *et al.* 2014). Seed germination is the most critical and sensitive stage in the life cycle of plants (Ahmad *et al.* 2009)

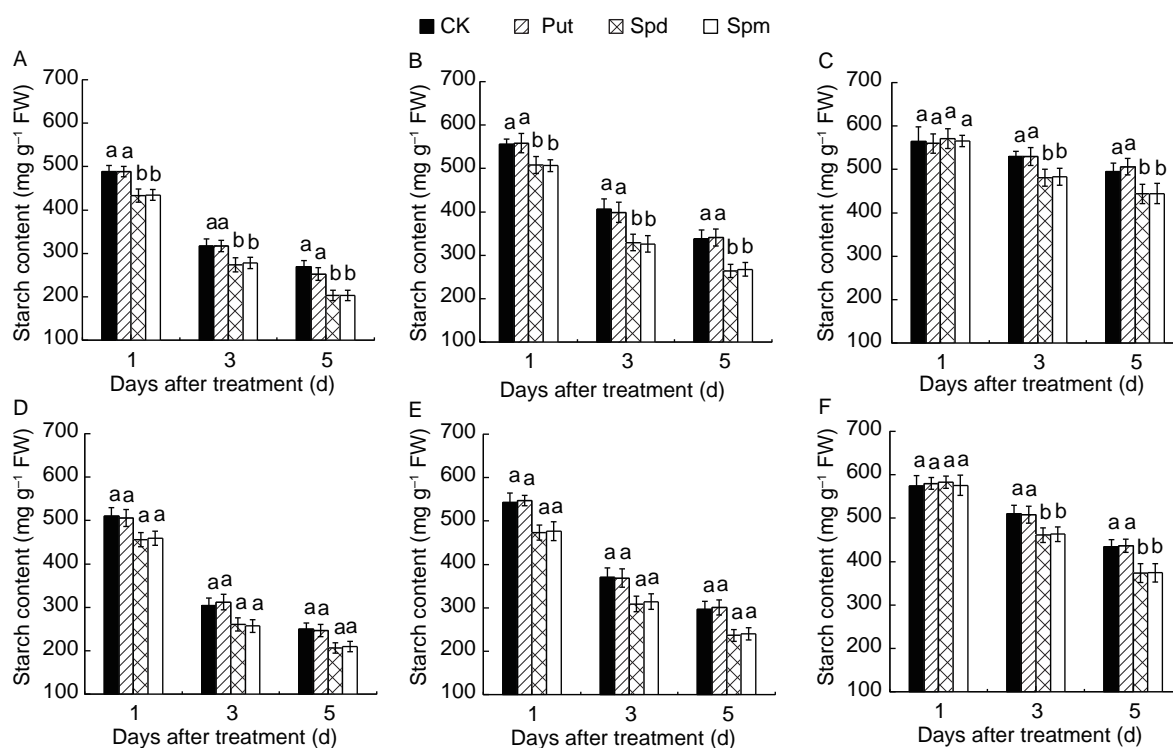


**Fig. 8** Effect of PA soaking on the change of zeatin (Z)+zeatin riboside (ZR) contents in wheat seeds during seed germination of different wheat cultivars (A, B and C, Luomai 18; D, E and F, Changhan 58) under drought stress. A and D, distilled water was used for seed germination. B and E, 15% PEG-6000 was used for seed germination. C and F, 20% PEG-6000 was used for seed germination.





**Fig. 9** Effect of PA soaking on the change of total soluble sugar content in wheat seeds during seed germination of different wheat cultivars (A, B and C, Luomai 18; D, E and F, Changhan 58) under drought stress. A and D, distilled water was used for seed germination. B and E, 15% PEG-6000 was used for seed germination. C and F, 20% PEG-6000 was used for seed germination.



**Fig. 10** Effect of PA soaking on the change of starch content in wheat seeds during seed germination of different wheat cultivars (A, B and C, Luomai 18; D, E and F, Changhan 58) under drought stress. A and D, distilled water was used for seed germination. B and E, 15% PEG-6000 was used for seed germination. C and F, 20% PEG-6000 was used for seed germination.



and is a process that is inhibited or even entirely prevented by drought (Hubbard *et al.* 2012). PAs are closely associated with plant resistance to drought stress (Groppa and Benavides 2008). The manipulation of endogenous PA metabolism or external application of PA could affect the resistance of crops to drought stress (Capell *et al.* 2004; Farooq *et al.* 2009). In the present study, there was no significant difference observed in the free Spd or Put contents in seeds during seed germination between different cultivars under normal conditions. However, the free Spd content in seeds of drought-resistant cultivars was significantly higher than that of drought-susceptible cultivars under drought treatment during seed germination, but the free Put content in seeds of drought-resistant cultivars was significantly lower than that of drought-susceptible cultivars during seed germination under drought treatment. These results suggested that free Spd accumulation in seeds during the germination period favors wheat seed germination under drought stress; however, free Put accumulation in seeds during the seed germination period may work against wheat seed germination under drought stress. In addition, presoaking the seeds in Spd significantly promoted the wheat seed germination and coleoptile and radicle growth under drought stress. Presoaking the seed in Put had no significant effect on seed germination and even inhibited the coleoptile and radicle growth under drought stress. Put is the precursor of Spd synthesis, but the effects of Spd and Put on plant growth and development can differ (Yang *et al.* 2008; Liu *et al.* 2013). Previous studies suggested that the Spd could relieve drought inhibition of plant growth. Overexpression of Spd synthase enhances tolerance to multiple environmental stresses including drought and up-regulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana* (Kasukabe *et al.* 2004).

A transgenic rice plant, in which the PA biosynthetic gene was modulated to promote the Spd content in plants, had better drought tolerance (Capell *et al.* 2004). The free Spd content in flag leaves had a significant and positive correlation to the yield maintenance ratio of rice (Yang *et al.* 2007). In contrast to Spd, the role of Put in drought stress responses of plants is also unclear (Bouchereau *et al.* 1999; Chen and Zhang 2000; Capell *et al.* 2004).

Previous studies suggest that a high accumulation of Put at the early stages of drought is necessary for plants to adapt to stress. However, excessive or long-term accumulation of Put may promote oxidation products and may be toxic to plants (DiTomaso *et al.* 1989; Watson and Malmberg 1996; Richard and Alexandra 1997). The promotion of the synthesis of Spd and Spm from Put can improve the drought resistance of transgenic rice plants. In contrast, wild-type plants were drought-sensitive because drought is

insufficient to trigger the conversion of Put to Spd and Spm, even though the wild-type plants had high Put accumulation under drought stress (Capell *et al.* 2004). Combined with previous studies, we suggest that the reason for the results of the present study may be that the synthetic route from Put to Spd in drought-susceptible wheat cultivars may be inhibited by drought and that this route of drought-resistant cultivars may have more resistance to drought stress than that of drought-susceptible cultivars. The drought-susceptible wheat cultivars accumulated more free Put than the drought-resistant cultivars, and the free Spd content of drought-susceptible wheat cultivars was significantly less than that of drought-resistant cultivars. The seed germination of drought-resistant cultivars had more resistance for drought stress than that of drought-susceptible cultivars.

The previous study suggested that like Spd, Spm could notably relieve the inhibition of plant growth by drought stress (Yang *et al.* 2007). However, in the present study, we found an interesting result, endogenous Spm cannot be detected during the seed germination of wheat. However, external presoaking of seeds in Spm significantly promoted seed germination under drought stress. This result suggested that Spm could relieve the inhibition of wheat seed germination by drought stress; however, it is not the dominant factor for wheat seed germination.

In addition to free PA, soluble- and insoluble-conjugated PAs are also involved in the regulation of plant growth (Yang *et al.* 2007). In the present study, the soluble- and insoluble-conjugated Spd and Put in seeds showed no significant difference between the various cultivars during seed germination under drought stress. In addition, the values of the soluble- and insoluble-conjugated Spd and Put were significantly less than those of free Spd and Put. We suggest that the soluble- and insoluble-conjugated PA may be involved in the regulation of seed germination. However, they are not the main factors governing seed germination under drought stress.

It is reported that PA and hormones interact to regulate the growth and development of plants (Smith *et al.* 1985; Kusano *et al.* 2007). Yang *et al.* (2008) indicated that external Spd and Spm promoted the endogenous CTK content of rice grains. Steiner *et al.* (2007) indicated that PA increased endogenous ABA levels. Our previous study found that external PA significantly increased the IAA content in wheat grains (Liu *et al.* 2013). Plant hormones significantly affected seed germination. ABA inhibited the seed germination of plants such as *Arabidopsis thaliana* (Kucera *et al.* 2005; Muller *et al.* 2006). GA antagonized the effect of ABA on seed germination (Miransari and Smith 2014). In addition, auxins and CTK also regulated seed germination (Chiwocha *et al.* 2005; Nikolic *et al.* 2006; Riefler *et al.* 2006; Liu *et al.*



2007). The results of the present study suggested that the promotion of the seed germination of wheat by Spd or Spm might relate to their effects on the endogenous hormones. External Spd and Spm significantly increased endogenous IAA, Z+ZR and GA contents in seeds during seed germination and may promote the wheat seed germination and coleoptile and radicle growth under drought stress. In addition, Put, Spd and Spm all significantly increased the ABA content in seeds under drought stress; however, the ABA content in seeds after Put treatment was significantly higher than that of Spd and Spm treatments under drought stress. ABA accumulation can promote the drought resistance of plants; however, the excessive accumulation of ABA in plants may reduce the drought resistance of plants (Yang *et al.* 2006). We suggested that the promotion effect of seed germination under drought stress by Spd and Spm might relate to the promotion of ABA accumulation in seeds. However, Put treatment lead to excessive ABA accumulation in seeds and reduced the drought resistance of the seed germination of wheat. The previous study suggested that IAA significantly promoted the seed germination and growth (Jung and Park 2011; Park *et al.* 2011). The present study suggested that the Spd, Spm and Put all significantly increases the IAA contents in seeds under drought. However, there was a notably difference for the effects of Spd, Spm and Put on the seed germination under drought stress. This means that the seed germination and growth of wheat was not be only regulated by IAA. In the present study, IAA, CTK, ABA, Gas, and polyamines were all involved in the regulation of seed germination under drought stress. This means that the balance of hormones, rather than the individual hormones, regulated the seed germination of wheat under drought stress.

In addition, the external Spd and Spm exerted considerable effects on starch metabolism under drought stress. The promotion of seed germination by Spd and Spm was closely associated with rapid seed starch degradation along with a fast increase in the concentration of soluble sugars in germinating seeds under drought. The previous study suggested that Spd and Spm significantly affect the starch metabolism of grains during rice grain filling (Yang *et al.* 2008). In addition, a previous study suggested that various hydrolytic enzymes are synthesized and involved in starch mobilization during seed germination (Zhang *et al.* 2005) and that the production of most hydrolytic enzymes is GA-dependent (Lovegrove and Hooley 2000; Zhang *et al.* 2010). In the present study, external Spd and Spm significantly increased the endogenous GA content in seeds. The effects of Spd and Spm on starch degradation may relate to GAs, or Spd and Spm immediately affect starch degradation. Further study is needed to answer this question.

## 5. Conclusion

Free Spd accumulation in seeds during the seed germination period favored wheat seed germination under drought stress; however, free Put accumulation in seeds during the seed germination period may reduce wheat seed germination under drought stress. In addition, soaking seeds in Spd and Spm significantly relieve the inhibition of seed germination by drought stress. However, soaking seeds in Put had no significant effect on seed germination under drought. External Spd and Spm significantly increased the endogenous IAA, Z+ZR, ABA, and GA contents of seeds and accelerated seed starch degradation and increased the concentration of soluble sugars in seeds during seed germination, which may promote wheat seed germination under drought stress.

## Acknowledgements

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# Effect of non-structural carbohydrate accumulation in the stem pre-anthesis on grain filling of wheat inferior grain



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## ARTICLE INFO

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## ABSTRACT

To promote the grain weight of wheat, the key is to improve the grain filling of inferior grain. The remobilization of non-structural carbohydrate (NSC) stored in reserve in the stem (sheath + culm) pre-anthesis is important for the grain filling of cereal. However, the mechanism that regulates the NSC reserve in the stem pre-anthesis on grain filling of inferior grains of wheat remains unclear. In the present study, six wheat cultivars with different grain weight were used. Additionally, different rates of nitrogen (N) fertilizer were examined. The objective of the present study was to investigate the relation between the NSC reserve in the stem pre-anthesis and grain filling of inferior grain of wheat and determine the mechanism of that relation. The results showed that the grain weight of inferior grain was significantly lower than that of superior grain, and the inferior grain was more affected by environmental factors than the superior grain. The superior grain had higher content of zeatin (Z) + zeatin riboside (ZR) and activities of synthase (SS) and ADP-glucose pyrophosphorylase (AGPP) and lower rate of ethylene (ETH) evolution than those of inferior grain. Because of these differences, the superior grain had higher sink strength and grain-filling rate and therefore had higher grain weight than that of inferior grain. With a high NSC reserve stored in the stem pre-anthesis, the Z + ZR content increased and the ETH evolution rate decreased in inferior grain significantly, which promoted the sink strength and grain-filling rate of inferior grain of wheat. Suitable nitrogen (N) fertilizer application notably increased the NSC reserve stored in the stem pre-anthesis, which promoted the sink strength and grain-filling rate of inferior grain. However, excess N fertilizer significantly decreased the NSC accumulation in the stem pre-anthesis and inhibited the sink strength and grain-filling rate of inferior grain of wheat.

## 1. Introduction

Wheat (*Triticum aestivum* L.) is an important crop in China. With increasing population and decreasing area of agricultural land, promoting crop production is essential in China. For wheat, the grain yield is separated into three components, and an effective approach to promote the grain yield of wheat is to increase the contributions of these components. However, the panicle number per plant has likely reached the highest level and continuing to increase the number may lead to serious problems such as lodging, premature senescence, and increased damage by diseases and insects, among others. Thus, the inevitable approach to increase the grain yield of wheat is to increase the grain weight or grain number per panicle, based on a suitable panicle number per area. Grain filling of cereals determines the grain weight, and therefore, improving grain filling is important for high grain weights and grain yields of cereals such as wheat (Chen et al., 2013; Yang and

Zhang, 2010; Kato et al., 2007).

According to the grain filling characteristics, the grain of wheat is divided into two types, superior and inferior grain. Superior grain primarily consists of earlier flowering flowerets and is located at the bottom of a spikelet, and the inferior grain primarily consists of later flowering flowerets and is located at the top of a spikelet of wheat (Jiang et al., 2003). In a previous study, inferior grain had higher temporal and spatial variation than that of superior grain, and the inferior grain was more sensitive to environmental factors (Yang, 2010). The poor grain filling of inferior grain is the primary explanation for the inability of large panicle rice cultivars to reach their high yield potentials (Yang and Zhang, 2010). Thus, the key to promote wheat grain weight and grain yield is to improve the grain filling of inferior grain.

Mechanisms have been proposed to explain the poor grain filling of inferior grain. Some previous studies suggest a priority in the photosynthate supply to the superior grain, which may lead to a deficiency of

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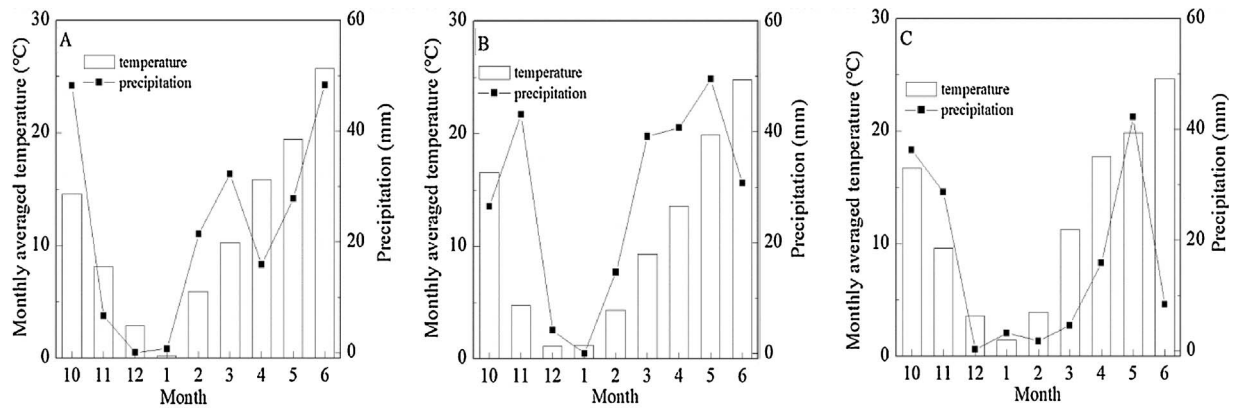


Fig. 1. The average monthly temperature and precipitation during the wheat growing seasons in experimental fields at Yangling (A. 2014–2015; B. 2015–2016) and Doukou Experimental Station (C. 2015–2016).

**Table 1**  
Grain yield and yield components of different wheat cultivars.

Year	Cultivars	No. of panicles ( $\times 10^4 \text{ hm}^{-2}$ )	Spikelets per panicle	Grain weight (mg)	Grain yield ( $\text{t hm}^{-2}$ )
2014–2015	Shuangda 1	370.02c	38.95a	57.84a	7.55ab
	Fugao 1	525.03b	37.37a	45.95b	7.91a
	Zhoumai 22	510.51b	37.58a	47.83b	7.96a
	Xiaoyan 6	615.34a	33.16b	35.85c	7.13b
	Xiaoyan 22	630.81a	36.18ab	36.34c	7.44ab
	Xinong 538	674.86a	34.56b	37.57c	7.70a
2015–2016	Shuangda 1	327.62c	40.31a	50.16a	6.95ab
	Fugao 1	493.85b	39.35a	41.24b	7.84a
	Zhoumai 22	451.46b	38.46a	42.11b	7.38a
	Xiaoyan 6	584.11a	31.67b	31.57c	6.02b
	Xiaoyan 22	601.95a	33.95b	34.61c	6.81b
	Xinong 538	612.79a	32.57b	30.15c	6.25b
F Value	Year (Y)	137.57*	0.17	57.24*	37.29*
	Cultivar (C)	619.14*	15.42*	90.070*	10.11*
	Y $\times$ C	2.792*	1.579	1.689	2.604

Values within a column and for the same year followed by different letters are significantly different at  $P = 0.05$ .

\* F values significant at the  $P = 0.05$  level.

photosynthate for the grain filling of inferior grain in rice (Murty and Murty, 1982). Kato (2004) suggest that the deficiency of sink size is the primary limitation of the grain filling of inferior grains of rice. Additionally, some studies suggest that lower activity of the enzymes that regulate the sucrose to starch conversion limits the grain filling of inferior grain, compared with the enzyme activity of superior grain (Ishimaru et al., 2005; Wang et al., 2008). However, the mechanism of the poor grain filling of inferior grain of cereals remains unclear.

Carbon and nitrogen are the primary constituent elements of wheat grain (Yu, 2003). The carbohydrates used for grain filling are supplied from two sources: current assimilation post-anthesis and remobilization of reserves stored in the stem pre-anthesis. The remobilization of non-structural carbohydrate (NSC) reserves stored in the stem (sheath + culm) pre-anthesis contributes approximately 20–30% of grain weight of wheat (Yang et al., 2004a). Horie et al. (2005) found that the reserve of NSC in the stem pre-anthesis was significantly correlated with rice growth at the early grain filling state, and Fu et al. (2011) suggested that the reserve of NSC in the stem pre-anthesis promoted the grain filling of inferior grains of rice. However, whether increasing the reserve of NSC in the stem pre-anthesis could improve the grain filling of inferior grains of wheat was unclear, and the mechanism for the regulation of reserves of NSC in the stem pre-anthesis on grain filling of inferior grains of wheat has also not been determined.

The cytokinins and ethylene (ETH) play important roles in the regulation of the grain filling of cereals. A large transient increase of

zeatin (Z) and zeatin riboside (ZR) significantly promoted the endosperm cell division of grains of wheat (Morris et al., 1993). Superior grains had a higher level of ethylene (ETH) than that of inferior grains of wheat (Yang et al., 2006). Our previous study found that plastic-covered ridge and furrow planting significantly affected the Z + ZR and ETH levels in grains, which promoted the grain filling of inferior grain of wheat (Liu et al., 2013a).

Starch is the primary component of wheat grain, accounting for approximately 65–74% of grain weight. The starch of wheat grain is derived from the NSC stored in the stem, and sucrose is the primary form of stored NSC (Yang et al., 2004b). In a previous study, sucrose synthase (SS) and ADP-glucose pyrophosphorylase (AGPP) were notably involved in the regulation of the biosynthesis from sucrose to starch in wheat grain (Jiang et al., 2003). The activities of AGPP and SS in superior grains were notably higher than those in inferior grains of rice and wheat, and AGPP and SS activities were significantly correlated with grain weight of rice and wheat (Yang et al., 2001a; Jiang et al., 2003).

In these studies, the hormones and the activities of AGPP and SS in grains were all significantly correlated with the grain filling of wheat. However, the relationship between the reserve of NSC in the stem pre-anthesis and the changes in hormones and activities of AGPP and SS in grains during grain filling of wheat remains unclear, in addition to whether the effect of the reserve of NSC in the stem pre-anthesis on grain filling of inferior grains is related to the hormones and activities of AGPP and SS in grains. In the present study, six wheat cultivars that were different for grain filling and grain weight were used, and the reserve of NSC in the stem pre-anthesis and the changes in hormones and activities of AGPP and SS in grains during grain filling were measured. Additionally, different rates of nitrogen (N) were applied to the wheat cultivars, and the activities of AGPP and SS in grains during grain filling were measured. The objective of this study was to investigate the effect of the reserve of NSC in the stem pre-anthesis on grain filling of inferior grains of wheat and determine whether an effect was related to hormones and the activities of AGPP and SS in inferior grains of wheat.

## 2. Materials and methods

### 2.1. Experiment design

#### 2.1.1. First experiment

This study was conducted from 2014 to 2016 at Northwest A & F University, Yangling, Shaanxi Province, China ( $34^{\circ}17' \text{ N}$ ,  $108^{\circ}05' \text{ E}$ ). The organic matter content and available nitrogen (N), phosphorus (P) and potassium (K) of the 0–20 cm of topsoil in the cropland were  $12.39 \text{ g kg}^{-1}$ ,  $49.85 \text{ mg kg}^{-1}$ ,  $24.63 \text{ mg kg}^{-1}$  and  $110.14 \text{ mg kg}^{-1}$ , respectively.

Six winter wheat cultivars, Shuangda 1, Fugao 1, Zhoumai 22,

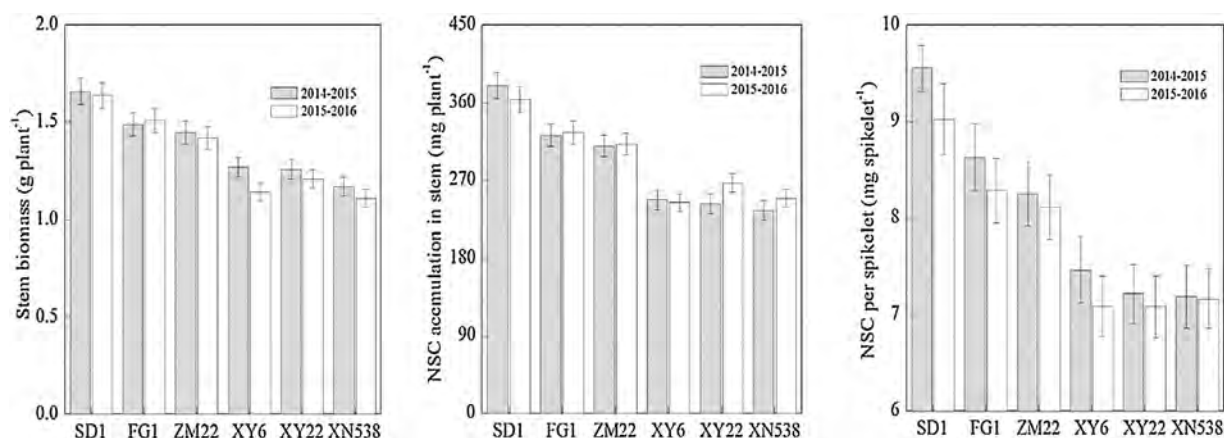


**Table 2**  
Grain-filling characteristics of different wheat cultivars.

Year	Cultivar	Superior grain			Inferior grain		
		MGR (mg grain <sup>-1</sup> d <sup>-1</sup> )	AGP (d)	GW (mg)	MGR (mg grain <sup>-1</sup> d <sup>-1</sup> )	AGP (d)	GW (mg)
2014–2015	Shuangda 1	1.94a	31.91a	62.01a	1.53a	32.28a	49.4a
	Fugao 1	1.56b	33.42a	52.08b	1.26b	33.02a	41.73b
	Zhoumai 22	1.50b	33.47a	50.36b	1.16bc	33.83a	39.09b
	Xiaoyan 6	1.26c	33.03a	41.47c	0.99cd	32.75a	32.30c
	Xiaoyan 22	1.23c	34.38a	42.25c	1.00cd	33.36a	33.25c
	Xinong 538	1.20c	33.85a	40.56c	0.93d	32.88a	30.60c
2015–2016	Shuangda 1	1.92a	31.88a	60.25a	1.34a	30.45a	40.80a
	Fugao 1	1.59b	33.03a	51.86b	1.12b	30.37a	34.01b
	Zhoumai 22	1.49b	32.99a	49.16b	1.08c	31.17a	33.66b
	Xiaoyan 6	1.27c	33.16a	40.90c	0.88d	30.64a	26.96c
	Xiaoyan 22	1.23c	33.92a	41.72c	1.02bc	29.07ab	29.65c
	Xinong 538	1.19c	33.13a	39.42c	0.85d	27.56b	23.43d
Mean	2014–2015	1.45	33.34	48.12	1.14	33.02	37.73
	2015–2016	1.45	33.02	47.22	1.05	29.88	31.42
	CV	0.01	0.69	1.34	6.17	7.06	12.91
F Value	Year (Y)	0.012	0.539	1.971	42.410*	56.008*	180.324*
	Cultivar (C)	137.755*	1.838	108.651*	121.077*	2.115	130.581*
	Y × C	0.160	0.085	0.128	3.772*	1.761	2.571

Values within a column and for the same year followed by different letters are significantly different at  $P = 0.05$ . MGR: mean grain filling rate; AGP: active grain filling period; GW: Grain weight.

\* F values significant at the  $P = 0.05$  level.



**Fig. 2.** The stem biomass, NSC accumulation in the stem and NSC per spikelet at anthesis of different wheat cultivars in the first experiment. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ). SD1, FG1, ZM22, XY6, XY22 and XN 538 are the cultivars Shuangda 1, Fugao1, Zhengmai 22, Xiaoyan 6, Xiaoyan 22 and Xinong 538, respectively.

Xiaoyan 6, Xiaoyan 22 and Xinong 538, were sown on October 16 in 2014 and 2015. The sowing rate was 150 kg ha<sup>-1</sup>, and the row spacing was 0.25 m. Urea and (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> were applied at 375 kg ha<sup>-1</sup>. All fertilizer use was at a basal level. The experiment was conducted with a complete randomized design. Each treatment had three replicates. The area of each plot was 6 m<sup>2</sup> (3 m × 2 m).

### 2.1.2. Second experiment

This study was conducted from 2015 to 2016 at the Doukou Wheat and Maize Experimental Farm of Northwest A & F University, Shaanxi Province, China (34°36' N, 108°52' E). The organic matter content and available N, P and K of the 0–20 cm of topsoil of the cropland were 15.24 g kg<sup>-1</sup>, 40.26 mg kg<sup>-1</sup>, 18.56 mg kg<sup>-1</sup> and 186.75 mg kg<sup>-1</sup>, respectively.

Five levels of N were applied: 0, 120, 180, 240 and 300 kg N ha<sup>-1</sup>. The N fertilizer was urea, which was applied at basal levels. Two wheat cultivars, Zhoumai 22 and Xinong 538, were sown on Oct 14, 2015. The sowing rate was 225 kg ha<sup>-1</sup>, and the row spacing was 0.25 m. Ordinary superphosphate at 150 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> was applied at basal levels. The experiment was conducted with a complete randomized block design. Each treatment had three replicates. The area of each plot

was 12 m<sup>2</sup> (4 m × 3 m).

The average monthly temperature and precipitation of the two experiment stations during the experimental period are shown in Fig. 1.

### 2.2. Sampling and measurement

At anthesis, twenty wheat plants from each plot were sampled. The spike, leaf and stem (sheath + culm) were separated and put into different paper bags. The spikelet per spike was measured, and then the spike, leaf and stem were deactivated of enzymes and dried at 70 °C to a constant weight and weighed. The spike, leaf and stem were ground for measurement of NSC.

Three hundred ears that headed on the same day were tagged in each plot, and 20 ears were sampled at 4-day intervals during grain filling period. The grains on an ear were divided into superior grains and inferior grains (Jiang et al., 2003). Some grains were used for the hormone and enzyme measurements and the others were used to determine the grain weight. Yield and yield components were determined according to our previous study (Liu et al., 2016).



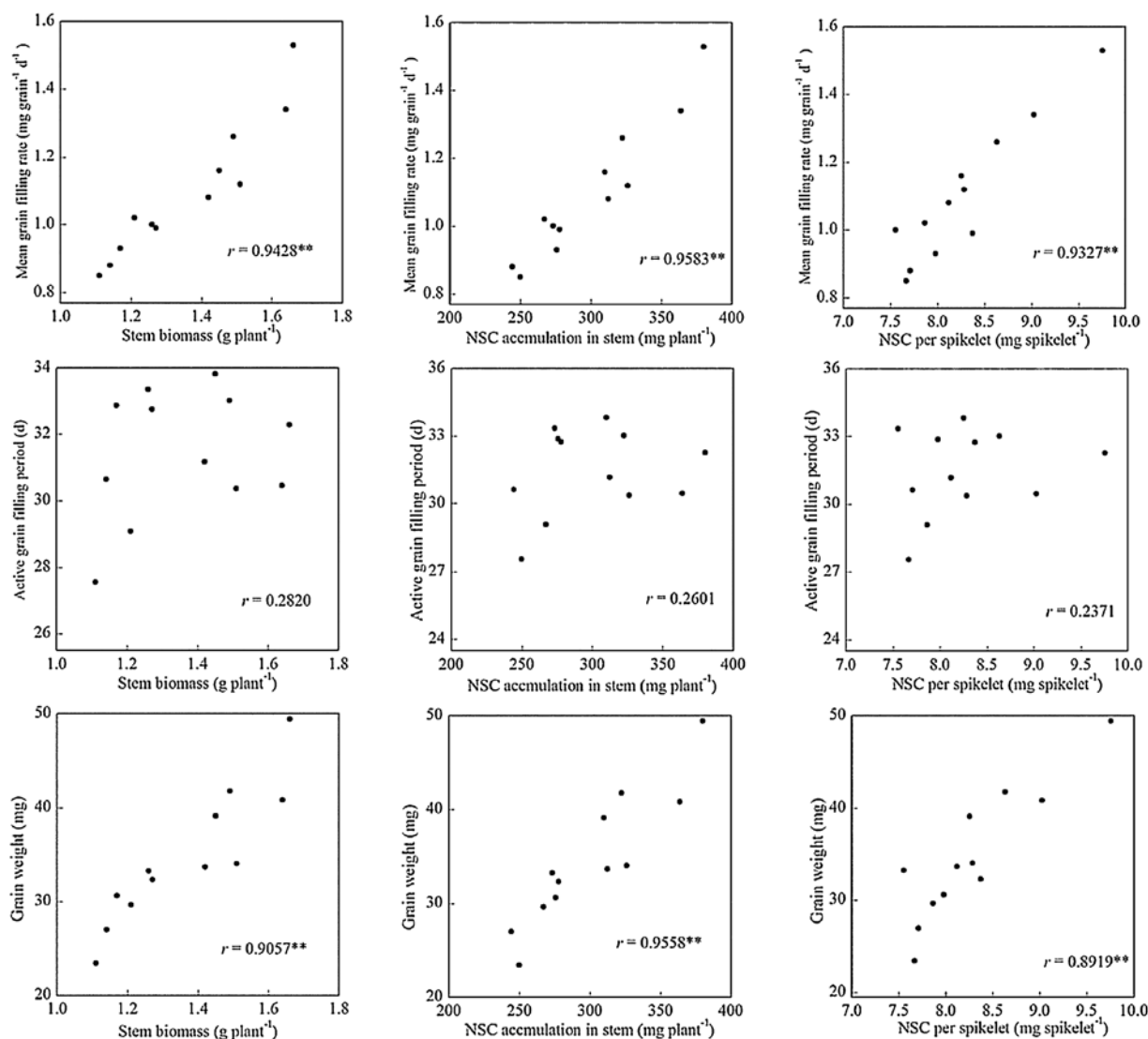


Fig. 3. Correlations of stem biomass, NSC accumulation in the stem and NSC per spikelet at anthesis with the mean grain-filling rate, active grain-filling period and grain weight of inferior grain of wheat in the first experiment. Correlation coefficients ( $r$ ) are presented, and \*\* indicates significance at the 0.01 probability level ( $n = 12$ ).

### 2.2.1. Grain-filling process

The grain-filling process was fitted by the Richards' (1959) growth equation as described by Zhu et al. (1988).

### 2.2.2. Hormones

The endogenous Z + ZR was extracted with 80% (v/v) methanol and quantified by enzyme-linked immunosorbent assay (ELISA) according to previous studies (Liu et al., 2011; Yang et al., 2001a). The Phytohormones Research Institute, China Agricultural University, manufactured the ELISA kits. The recovery rate was  $94.3 \pm 2.7\%$ . The ETH evolved from the grains was determined according to our previous study (Liu et al., 2016).

### 2.2.3. Activities of SS and AGPP

The activities of SS and AGPP were measured according to Jiang et al. (2003). The frozen grains (stored at  $-40^{\circ}\text{C}$ ) were homogenized in 50 mM Hepes-NaOH (pH 7.5) buffer solution. The SS and AGPP activities were measured using a spectrophotometer (2600 UV/vis; Unic Corp., China) at 480 and 340 nm, respectively.

### 2.3. Statistical analyses

The SPSS 16.0 statistical software package was used to conduct

ANOVAs. The data from each sampling were analyzed separately. The means were tested by the least significant difference at  $P_{0.05}$  ( $\text{LSD}_{0.05}$ ). The hormone levels and enzyme activity of the first experiment were sampled in 2015–2016.

## 3. Results

### 3.1. Grain yield and grain filling of different wheat cultivars

The six cultivars had different components of grain yield (Table 1). The panicles per area of XY 6, XY 22 and XN 538 was significantly higher than that of SD 1, FG 1 and ZM 22, and the panicles per area of FG 1 and ZM 22 was significantly higher than that of SD1. However, the spikelets per panicle and grain weight of SD 1, FG 1 and ZM 22 were significantly higher than those of XY 6, XY 22 and XN 538, and the grain weight of SD1 was significantly higher than that of FG 1 and ZM 22. The grain-filling rate and grain weight of superior grains and inferior grains showed a similar trend: SD 1 > FG 1 and ZM 22 > XY 6, XY 22 and XN 538 (Table 2). However, no significant differences were observed for the active grain-filling period among the six cultivars.



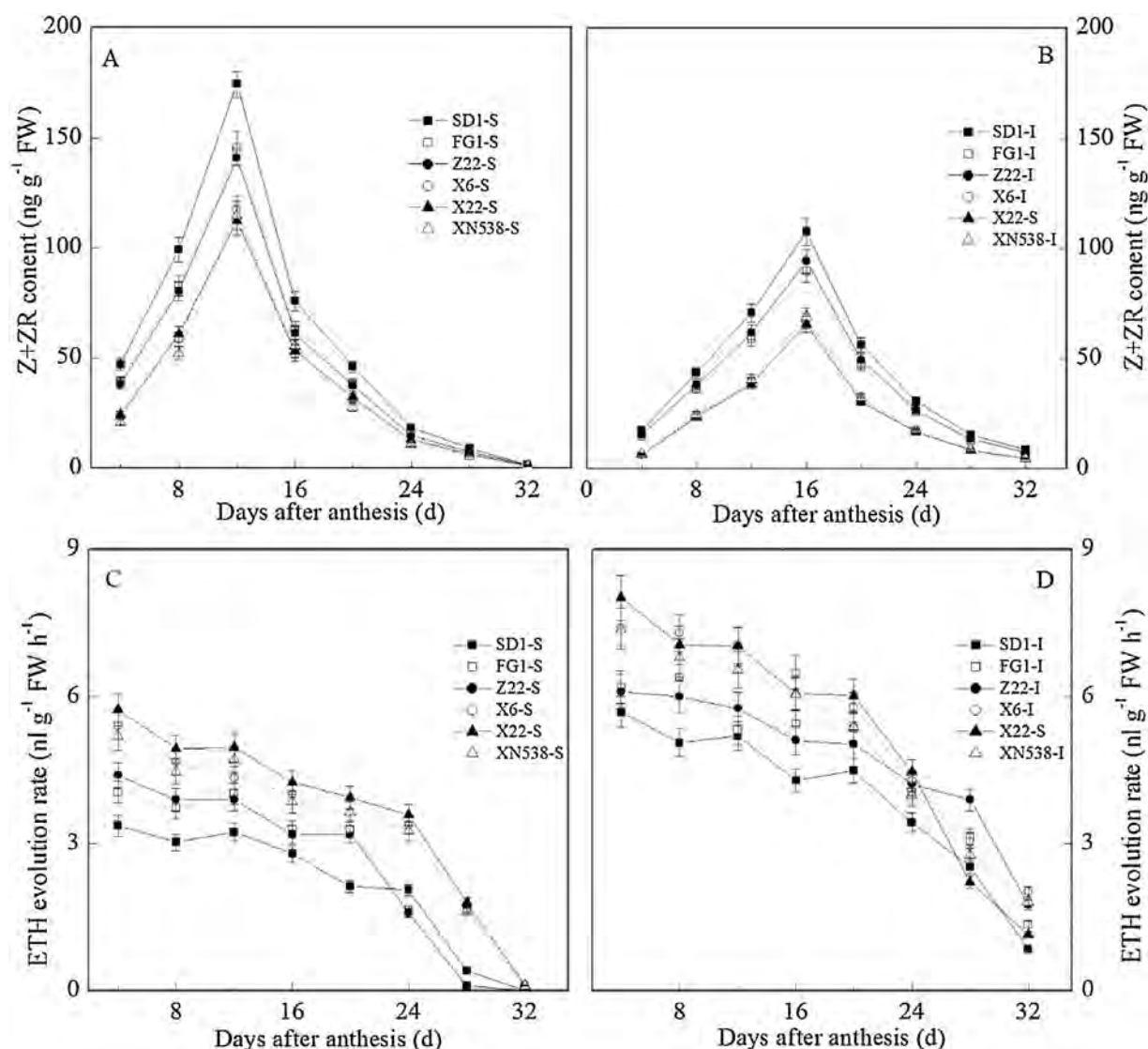


Fig. 4. Changes in Z + ZR and ETH levels in superior grain (A and C) and inferior grain (B and D) during grain filling of different wheat cultivars in the first experiment. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ). SD1, FG1, ZM22, XY6, XY22 and XN538 are the cultivars Shuangda 1, Fugao1, Zhengmai 22, Xiaoyan 6, Xiaoyan 22 and Xinong 538, respectively. S: superior grain and I: inferior grain.

### 3.2. NSC accumulation in the stem

The stem biomass, reserve of NSC in the stem pre-anthesis and NSC per spikelet (NSCPS) showed a similar trend: SD 1 > ZM 22 and FG 1 > XY 6, XY 22 and XN538 (Fig. 2). No significant differences were detected for the stem biomass, reserve of NSC in the stem pre-anthesis and NSCPS between 2014 and 2015 and 2015–2016. Based on regression analyses, the stem biomass, reserve of NSC in the stem pre-anthesis and NSCPS were significantly and positively correlated with the grain-filling rate and grain weight of inferior grains. However, these same parameters were not significantly correlated with the active grain-filling period of inferior grain (Fig. 3).

### 3.3. Hormones

With the grain filling, the Z + ZR content in grains increased initially and then decreased (Fig. 4). The peak value of the Z + ZR content in superior grains and inferior grains occurred separately at 12 and 16 days post anthesis, respectively. Additionally, the Z + ZR content in superior grains was significantly higher than that in inferior grains during the early and middle grain-filling stages. The Z + ZR content in grain of SD1 was significantly higher than that of ZN 22 and FG 1, and

the Z + ZR content in grain of ZN 22 and FG 1 was significantly higher than that of XY 6, XY 22 and XN 538 during the early and middle grain-filling stages.

Different than the contents of Z + ZR, the ETH evolution rate in grains continually decreased during the grain-filling stage, and the ETH evolution rate in superior grains was significantly lower than that in inferior grains. The ETH evolution rates in grains of SD 1, FG 1 and ZM 22 were significantly lower than those of XY 6, XY 22 and XN 538 during the early and middle grain-filling stages.

### 3.4. Activities of SS and AGPP

The activities of SS and AGPP of grain at 4 days post anthesis were significantly lower than those at 16 days post anthesis (Fig. 5). The SS and AGPP activities of superior grain were significantly higher than those of inferior grains. The genotypic differences of SS and AGPP activities of grains showed that SD 1 > ZM22 and FG1 > XY6, XY22 and XN538.

### 3.5. Effect of N on grain yield and grain filling of grain

With the increase in level of N fertilizer, the grain yield of wheat



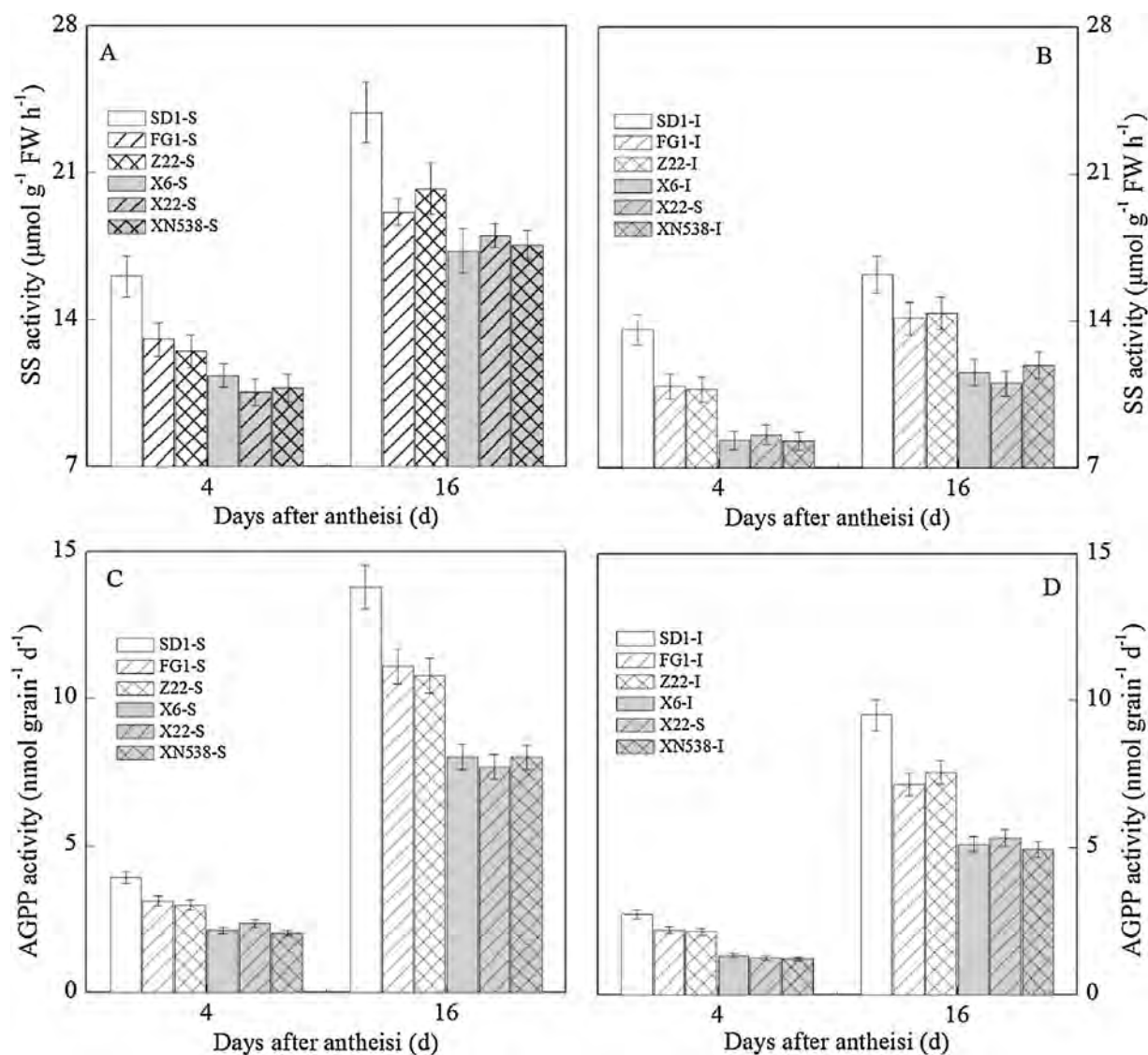


Fig. 5. Changes in SS and AGPP activities in superior grain (A and C) and inferior grain (B and D) during grain filling of different wheat cultivars in the first experiment. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ). SD1, FG1, ZM22, XY6, XY22 and XN 538 are the cultivars Shuangda 1, Fugao1, Zhengmai 22, Xiaoyan 6, Xiaoyan 22 and Xinong 538, respectively. S: superior grain and I: inferior grain.

Table 3

Effect of nitrogen on grain yield and yield components of wheat.

Cultivar	Treatment	No. of panicles ( $\times 10^4 \text{ hm}^{-2}$ )	Spikelets per panicle	Grain weight (mg)			Grain yield ( $\text{t hm}^{-2}$ )
				Mean	Superior grain	Inferior grain	
Zhoumai 22	N0	314.93d	28.13c	35.36c	47.64a	23.98d	3.21d
	N1	368.85c	33.64b	38.17b	47.91a	27.70c	4.65c
	N2	462.63b	38.95a	42.35a	48.53a	32.53a	7.52a
	N3	513.67a	36.79a	40.11ab	47.86a	29.98b	7.37a
	N4	524.78a	33.92b	38.26b	48.00a	27.92c	6.61b
Xinong 538	N0	504.81d	25.35c	26.68d	39.39a	18.24d	3.37d
	N1	536.59c	30.25b	28.19c	39.54a	21.50c	4.48c
	N2	613.42b	33.64a	31.23b	39.66a	25.70b	6.37b
	N3	670.47ab	34.77a	33.24a	38.96a	27.94a	7.62a
	N4	706.88a	34.68a	30.02b	39.30a	24.38b	7.18ab

Values within a column and for the same cultivar followed by different letters are significantly different at  $P = 0.05$ . N0, N1, N2, N3, N4 means the nitrogen rate is 0, 75, 150, 225, 300  $\text{kg hm}^{-2}$ , respectively.



**Table 4**  
Effect of nitrogen on grain filling characteristics of inferior grain wheat.

Cultivar	Treatment	Inferior grain		
		MGR (mg grain <sup>-1</sup> d <sup>-1</sup> )	AGP (d)	GW (mg)
Zhoumai 22	N0	0.83c	28.89b	23.98d
	N1	0.92b	30.11a	27.70c
	N2	1.04a	31.28a	32.53a
	N3	0.97ab	30.91a	29.98b
	N4	0.93b	30.02a	27.92c
Xinong 538	N0	0.72d	25.34c	18.24d
	N1	0.78c	27.56b	21.50c
	N2	0.91ab	28.24ab	25.70b
	N3	0.95a	29.41a	27.94a
	N4	0.84b	29.02a	24.38b

Values within a column and for the same cultivar followed by different letters are significantly different at  $P = 0.05$ . N0, N1, N2, N3, N4 means the nitrogen rate is 0, 75, 150, 225, 300 kg hm<sup>-2</sup>, respectively. MGR: mean grain filling rate; AGP: active grain filling period; GW: Grain weight.

first increased and then decreased (Table 3). However, the response of ZM 22 and XN 538 to N fertilizer was different; the maximum grain yield of ZM 22 occurred in the N2 treatment, whereas the maximum grain yield of XN 538 occurred in the N3 treatment. With the increase in level of N fertilizer, the panicles per plant continually increased. However, the spikelets per panicle and grain weight increased initially and then decreased. The maximum spikelets per panicle and grain weight of ZM 22 were observed in the N2 treatment, whereas those of XN 538 occurred in the N3 treatment.

Nitrogen fertilizer had no significant effect on the grain weight of superior grains, but the grain weight of inferior grains was significantly affected. With the increase in level of N application, the grain weight of inferior grains initially increased and then decreased (Table 4). The maximum grain weight of inferior grains was in the N2 and N3 treatments for ZM 22 and XN 538, respectively. Nitrogen fertilizer significantly increased the active grain-filling period. However, the mean grain-filling rate first increased and then decreased with the increase in level of N application. Additionally, the grain weight of inferior grain was significantly and positively correlated with the mean grain-filling rate ( $r = 0.9900^{**}$ ) and active grain-filling period ( $r = 0.9568^{**}$ ). Thus, by regulating the mean grain-filling rate and active grain-filling period, the N fertilizer regulated the grain weight of inferior grain.

### 3.6. Effect of N on NSC accumulation in the stem

Nitrogen significantly affected the stem biomass, reserve of NSC in the stem pre-anthesis and NSCPS (Fig. 6). With the increase in level of N, the stem biomass, reserve of NSC in the stem pre-anthesis and NSCPS

first increased and then decreased. The peak values of stem biomass, reserve of NSC in the stem pre-anthesis and NSCPS of ZM 22 occurred in the N3 treatment, whereas those of XN 538 were observed in the N4 treatment.

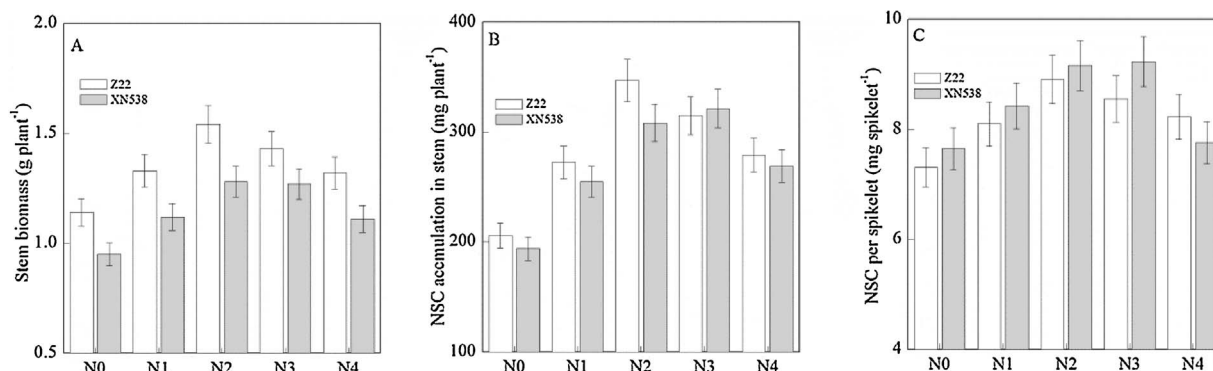
### 3.7. Effect of N on hormones and activities of SS and AGPP in inferior grains

Nitrogen fertilizer significantly increased the content of Z + ZR and the activities of SS and AGPP and decreased the rate of ETH evolution in inferior grains (Figs. 7 and 8). The content of Z + ZR and activities of SS and AGPP in inferior grain first increased and then decreased with the increase in level of N, and the peak values were in the N2 treatment. In contrast to these results, the rate of ETH evolution in inferior grain decreased with the increase in level of N.

## 4. Discussion

The poor grain filling of inferior grain is the primary factor that inhibits the promotion of the grain weight of cereals; thus, the key to promote the grain weight of cereals is to improve the grain filling of inferior grain (Yang and Zhang, 2010). In the present study, the grain weight of inferior grain was significantly lower than that of superior grain. However, the coefficients of variation of mean grain-filling rate, active grain-filling period and grain weight of inferior grains in the two years of the study were notably higher than those of superior grains. The N fertilizer significantly affected the grain filling of inferior grains but had no significant effect on that of superior grains of wheat. Based on these results, the inferior grain was more sensitive to environmental factors than the superior grain, and promoting the grain filling of inferior grain might be the key measurement to promote the grain weight of wheat.

In a previous study, low sink strength was implicated as a primary reason for the poor grain filling of inferior grain, and a hypothesis was proposed that high levels of enzymes involved in the regulation of the synthesis from sucrose to starch would promote the sink activity of rice (Fu et al., 2011; Liang et al., 2001). The important enzymes that regulate the synthesis from sucrose to starch are SS and AGPP: SS is the primary enzyme that regulates the sucrose cleavage in wheat grains, and AGPP is one of the key enzymes involved in starch synthesis in wheat grain (Jiang et al., 2003). In the present study, the activities of SS and AGPP in superior grain were significantly higher than those in inferior grain, and the activities of these two enzymes in the high grain weight cultivars were significantly higher than those of low grain weight cultivars. Thus, the superior grain had higher sink strength than that of inferior grain, which might be one of the primary reasons that the grain-filling rate and grain weight of superior grain were significantly higher than those of inferior grain.



**Fig. 6.** Effects of nitrogen fertilizer on stem biomass, NSC accumulation in the stem and NSC per spikelet at anthesis in the second experiment. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ). ZM22 and XN 538 are the cultivars Zhengmai 22 and Xinong 538, respectively. N0, N1, N2, N3 and N4 indicate N fertilizer levels of 0, 120, 180, 240 and 300 kg N ha<sup>-1</sup>, respectively.



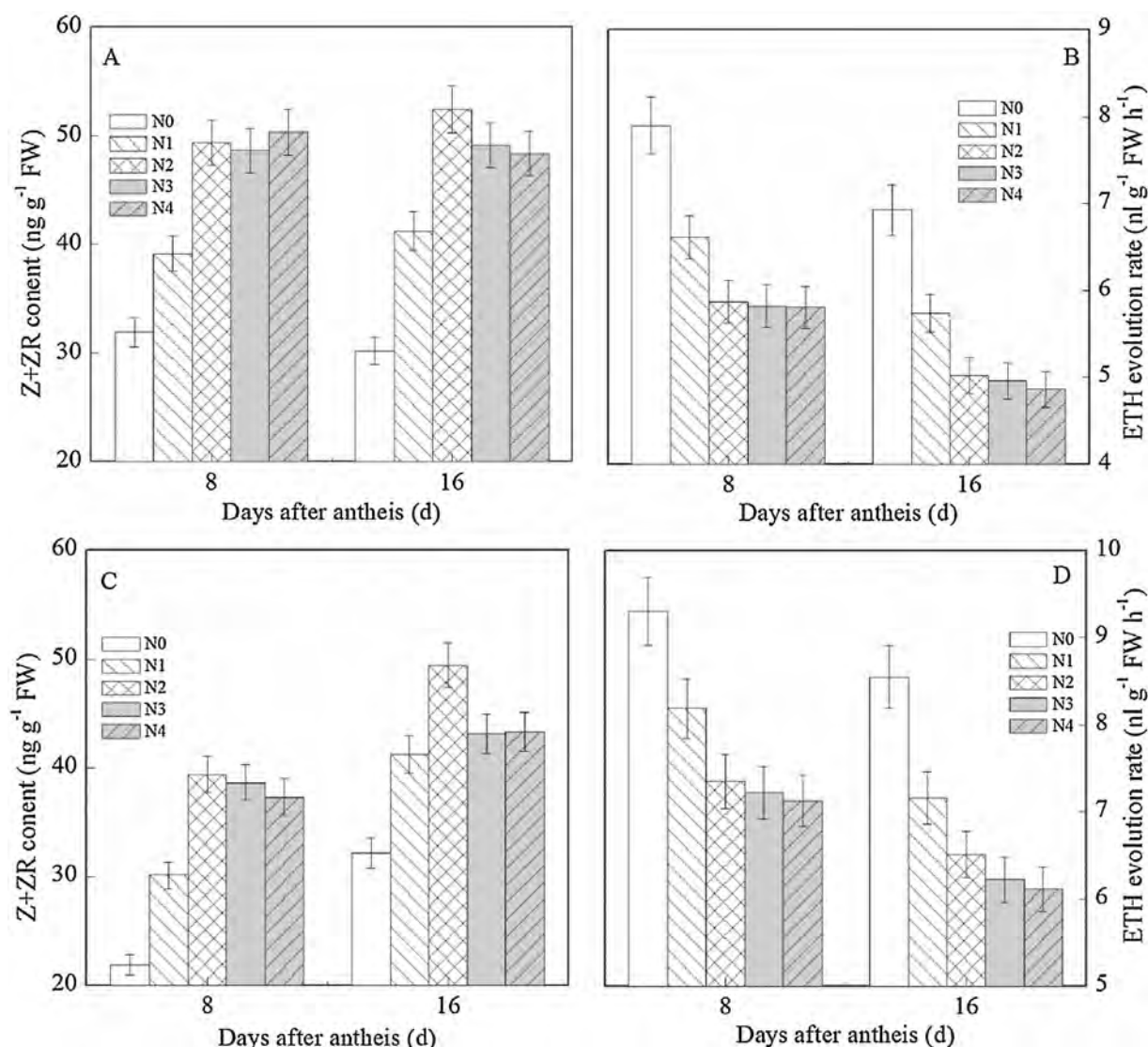


Fig. 7. Effects of nitrogen fertilizer on Z + ZR content and ETH evolution rate in inferior grain in the second experiment. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ). A and B: Zhengmai 22; C and D: Xinong 538. N0, N1, N2, N3 and N4 indicate the N fertilizer levels of 0, 120, 180, 240 and 300 kg N ha<sup>-1</sup>, respectively.

In addition to the activities of SS and AGPP, the hormonal levels in grains are significantly correlated with the grain-filling rate, and multiple hormones are involved in regulating the grain-filling rate of cereals (Yang and Zhang, 2006). The levels of CTK in rice grains are significantly correlated with grain filling (Yang et al., 2002; Zhang et al., 2009), and studies on barley (*Hordeum vulgare* L.), maize, rice and wheat suggest that CTK is indispensable for cell division during the early grain development phase (Dietrich et al., 1995; Yang et al., 2000). In the present study, the superior grain had higher Z + ZR content than that in the inferior grain, and the Z + ZR contents in grain of high grain weight cultivars were notably higher than those of low grain weight cultivars. In previous studies, CTK was an important regulating factor for the differentiation and division of endosperm cells of rice and notably promoted the synthesis of starch in inferior grain of rice, thereby promoting the grain filling of inferior grain (Yang et al., 2000; Yang et al., 2008; Chen et al., 2013). In the present study, the Z + ZR contents in grain were significantly and positively correlated with the SS and AGPP activities in grain ( $r = 0.6079^{**}$  and  $0.7859^{**}$ , respectively). Therefore, a high Z + ZR content in grain promoted the activities of SS and AGPP in grain and the synthesis of starch, which promoted the grain filling of wheat.

In contrast to CTK, the rate of ETH evolution of the superior grains is significantly lower than that of the inferior grains in rice and wheat,

and low ETH in grains is associated with a higher filling rate in the superior grains (Yang et al., 2006; Xu et al., 2007). Thus, ETH inhibits the grain filling of cereal. In previous studies, ETH significantly decreases the activities of SS and AGPP and inhibits starch synthesis and therefore, decreases grain weights of rice and wheat (Liu et al., 2008; Yang et al., 2006; Yang et al., 2014). In the present study, the superior grain had lower rates of ETH evolution than those of inferior grain, and the ETH evolution rates in grain of high grain weight cultivars were notably lower than those of low grain weight cultivars. Additionally, the ETH evolution rate in grain was significantly and negatively correlated with the SS and AGPP activities in grain ( $r = -0.8935^{**}$  and  $-0.7370^{**}$ , respectively). Therefore, in contrast to CTK, a high ETH evolution rate in grain inhibits the activities of SS and AGPP and the synthesis of starch, which inhibit the grain filling of wheat. From these results, we suggest that increasing the CTK content and decreasing the rate of ETH evolution in grain might be a key approach to promote the grain filling of inferior grain of wheat.

Based on a previous study, a deficiency of carbohydrates for grain filling is the primary cause of the poor grain filling of inferior grain (Fu et al., 2011). The carbohydrates that are used for grain filling originate from two sources: current assimilation post-anthesis and remobilization of reserves stored in the stem pre-anthesis (Yang et al., 2004a). According to Horie et al. (2005), the reserve of NSC in the stem pre-



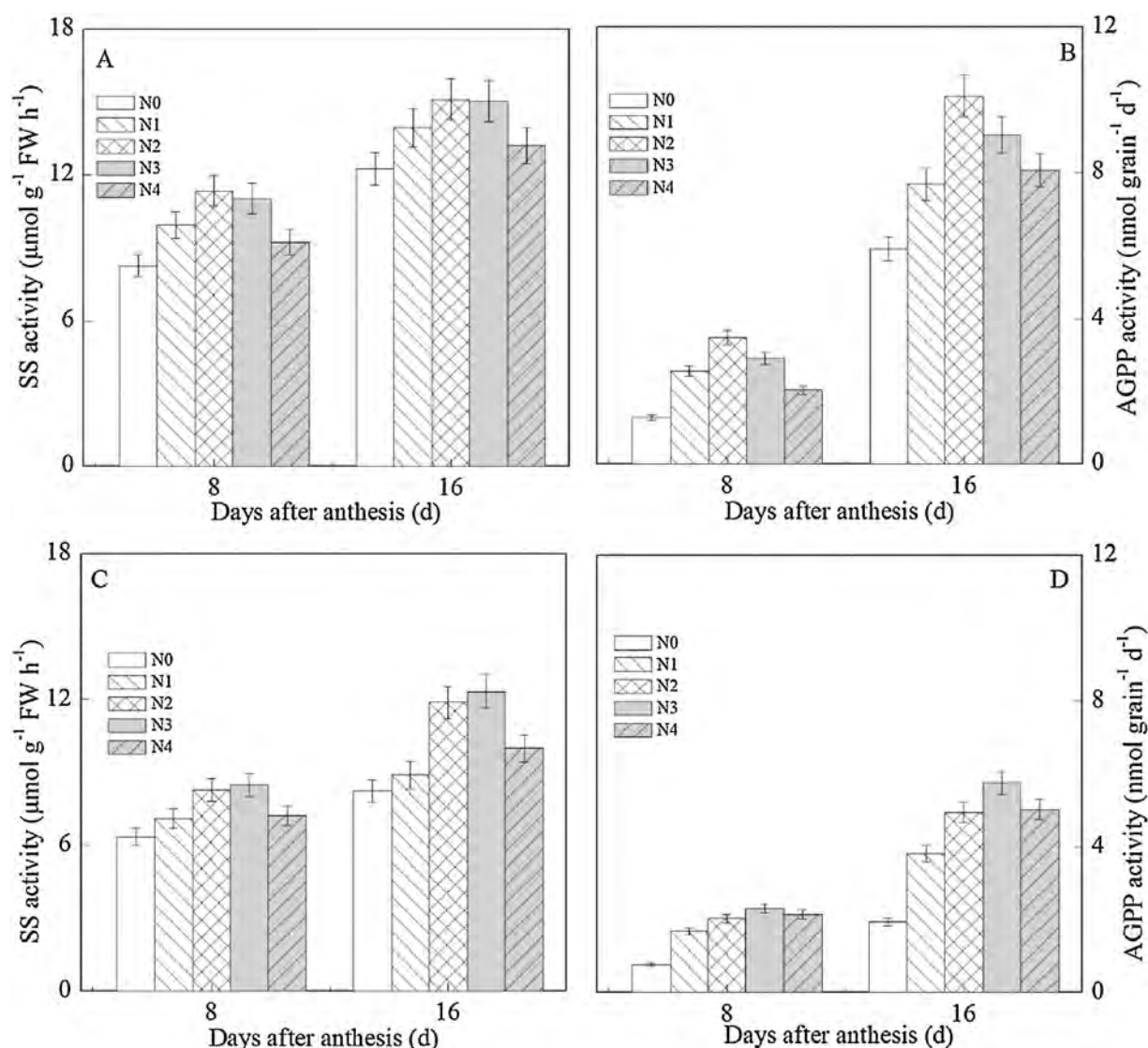


Fig. 8. Effects of nitrogen fertilizer on SS and AGPP activities in inferior grain in the second experiment. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ). A and B: Zhengmai 22; C and D: Xinong 538. N0, N1, N2, N3 and N4 indicate the N fertilizer levels of 0, 120, 180, 240 and  $300 \text{ kg N ha}^{-1}$ , respectively.

anthesis was significantly correlated with rice growth at the early grain-filling stage. [Fu et al. \(2011\)](#) found that the reserve of NSC in the stem pre-anthesis significantly promoted the grain filling of inferior grains of rice. According to [Zheng et al. \(2010\)](#), at the early grain-filling stage of rice, the current assimilation post-anthesis may not supply sufficient NSC for grain filling, because the initiation of grain filling requires a large supply of NSC. In the case of insufficient supply, the NSC reserves stored in the stem pre-anthesis may be transported from stem to spikelet to supply the grain filling. The remobilization of NSC reserves stored in the stem pre-anthesis may be a signal to begin grain filling of inferior grain of rice ([Zheng et al., 2010](#)). In the present study, the wheat cultivars that had high grain-filling rates and grain weight had more NSC reserves stored in the stem pre-anthesis and had high NSCPS. Based on regression analyses, the NSC reserve stored in the stem pre-anthesis was significantly and positively correlated with the mean grain-filling rate and grain weight of inferior grains. However, the NSC reserve was not significantly correlated with the active grain-filling period. Therefore, the NSC reserve stored in the stem pre-anthesis increased the grain weight by promoting the grain-filling rate of inferior grain but had no significant effect on the active grain-filling period of inferior grain.

Based on regression analyses, the NSC reserve stored in the stem pre-anthesis was significantly and positively correlated with the contents of Z + ZR and the activities of SS and AGPP in inferior grains.

However, the NSC reserve stored in the stem pre-anthesis was significantly and negatively correlated with the rate of ETH evolution in inferior grain. Therefore, the NSC reserve stored in the stem pre-anthesis increased the Z + ZR content and decreased the rate of ETH evolution in inferior grain of wheat. From this conclusion, we suggest that the NSC reserve stored in the stem pre-anthesis may act alone to regulate the hormone balance in inferior grain, thereby promoting the activities of SS and AGPP. Through this regulation, the NSC reserve promotes the sink strength, which can promote the transport of carbohydrates from current assimilation post-anthesis from stem to inferior grain and thereby increase the grain-filling rate of inferior grain. In contrast to rate of grain filling, the active grain-filling period is primarily regulated by the senescence of plants ([Liu et al., 2013b](#)). However, the senescence of leaves primarily correlates with the assimilation and transport of carbohydrates post-anthesis ([Yang et al., 2001b](#)), and NSC reserves stored in the stem pre-anthesis may have no significant effect on the senescence of plant. Therefore, in this study, the NSC reserves stored in the stem pre-anthesis had no significant effect on the active grain-filling period of inferior grain.

Nitrogen fertilizer is an important agricultural component in crop production and significantly affects the grain weight of cereal. However, different studies report different results. In some studies, N significantly increases the grain weight of cereal ([Yan et al., 2016](#)),



whereas in others, high levels of N fertilizer had no significant effect on grain weight or even decreased the grain weight of cereal (Jiang et al., 2016). Thus, the effect of N is complex on the grain weight of cereals. In the present study, we found that with the increase in level of N fertilizer, the grain weight of wheat first increased and then decreased. In a previous study, N fertilizer notably affected the carbon metabolism of plants (Gong et al., 2009). Zheng et al. (2010) found that suitable N fertilizer application significantly increased the NSC accumulation in stems of rice, because the N significantly promoted photosynthesis of leaves; however, excess N application significantly decreased the NSC accumulation in stems of rice, because the growth of vegetative organs such as leaves was significantly promoted, which was a process that consumed much of the NSC from photosynthesis and led to the low NSC accumulation in stems. In the present study, with the increase in N fertilizer, the NSC accumulation in stems was significantly affected. With the increase in N, the accumulation of NSC in the stem pre-anthesis first increased and then decreased. Additionally, the peak values of NSC accumulation in the stem pre-anthesis and the grain weight of inferior grain occurred at the same level of N. Based on regression analyses, the accumulation of NSC in the stem pre-anthesis was significantly and positively correlated with the grain-filling rate and grain weight of inferior grain ( $r = 0.8952^{**}$  and  $0.8570^{**}$ , respectively). Thus, the NSC also significantly affected the grain filling of inferior grain under N fertilizer application, and suitable N fertilizer application is important for the production of wheat.

Additionally, in the present study, N fertilizer had different effects on the grain weight of superior grain and inferior grain. Nitrogen fertilizer had no significant effect on the grain weight of superior grains but significantly affected that of inferior grains. Our analysis suggests that this difference between grain types is because the superior grain had “prior dominant”; thus, the NSC accumulation in the stem is priority transported to superior grains (Zheng et al., 2010), which obtain sufficient NSC for grain filling of those grains. However, because of this priority, the inferior grains cannot obtain sufficient NSC for grain filling when the accumulation of NSC in the stem is insufficient. Therefore, in the present study, because the inferior grain could not obtain sufficient NSC in the N0 and N4 treatments, the grain weights of those two treatments were notably lower than those of the N2 and N3 treatments. We also found that the N fertilizer significantly affected the Z + ZR content, ETH evolution rate and SS and AGPP activities in inferior grain. Based on regression analyses, the contents of Z + ZR and activities of SS and AGPP in grain were significantly and positively correlated with the NSC accumulation in the stem pre-anthesis ( $r = 0.7532^{**}$ ,  $0.7478^{**}$  and  $0.7296^{**}$ , respectively), and the rate of ETH evolution in grain was significantly and negatively correlated with the NSC accumulation in the stem pre-anthesis ( $r = -0.8143^{**}$ ) under N treatments. Thus, the N fertilizer significantly affected the NSC accumulation in the stem pre-anthesis, which promoted the sink strength and the grain-filling rate of inferior grain of wheat. Therefore, the agronomic practices that significantly increase the NSC accumulation in the stem pre-anthesis, such as N fertilizer application, can significantly promote the grain filling of inferior grain of wheat.

## 5. Conclusions

The superior grain had higher Z + ZR content and SS and AGPP activities and lower ETH evolution rate than those in inferior grain, which led to greater sink strength and higher rate of grain filling and resulted in higher grain weight in the superior grain than in the inferior grain. High NSC reserves stored in the stem pre-anthesis significantly increased the Z + ZR content and decreased the ETH evolution rate in inferior grain, which promoted the sink strength and grain-filling rate of inferior grain of wheat. Suitable N fertilizer significantly increased the NSC reserves stored in the stem pre-anthesis, which promoted the sink strength and grain-filling rate of inferior grain; however, excess N fertilizer significantly decreased the NSC accumulation in the stem pre-

anthesis, which inhibited the sink strength and grain-filling rate of inferior grain of wheat.

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# Effect of potassium foliage application post-anthesis on grain filling of wheat under drought stress



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## ABSTRACT

Potassium foliage application (KFA) is widely used for wheat production in China. The objective of this study was to investigate the effect of KFA on grain filling of wheat under different soil moisture conditions and the underlying mechanisms. The results indicate that KFA increased the zeatin (Z), Z riboside (ZR), and abscisic acid (ABA) contents and the ethylene (ETH) evolution rate in inferior grains during the early and middle grain filling stages, which promoted sink strength. However, KFA decreased the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), as well as the soil-plant analyses development (SPDA) value and increased the malondialdehyde (MDA) content in the flag leaves. The effect of KFA on grain filling also exhibited a notable genotypic difference. In the heavy-panicle cultivar, KFA had no significant effect on grain filling under the well-watered (WW) treatment, but it decreased the rate and active period of the grain filling of inferior grains and significantly decreased the grain weight following soil-dried (SD) treatment. In the light-panicle cultivar, KFA significantly promoted the grain filling rates of inferior grains and increased the grain weight under the WW treatment. However, KFA significantly decreased the active grain filling period but increased the grain filling rate and, therefore, had no significant effect on the grain weight under the SD treatment.

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## 1. Introduction

Drought is one of the main abiotic stresses limiting wheat (*Triticum aestivum* L.) production in northern China (Jiang and Zhang, 2004; Li et al., 2000). Grain filling, an important growth stage of cereal, determines the grain weight (Yang and Zhang, 2006). In cereals such as wheat, the booting and grain filling stages are most sensitive to the water supply (Yu, 2003). Drought stress during the grain filling stage usually seriously reduces the grain weight and yield (Kobata et al., 1992; Zhang et al., 1998). Thus, reducing the inhibitory effect of drought stress on wheat grain filling is important for improving wheat production in northern China.

Potassium (K) is an important nutrient element that significantly affects the grain weight and yield of cereals (Brennan and Bolland, 2009; Kunzová and Hejman, 2010; Wani et al., 2014). Previous studies have indicated that K fertilizer promotes the grain filling of wheat in dryland (Chen et al., 2006; Hu et al., 2014). Wang et al. (2003) suggested that K significantly promoted the accumulation and transfer of sucrose in stems and increased the starch

content in wheat grain. Zou et al. (2007) suggested that the appropriate application of K fertilizer increased the net photosynthetic rate of flag leaves during the grain filling stage. In addition, K significantly affected the drought resistance of wheat (Raza et al., 2014). Wei et al. (2013) suggested that external K ameliorated drought stress in wheat while Damon et al. (2011) found that the endogenous K content of a drought-resistant variety was significantly higher than that of a drought-sensitive variety of wheat was. These results suggest that K significantly affected the grain weight and drought resistance of wheat. However, the effects of K on grain filling of wheat under drought stress conditions are unclear.

In northern China, N and P fertilizers are the main types used for wheat production. K fertilizer is rarely used in wheat production because the K level in the soil is traditionally considered sufficient for wheat production in this region (Guo et al., 2010). However, increase wheat grain yields are accompanied by increased K absorption from the soil by the wheat plants to maintain growth, which would lead to a K deficiency for subsequent wheat production if the levels in the soil are not supplemented (Chen et al., 2006). Because the price of K fertilizers has increased over the years, farmers have not been encouraged to increasing their use in wheat production. In addition, wheat roots exhibit a gradual senescence in the later growth period, which weakens their ability to absorb nutrients (Zhao and Si, 2015). For these reasons, K foliage applica-

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tion (KFA) is mainly used for wheat production in northern China because of its low cost and ease of use compared to that of soil fertilizer. However, the previously reported effects of KFA on wheat grain weight and yield differed, and some studies suggest that KFA significantly increased grain weight (Lv et al., 2011; Sun et al., 2006) while others found no significant effect on wheat yield or even an increased yield (Cao et al., 2015; Yan and Pei, 2014). Therefore, the effect of KFA on wheat grain weight is complex, and the relationship of its observed differential effects with soil moisture is unclear.

Plant hormones play an important role in regulating the grain filling of cereals including cytokinin (CTK), which is important for the endosperm cell division of wheat grains (Morris et al., 1993). The abscisic acid (ABA) levels of superior wheat grains were significantly higher than those of inferior wheat grains, while the ethylene (ETH) levels showed an opposite trend (Yang et al., 2006). Indole-3-acetic acid (IAA) levels in superior rice grains were higher than those in inferior rice grains (*Oryza sativa* L.) (Xu et al., 2007). Furthermore, the maximal grain filling rate was negatively correlated with gibberellic acids (GAs) in rice grains (Yang et al., 2001a).

Although it is well known that hormones affect grain filling in wheat, the relationship between hormonal changes and the KFA-induced grain filling is unclear. The objectives of this study were to investigate the effect of K foliage application on the grain filling of wheat under different soil moisture conditions and determine how the changes in endogenous hormones in the developing wheat grains under KFA are related to the grain filling process under drought stress conditions. Drought stress was applied during the grain filling stage of wheat and potassium chloride (KCl), the main type of KFA used for wheat production in northern China, was used as a foliage application at the anthesis stage. Furthermore, changes in IAA, ABA, zeatin (Z) plus zeatin riboside (ZR), GAs, and ETH in the wheat grain were measured during the grain filling process.

## 2. Materials and methods

### 2.1. Experimental design and treatments

The experiment was conducted in mobile waterproof sheds using 3 m × 4 m plots, which were divided by a concrete wall. The soil is Eum-Orthosols (Chinese soil taxonomy), and the readily available N, P and K concentrations were 51.23 mg kg<sup>-1</sup>, 20.01 mg kg<sup>-1</sup>, and 105.37 mg kg<sup>-1</sup>, respectively. The organic matter concentration of the 0–20 cm topsoil was 11.96 g kg<sup>-1</sup>, and the pH was 7.02. Two wheat cultivars, Zhoumai 22 and Xinong 538, were grown. The seeds were sown on October 20 and October 18 during the 2013–2014 and 2014–2015 growth years, respectively. The seedling rate was 150 kg ha<sup>-1</sup> with a row spacing of 0.20 m. The fertilizer was applied at basal levels of 150 kg ha<sup>-1</sup> each of urea and diammonium orthophosphate. The experiment was based on a 2 × 2 × 2 factorial design (two soil moisture levels, two KFA application rates, and two cultivars), with eight treatment combinations. Each treatment was applied to three plots as replicates in a complete randomized block design.

From anthesis to maturity, two levels of soil moisture were maintained, and the moisture treatments were based on those of our previous study (Liu et al., 2016). The well-watered (WW) and soil-dried (SD) treatments maintained the soil water potential of the 15–20 cm soil layer at  $-20 \pm 5$  and  $-60 \pm 5$  kilopascals (kPa), respectively. Five tension meters (SWP-100, Soil Science Research Institute, China Academy of Sciences, Nanjing, China) were installed in each plot and readings were recorded at 11:00–12:00 each day. When the reading dropped to a certain value, appropriate amounts of water were added. Before anthesis, the soil water potential was maintained at  $-20 \pm 5$  kPa.

Under each soil moisture condition at anthesis, 30 mmol L<sup>-1</sup> KCl was sprayed on the leaves using a sprayer (T1) daily for 4 days at a rate of 750 kg hm<sup>-2</sup> at each application. The same volume of deionized water was applied to the control plants (CK).

### 2.2. Measurements

Four hundred spikes that flowered on the same day in each plot were tagged and sampled from anthesis to maturity on a 4-day interval for each plot while 20 spikes were sampled at each sampling stage. Grains on a spike were divided into superior and inferior grains according to Jiang et al. (2003). One half of the sampled grains was used to measure the hormones, while the other half was dried at 70 °C to a constant weight, which was recorded. On the same sampling day, 20 flag leaves were sampled from each plot, stored at  $-40$  °C, and were used to measure the superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities, as well as malondialdehyde (MDA) content. At 8-day intervals from anthesis to maturity, the soil-plant analyses development (SPAD) value of the flag leaves was measured with an SPAD-502 chlorophyll meter model (Minolta Camera Co., Osaka, Japan).

#### 2.2.1. Grain filling process

The grain filling process was simulated using Richards's (1959) growth equation and according to Yang et al. (2006):

$$W = \frac{A}{(1 + Be^{-kt})^{\frac{1}{N}}} \quad (1)$$

The grain filling rate (G) was calculated using a derivation of Eqn. 1:

$$G = \frac{AkBe^{-kt}}{(1 + Be^{-kt})^{\frac{N+1}{N}}} \quad (2)$$

where,  $W$  is the grain weight (mg);  $A$  is the final grain weight (mg);  $t$  is the time after anthesis (d); and  $B$ ,  $k$ , and  $N$  are coefficients determined using regression.

The active grain filling period was defined as the period when  $W$  was between 5% ( $t_1$ ) and 95% ( $t_2$ ) of  $A$ . Therefore, the average grain filling rate during this period was calculated from  $t_1$  to  $t_2$  (Yang et al., 2006).

#### 2.2.2. Hormones

Approximately 0.5 g of the free weight (FW) sample was collected, and the endogenous Z, ZR, GAs ( $GA_1 + GA_4$ ), IAA, and ABA were extracted according to a previously reported method (Yang et al., 2001a). The samples were homogenized with 5 mL 80% (v/v) methanol containing 1 mmol L<sup>-1</sup> butylated hydroxytoluene (BHT). The extracting solution was passed through Chromosep C18 columns (C18 Sep-Park Cartridge, Waters Corp., Milford, MA, USA), the fractions were vacuum-dried at 40 °C, and dissolved in 1 mL phosphate-buffered saline (PBS) containing 0.1% (v/v) Tween 20 and 0.1% (w/v) gelatin (pH 7.5) for the enzyme-linked immunosorbent assay (ELISA). The ELISA kits were manufactured by the Phytohormones Research Institute, China Agricultural University. The quantification of Z + ZR, GAs ( $GA_1 + GA_4$ ), IAA, and ABA was performed using an ELISA as previously described (Yang et al., 2001a). The recovery rates for IAA, Z + ZR, ABA, and GAs were  $85.4 \pm 4.7\%$ ,  $93.1 \pm 6.2\%$ ,  $89.5 \pm 3.2\%$ , and  $78.2 \pm 5.4\%$ , respectively.

The ETH generated by the grains was determined according to the methods of Beltrano et al. (1994) and Yang et al. (2008). The ETH was assayed using a gas chromatography (GC) system (Trace GC Ultra™, Thermo Fisher Scientific, USA) according to our previous study (Liu et al., 2016).



**Table 1**

Variation analysis of cultivar, soil moisture and foliage application on grain yield, grain filling characteristics, hormones, antioxidant enzyme activity of wheat.

Factor	No. of spikes	Spikelets per spike	Grain weight	Grain yield	W-S	G-S	A-S	W-I	G-I	A-I	SOD	POD
Cultivar (C)	**	**	**	**	**	**	**	**	**	**	**	**
Soil moisture (M)	ns	ns	**	**	**	**	ns	**	**	**	**	**
Foliage application (F)	ns	ns	**	*	ns	ns	ns	**	*	**	**	**
C × M	ns	ns	*	ns	ns	**	ns	ns	ns	ns	ns	**
C × F	ns	ns	*	ns	ns	ns	ns	**	**	*	ns	*
M × F	ns	ns	**	*	ns	**	ns	**	**	**	*	ns
C × M × F	ns	ns	ns	ns	ns	*	ns	ns	*	ns	ns	ns

Factor	IAA-S	Z + ZR-S	ABA-S	ETH-S	IAA-I	Z + ZR-I	ABA-I	ETH-I	CAT	MDA	SPAD
Cultivar (C)	ns	**	**	**	**	**	**	**	**	**	ns
Soil moisture (M)	**	**	**	**	**	**	**	**	**	**	**
Foliage application (F)	ns	ns	ns	ns	ns	**	**	**	**	**	**
C × M	*	ns	ns	ns	ns	**	**	**	ns	**	*
C × F	ns	ns	ns	ns	**	ns	ns	ns	ns	**	ns
M × F	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns
C × M × F	ns	ns	ns	ns	**	**	**	*	ns	ns	ns

\* and \*\*, significantly at 0.05 and 0.01 probability level. ns, no significant at  $P=0.05$ . W: the final grain weight; G: mean grain-filling rates; A: Active grain-filling period; S: superior grain; I: inferior grain. Data of grain yield and grain filling characteristics used for variation analysis is the mean of the data of 2013–2014 and 2014–2015. Data of hormones and antioxidant enzyme activity used for variation analysis is the mean of that during grain filling stage of the each treatment.

### 2.2.3. Activities of SOD, POD, and CAT, and MDA content in flag leaves

For enzyme extraction, 500 mg of the fresh leaves was ground in 5 mL extraction buffer consisting of 100 mM potassium phosphate buffer (pH 7.0), 1 mM ethylenediaminetetraacetic acid (EDTA), and 1% polyvinylpyrrolidone (PVPP). The extract was centrifuged at 20,000 rpm for 20 min at 4 °C, the supernatant was collected, and was used in all the enzyme analyses.

The activity of SOD (EC 1.15.1.1) was evaluated based on its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT), as proposed by Wang and Huang (2000). Measurements were recorded at 560 nm, and 1 unit of SOD corresponded to the amount of enzyme capable of inhibiting 50% of the NBT photoreduction under the experimental conditions. CAT (EC 1.11.1.6) activity was determined by measuring the hydrogen peroxide  $H_2O_2$  consumption at 240 nm over 3 min (Wang and Huang, 2000). POD (EC 1.11.1.7) activity was determined by measuring guaiacol oxidation at 470 nm according to the method of Wang and Huang (2000) with modifications.

Samples containing 500 mg of leaf tissue were homogenized in 5 mL 0.1% trichloroacetic acid (TCA), centrifuged at 20,000 × g for 20 min at 4 °C, and the supernatant was used for MDA content determination using the method of Wang and Huang (2000) based on the thiobarbituric acid reaction. MDA content was calculated according to the following formula: MDA content ( $\eta M$ ) =  $[(A535 - A600)/1.56] \times 105$ .

### 2.3. Statistical analysis

The results were analyzed using an analysis of variance (ANOVA) using the statistical package for the social science (SPSS) 16.0 for Windows. The analysis used complete randomized block design. The means were analyzed using the least significant difference (LSD) method at  $P=0.05$  (LSD 0.05).

## 3. Results

### 3.1. Grain yield

Table 1 shows that the drought stress post-anthesis and KFA had no significant effects on the number of spikes and spikelets per spike of the two cultivars. In contrast, drought stress significantly decreased the grain weight and yield of both cultivars (Table 2). The cultivars, KFA and soil moisture had significant interaction effect on

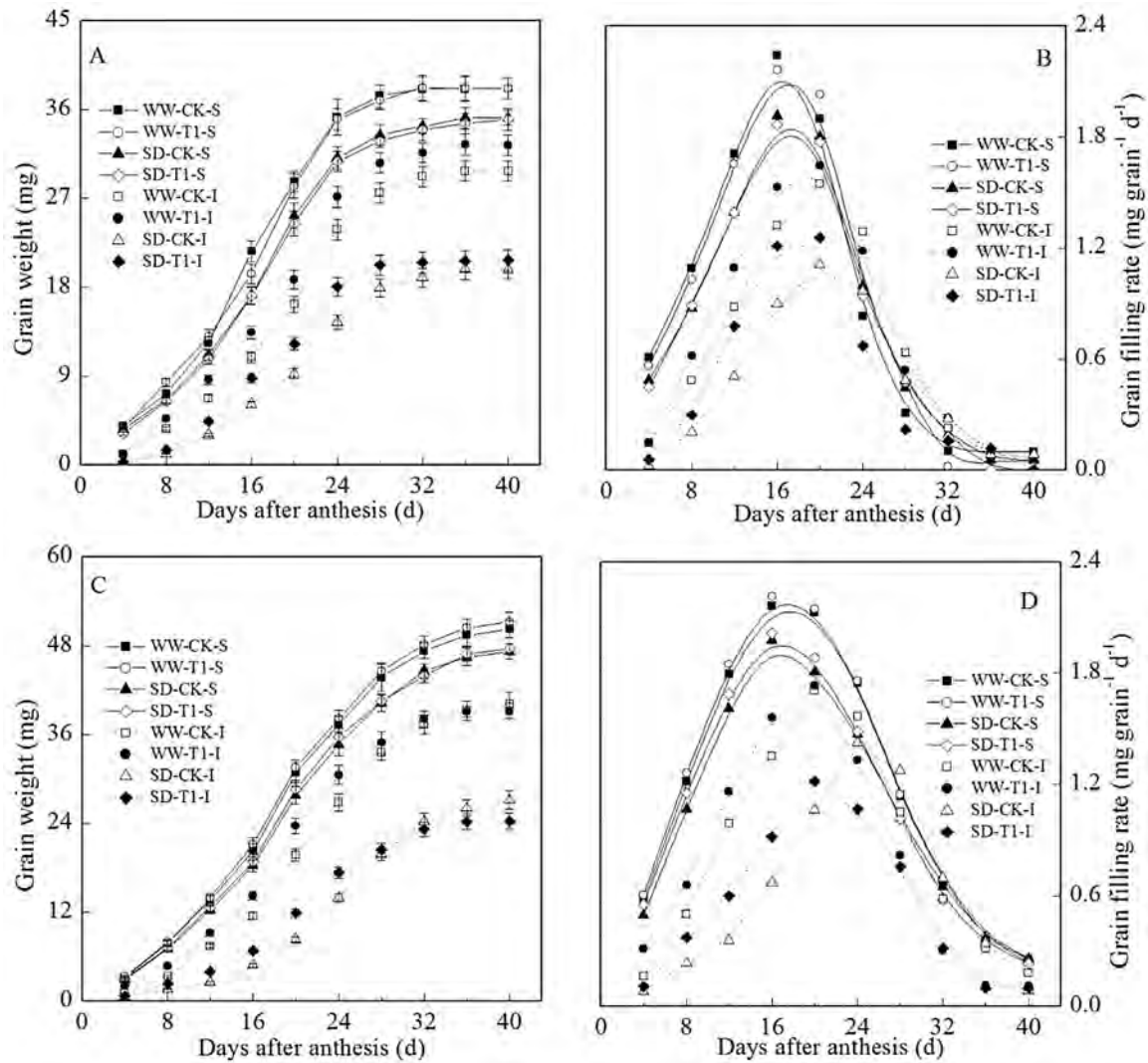
the regulation of grain weight. For Xinong 538, the KFA significantly promoted the grain weight of that, but the KFA had no significant effect on the grain weight of Zhoumai 22. Under WW condition, the KFA significantly increased the grain weight, but the KFA had opposite effect under the SD condition.

### 3.2. Grain filling

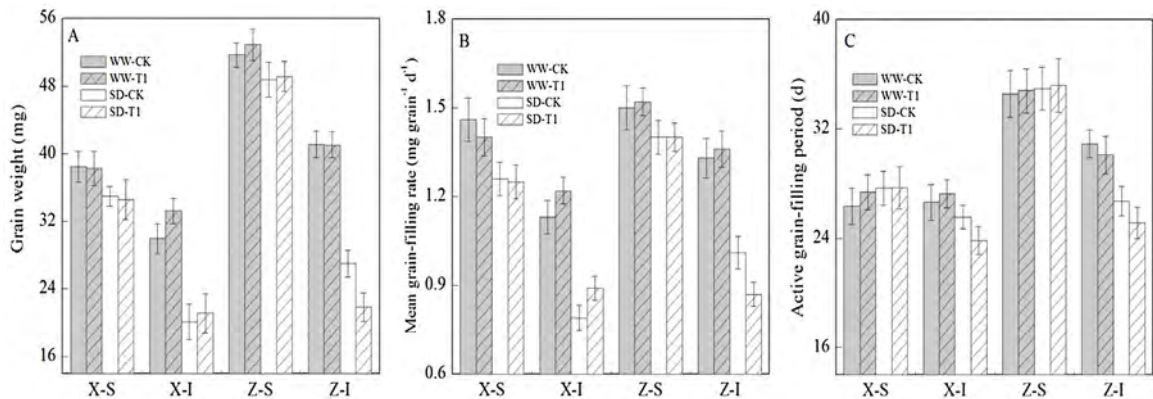
KFA had no significant effect on the maximum grain weight and the maximum and mean grain filling rates of superior grains (Figs. 1 and 2). However, drought and KFA significantly affected the maximum grain weight and the maximum and mean grain filling rates of inferior grains. Furthermore, drought significantly decreased the maximum grain weight and the maximum and mean grain filling rates of inferior grains of the two cultivars. However, KFA had different effects on the grain filling rate and grain weight of the two cultivars. Variance analysis suggested that the KFA had no significant effect on the number of spikes and spikelets per spike, but it significantly affected the grain weight and grain yield (Table 1). So in this experiment, the KFA through affects the grain weight to affects the grain yield of wheat.

For Xinong 538, KFA significantly promoted the maximum grain weight and the maximum and mean grain filling rates of inferior grains under the WW treatment and the maximum and mean grain filling rates of inferior grains under the SD treatment. However, KFA had no significant effect on the active grain filling period of inferior grains under the WW treatment and significantly decreased the active grain filling period of inferior grains under the SD treatment. KFA significantly increased the maximum and mean grain filling rates of inferior grains but had no significant effect on the maximum grain weight under SD treatment. For Zhoumai 22, KFA had no significant effect on the maximum grain weight and the active grain filling period or the maximum and mean grain filling rates of inferior grains under WW treatment. However, KFA significantly decreased the maximum and mean grain filling rates and active grain filling period of inferior grains under the SD treatment and, therefore, significantly decreased the maximum grain weight of inferior grains under the SD treatment. Variance analysis suggested that the cultivar, spikelet categories, soil moisture, and KFA all significantly affected the grain filling characteristics of wheat (Table 2). Moreover, the interaction between cultivar and KFA, spikelet categories and KFA, spikelet categories and soil moisture, and soil moisture and KFA significantly affected the grain filling characteristics of wheat.





**Fig. 1.** Effects of drought and K foliage application (KFA) on grain weights of (A) Xinong 538 and (C) Zhoumai 22 and grain filling rates of (B) Xinong 538 and (D) Zhoumai 22 wheat cultivars. Results are mean  $\pm$  standard deviation (vertical bars,  $n=3$ ). Some vertical bars may be too small to be visible. WW: soil water potential maintained at  $-20 \pm 5$  kPa after anthesis. SD: soil water potential maintained at  $-60 \pm 5$  kPa after anthesis. T1:  $30 \text{ mmol L}^{-1}$  KCl was sprayed on the leaves at anthesis. CK: deionized water was sprayed on the leaves at anthesis. S: superior grain; I: inferior grain.



**Fig. 2.** Effect of drought and K foliage application (KFA) on grain filling characteristics of wheat. Results are mean  $\pm$  standard deviation (vertical bars,  $n=3$ ). WW: soil water potential maintained at  $-20 \pm 5$  kPa after anthesis. SD: soil water potential maintained at  $-60 \pm 5$  kPa after anthesis. T1:  $30 \text{ mmol L}^{-1}$  KCl was sprayed on the leaves at anthesis. CK: deionized water was sprayed on the leaves at anthesis. S: superior grain; I: inferior grain. X: Xiaoyan 22. Z: Zhoumai 22.



**Table 2**

Effects of drought and K foliage application on grain yield and yield components of two wheat cultivars.

a. Effect of cultivar o grain yield and yield components					
Cultivar (C)	No. of spikes ( $\times 10^4 \text{ hm}^{-2}$ )		Spikelets per spike	Grain weight (mg)	Grain yield ( $\text{t hm}^{-2}$ )
Xinong 538	544.2a		31.5b	30.3b	5.2b
Zhoumai 22	456.4b		40.9a	41.4a	7.7a
b. Grain weight as affected by C $\times$ M, C $\times$ F and F $\times$ M interactions					
	Moisture (M)			Fertilizer (F)	
Cultivar (C)	WW	SD	Cultivar (C)	CK	T1
Xinong 538	33.8bA	26.9bB	Xinong 538	29.7bB	32.0bA
Zhoumai 22	45.7aA	37.2aB	Zhoumai 22	41.8aA	41.0aA
b. Grain weight as affected by C $\times$ M, C $\times$ F and F $\times$ M interactions					
	Moisture (M)				
Fertilizer (F)	WW	SD			
CK	38.9bA	32.9aB			
T1	40.5aA	31.2bB			
c. Grain yield as affected by F $\times$ M interaction					
	Moisture (M)				
Fertilizer (F)	WW	SD			
CK	7.0bA	5.8aB			
T1	7.6aA	5.4aB			

Values followed by different lowercase letters are significantly different at  $P=0.05$  within a column. Values followed by different uppercase letters are significantly different at  $P=0.05$  within a row. WW: maintained the soil water potential at  $-20 \pm 5 \text{ kPa}$  after anthesis. SD: maintained the soil water potential  $-60 \pm 5 \text{ kPa}$  after anthesis. T1:  $30 \text{ mmol L}^{-1}$  KCl was sprayed on the leaves at anthesis. CK: deionized water was sprayed on the leaves at anthesis.

### 3.3. Hormones

#### 3.3.1. IAA and Z + ZR

The change in IAA and Z + ZR contents in the grains exhibited similar trends, increasing at the early grain filling stage and reaching peak values 12 and 16 days post-anthesis for superior and inferior grains, respectively (Fig. 3). In addition, the IAA and Z + ZR contents of the superior grains were significantly higher than those of the inferior grains were during the early grain filling stage of the two cultivars.

Drought stress significantly decreased the IAA contents of superior and inferior grains at the early and middle grain filling stages. KFA had no significant effect on the IAA contents of the superior or inferior grains and the Z + ZR content of superior grains. However, KFA significantly increased the Z + ZR content of inferior grains under both the WW and SD treatments at the early and middle grain filling stages.

#### 3.3.2. ABA and ETH

The trends observed for changes in the ABA level of the grains were similar to those for the IAA and Z + ZR, but the peak ABA value of the grains occurred later than the peak IAA and Z + ZR values did. Peak values were achieved 16 and 20 days post-anthesis for the superior and inferior grains (Fig. 4), respectively. The ABA content of the superior grains was significantly higher than that of the inferior grains was in the early and middle grain filling stages. Drought stress significantly decreased the ABA contents of the superior and inferior grains at the early and middle grain filling stages. KFA had no significant effect on the ABA content of the superior grains; however, it significantly increased the ABA content of inferior grains at the early and middle grain filling stages.

In contrast to IAA, Z + ZR, and ABA, the ETH evolution rate of the grains decreased with grain filling and was significantly lower in the superior grains than it was in the inferior grains. Drought stress

significantly increased the ETH evolution rate of both the superior and inferior grains. KFA had no significant effects on the ETH evolution rate of the superior grains. In contrast, KFA significantly increased the ETH evolution rate of the inferior grains during the early and middle grain filling stages for the two cultivars.

### 3.4. Antioxidant enzyme activity, MDA content, and SPAD value

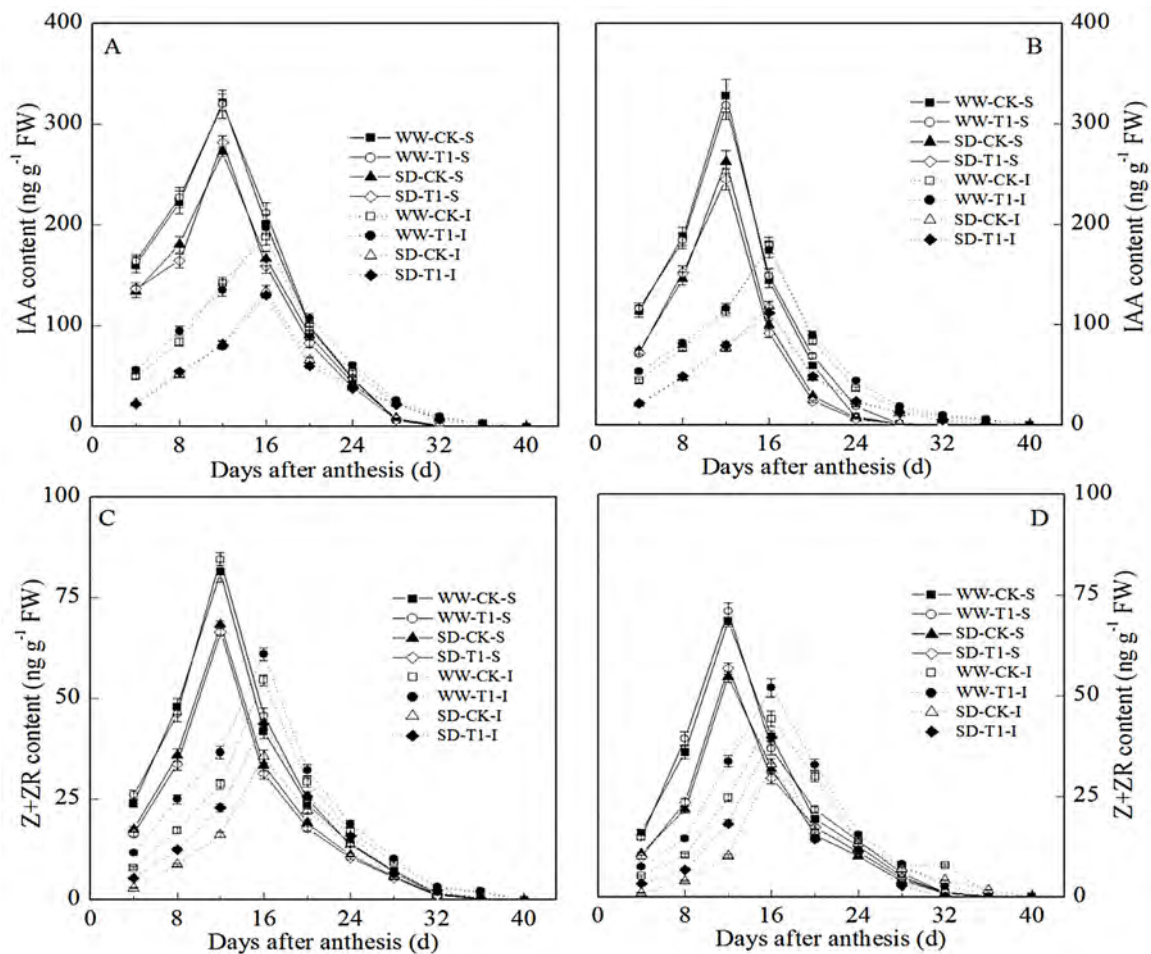
During the grain filling stage, the SOD, POD and CAT activities and SPAD value of the flag leaves decreased during the grain filling stage (Fig. 5). In contrast, the MDA content in flag leaves increased during the grain filling stage (Fig. 6). Drought stress and KFA significantly decreased the SOD, POD and CAT activities and SPAD value of the flag leaves and that significantly increased the MDA content in flag leaves during the grain filling stage.

## 4. Discussion

### 4.1. Effect of KFA on grain filling of wheat

Previous studies indicated that K fertilizer notably increases the grain weight of wheat (Chen et al., 2006; Hu et al., 2014). However, the results of our present study suggest that the effect of KFA on wheat grain filling was significantly related to the wheat cultivars. Under normal soil moisture conditions, KFA had no significant effect on the grain weight of Zhoumai 22, the large-sink cultivar, but significantly increased the grain weight of Xinong 538, a small-sink cultivar. In addition, the variance analysis suggested that KFA only significantly affected the grain filling of inferior grains and not that of the superior grain. Moreover, the variance analysis suggests that the interaction between cultivar and KFA, soil moisture and KFA significantly affected the grain filling characteristics of wheat. This indicates that cultivar and soil moisture are all involved in the regulatory effects of KFA on grain weight of wheat and a notable





**Fig. 3.** Effect of drought and K foliage application (KFA) on the Indole-3-acetic acid (IAA) and zeatin (Z) plus Z riboside (Z + ZR) contents of wheat grains (A and C) Zhoumai 22 and (B and D) Xinong 538. Results are mean  $\pm$  standard deviation (vertical bars,  $n = 3$ ). Some vertical bars may be too small to be visible. WW: soil water potential maintained at  $-20 \pm 5$  kPa after anthesis. SD: soil water potential maintained at  $-60 \pm 5$  kPa after anthesis. T1: 30 mmol L<sup>-1</sup> KCl was sprayed on the leaves at anthesis. CK: deionized water was sprayed on the leaves at anthesis. S: superior grain; I: inferior grain.

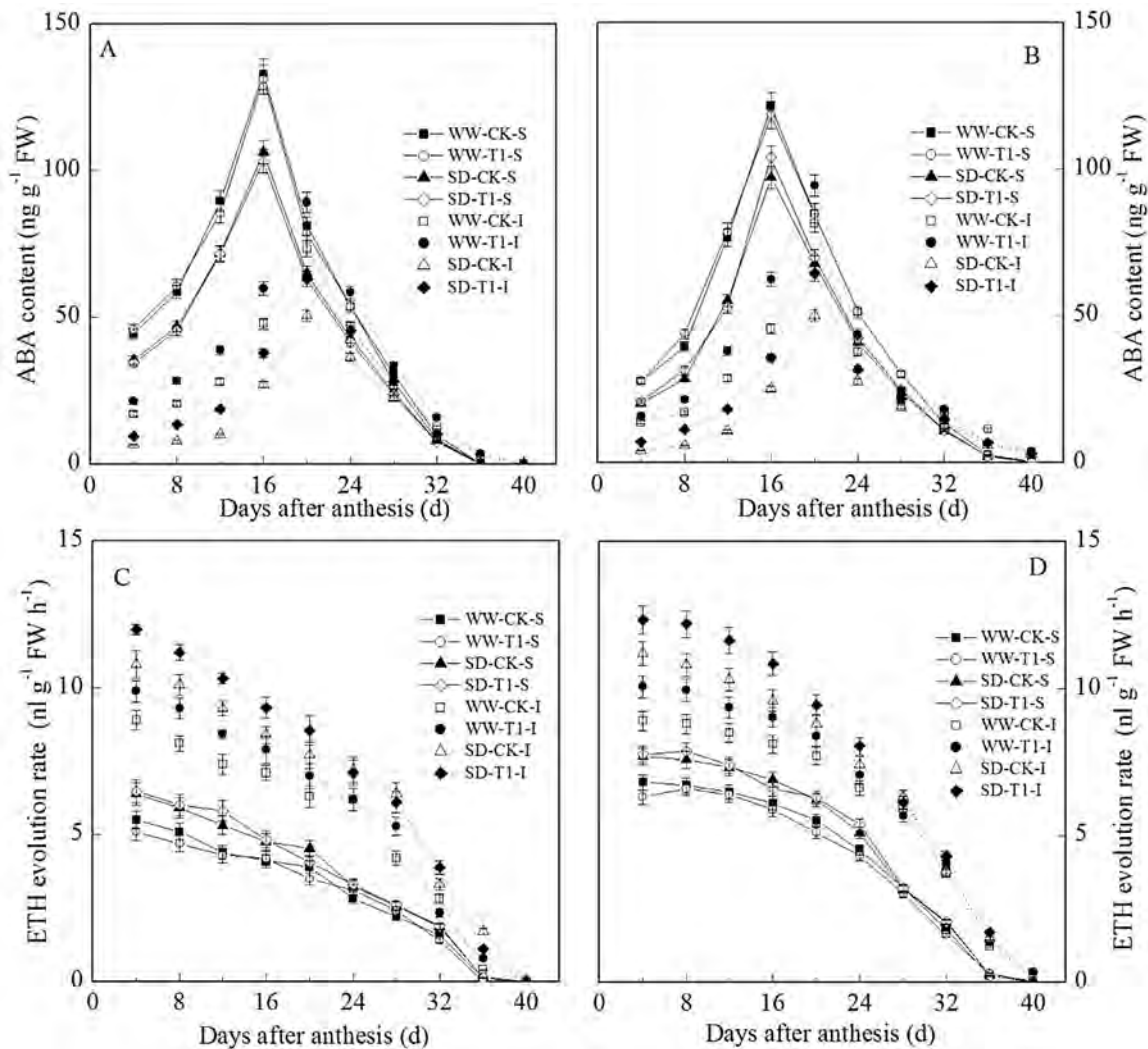
interaction occurs between these factors in the regulation of grain filling of wheat.

The grain filling of cereal was determined by the relationship between the sink and source (Yang and Zhang, 2010). A previous study suggested that a stronger source is necessary to ensure a sufficient carbohydrate supply for grain filling of cereals and high sink activity promotes the carbohydrate transport from source to sink (Fu et al., 2011). Plant hormones are significantly related to the sink activity of cereal grains, and multiple hormones are involved in regulating the sink activity of cereals (Yang and Zhang, 2006). CTK is regarded as indispensable for cell division during the early grain development phase and significantly promotes the endosperm cell division of cereal grain (Chen et al., 2013; Dietrich et al., 1995; Michael and Seiler-Kelbitsch, 1972; Morris et al., 1993; Saha et al., 1986). ABA can promote sink activity and carbohydrate transport from stems to grains of rice and wheat (Xu et al., 2007; Yang et al., 2006). ETH promotes plant senescence and a moderate increase in ETH evolution can promote carbohydrate transport from stems to grains of wheat (Yang et al., 2006). The present study suggests that KFA significantly increased the Z + ZR and ABA contents and ETH evolution rate in inferior grain. Based on these results, we suggest that the KFA-induced regulation of grain filling of inferior wheat grains was mediated by its modulation of the Z + ZR, ABA, and ETH levels in grains. Wang et al. (2003) suggested that K significantly promoted the accumulation and transfer of sucrose in stems. These results suggest that the increased Z + ZR content in grains induced

by KFA may have promoted endosperm cell division and consequently increased the sink capacity of inferior grains of wheat, thereby enhancing their potential to absorb more of the carbohydrate transported from the stem. In addition, KFA may increase the ABA content and ETH evolution rate in grains to promote the sink activity as well as the transport of carbohydrate from stem to grains and, thereby, enrich the sink capacity. This may be the main mechanism by which KFA promoted the grain filling of inferior grains of wheat during the early and middle grain filling stages in the present study. However, in the present study, we found that KFA significantly decreased the grain filling rate during the later stage of the inferior grains of the large-sink cultivar, Zhoumai 22. This is likely the main reason why KFA had no significant effect on the grain weight of Zhoumai 22. From this observation, we surmised that KFA might have aggravated the premature senescence of the wheat plants.

One of the important reasons for plant senescence is that active oxygen or free radicals affect the pericellular membrane, thereby inhibiting normal cell metabolism (Zhao et al., 2011). Antioxidants such as SOD, POD, and CAT eliminate active oxygen or free radicals and notably reduce their detrimental effects on the pericellular membrane, which relieves the plant senescence (Yamazaki and Kamimura, 2002). The present study found that KFA significantly decreased the activities of SOD, POD, and CAT and significantly increased the MDA content of the flag leaves, which decreased the SPAD value and the net photosynthetic rate of the leaves. This result





**Fig. 4.** Effect of drought and K foliage application (KFA) on abscisic acid (ABA) content and ethylene (ETH) evolution rate of wheat grains (A and C) Zhoumai 22 and (B and D) Xinong 538. Results are mean  $\pm$  the standard deviation (vertical bars,  $n=3$ ). Some vertical bars may be too small to be visible. WW: soil water potential maintained at  $-20 \pm 5$  kPa after anthesis. SD: soil water potential maintained at  $-60 \pm 5$  kPa after anthesis. T1: 30 mmol L<sup>-1</sup> KCl was sprayed on the leaves at anthesis. CK: deionized water was sprayed on the leaves at anthesis. S: superior grain; I: inferior grain.

indicates that KFA notably promoted the senescence of the wheat plants.

These results revealed that KFA significantly affected the hormones in the grains, which promoted the sink activity and capacity, thereby enhancing the transfer of carbohydrate reserves stored in the source to the sink. However, the effect of KFA might decrease the carbohydrate content of the leaves and stem, leading to senescence of the plant leaves (Yang and Zhang, 2010). For the large-sink cultivar, Zhoumai 22, the larger sink requires a greater carbohydrate supply from the source to meet the grain filling needs and the larger sink has a strong "attractive power" that promotes the transfer of carbohydrate from source to sink. This effect may intensify the "burden" on the source to supply sufficient carbohydrate to satisfy the grain filling requirement, leading to premature senescence of the plant (Yang and Zhang, 2010). A previous study found that a hybrid rice strain with a large panicle exhibited obvious premature senescence and poor grain filling (Yang and Zhang, 2010). Moreover, KFA enhances the carbohydrate transport from the source to the sink by regulating the hormonal changes in the grains. This effect may lead to an excessive shortage of carbohydrates in the source and cause severe premature senescence of the Zhoumai 22 plant. Therefore, KFA significantly decreased the grain filling rate during the later stage of the inferior Zhoumai 22 grains. This com-

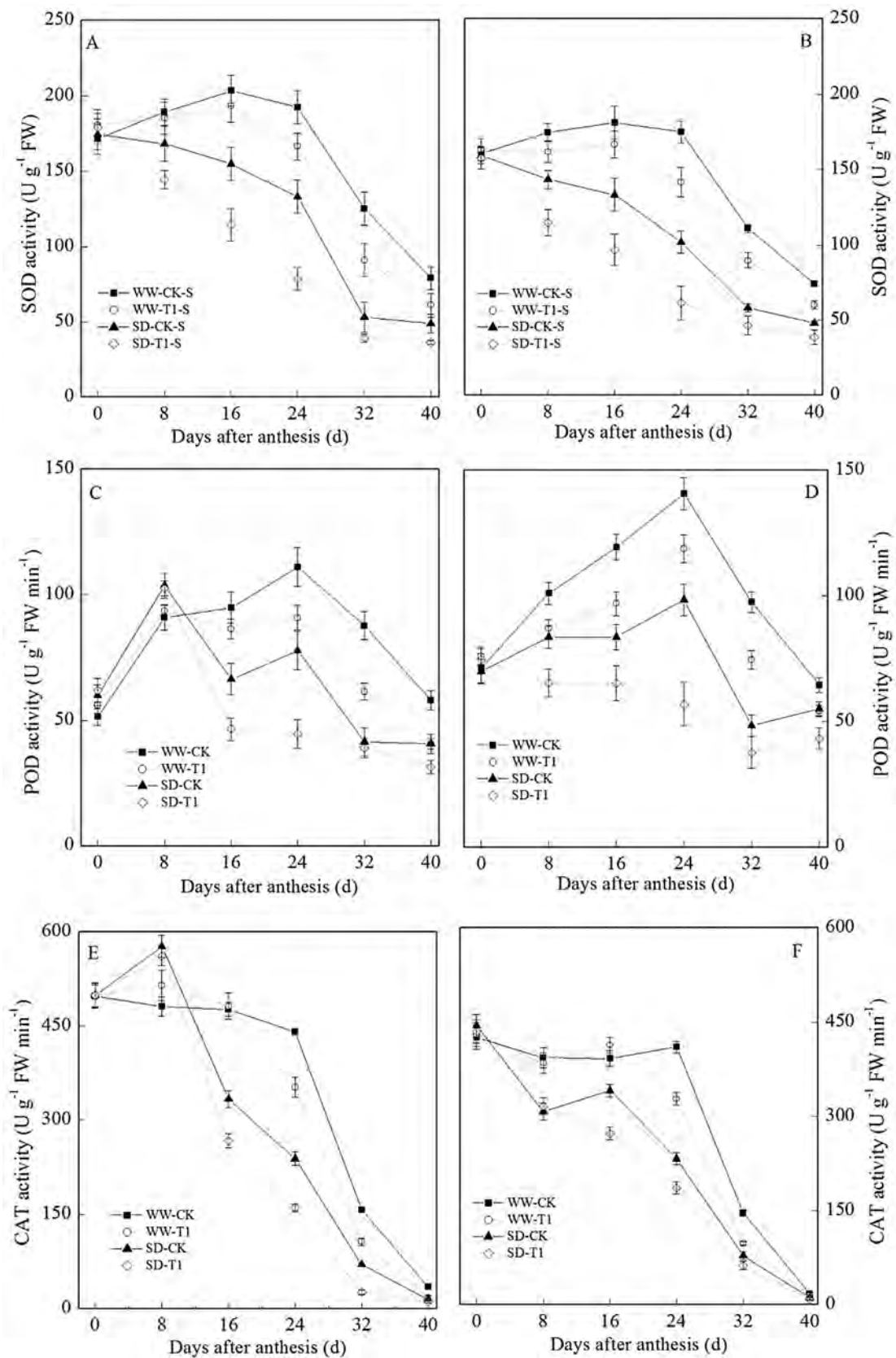
prehensive effect of KFA on the sink and source indicates it did not have a significant effect on the grain weight of Zhoumai 22.

Compared to Zhoumai 22, the sink of Xinong 538, which is a small-sink cultivar, requires less carbohydrate from the source, and the sink capacity and activity are the key limiting factors for the grain filling of these types of wheat and rice cultivars (Yang and Zhang, 2006). KFA promoted the sink capacity and activity as well as increased the carbohydrate transport from the source to the sink. Because the source of the small-sink cultivar was stronger than that of its sink, the source had sufficient carbohydrate to supply the grain filling. Therefore, the KFA did not notably decrease the grain filling rate during the later stage of the inferior grains of Xinong 538, although it significantly decreased the chlorophyll content and promoted the senescence of the plants at the later grain filling stage. Therefore, in contrast to its effects on Zhoumai 22, KFA significantly increased the grain filling rate of the inferior Xinong 538 grain, which increased its grain weight.

#### 4.2. Relationship between drought, KFA, and grain filling of wheat

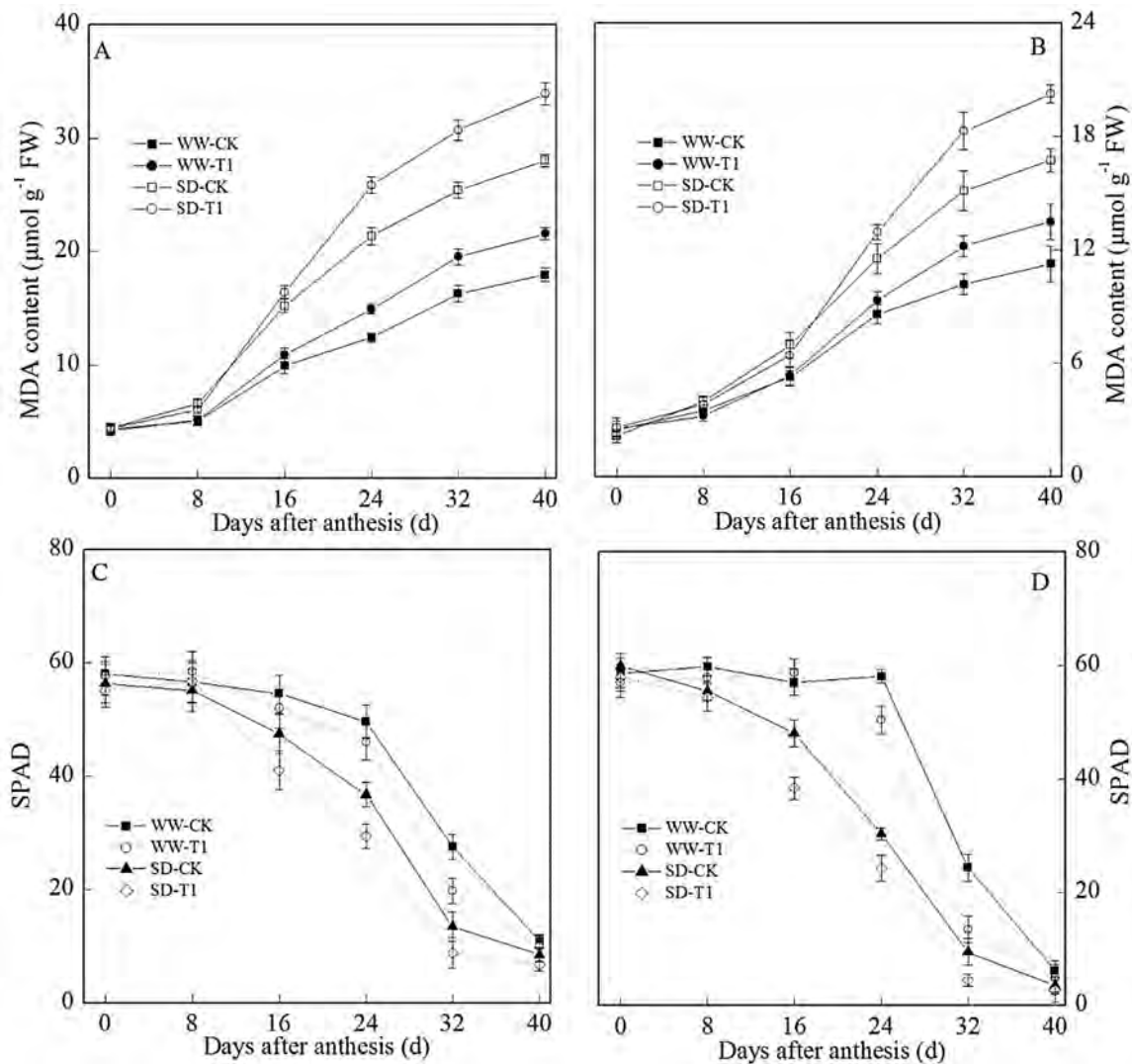
A previous study suggested that drought stress significantly inhibited the grain filling of wheat (Yang et al., 2006) and our present study showed a similar result. Moreover, the effect of KFA





**Fig. 5.** Effects of drought and K foliage application (KFA) on antioxidant enzyme activities of wheat flag leaves (A, C, and E) Zhoumai 22 and (B, D, and F) Xinong 538. Results are mean  $\pm$  standard deviation (vertical bars,  $n=3$ ). Some vertical bars may be too small to be visible. WW: soil water potential maintained at  $-20 \pm 5$  kPa after anthesis. SD: soil water potential maintained at  $-60 \pm 5$  kPa after anthesis. T1: 30 mmol L<sup>-1</sup> KCl was sprayed on the leaves at anthesis. CK: deionized water was sprayed on the leaves at anthesis.





**Fig. 6.** Effects of drought and K foliage application (KFA) on malondialdehyde (MDA) content and soil-plant analyses development (SPAD) value of the flag leaves (A and C) Zhoumai 22 and (B and D) Xinong 538. Results are mean  $\pm$  standard deviation (vertical bars,  $n = 3$ ). Some vertical bars may be too small to be visible. WW: soil water potential maintained at  $-20 \pm 5$  kPa after anthesis. SD: soil water potential maintained at  $-60 \pm 5$  kPa after anthesis. T1:  $30 \text{ mmol L}^{-1}$  KCl was sprayed on the leaves at anthesis. CK: deionized water was sprayed on the leaves at anthesis.

on grain filling of wheat differed between the WW and SD treatments. Under SD treatment, the KFA had no significant effect on the grain weight of Xinong 538, but it significantly decreased that of Zhoumai 22. We determined that this phenomenon was also related to the relationship between the sink and source.

KFA enhanced the sink capacity and activity of the inferior grain of the large-sink cultivar, Zhoumai 22, as well as its carbohydrate transport from source to sink. However, this process caused an excessive shortage of carbohydrates in the source and premature senescence of the plants. A previous study suggested that drought stress promotes the transfer of dry matter from the stem to the panicle and promotes plant senescence (Yang et al., 2001b). Therefore, the combined action of drought stress and KFA notably aggravated the senescence of plants and caused severe premature senescence of plants. Therefore, under SD conditions, KFA not only significantly decreased the grain filling rate during the later stage of the inferior grains, but it also significantly decreased the active grain filling period. This led to a notable decrease in the grain weight of the KFA-treated Zhoumai 22 compare that of the CK under SD conditions.

In contrast to its effects on Zhoumai 22, under the SD condition, KFA significantly decreased the active grain filling period of

the inferior Xinong 538 grains. However, because KFA enhanced the sink activity and transport of carbohydrate from the source to sink, it abrogated the key rate-limiting factors for grain filling of the small-sink cultivar, which are low sink capacity and activity. Furthermore, this activity of KFA significantly increased the grain filling rate of the inferior Xinong 538 grain under SD conditions. The KFA showed a considerable effect on the grain filling rate and active grain filling period of the inferior grain, which led its lack of a significant effect on the grain weight of Xinong 538 under SD conditions.

Based on these results, we concluded that the effect of KFA on grain weight of wheat was different from that of the application of K fertilizer to the soil. While KFA significantly enhanced the sink capacity and activity as well as the transport of carbohydrate from the source to sink, it also caused premature plant senescence. Therefore, the effect of KFA on grain weight differed with the various cultivars and conditions soil moisture. In wheat production, the effective use of KFA and preventing its enhancement of the premature senescence of wheat plants is important for the yield. Some studies have suggested that foliage application of N or P relieves plant senescence and prolongs the grain filling period of wheat (Li



et al., 2015; Xu et al., 2016). Therefore, mixed foliage application of K, N, and P may be an effective approach to promoting the grain filling of wheat on dryland production.

## 5. Conclusions

The results of the present study indicate that drought stress significantly inhibited grain filling and decreased the grain weight of inferior grains of wheat. The effects of KFA on the grain filling of wheat varied depending on the sink type. KFA significantly increased the Z+ZR and ABA contents and the ETH evolution rate of inferior grains during the early and middle grain filling stages, which promoted sink strength. However, KFA significantly decreased the activities of SOD, POD, and CAT as well as decreased the MDA content and SPDA value of the flag leaves, which aggravated the premature senescence of the plant. The effect of KFA on grain filling had a notable genotypic difference. KFA affected the grain filling of inferior grains but not that of superior grains. Furthermore, KFA showed no significant effect on the grain filling of the large-panicle cultivar under the WW treatment, while it significantly decreased the grain filling rate and active period of inferior grains, which significantly decreased the grain weight. KFA significantly promoted the maximum and mean grain filling rates of inferior grains of the light-panicle cultivar, which significantly increased the grain weight under the WW treatment. However, under the SD treatment, KFA significantly decreased the active grain filling period, although it increased the grain filling rate. Therefore, KFA had no significant effect on the weight of inferior grains under the SD treatment.

## Acknowledgements

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# Effect of phosphorus and potassium foliage application post-anthesis on grain filling and hormonal changes of wheat



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## ABSTRACT

Foliage application of fertilizer is an important supplement for soil application fertilizer. Phosphorus (P) and Potassium (K) are important nutrient elements that significantly affect the grain weight and grain yield of wheat, and foliage application of K and P is mainly used for wheat production in the northern dryland region of China. However, the mechanism underlying the regulation of grain filling by foliage application of K and P is not understood. In the present study, monopotassium phosphate ( $\text{KH}_2\text{PO}_4$ ), monosodium orthophosphate ( $\text{NaH}_2\text{PO}_4$ ) and potassium chloride (KCl) were used for foliage application at the anthesis of wheat, and the changes of IAA, ABA, Z + ZR, GAs and ETH in wheat grain were measured during the grain filling process. The objective of this study was to investigate the effect of P and K foliage application on the grain filling process of wheat and to determine how changes in the endogenous hormones of the developing grains of winter wheat under P and K foliage application are related to the grain filling process. The results indicate that the effect of P and K foliage application on the grain filling of wheat is the difference. The K foliage application significantly increased the Z + ZR and ABA contents and decreased the ETH evolution rate in inferior grains, which promoted sink strength and increased the grain filling rate and the grain weight. In comparison, the P foliage application significantly promoted the activities of SOD, POD, and CAT and decreased the MDA content in the flag leaves and relieved the premature senility of the plant, which increased the active grain-filling period and the grain weight. For the heavy panicle cultivar, foliage P had a reasonable effect on grain filling, and for the light panicle cultivar, foliage K had a reasonable effect on grain filling. The mixture of P and K had an additive effect and prevented P and K deficiencies. Foliage application of  $\text{KH}_2\text{PO}_4$  is an effectual measure for promoting the grain weight of wheat.

## 1. Introduction

The yield potential of wheat (*Triticum aestivum* L.) is dissected into three major components as follows: panicle number per plant, grain number per panicle and grain weight. Grain filling, the final stage of cereal growth, determines the grain weight (Yang and Zhang, 2006). Currently, the high yield crop production systems need high yield outputs, and because of this, improving grain filling has become more important than ever (Saini and Westgate 2000; Zahedi and Jenner 2003).

Phosphorus (P) and Potassium (K) are important nutrient elements that significantly affect the grain weight and grain yield of wheat (Kang et al., 2014; Zhan et al., 2015; Wani et al., 2014; Kunzova and Hejman, 2010; Brennan and Bolland, 2009). The northern dryland region is the largest wheat growing area in China. In this region, nitrogen (N) is the main fertilizer for wheat production. K fertilizer is rarely applied for wheat production in this region because the traditional view suggests

that the K in the soil is enough for wheat production in this region (Guo et al., 2010). In addition, P fertilizer is also often lacking in wheat production because of the cost of production (Meng et al., 2008). By increasing of the wheat grain yield, the wheat plants should absorb more P and K from the soil to maintain growth, and this may lead to a P and K deficiency for wheat production if there is not enough P and K fertilizer supplement in the soil (Chen et al., 2006). Previous studies indicate that an appropriate increase of P and K content in the soil notably promotes the wheat yield in this region (Chen et al., 2006; Hu et al., 2014). However, the price of P and K fertilizers has increased over the years, and this inhibits the farmers from increasing P and K fertilizers for wheat production. In addition, the basic fertilizer is the main fertilization pattern for wheat production in this region, and this fertilization pattern may lead to nutrient deficiency at the later growth period, especially the grain filling stage of wheat (Zhao and Si, 2015). A previous study suggested that a top dress can resolve this problem and notably promoted nutrient-use efficiency of the middle and later

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growth stage of wheat (Zhao and Si, 2015). However, a top dressing is difficult to apply in the northern dryland region in China because irrigation and rain are deficient during the wheat growth period in this region.

For these reasons, foliage application of K and P is mainly used for wheat production in the northern dryland region of China because of its lower cost compared to soil fertilizer, and it can be applied without regard to irrigation or rain. In China, monopotassium phosphate ( $\text{KH}_2\text{PO}_4$ ) is extensively used for foliage application at the anthesis stage of wheat. Previous studies suggest that  $\text{KH}_2\text{PO}_4$  significantly promotes the grain filling and grain weight of wheat (Lu et al., 2013). However, the mechanism underlying the regulation of grain filling by  $\text{KH}_2\text{PO}_4$  is not understood.

Plant hormones play an important role in regulating the grain filling of cereals. The large transient increase of zeatin (Z) and zeatin riboside (ZR) in grains is an important condition for seed setting and the endosperm cell division of grains of wheat (Morris et al., 1993). The abscisic acid (ABA) concentrations in superior grains were significantly higher than those of inferior grains, and the ethylene (ETH) concentrations showed an opposite trend (Yang et al., 2006). The content of indole-3-acetic acid (IAA) was higher in superior grains than in inferior ones at the early grain-filling stage of rice (*Oryza sativa* L.) (Xu et al., 2007). The maximal grain filling rate was negatively correlated with the Gibberellic acids (GAs) in the rice grains (Yang et al., 2001), but our previous study suggests that there is no significant correlation between GA content in grains and the grain filling rate and the grain weight of wheat (Liu et al., 2013a,b).

These studies indicate that the hormones notably affect the wheat grain filling. However, the relationship between hormonal changes and the grain filling induced by  $\text{KH}_2\text{PO}_4$  is unclear. In the present study,  $\text{KH}_2\text{PO}_4$ , monosodium orthophosphate ( $\text{NaH}_2\text{PO}_4$ ) and potassium chloride (KCl) were used for foliage application at the anthesis stage of wheat, and the changes of IAA, ABA, Z + ZR, GAs and ETH in the wheat grain were measured during the grain filling process. The objective of this study was to investigate the effect of P and K foliage application on the grain filling process of winter wheat and to determine how the changes in the endogenous hormones of the developing grains of winter wheat under P and K foliage application are related to the grain filling process.

## 2. Materials and methods

### 2.1. Study site description

This study was conducted from 2011 to 2014 at the experimental station of the Crop Specimen Farm in Northwest A & F University, Shaanxi Province, China, at an elevation of 466.7 m above sea level. The annual mean temperature was 12.9 °C, and the annual mean precipitation was 550 mm, 70% of which fell from June to September. The soil in the top 1.2 m was Eum-Orthosols (Chinese soil Taxonomy) with mean bulk density of 1.35 g cm<sup>-3</sup>. The readily available N, P and K of the cropland in this study were 53.21 mg kg<sup>-1</sup>, 21.34 mg kg<sup>-1</sup> and 105.68 mg kg<sup>-1</sup>, respectively. The organic matter content of 0–20 cm topsoil and the pH were 12.27 g kg<sup>-1</sup> and 7.32, respectively. The average monthly temperature and precipitation of the four years during the experimental period are shown in Fig. 1.

### 2.2. Experiment design and treatments

#### 2.2.1. The first experiment

Two winter wheat cultivars, Shuangda 1 and Xinong 538, were grown in the dryland field. The seeds were sown on Oct 20 for 2011 and Oct 19 for 2012. At anthesis, 10 mmol L<sup>-1</sup>, 30 mmol L<sup>-1</sup> and 50 mmol L<sup>-1</sup>  $\text{KH}_2\text{PO}_4$  (PK1, PK2 and PK3, respectively) was sprayed from above canopy using a sprayer. The chemicals were applied daily for 4 days at a rate of 750 kg h m<sup>-2</sup> at each application. All of the

solutions contained 0.01% (V/V) Tween-20. The same volume of deionized water containing the same concentration of Tween-20 was applied to the control plants (CK1). Each treatment had three replicates with a completely randomized block design, and the plot dimension was 3 m × 2 m.

#### 2.2.2. The second experiment

Two winter wheat cultivars, Shuangda 1 and Xinong 538, were grown in the dryland field. The seeds were sown on Oct 20 for 2013 and Oct 18 for 2014. At anthesis, 30 mmol L<sup>-1</sup>  $\text{KH}_2\text{PO}_4$  (PK), 30 mmol L<sup>-1</sup>  $\text{NaH}_2\text{PO}_4$  (P) and 30 mmol L<sup>-1</sup> KCl (K) was sprayed from above canopy, using a sprayer. The chemicals were applied daily for 4 days at a rate of 750 kg h m<sup>-2</sup> at each application. All of the solutions contained 0.01% (V/V) Tween-20. The same volume of deionized water containing the same concentration of Tween-20 was applied to the control plants (CK2). Each treatment had three replicates with a completely randomized block design and the plot dimension was 3 m × 4 m.

For the two experiments, the sowing density was 150 kg ha<sup>-1</sup> with a row spacing of 0.25 m. We applied 150 kg ha<sup>-1</sup> of urea and 100 kg ha<sup>-1</sup> of diammonium orthophosphate at basal levels. Irrigation was provided at winter and jointing stage, respectively, and the irrigation amount was 60 mm at the each stage.

### 2.3. Sampling and measurement

Four hundred spikes that flower on the same day were chosen and tagged in each plot. Twenty tagged spikes from each plot were sampled at 4-day intervals from anthesis to maturity. All of the grains from each spike were removed. The grains on a spike were divided into superior grains and inferior grains. The most basal grains in the middle spikelets (four to 12 spikelets) from the bottom of a spike were considered superior grains, and the most distal grains in the middle spikelets (four to 12 spikelets) from the bottom of a spike were considered inferior grains. Half of the sampled grains were used for the hormone measurements. The other half of the grains were dried at 70 °C to a constant weight and were weighed. Twenty flag leaves of the tagged spikes from each plot were sampled at 8-day intervals from anthesis to maturity. The flag leaves were freeze by liquid nitrogen for 15 min and then store at -40 °C refrigerator.

#### 2.3.1. Grain-filling process

The grain-filling process was fitted by the Richards's (1959) growth equation as described by Zhu et al. (1988):

$$W = \frac{A}{(1 + Be^{-kt})^{\frac{1}{N}}} \quad (1)$$

The grain-filling rate (G) was calculated as the derivative of Eq. (1):

$$G = \frac{AkBe^{-kt}}{(1 + Be^{-kt})^{\frac{N+1}{N}}} \quad (2)$$

[W, the grain weight (mg); A, the final grain weight (mg); t, time after anthesis (d); B, k and N, coefficients determined by regression.]

The active grain-filling period was defined as the period when W was between 5% ( $t_1$ ) and 95% ( $t_2$ ) of A. The average grain-filling rate during this period was therefore calculated from  $t_1$  to  $t_2$ .

#### 2.3.2. Hormones

Approximately 0.5 g of the FW sample was taken, and the endogenous Z + ZR, GAs ( $\text{GA}_1 + \text{GA}_4$ ), IAA, and ABA were extracted according to previous studies (Yang et al., 2001; Liu et al., 2011). The samples were homogenized with 5 mL of 80% (v/v) methanol, which contained 1 mmol L<sup>-1</sup> butylated hydroxytoluene (BHT). The extracting solution was passed through Chromosep C18 columns (C18 Sep-Park Cartridge, Waters Corp., Milford, MA, USA), and the fractions were vacuum dried at 40 °C and dissolved in 1 mL of phosphate-buffered



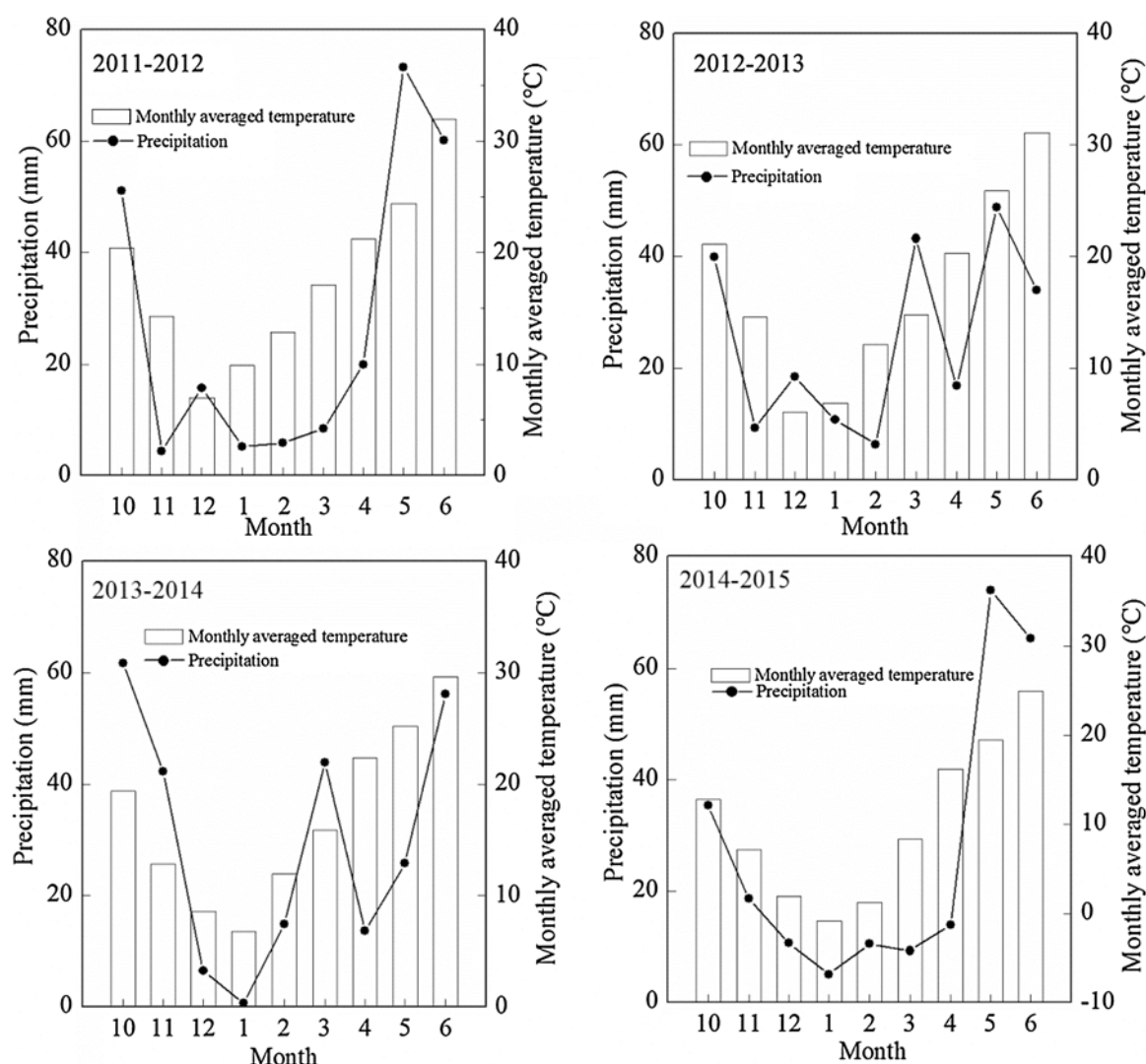


Fig. 1. The average monthly temperature and precipitation during the four wheat growing seasons in experimental field.

saline (PBS) containing 0.1% (v/v) Tween 20 and 0.1% (w/v) gelatin (pH 7.5) for enzyme-linked immunosorbent assay (ELISA). The ELISA kits were manufactured by the Phytohormones Research Institute, China Agricultural University. The quantification of Z + ZR, GAs ( $G_A_1 + G_A_4$ ), IAA, and ABA was performed by ELISA as previously described (Yang et al., 2001; Liu et al., 2011). The recovery rates for IAA, Z + ZR, ABA, and GAs were  $(85.4 \pm 4.7) \%$ ,  $(93.1 \pm 6.2) \%$ ,  $(89.5 \pm 3.2) \%$ , and  $(78.2 \pm 5.4) \%$ , respectively.

The ethylene evolved from the grains was determined according to Beltrano et al. (1994) and Yang et al. (2008). The ethylene was assayed using a gas chromatograph (Trace GC Ultra™, Thermo Fisher Scientific, USA) equipped with a Porapak Q column (0.3 cm × 200 cm, 0.18–0.30 mm) and a flame ionization detector (FID). The temperatures for the injection port, the column and the detector were kept constant at 70, 70 and 150 °C, respectively. Nitrogen was used as a carrier at a flow rate of 40 Kpa, and hydrogen and air were used for the FID at rates of 35 and 350 mL min<sup>-1</sup>, respectively. The rate of ethylene evolution is expressed as a function of per unit fresh weight (FW).

#### 2.4. Yield and yield components

Plants (except the border) from a 1-m<sup>2</sup> site from each plot were harvested at maturity for determination of the yield components, i.e., the spikes per square meter, the spikelets number per spike, and the grain weight and grain yield.

#### 2.5. Antioxidant enzyme activity, malonaldehyde (MDA) content

Enzyme extraction was carried out by 500 mg of fresh leaves were ground in 5 mL extraction buffer constituted of 100 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA and 1% PVPP. The extract was centrifuged at 20,000 rpm per 20 min at 4 °C and the supernatant was collected. The collected supernatants were used in all enzyme analyses.

The activity of superoxide dismutase (SOD, EC 1.15.1.1) was evaluated by its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT), as proposed by Wang and Huang (2000). Measurements were taken at 560 nm and one unit of SOD corresponded to the amount of enzyme capable of inhibiting 50% of NBT photoreduction in the experimental conditions. Catalase activity (CAT, EC 1.11.1.6) was determined by H<sub>2</sub>O<sub>2</sub> consumption at 240 nm during 3 min (Wang and Huang, 2000). Guaiacol peroxidase activity (POD, EC 1.11.1.7) was determined by guaiacol oxidation at 470 nm according to Wang and Huang (2000) with modifications.

Samples containing 500 mg of leaf tissue were homogenized in 5 mL 0.1% trichloroacetic acid (TCA), and centrifuged at 20,000g per 20 min at 4 °C. The supernatant was used for MDA content determination. The method used followed that of Wang and Huang (2000) and was based on thiobarbituric acid reaction. MDA content was calculated according to the formula MDA content (ηM) =  $[(A_{535} - A_{600})/1.56] \times 105$ .



## 2.6. SPAD value and net photosynthetic rate of flag leaves

The SPAD value and net photosynthetic rate were measured at 8-day intervals from anthesis to maturity. Ten flag leaves of the tagged plants from each plot were sampled. The SPAD measurements were performed by SPAD-502 Chlorophyll Meter (Minolta Co. Ltd., Osaka, Japan). The net photosynthetic rate was measured by the portable photosynthesis system (LI-6400XT, Li-Cor Co. Ltd., UAS).

## 2.7. Statistical analyses

SPSS 16.0 was used for the ANOVA. The data from each sampling were analyzed separately. The means were tested by the least significant difference at  $P_{0.05}$  (LSD  $_{0.05}$ ).

## 3. Results

### 3.1. Yield and yield component

Foliage-applied  $\text{KH}_2\text{PO}_4$  significantly affected the grain yield of the wheat; however, the response of the grain yield of the two cultivars to  $\text{KH}_2\text{PO}_4$  was different (Table 1, the first experiment). For Xinong 538, the grain yields of the PK1, PK2 and PK3 treatments were all significantly higher than those of the CK1 treatment. And the grain yields of the PK2 and PK3 treatments were significantly higher than that of PK1. For Shuangda 1, the grain yields of the PK2 and PK3 treatments were significantly higher than that of CK1, but there was no significant difference between the grain yields of the PK1 and CK1 treatments of Shuangda 1.

Foliage-applied  $\text{KH}_2\text{PO}_4$  did not significantly affect the spikes per ha and spikelets per spike for the two cultivars. However, it did significantly affect the grain weight of the wheat, and the effect of  $\text{KH}_2\text{PO}_4$  on grain weight was similar to that of the grain yield. This means that the  $\text{KH}_2\text{PO}_4$ , through regulation of the grain weight, affects the grain yield of the wheat. In addition, 30  $\text{mmol L}^{-1}$  and 50  $\text{mmol L}^{-1}$   $\text{KH}_2\text{PO}_4$  similarly promote the grain weight and grain yield of the wheat, and the promoting effects on grain weight and grain yield were notably higher than 10  $\text{mmol L}^{-1}$   $\text{KH}_2\text{PO}_4$ .

Table 2 shows that external  $\text{KH}_2\text{PO}_4$ ,  $\text{NaH}_2\text{PO}_4$  and KCl did not significantly affect the panicles per ha and spikelets per panicle. In addition, the foliage-applied  $\text{KH}_2\text{PO}_4$ ,  $\text{NaH}_2\text{PO}_4$  and KCl had different effects on grain weight and grain yield for the two cultivars. For Shuangda 1, the foliage-applied  $\text{NaH}_2\text{PO}_4$  significantly promoted the

grain weight and grain yield, and the foliage-applied KCl did not significantly affect the grain weight and grain yield. For Xinong 538, the foliage-applied KCl significantly promoted the grain weight and grain yield, and the foliage-applied  $\text{NaH}_2\text{PO}_4$  did not significantly affect the grain weight and grain yield. In addition, the foliage-applied  $\text{KH}_2\text{PO}_4$  significantly promoted the grain weight and grain yield of the two cultivars, and the grain weight and grain yield of the PK treatment was significantly higher than the P and K treatments.

### 3.2. Grain filling

The external  $\text{KH}_2\text{PO}_4$ ,  $\text{NaH}_2\text{PO}_4$  and KCl had different effects on the grain filling of superior grains and inferior grains (Table 3, Fig. 2). External  $\text{KH}_2\text{PO}_4$ ,  $\text{NaH}_2\text{PO}_4$  and KCl all did not significantly affect the grain filling of the superior grains, and external  $\text{NaH}_2\text{PO}_4$  did not significantly affect the grain filling of inferior grains of Xinong 538. Beside this, the external  $\text{KH}_2\text{PO}_4$  significantly promoted the grain filling of the inferior grains of the two cultivars and KCl significantly promoted the grain filling of inferior grains of Shuangda 1. The maximum grain weight and the maximum and mean grain-filling rates of the inferior grains of the K and PK treatments were significantly higher than those of the CK2 for the two cultivars. However, the K treatment notably decreased the active grain-filling period, and by this, the maximum grain weight of the K treatment was significantly lower than that of the PK treatment.

### 3.3. Hormonal changes

#### 3.3.1. IAA and Z + ZR

The IAA and Z + ZR contents in the grains had a similar trend, and they increased at the early grain filling stage and reached a peak value at 12 and 16 days post-anthesis for the superior grains and inferior grains, respectively (Fig. 3). The IAA and Z + ZR contents in the grains of Shuangda 1 were significantly higher than those of Xinong 538. Moreover, the IAA and Z + ZR contents in the superior grains were significantly higher than those in the inferior grains during 0–16 days post-anthesis for Shuangda 1 and during 0–12 days for Xinong 538.

The external spray of  $\text{KH}_2\text{PO}_4$ ,  $\text{NaH}_2\text{PO}_4$  and KCl did not significantly affect the IAA contents in the superior grains and inferior grains and the Z + ZR contents in the superior grains. Contrary to this, these notably affected the Z + ZR content in the inferior grains. The external spray of  $\text{KH}_2\text{PO}_4$  and KCl significantly increased the Z + ZR content in the inferior grains at 4–16 days post-anthesis. However, the

**Table 1**  
Effect of  $\text{KH}_2\text{PO}_4$  foliage application on the grain yield and yield component of wheat.

Year	Cultivar	Treatments	No. of spikes ( $\times 10^4 \text{ h m}^{-2}$ )	Spikelets per spike	Grain weight (mg)	Grain yield ( $\text{t h m}^{-2}$ )
2011–2012	Shuangda1	CK1	311.0a	43.7a	50.53b	6.86b
		PK1	307.7a	43.3a	51.83ab	6.91ab
		PK2	314.2a	43.4a	52.73a	7.18a
		PK3	309.7a	44.2a	52.80a	7.23a
	Xinong538	CK1	598.3a	33.9a	34.47c	6.99c
		PK1	592.3a	34.0a	36.63b	7.37b
		PK2	603.1a	33.9a	39.10a	7.98a
		PK3	592.0a	33.6a	39.33a	7.83a
2012–2013	Shuangda1	CK1	348.9a	43.5a	50.87b	7.72b
		PK1	352.3a	43.4a	51.88ab	7.93ab
		PK2	350.7a	44.6a	52.90a	8.28a
		PK3	352.7a	43.6a	53.03a	8.02a
	Xinong538	CK1	612.3a	31.0a	36.70c	6.97c
		PK1	617.7a	31.7a	38.13b	7.46b
		PK2	614.3a	32.4a	39.50a	7.86a
		PK3	613.0a	32.7a	39.80a	7.70a

Values within a column and for the same year and the same cultivar followed by different letters are significantly different at  $P = 0.05$ . PK1, PK2 and PK3 represent 10  $\text{mmol L}^{-1}$ , 30  $\text{mmol L}^{-1}$  and 50  $\text{mmol L}^{-1}$   $\text{KH}_2\text{PO}_4$ , respectively, that was sprayed on leaves at anthesis. CK1 means that deionized water was sprayed on leaves at anthesis.



**Table 2**

Effects of P and K foliage application on the grain yield and yield component of wheat.

Year	Cultivar	Treatment	No. of spikes ( $\times 10^4 \text{ h m}^{-2}$ )	Spikelets per spike	Grain weight (mg)	Grain yield ( $\text{t h m}^{-2}$ )
2013–2014	Shuangda1	CK2	363.2a	44.2a	46.42c	7.45c
		P	364.5a	45.2a	48.68b	8.02b
		K	366.5a	44.5a	46.17c	7.53c
		PK	368.7a	45.9a	50.18a	8.49a
	Xinong538	CK2	596.6a	33.7a	36.41c	7.32c
		P	603.2a	33.4a	37.04bc	7.46bc
		K	594.1a	34.3a	38.34ab	7.81ab
2014–2015	Shuangda1	CK2	367.4a	42.1a	49.03c	7.58c
		P	369.1a	42.3a	51.32b	8.01b
		K	370.2a	41.7a	49.31c	7.61c
		PK	370.4a	42.8a	52.99a	8.40a
	Xinong538	CK2	633.6a	31.5a	37.41c	7.47c
		P	629.5a	32.2a	38.28bc	7.76bc
		K	631.4a	32.3a	39.86b	8.13b
		PK	638.2a	33.1a	41.52a	8.77a

Values within a column and for the same year and the same cultivar followed by different letters are significantly different at  $P = 0.05$ . P, K and PK represent  $30 \text{ mmol L}^{-1} \text{ KH}_2\text{PO}_4$ ,  $30 \text{ mmol L}^{-1} \text{ NaH}_2\text{PO}_4$  and  $30 \text{ mmol L}^{-1} \text{ KCl}$ , respectively, that was sprayed on leaves at anthesis. CK2 means that deionized water was sprayed on leaves at anthesis.

**Table 3**

Effect of P and K foliage application on the grain-filling characteristics of wheat.

Year	Cultivar	Spikelet categories	Treatment	Wmax mg	Gmax $\text{mg grain}^{-1} \text{ d}^{-1}$	Gmean $\text{mg grain}^{-1} \text{ d}^{-1}$	D d
2012–2013	Shuangda1	S	CK2	52.07a	3.18a	1.98a	26.3a
			P	52.40a	3.22a	1.97a	26.6a
			K	51.68a	3.24a	1.95a	26.5a
			PK	51.87a	3.24a	1.95a	26.6a
		I	CK2	40.76c	2.21b	1.65b	24.7b
			P	42.80b	2.23b	1.64b	26.1a
			K	40.59c	2.57a	1.82a	22.3c
			PK	46.55a	2.58a	1.84a	25.3ab
	Xinong538	S	CK2	40.00a	2.42a	1.63a	24.5a
			P	40.71a	2.44a	1.64a	24.8a
			K	41.01a	2.46a	1.67a	24.6a
			PK	41.38a	2.39a	1.68a	24.6a
		I	CK2	31.35c	1.57b	1.26b	24.9a
			P	31.87c	1.54b	1.23b	25.9a
			K	34.10b	1.76a	1.38a	24.7a
			PK	35.67a	1.79a	1.39a	25.7a
2013–2014	Shuangda1	S	CK2	54.67a	3.27a	2.04a	26.8a
			P	54.47a	3.33a	2.01a	27.1a
			K	54.25a	3.31a	1.98a	27.4a
			PK	54.61a	3.34a	2.03a	26.9a
		I	CK2	42.83c	2.36b	1.77b	24.2b
			P	45.76b	2.43b	1.76b	26.0a
			K	42.37c	2.68a	1.90a	22.3c
			PK	50.23a	2.71a	1.91a	26.3a
	Xinong538	S	CK2	41.53a	2.51a	1.67a	24.9a
			P	42.21a	2.48a	1.66a	25.4a
			K	42.03a	2.41a	1.64a	25.6a
			PK	41.82a	2.43a	1.66a	25.2a
		I	CK2	34.01c	1.68b	1.31b	25.1a
			P	35.15c	1.71b	1.29b	26.2a
			K	36.51b	1.93a	1.46a	25.0a
			PK	37.80a	2.00a	1.45a	26.1a

Values within a column and for the same cultivar and same grain type followed by different letters are significantly different at  $P = 0.05$ . P, K and PK represent  $30 \text{ mmol L}^{-1} \text{ KH}_2\text{PO}_4$ ,  $30 \text{ mmol L}^{-1} \text{ NaH}_2\text{PO}_4$  and  $30 \text{ mmol L}^{-1} \text{ KCl}$ , respectively, that was sprayed on leaves at anthesis. CK2 means that deionized water was sprayed on leaves at anthesis. Wmax: the final grain weight; Gmax: maximum grain-filling rates; Gmean: mean grain-filling rates; D: active grain-filling period S: superior grain; I: inferior grain.

external spray of  $\text{NaH}_2\text{PO}_4$  did not significantly affect the Z + ZR contents in the inferior grains.

### 3.3.2. ABA and ETH

The change in the trend of ABA in the grains was similar with IAA and Z + ZR, but the peak value of ABA in the grains was later than IAA

and Z + ZR. It reached the peak value at 16 and 20 days post-anthesis for the superior grains and inferior grains, respectively (Fig. 4). The ABA content in the superior grains was significantly higher than that in the inferior grains at the early and middle grain filling stages. The external spray of  $\text{KH}_2\text{PO}_4$ ,  $\text{NaH}_2\text{PO}_4$  and KCl did not significantly affect the ABA content in the superior grains and inferior grains. Contrary to



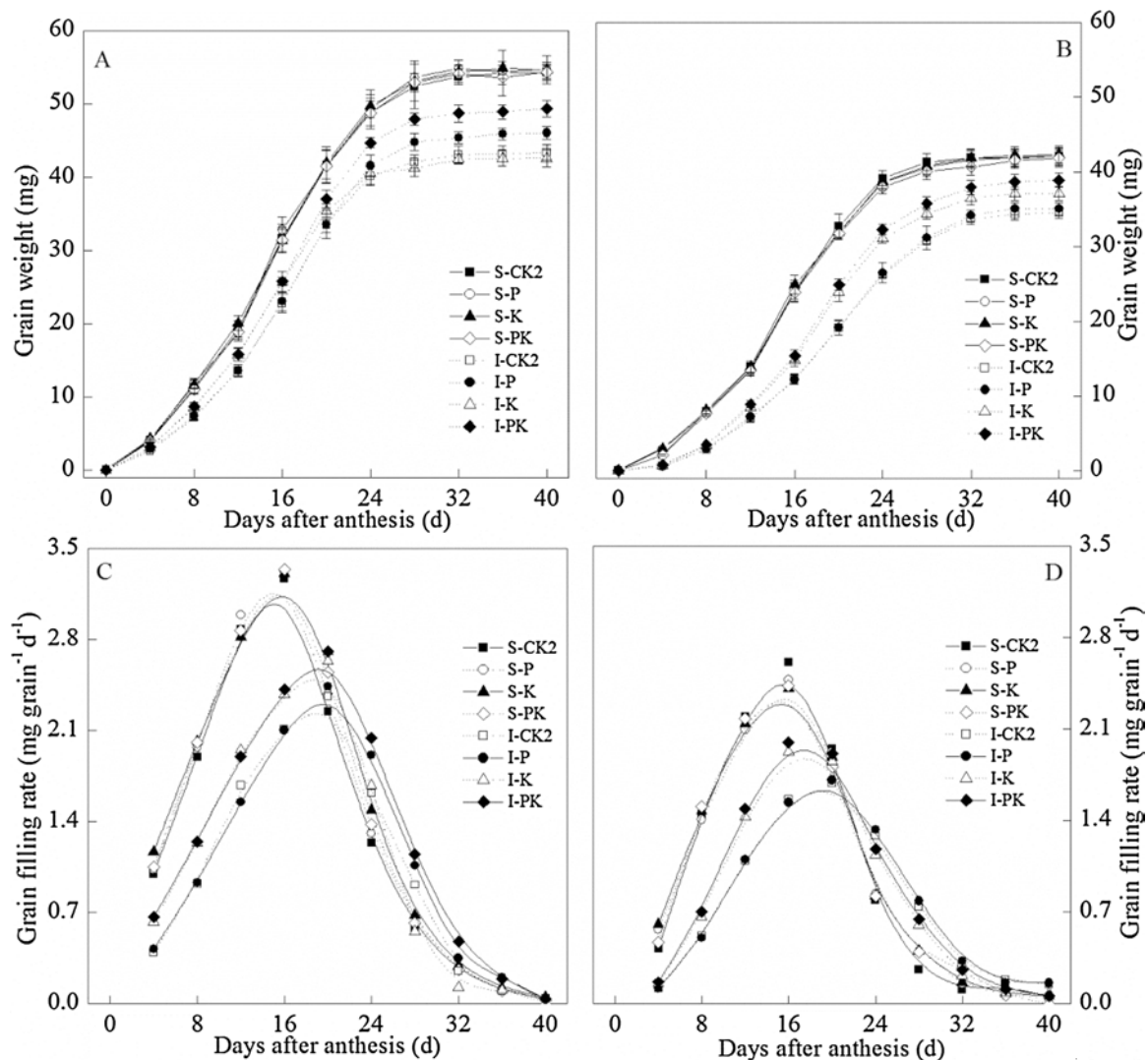


Fig. 2. Effects of P and K foliage application on grain weights (A: Shuangda 1; B: Xinong 538) and grain filling rates (C: Shuangda 1; D: Xinong 538) of wheat. S: superior grain; I: inferior grain. Vertical bars represent  $\pm$  the standard deviation of the mean ( $n = 6$ ). P, K and PK represent  $30 \text{ mmol L}^{-1} \text{ KH}_2\text{PO}_4$ ,  $30 \text{ mmol L}^{-1} \text{ NaH}_2\text{PO}_4$  and  $30 \text{ mmol L}^{-1} \text{ KCl}$ , respectively, that was sprayed on leaves at anthesis. CK2 means that deionized water was sprayed on leaves at anthesis.

this, the external spray of  $\text{KH}_2\text{PO}_4$  and  $\text{KCl}$  significantly increased the ABA content in the inferior grains during 4–20 days post-anthesis for Shuangda 1 and Xinong 538.

Contrary to the fact that the IAA, Z + ZR and ABA, the ETH evolution rates in the grains are continuing decrease with grain filling, and the ETH evolution rate in the superior grains was significantly lower than that in the inferior grains. Similar to ABA, the external spray of  $\text{KH}_2\text{PO}_4$ ,  $\text{NaH}_2\text{PO}_4$  and  $\text{KCl}$  had no significant effects on the ETH evolution rate in the superior grains and the inferior grains. In contrast, the external spray of  $\text{KH}_2\text{PO}_4$  and  $\text{KCl}$  significantly increased the ETH evolution rate in inferior grains during 4–16 days post-anthesis for Shuangda 1 and Xinong 538.

#### 3.4. Antioxidant enzyme activity

During the grain filling stage, the SOD and POD activities of flag leaves first increased and then decreased, and they reached a peak value at 16 and 24 days post-anthesis, respectively (Fig. 5). Contrary to this, the CAT activity of the flag leaves decreased during the grain filling stage. The external  $\text{KH}_2\text{PO}_4$ ,  $\text{NaH}_2\text{PO}_4$  and  $\text{KCl}$  had a different effect on the antioxidant enzymes activities of the flag leaves. At the early grain filling stage, they did not significantly affect the antioxidant enzymes activities of the flag leaves. After 16 days post-anthesis, the

external  $\text{KH}_2\text{PO}_4$  and  $\text{NaH}_2\text{PO}_4$  significantly promoted the SOD, CAT and POD activities of the flag leaves compared to the CK2 plants, but the external  $\text{KCl}$  significantly decreased the SOD, CAT and POD activities of the flag leaves.

#### 3.5. MDA, SPAD and photosynthesis

With grain filling, the MDA content of the flag leaves continued to increase and the net photosynthetic rate and the SPAD value of the flag leaves continued to decrease (Fig. 6). The external  $\text{KH}_2\text{PO}_4$  and  $\text{NaH}_2\text{PO}_4$  significantly decreased the MDA content of the flag leaves and significantly increased the net photosynthetic rate and the SPAD value of the flag leaves during the middle and later grain filling stage compared to the CK2 plants. Contrary to this, the external  $\text{KCl}$  significantly increased the MDA content of the flag leaves and significantly decreased the net photosynthetic rate and the SPAD value of the flag leaves during the middle and later grain filling stages.

#### 4. Discussion

To analyze the mechanism underlying the regulation of grain filling by  $\text{KH}_2\text{PO}_4$ ,  $\text{NaH}_2\text{PO}_4$  and  $\text{KCl}$  were used for foliage application to study the effects of P and K foliage application, respectively, on the grain



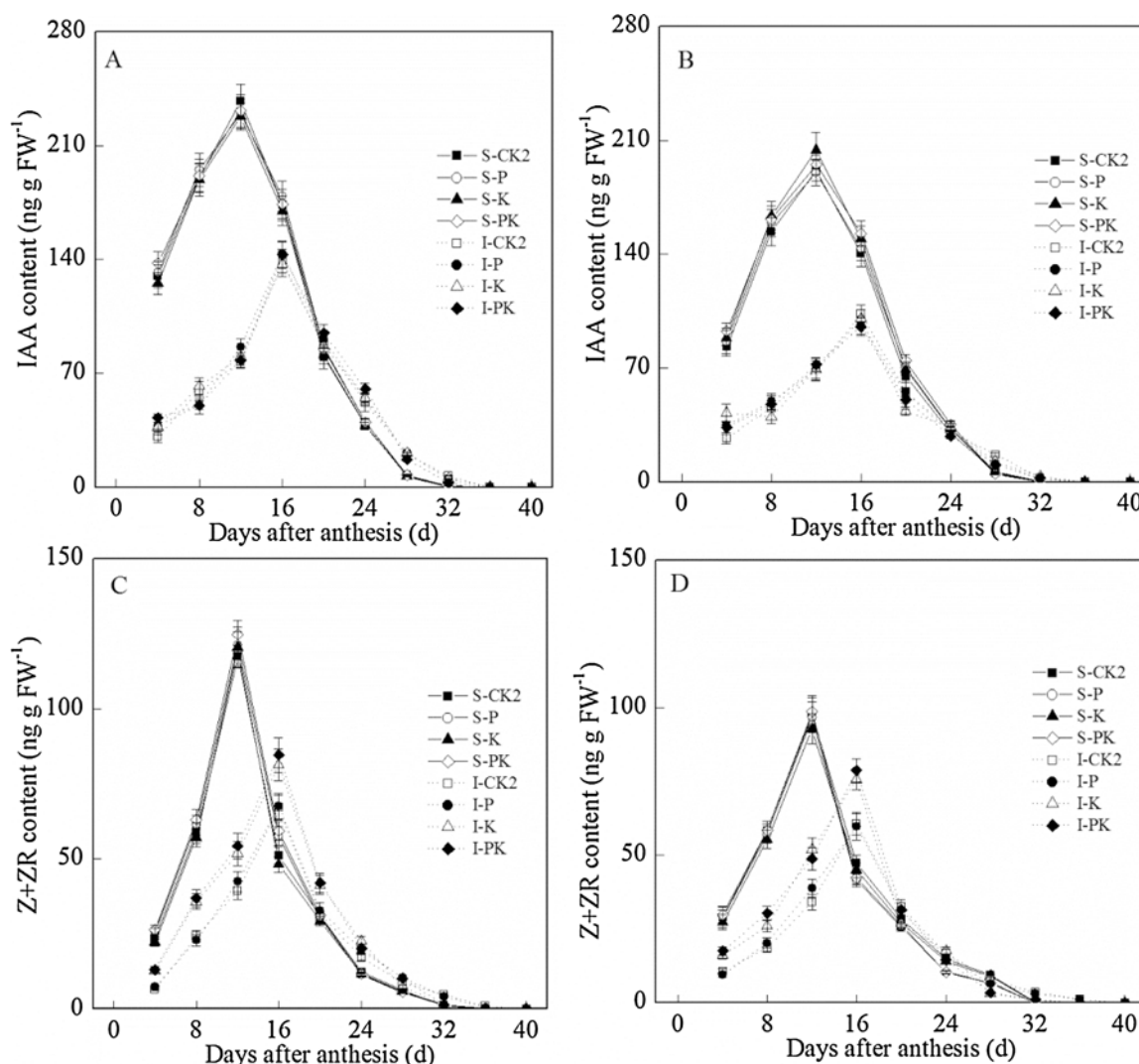


Fig. 3. Effect of P and K foliage application on the IAA and Z + ZR content in the wheat grains (A and C: Shuangda 1; B and D: Xinong 538). S: superior grain; I: inferior grain. Vertical bars represent  $\pm$  the standard deviation of the mean ( $n = 6$ ). P, K and PK represent  $30 \text{ mmol L}^{-1} \text{ KH}_2\text{PO}_4$ ,  $30 \text{ mmol L}^{-1} \text{ NaH}_2\text{PO}_4$  and  $30 \text{ mmol L}^{-1} \text{ KCl}$ , respectively, that was sprayed on leaves at anthesis. CK2 means that deionized water was sprayed on leaves at anthesis.

filling of wheat. K foliage application significantly promoted the grain weight of the light panicle cultivar Xinong 538, and P foliage application significantly promoted the grain weight of the heavy panicle cultivar Shuangda 1. However, the P and K foliage application together promoted the grain filling of all the two cultivars. This means that P and K had an additive effect on the grain filling of wheat. Beside this, the present study suggests that the P and K foliage application is through regulated the grain filling of the inferior grains to regulate the grain filling of the wheat.

The grain filling rate and the grain-filling period determined the grain weight of the cereals. The present study found that the K foliage application significantly promoted the maximum and mean grain-filling rates of the inferior grains of the two cultivars, and the P foliage application significantly promoted the active grain-filling period of Shuangda 1. In addition, the foliage application of K significantly promoted the grain-filling rate of the early and middle grain filling stage, and it had no notable effects on the grain-filling rate of the later grain filling stage. From this, we suggest that the K foliage application mainly affects the grain-filling rate of the early and middle grain filling stages to regulate the grain weight, and the P application, mainly through its effects on the grain-filling period, regulates the grain weight of the wheat.

The hormones are involved in regulating the grain-filling rate of

cereals (Yang and Zhang, 2006). Studies about barley (*Hordeum vulgare* L.), maize, rice and wheat suggest that cytokinin (CTK) is indispensable for cell division during the early grain development phase (Michael and Seiler-Kelbitsch, 1972; Saha et al., 1986; Morris et al., 1993; Dietrich et al., 1995; Yang et al., 2000). The study about rice and wheat suggests that the ABA content of the superior grains was significantly higher than the inferior grains, and the ETH evolution rate of the superior grains was significantly lower than the inferior grains, and the high ABA and low ETH in the grains are associated with a higher filling rate in the superior grains (Yang et al., 2006; Xu et al., 2007). The present study suggests that the foliage application of KCl and  $\text{KH}_2\text{PO}_4$  significantly increases the Z + ZR level in the inferior grains during the early and middle grain filling stage. Beside this, the regression analysis of the present study demonstrated that the maximum and mean grain-filling rates were significantly and positively correlated with the maximum Z + ZR concentrations in the grains ( $R = 0.896^{**}$  and  $0.907^{**}$ , respectively). This means that the Z + ZR play an important role in the grain filling of wheat induced by K foliage application. Moreover, in the present study, the foliage application of KCl and  $\text{KH}_2\text{PO}_4$  significantly promoted all of the ABA and ETH levels in the inferior grains. Yang et al. (2006) suggests that the moderate drought stress notably increased the ratio of ABA/ETH and promoted the grain filling of wheat grain. The results of the present study suggest that the foliage



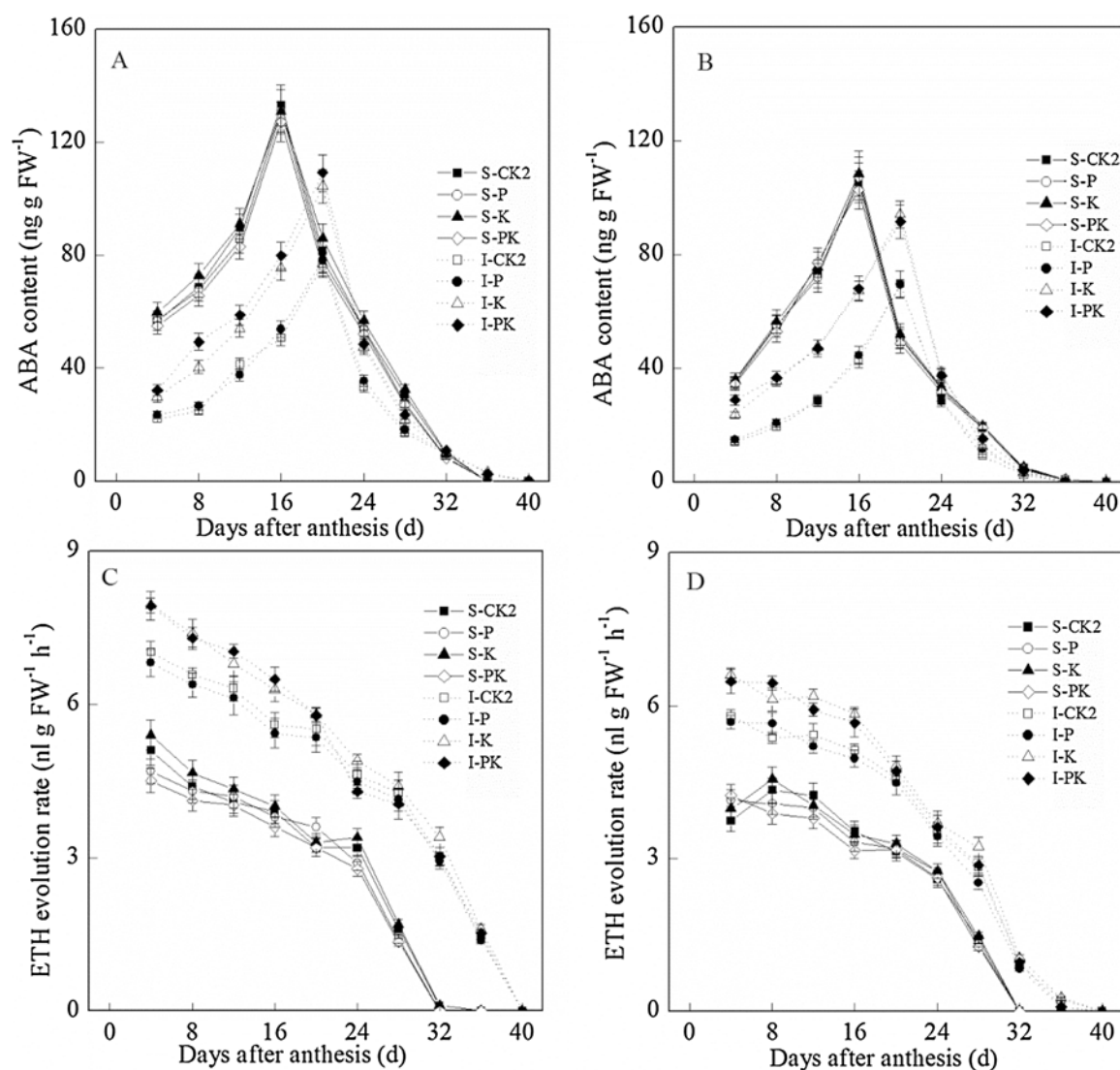


Fig. 4. Effect of P and K foliage application on the ABA content and ETH evolution rate in the wheat grains (A and C: Shuangda 1; B and D: Xinong 538). S: superior grain; I: inferior grain. Vertical bars represent  $\pm$  the standard deviation of the mean (n = 6). P, K and PK represent 30 mmol L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 30 mmol L<sup>-1</sup> NaH<sub>2</sub>PO<sub>4</sub> and 30 mmol L<sup>-1</sup> KCl, respectively, that was sprayed on leaves at anthesis. CK2 means that deionized water was sprayed on leaves at anthesis.

application of KCl and KH<sub>2</sub>PO<sub>4</sub> increases the ratio of ABA/ETH in the inferior grains (Fig. 7), and the regression analysis demonstrates that the maximum and mean grain-filling rates are significantly and positively correlated with the ratio of ABA/ETH in the inferior grains ( $R = 0.871^{**}$  and  $0.882^{**}$ , respectively). These results suggest that multiple hormones, rather than a special hormone, are involved in regulating the grain filling rate of wheat induced by the foliage application of K. The foliage application of K, through the regulation of endogenous ABA, ETH and Z + ZR, affects the grain-filling rate of wheat.

Although the foliage application of K significantly promoted the grain filling rate of the inferior grains of the two cultivars, it only significantly promoted the grain weight of Xinong 538. We found that the reason of this is that the foliage application of K significantly decreased the active grain-filling period and the grain-filling rate of the later grain filling stage of Shuangda 1. This means that the foliage application of K may aggravate the premature senescence of the plants of Shuangda 1. One of the important reasons for the plant senescence is that the active oxygen or free radical detriment the pericellular membrane inhibits normal cell metabolism (Zhao et al., 2011). An antioxidase, such as SOD, POD and CAT, eliminates the active oxygen or free radical and notably relieves the detrimental effects of active oxygen or free radical

on the pericellular membrane and relieves the senescence of the plants (Yamazaki and Kamimura, 2002). The present study found that the foliage application of K significantly decreased the activities of SOD, POD and CAT of the flag leaves and significantly increased the MDA content in the flag leaves, which decreased the SPAD value and the net photosynthetic rate of the leaves. This means that the foliage application of K promoted the senescence of the wheat plants. Compared to K, the foliage application of P had an opposite effect and relieves the senescence of the wheat plants.

Although the foliage application of K significantly promoted the grain-filling rate of the inferior grains of the two cultivars, it significantly decreased the activity of the grain-filling period of Shuangda 1, and because of this, the grain weight of the K treatment was not significantly different than the CK2 treatment. However, the foliage application of K did not significantly affect the activity of the grain-filling period of Xinong 538, and the foliage application of K significantly promoted the grain weight of the inferior grains of Xinong 538. The foliage application of P significantly increased the SPAD value and the net photosynthetic rate of the flag leaves and relieved the senescence of the plants but had no significant effect on the active grain-filling period of the inferior grains of Xinong 538. However, the foliage application of P significantly increased the active grain-filling period of



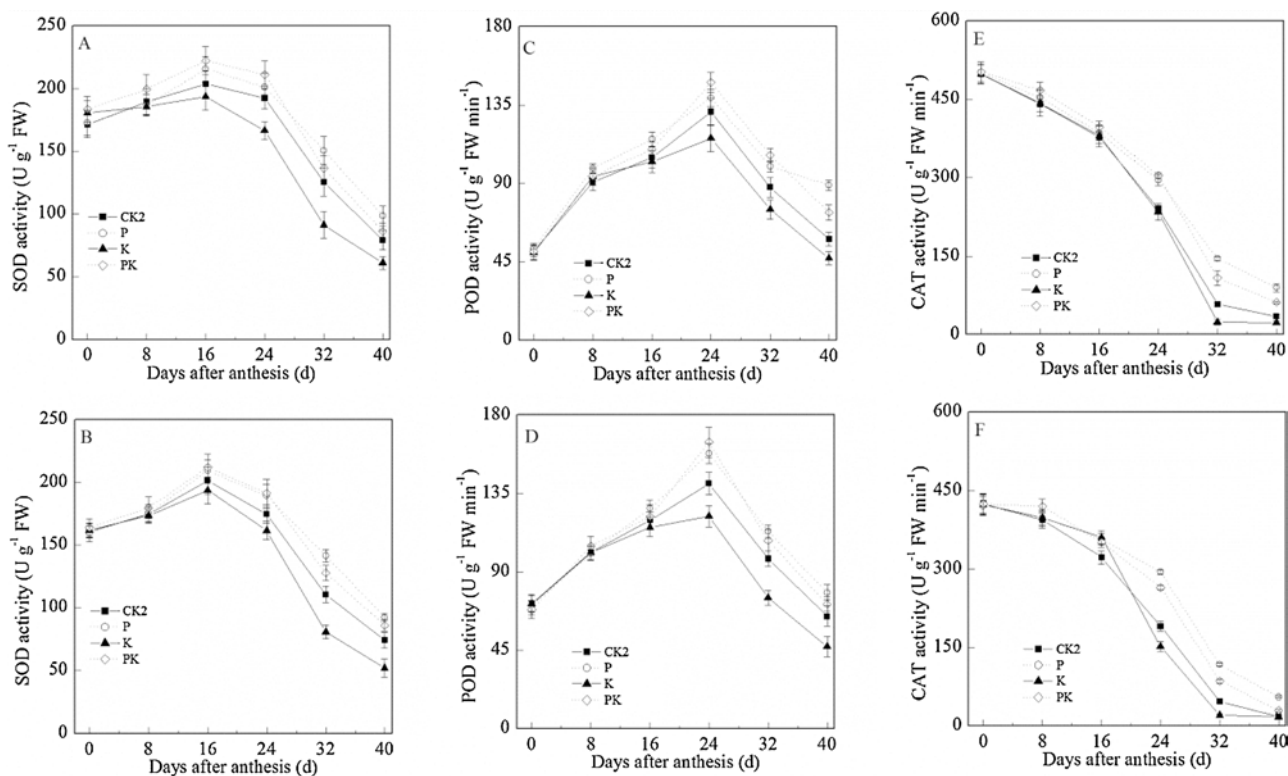


Fig. 5. Effects of P and K foliage application on the antioxidant enzyme activities of the flag leaves of the wheat (A, C and E: Shuangda 1; B, D and F: Xinong 538). Vertical bars represent ± the standard deviation of the mean (n = 6). P, K and PK represent 30 mmol L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 30 mmol L<sup>-1</sup> NaH<sub>2</sub>PO<sub>4</sub> and 30 mmol L<sup>-1</sup> KCl, respectively, that was sprayed on leaves at anthesis. CK2 means that deionized water was sprayed on leaves at anthesis.

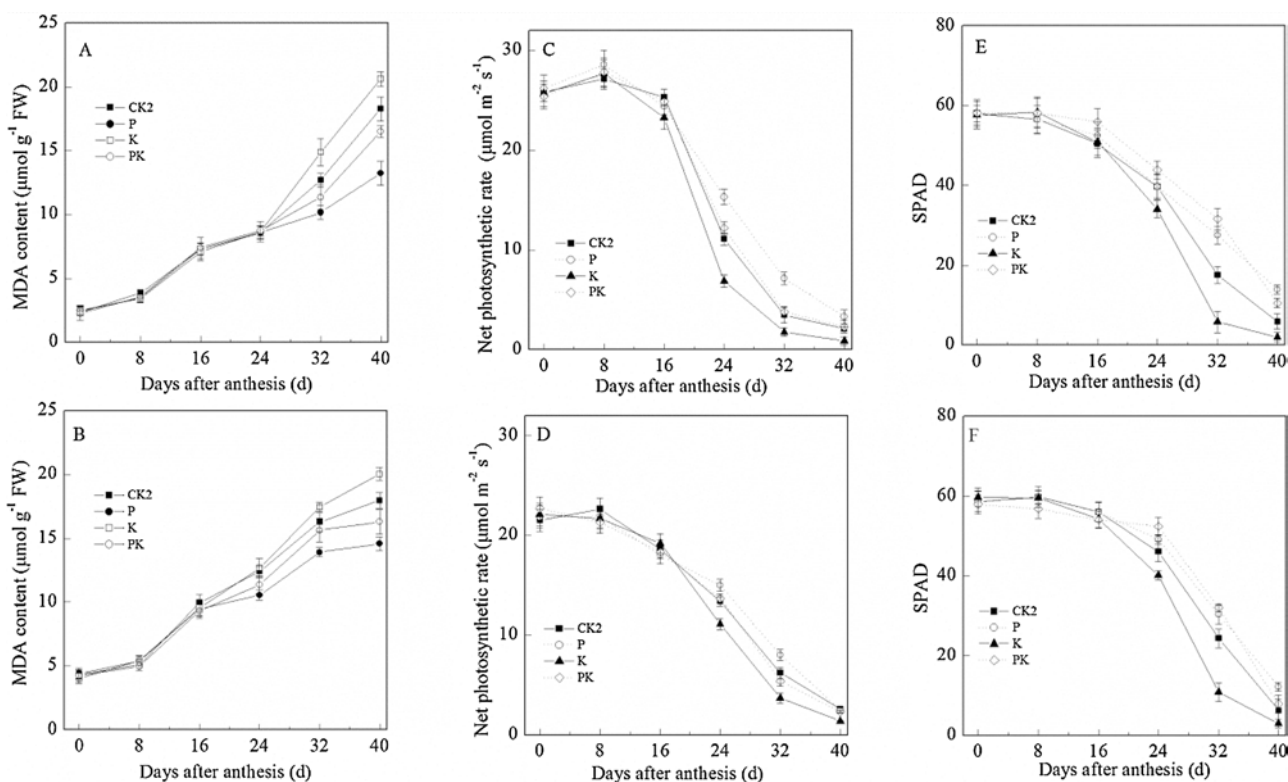


Fig. 6. Effects of P and K foliage application on the MDA content, SPAD value and net photosynthetic rate of the flag leaves of the wheat (A, C and E: Shuangda 1; B, D and F: Xinong 538). Vertical bars represent ± the standard deviation of the mean (n = 6). P, K and PK represent 30 mmol L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 30 mmol L<sup>-1</sup> NaH<sub>2</sub>PO<sub>4</sub> and 30 mmol L<sup>-1</sup> KCl, respectively, that was sprayed on leaves at anthesis. CK2 means that deionized water was sprayed on leaves at anthesis.



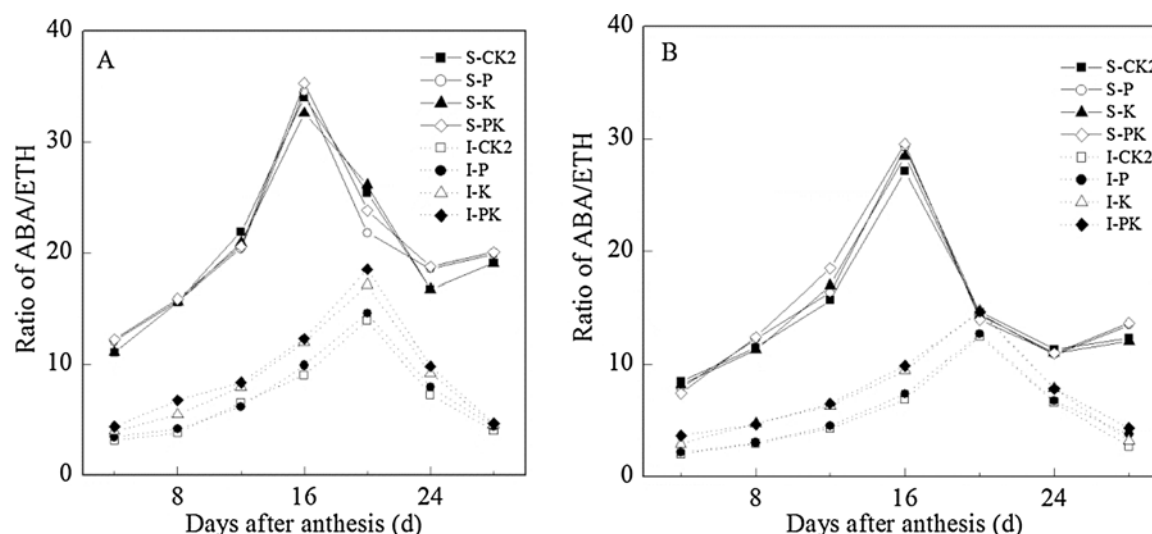


Fig. 7. Effects of P and K foliage application on the ratio of ABA/ETH in the grains of the wheat (A: Shuangda 1; B: Xinong 538). S: superior grain; I: inferior grain. P, K and PK represent  $30 \text{ mmol L}^{-1} \text{ KH}_2\text{PO}_4$ ,  $30 \text{ mmol L}^{-1} \text{ NaH}_2\text{PO}_4$  and  $30 \text{ mmol L}^{-1} \text{ KCl}$ , respectively, that was sprayed on leaves at anthesis. CK2 means that deionized water was sprayed on leaves at anthesis.

the inferior grains of Shuangda 1. From this study, we found that the foliage application of K significantly affected the hormones in the gains, which notably promoted the sink strength and might lead to more carbohydrate reserves stored in the source that are transferred to the sink. However, the effect of K might decrease the carbohydrate content in the leaves and stem and might lead to leave senility of the plant (Yang and Zhang, 2010). For the heavy panicle cultivar Shuangda 1, a bigger sink is needed for the transfer of much carbohydrate supply from the source to maintain the grain filling need, and an insufficient carbohydrate supply from the source for the sink-filling is the main limiting factor for the grain filling of these types of wheat and rice cultivars (Yang and Zhang, 2010). The foliage application of K intensifies the carbohydrate transport from the source to the sink. This may lead to an excessive shortage of carbohydrates in the source and lead to severe premature senility of the plant of Shuangda 1. Thus, the foliage application of K significantly decreases the grain-filling rate during the later grain filling stage and the active grain-filling period of the inferior grains of Shuangda 1. Compared to Shuangda 1, the sink of Xinong 538, which is the light panicle cultivar, needs less of a carbohydrate supply from the source, and the sink strength might be the key limiting factor for the grain filling of these types of wheat and rice cultivars (Yang and Zhang, 2006). The foliage application of K promoted the sink strength and might increase the carbohydrate transport from the source to the sink. Due to the stronger source of the light panicle cultivar compared to the sink, the source had a sufficient carbohydrate to supply the grain filling. Thus, the foliage application of K did not notably decrease the active grain-filling period of the inferior grains of Xinong 538, although it significantly decreased the chlorophyll content and promoted the senescence of the plants at the later grain filling stage. Thus, in contrast to Shuangda 1, the foliage application of K significantly increased the grain-filling rate of the inferior grains, which increased the grain weight of the inferior grains of Xinong 538.

Compared to K, the foliage application of P significantly relieved the senescence of the plants. However, the foliage application of P did not significantly affect the hormone levels and the grain-filling rate of the inferior grains. For Shuangda 1, the insufficient carbohydrate supply from the source for the sink-filling is the main limiting factor for the grain filling, and the foliage application of P notably promotes the net photosynthetic rate of the flag leaves and promotes the carbohydrate supply, which significantly increased the active grain-filling period and the grain weight of Shuangda 1. In contrast, the carbohydrate supply from the source for the sink filling is sufficient, and thus, the foliage

application of P did not significantly affect the active grain-filling period and the grain weight of Xinong 538, although it significantly increased the SPAD value and the net photosynthetic rate of the flag leaves.

## 5. Conclusions

The present study indicated that the effect of P and K foliage application on the grain filling of wheat is the difference. The K foliage application significantly increased the Z + ZR and ABA contents and decreased the ETH evolution rate in inferior grains, which promoted sink strength and increased the grain filling rate and the grain weight. In comparison, the P foliage application significantly promoted the activities of SOD, POD, and CAT and decreased the MDA content in the flag leaves and relieved the premature senility of the plant, which increased the active grain-filling period and the grain weight. For the heavy panicle cultivar, foliage P had a reasonable effect on grain filling, and for the light panicle cultivar, foliage K had a reasonable effect on grain filling. The mixture of P and K had an additive effect and prevented P and K deficiencies. Foliage application of  $\text{KH}_2\text{PO}_4$  is an effective measure for promoting the grain weight of wheat.

## Acknowledgements

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# Spermidine Increases the Sucrose Content in Inferior Grain of Wheat and Thereby Promotes Its Grain Filling

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The improvement of grain filling is the key issue for promoting wheat thousand grain weight and grain yield. The levels of polyamines (PAs) significantly affect grain filling in cereals, but the mechanism by which PAs affect grain filling in wheat is unclear. In the present study, six wheat cultivars whose grain filling differed were used, and their grain-filling characteristics and endogenous PA contents were measured. In addition, exogenous PAs were supplied during the wheat grain-filling period. The grain-filling characteristics, hormone levels, starch contents, and gene expression [based on RNA sequencing (RNA-seq)] in the grain were analyzed. The objective of the present study was to investigate the effects of PAs on grain filling in wheat. The results suggested that the direct synthetic pathway from putrescine (Put) to spermidine (Spd) in the grain was a key factor in promoting grain filling and thousand grain weight in wheat. Spd through regulates the grain-filling rate of inferior grain during the early grain-filling period to affecting the grain filling and thousand grain weight of wheat. The promotive effect of Spd on the grain filling of inferior wheat grain was notably related to carbohydrate metabolism in that grain. Spd significantly increased the zeatin (Z) + zeatin riboside (ZR) contents but reduced the ethylene (ETH) evolution rate in the inferior grain. In addition, Spd significantly increased the sucrose synthase (SS) and acid invertase (AI) activities in the inferior grain. These effects of Spd led to increased sucrose content in the inferior grain. These reasons might explain why Spd significantly promoted the filling and weight of inferior wheat grain.

**Keywords:** polyamine, grain filling, wheat, carbohydrate transport, hormone

## INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important global cereal crop species worldwide, including within China, and promoting wheat grain production is essential for food security in China. The grain yield of wheat can be divided into three components: the number of spikes per unit area, the number of spikelets per spike, and thousand grain weight (Yu, 2011). In China, high wheat grain yield relies mainly on high numbers of spikes per unit area (Cai et al., 2014). However, the high numbers of spikes may lead to severe problems, such as lodging, premature senescence, and increased damage from disease and insects (Sicher and Bunce, 1998; Robert., 2002; Kelbert et al., 2004). Therefore, increasing the thousand grain weight or grain number per spike based on a suitable panicle number per area is an inevitable approach to promote wheat grain yield. In cereals, grain filling determines



the thousand grain weight, so improving grain filling is important for achieving increased wheat thousand grain weight and grain yield (Kato et al., 2007).

Wheat grain can be divided into two types: superior grain and inferior grain. Superior grain consists mainly of early flowering types, usually located on basal of the middle spikelets, which produce larger and heavier grains, while inferior grain consists mainly of late-flowering types, usually located on distal of the middle spikelets and the basal and the distal spikelets, poorly to produce grains (Jiang et al., 2003; Yang and Zhang, 2010). Compared with superior grain, inferior grain requires more energy for grain filling (Peng et al., 2011), and inferior grain is more sensitive to environmental factors, such as water, temperature, and fertilizer (Peng et al., 2013). Therefore, the weight of inferior grain is low and notably varies from year to year (Yang and Zhang, 2010). Previous studies suggested that variations in the grain weight of cereals such as rice (*Oryza sativa* L.) and wheat are caused mainly by inferior grain (Yang and Zhang, 2006). Thus, improving the filling of inferior grain is key for promoting wheat grain weight and grain yield.

Carbohydrates are the main components of wheat grain and account for more than 70% of grain dry weight (Yang et al., 2004). The carbohydrates that accumulate in wheat grain are derived mainly from the transportation of nonstructural carbohydrates (NSCs) stored in the stem (Sikder and Gupta, 1976). Previous studies have suggested that superior grain and inferior grain notably differed in their ability to use NSCs stored in the stem (Murty and Murty, 1982). Compared to those of superior grain, the lower sink size and sink strength of inferior grain limit the transport of NSCs from the stem; this phenomenon leads to the inferior grain being a poor sink for NSCs stored in the stem, which is one of the main reasons why in cereals, compared with superior grain, inferior grain experiences poor filling and weighs less (Fu et al., 2011; Liang et al., 2017). Appropriate nitrogen (N) and potassium (K) fertilizer applications significantly promoted NSC transport from the stem to inferior grain and notably increased the weight of inferior grain of wheat and rice (Fu et al., 2011; Yang and Zhang, 2010; Liang et al., 2017). This finding means that promoting NSC transport from the stem to inferior grain may be an effective way to improve the grain filling of inferior grain in cereals.

Polyamines (PAs) are important endogenous plant growth regulators and notably regulate the grain filling of cereals such as wheat, rice, and maize (Kusano et al., 2007; Yang et al., 2008; Liu et al., 2013). Previous studies have suggested that PA biosynthesis is significantly related to the grain-filling rate and grain weight in rice; high spermidine (Spd, a type of PA) and spermine (Spm, a type of PA) levels in grain were beneficial to the grain filling of inferior spikelets in rice (Yang et al., 2008; Chen et al., 2013). Severe water deficit significantly reduced Spd levels in grain and inhibited grain filling in wheat (Liu et al., 2016; Yang et al., 2017). External Spd and Spm promoted wheat grain filling (Liu et al., 2013). These findings indicated that PAs affect grain filling in cereals. However, the mechanism by which PAs affect grain filling in wheat is unclear.

Previous study has suggested that PAs are notably related to carbohydrate metabolism in plants (Oufir et al., 2008).

Chen et al. (2013) suggested that PAs promote starch synthesis and consequently promote grain filling in rice. Liu et al. (2013) suggested that PAs increase both the photosynthetic rate in flag leaves and carbohydrate accumulation in plants, thereby promoting grain filling in wheat. However, whether the effect of PAs on the grain filling of wheat is related to NSC transport from the stem to the grain is also unclear. The previous study suggested that the superior grains of wheat showed generally higher activity of sucrose synthase (SS, EC 2.4.1.13) than inferior grains, and this may relate to the higher starch accumulation rates and grain weight of superior grain (Jiang et al., 2003). Wang et al. (2017) suggested that sucrose-phosphate synthase (SPS, EC 2.4.1.14) activity in straws was important for carbon reserve remobilization of rice. Beside this, the acid invertase (AI, EC 3.2.1.26) was related to the sucrose unloading in grain, and the nitrogen application notably promoted the AI activity in wheat grain and increased the sugar content in grains (Zhang et al., 2014). These mean that these enzymes were notably involved in the sugar transport of cereals. But, whether the PA through regulated the activities of these enzymes, such as SS, SPS and AI, to affect the grain filling of wheat, is also unclear. In addition to carbohydrates, hormones, plant senescence, and N metabolism are also considered to regulate grain filling in cereals (Takahashi et al., 1996; Kim et al., 2011; Wilkinson et al., 2012). Previous studies have suggested that the effect of PAs on the grain filling of cereals was related to hormone and plant senescence (Yang et al., 2008), which means that there may be an interaction among PAs, hormones, carbohydrate metabolism, and plant senescence in the regulation of wheat grain filling. However, little is known about the regulatory network of PAs on the grain filling of inferior wheat grain. RNA sequencing (RNA-seq) has become an essential method for large-scale analysis of genes in various fields of plant biology, including grain filling in cereal crops. Li et al. (2014) identified 7713 differentially expressed genes (DEGs) in grain-filling caryopses between the rice *sugary* mutant and the wild-type strain based on RNA-seq data. Moreover, using RNA-seq, Jeong et al. (2017) studied phosphorus (P) remobilization from rice flag leaves during grain filling, and Peng et al. (2011) suggested that the expression and function of miRNA partly explain the slow grain-filling rate of inferior spikelets. However, little is known about the mechanism of grain filling in wheat *via* RNA-seq analysis.

In the present study, six wheat cultivars whose grain filling differed were used, and the grain-filling characteristics, endogenous PA content, and the activities of the enzymes that regulated PA synthesis were measured. In addition, exogenous Spd and putrescine (Put) were supplied during the wheat grain-filling period. The objective of the present study was to investigate the effect of PAs on the grain filling of inferior grain in wheat. For this, the grain-filling characteristics of the grain and gene expression (based on RNA-seq) were analyzed, and the endogenous zeatin (Z) and zeatin riboside (ZR) contents, the ethylene (ETH) evolution rate, the starch and sucrose contents, the activities of the enzymes involved in sucrose–starch synthesis, and carbohydrate transport from stem to grain were measured.



## MATERIALS AND METHODS

### Experimental Design

Two experiments were conducted at the experimental site of Northwest A&F University, Yangling, Shaanxi Province, China (34°17' N, 108°05' E) in 2014–2016, and the two experiments were conducted in the same experimental field. The soil at the experimental site is an Eum-Orthrosol (Chinese soil taxonomy). The organic matter content and available N, P, and K in the 0–20 cm of topsoil in the cropland were 12.39 g kg<sup>-1</sup>, 49.85 mg kg<sup>-1</sup>, 24.63 mg kg<sup>-1</sup>, and 110.14 mg kg<sup>-1</sup>, respectively.

#### The First Experiment

Six winter wheat cultivars, Shuangda 1 (SD 1), Fugao 1 (FG 1), Zhoumai 22 (ZM 22), Xiaoyan 6 (XY 6), Xiaoyan 22 (XY 22), and Xinong 538 (XN 538), were sown at a rate of 150 kg ha<sup>-1</sup>, and the row spacing was 0.25 m. The sow date was October 16 in 2014–2015 and 2015–2016. Urea and (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> were applied and each of them applied at 375 kg ha<sup>-1</sup>. All fertilizer applied was at a basal level. The experiment was conducted in accordance with a completely randomized design, and each treatment consisted of three replicates. The area of each plot was 6 m<sup>2</sup> (3 × 2 m).

#### The Second Experiment

Two wheat cultivars, SD 1 and XN 538, were sown. The planting method was the same as that of the first experiment. At anthesis, four treatments were applied to each cultivar: SPD, the spikes were sprayed with 1 mmol L<sup>-1</sup> Spd at anthesis; Put, the spikes were sprayed with 2 mmol L<sup>-1</sup> Put at anthesis; MGBG (Yang et al., 2008), the spikes were sprayed with 5 mmol L<sup>-1</sup> methylglyoxal-bis(guanyldihydrazone) (MGBG, an inhibitor of Spd) at anthesis; and CK, the spikes were sprayed with water at anthesis. Spd, Put, MGBG, and water were applied daily for four days at a rate of 5 ml spike<sup>-1</sup> for each application. All of the solutions contained 0.1% (V/V) ethanol and 0.01% (V/V) Tween-20. For the CK, the water contained the same concentrations of ethanol and Tween-20. Each treatment was replicated three times in a split-plot experimental design. The area of each plot was 5 m<sup>2</sup> (2.5 × 2 m). Two cultivars, four chemical application treatments, and three replicates gave 24 individual plots. The Spm, Spd, and MGBG were purchased from Sigma-Aldrich (USA).

### Sampling and Measurements

For each treatment, the spikes that flowered on the same day were tagged and sampled from anthesis to maturity at 4-day intervals. Forty spikes were sampled at each sampling stage for each plot.

The grain on an ear was divided into superior grain and inferior grain according to the methods of Jiang et al. (2003). Half of the sampled grain was quickly frozen in liquid N and then stored at -80°C, and the enzymes within the other half were deactivated by heating at 105°C for 30 min, after which the grain was then dried at 70°C to a constant weight and subsequently weighed. The yield and yield components were determined according to our previous methods (Liu et al., 2016).

### Grain-Filling Process

The grain-filling process was fitted by Richards' (1959) growth equation as described by Zhu et al. (1988).

$$W = \frac{A}{\left(1 + Be^{kt}\right)^{\frac{1}{N}}} \quad (1)$$

The grain filling rate ( $G$ ) was calculated using a derivation of Equation 1:

$$G = \frac{AkBe^{-kt}}{\left(1 + Be^{-kt}\right)^{\frac{N+1}{N}}} \quad (2)$$

where  $W$  is the grain weight (mg);  $A$  is the final grain weight (mg);  $t$  is the time after anthesis (d); and  $B$ ,  $k$ , and  $N$  are coefficients determined using regression.

The active grain filling period was defined as the period when  $W$  was between 5% ( $t_1$ ) and 95% ( $t_2$ ) of  $A$ . Therefore, the average grain filling rate during this period was calculated from  $t_1$  to  $t_2$  (Yang et al., 2006).

### PAs

Spd, Spm, and Put were extracted and measured according to the methods of Liu et al. (2002). Briefly, approximately 0.5 g fresh weight (FW) of samples was homogenized in 3–5 ml of 5% (v/v) perchloric acid (PCA) in a prechilled mortar and pestle. The Spd, Spm, and Put were measured according to the methods Liu et al. (2016) and quantified *via* a high-performance liquid chromatography system (Waters 1525 Binary HPLC Pump/2489 UV Detector, Waters, USA).

### PA Biosynthetic Enzyme Activity

The activities of arginine decarboxylase (ADC, EC 4.1.1.19), S-adenosylmethionine decarboxylase (SAMDC, EC 4.1.1.50), and Spd synthase (SpdSy, EC 2.5.1.16) were measured according to the methods of Yang et al. (2008). The activities of ADC and SAMDC were determined by measuring the CO<sub>2</sub> evolution as described by Lee et al. (1997), and the SpdSy activity was assayed according to the methods of Kasukabe et al. (2004).

### RNA Extraction, Library Preparation, and RNA-Seq

At 8 and at 16 days post-anthesis, four tagged spikes in each plot were used, respectively. The inferior grain of these spikes was sampled, after which it (the inferior grain of 8 and 16 days post-anthesis) was mixed together and then used for RNA-seq analysis. The RNA extraction, library preparation, and RNA-seq were performed by Novel Bioinformatics Ltd., Co. (Shanghai, China). The total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA), and the RNA quality was confirmed using a microspectrophotometer (NanoDrop™ 2000, Thermo Fisher Scientific, MA, USA).

A sequencing library of each RNA sample was prepared by using an Illumina TruSeq RNA Library Prep Kit v2 according to the protocol provided by the manufacturer (Illumina, USA). FAST-QC was then used to evaluate the quality of the sequencing data (Langmead et al., 2009; Trapnell et al., 2009). Raw reads after quality control testing were subsequently mapped to the reference wheat genome using the HISAT2 algorithm with the default parameters (Pertea et al., 2016).



A differential expression analysis was performed in which the EBSeq algorithm was used to filter DEGs by a log-fold expression change ( $\log_2$  fold change (FC) > 0.585 or < -0.585) with a false discovery rate (FDR) threshold of <0.05. The DEGs were queried *via* BLAST using the tools and databases for the *T. aestivum* L. IWGSC1+popseq.31 genome assembly hosted on the EnsemblPlants website ([http://plants.ensembl.org/Triticum\\_aestivum/Info/Index](http://plants.ensembl.org/Triticum_aestivum/Info/Index)). Gene Ontology (GO) analysis was applied to analyze the main functions of the DEGs according to the GO database, which provides key functional classifications for genes (Ashburner et al., 2000). Pathway assignments were carried out based on the Kyoto Encyclopedia of Genomes and Genomes (KEGG) database (<http://www.genome.ad.jp/kegg/>). The RNA-seq analysis had three biological replicates, and the RNA-seq data were deposited in the Sequence Read Archive of NCBI; the accession number is SRP217735.

### Sucrose and Starch Contents and the Activities of Enzymes Involved in Starch Biosynthesis in the Grain

Sucrose was extracted from the grain by 80% ethyl alcohol and measured by the resorcinol-HCl method (Wang and Huang, 2000). After sucrose was extracted, the residue was extracted by 36 mol L<sup>-1</sup> PCA, and then extracted by 18 mol L<sup>-1</sup> PCA, and the extracting solutions were mixed and used for starch measured. The starch concentration was measured *via* the anthrone method (Liu et al., 2011).

The activities of SS, SPS, and AI in the grain or stems were measured according to the methods of Jiang et al. (2003) and Yang et al. (2004).

### Hormones

Endogenous Z+ZR and abscisic acid (ABA) were extracted according to previous reports (Liu et al., 2011), using 80% (v/v) methanol. Z+ZR and ABA were quantified *via* by enzyme-linked immunosorbent assays (ELISAs) (Liu et al., 2011). The recovery rates for Z+ZR and ABA were  $94.8 \pm 5.7\%$  and  $92.4 \pm 8.8\%$ , respectively.

The ETH generated by the grains was determined according to the methods of Beltrano et al. (1994) and Yang et al. (2008). The ETH was assayed using a gas chromatography (GC) system (Trace GC Ultra™, Thermo Fisher Scientific, USA) according to our previous study (Liu et al., 2016).

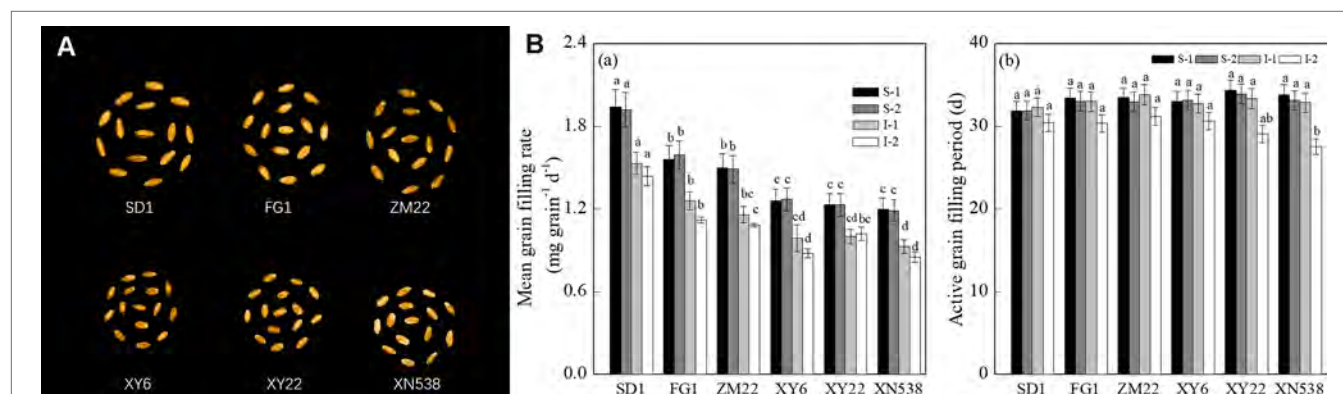
## Statistical Analyses

The SPSS 16.0 statistical software package was used to conduct ANOVAs. The data from each sampling were analyzed separately. The analysis used completely randomized design and split-plot experimental design, respectively, for experiments 1 and 2. The means were tested by the Tukey HSD test.

## RESULTS

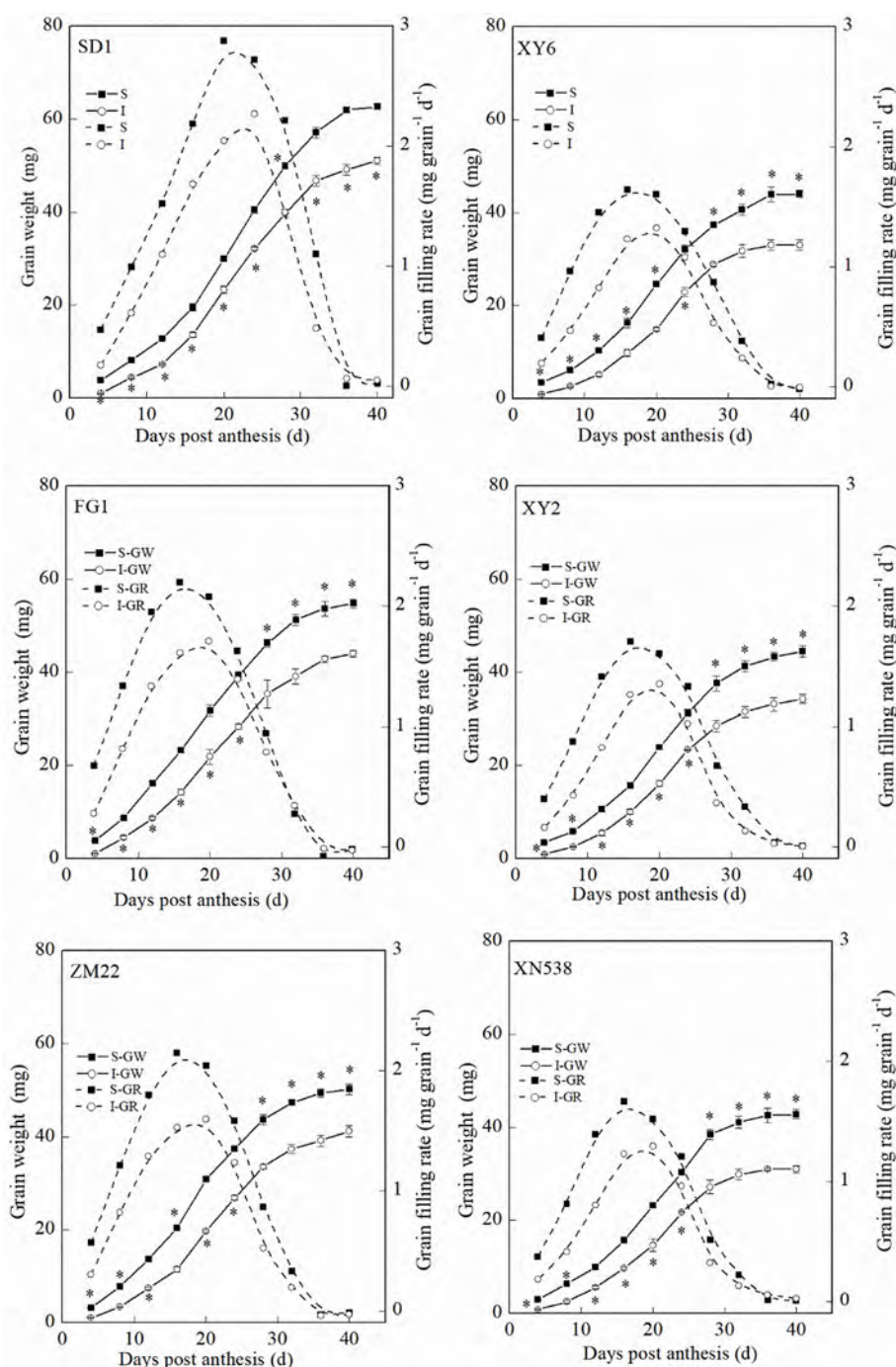
### Grain Filling

Figure 1 and Supplemental Table 1 show that the thousand grain weights of the six cultivars were significantly different. The mean grain-filling rate and thousand grain weight among the cultivars followed the order of SD 1 > FG 1 and ZM 22 > XY 6, XY 22, and XN 538 (Figures 1A, B-a). However, no significant differences in the active grain-filling period were observed between the six cultivars (Figure 1B-b). Among the six cultivars, the grain-filling rate and weight of the superior grain were significantly higher than those of the inferior grain of each cultivar (Figure 2). However, the trends of the active grain-filling period between the superior grain and inferior grain differed between the two years. In 2014–2015, no significant differences were observed for the active grain-filling period between the superior grain and inferior grain for any of the six cultivars. However, the active grain-filling period of the inferior grain was significantly lower than that of the superior grain in 2015–2016. These results suggested that compared with the superior grain, the significant low grain weight of inferior grain for these six cultivars seems due to the lower level of grain filling rate.



**FIGURE 1 |** Grain-filling characteristics of different wheat cultivars. **(A)** The mature grains of the six wheat cultivars. **(B)** The mean grain-filling rate (a) and active grain-filling period (b) of the six wheat cultivars. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ,  $n$  represents the biological replicates). Values for the same year and same grain type followed by different letters are significantly different ( $P < 0.05$ ). \* represents the significant difference ( $P < 0.05$ ) between superior grain and inferior grain for the same year. SD 1, FG 1, ZM 22, XY 6, XY 22, and XN 538 are the cultivars Shuangda 1, Fugao 1, Zhengmai 22, Xiaoyan 6, Xiaoyan 22, and Xinong 538, respectively. S, superior grain; I, inferior grain; 1 and 2 represent 2014–2015 and 2015–2016, respectively.



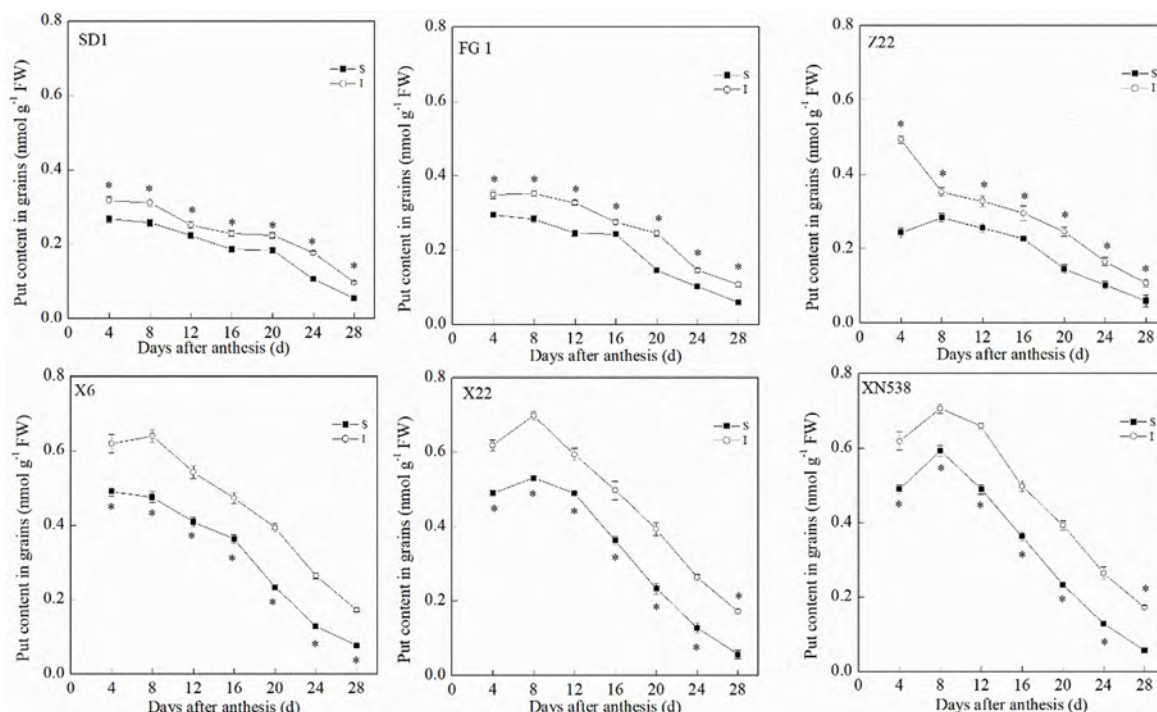


**FIGURE 2 |** The changes of grain weight and grain-filling rate characteristics during grain-filling period. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ,  $n$  represents the biological replicates). \* represents the significant difference ( $P < 0.05$ ) between superior grain and inferior grain at the same day. The solid line and dashed line represent the grain weight and grain-filling rate, respectively. SD 1, FG 1, ZM 22, XY 6, XY 22, and XN 538 are the cultivars Shuangda 1, Fugao 1, Zhengmai 22, Xiaoyan 6, Xiaoyan 22, and Xinong 538, respectively. S, superior grain; I, inferior grain.

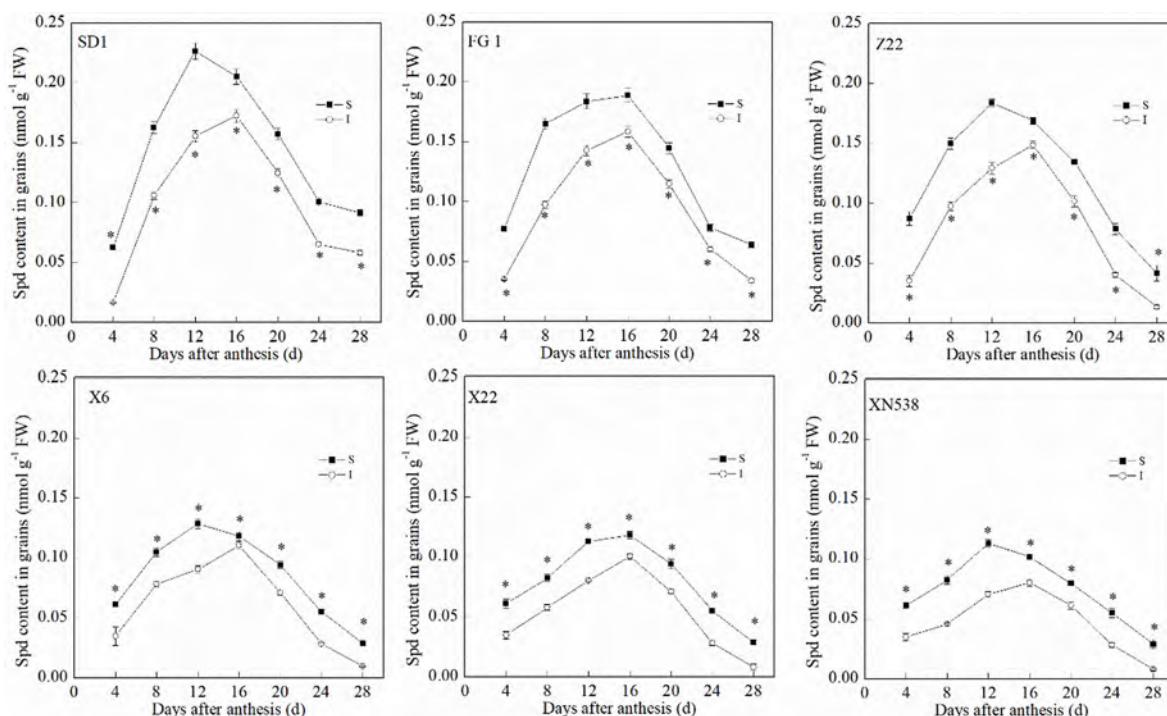
## PA Contents

During the grain-filling period, the trends of the endogenous Put and Spd levels in the grain differed (**Figures 3 and 4**). The Put level in the grain decreased during grain filling (**Figure 3**). In addition, the Put level in the superior grain was notably lower

than that in the inferior grain during the grain-filling period. With respect to the low-grain-weight cultivars, XY 6, XY 22, and XN 538, the Put levels in the grain were significantly greater than those of the high-grain-weight cultivars, SD 1, FG 1, and ZM 22, on the same day during the grain-filling period. In contrast to Put,



**FIGURE 3 |** The changes of putrescine (Put) contents in wheat grains during grain-filling period. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ,  $n$  represents the biological replicates). \* represents the significant difference ( $P < 0.05$ ) between superior grain and inferior grain at the same day. SD 1, FG 1, ZM 22, XY 6, XY 22, and XN 538 are the cultivars Shuangda 1, Fugao 1, Zhengmai 22, Xiaoyan 6, Xiaoyan 22, and Xinong 538, respectively. S, superior grain; I, inferior grain.



**FIGURE 4 |** The changes of spermidine (Spd) contents in wheat grains during grain-filling period. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ,  $n$  represents the biological replicates). \* represents the significant difference ( $P < 0.05$ ) between superior grain and inferior grain at the same day. SD 1, FG 1, ZM 22, XY 6, XY 22, and XN 538 are the cultivars Shuangda 1, Fugao 1, Zhengmai 22, Xiaoyan 6, Xiaoyan 22, and Xinong 538, respectively. S, superior grain; I, inferior grain.



the Spd in the grain first increased and then decreased during the grain-filling period, and the Spd peaked at 16 days post-anthesis (Figure 4). The Spd levels in the grain of the low-grain-weight cultivars, XY 6, XY 22, and XN 538, were significantly lower than those of high-grain-weight cultivars, SD 1, FG 1, and ZM 22, on the same day during the grain-filling period. The Spd levels in the superior grain were significantly greater than those in the inferior grain in all six cultivars. Correlation analysis revealed that the Spd levels in the grain were positively and significantly correlated with the grain-filling rate and thousand grain weight, but the Put levels in the grain were negatively and significantly correlated with the grain-filling rate and thousand grain weight (Table 1). However, the Put and Spd levels in the grain were not significantly correlated with the active grain-filling period.

The exogenous PAs affected the endogenous PAs' concentration in grains (Supplemental Table 2). The exogenous Spd significantly increased the endogenous Spd concentration in inferior grain. Beside this, the exogenous MGBG significantly decreased the Spd concentration in superior grain and inferior grain.

## PA Biosynthetic Enzyme Activity

The ADC, SAMDC, and SpdSy activities in the grain notably differed between the six cultivars (Figure 5). The ADC activity in the grain decreased during grain filling, and the ADC activity was significantly higher in the inferior grain than in the superior grain on the same day post-anthesis. In the superior grain, XY 6 exhibited the highest ADC activity at 8 days post-anthesis, but the highest ADC activity in the inferior grain on the same day occurred in ZM 22. In contrast to the ADC activity, the SAMDC and SpdSy activities exhibited a similar trend among the cultivars. Compared with the low-grain-weight cultivars, XY 6, XY 22, and XN 538, the high-grain-weight cultivars, SD 1, FG 1, and ZM 22, had higher SAMDC and SpdSy activities in their grain. In addition, the SAMDC and SpdSy activities were significantly lower in the inferior grain than in the superior grain on the same day post-anthesis.

## EFFECTS OF EXOGENOUS PAS ON GRAIN FILLING

The results of 2-year experiments suggested that exogenous PAs significantly affected the thousand grain weight of the two

cultivars (Supplemental Table 3). The exogenous PAs had no significant effect on the weight of superior wheat grain (Figure 6). However, exogenous Spd significantly promoted the increase in inferior thousand grain weight, and exogenous MGBG, an Spd synthesis inhibitor, significantly reduced the weight of the inferior grain. However, exogenous Put had no significant effect on the weight of the inferior grain.

Exogenous Spd differentially affected the mean grain-filling rate and active grain-filling period (Figure 7 and Table 2). Exogenous Spd and MGBG had no significant effect on the active grain-filling period of either the superior grain or inferior grain. However, exogenous Spd significantly promoted the mean grain-filling rate of the inferior grain, whereas exogenous MGBG had the opposite effect. The grain-filling rate of the inferior grain in the SPD treatment was greater than that in the CK treatment during 4–24 days post-anthesis.

## GENE EXPRESSION OF RNA-SEQ DATA

Compared to CK, the external Spd upregulated 167 genes' expression, and it downregulated 282 genes' expression (Figure 8A). KEGG analysis revealed that 317 DEGs were assigned to 10 KEGG pathways (FDR < 0.05) (Figure 8B). These KEGG pathways involve mainly starch and sucrose metabolism, plant hormone signal transduction, phenylpropanoid biosynthesis, secondary metabolite biosynthesis, fatty acid elongation, etc.

GO analysis revealed that DEGs associated with the biological process (BP) term were largely associated with signal transduction, the ETH biosynthetic process, transport, the starch biosynthetic process, cell differentiation, and cell response to cytokinin (CTK) stimuli, while concerning the molecular function (MF) term, the DEGs were found to be associated with nucleotides; DNA and protein binding; and hydrolase, catalytic, transferase, transporter, and kinase activities (Figure 9). In addition, the DEGs associated with the cellular component (CC) term were largely associated with chromosomes, nuclei, the cytoplasm, CCs, the plasma membrane, and the extracellular region.

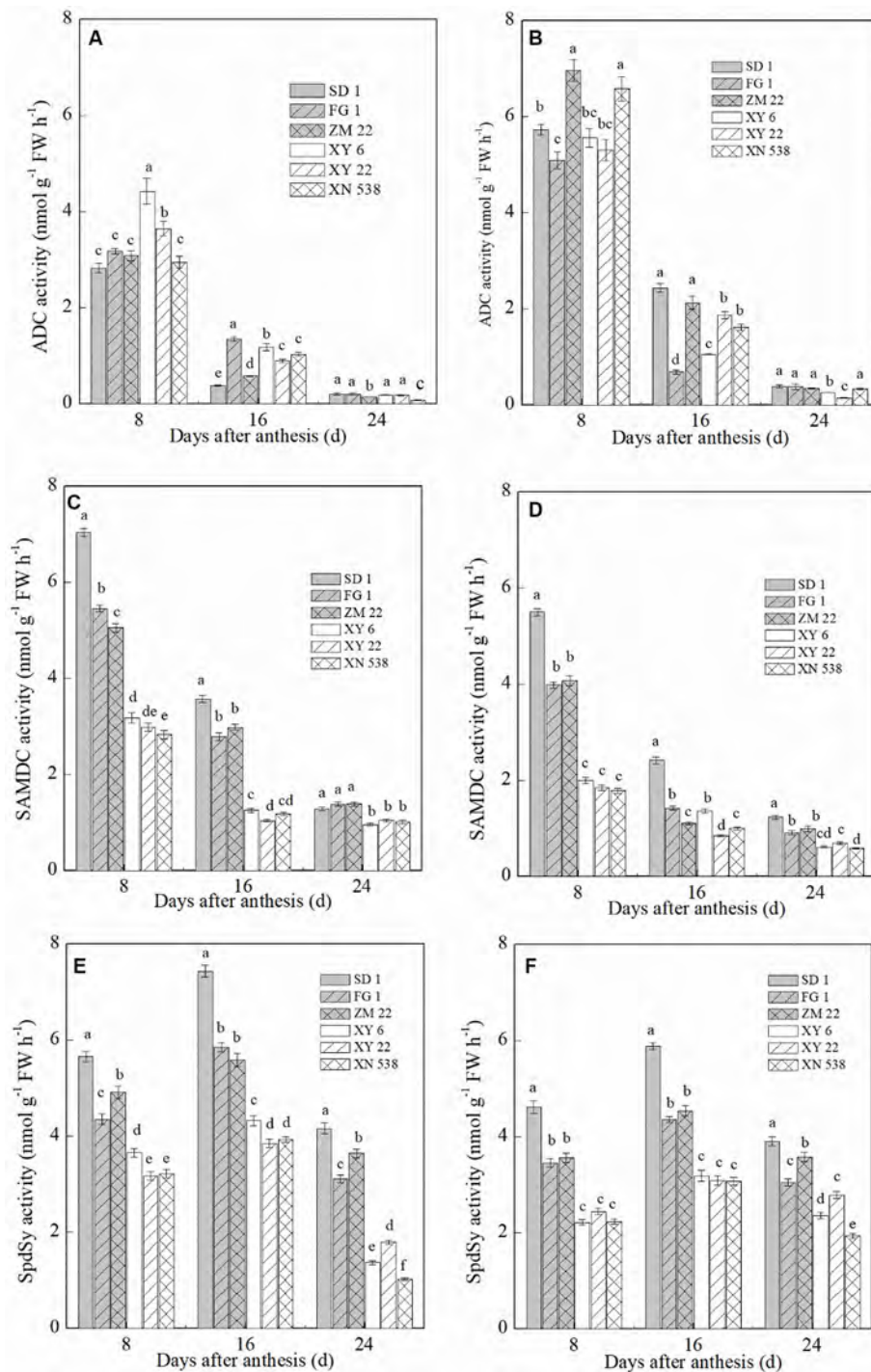
## Carbohydrate in Grain

During the grain-filling period, the sucrose content in the grain continually decreased, and the starch content in grain continually increased (Figures 10A–D). At 4 and 8 days post-anthesis, the sucrose content in the inferior grain was significantly lower than

**TABLE 1** | Correlation coefficients of peak polyamine (PA) contents in wheat grain for the two grain types (superior grain and inferior grain) and six cultivars with the mean grain-filling rate (MGR), active grain-filling period (AGP), and final grain weight (GW) of wheat.

	MGR	AGP	GW	Spd	Put
MGR	1				
AGP	0.5536	1			
GW	0.9865**	0.5680	1		
Spd	0.8838**	0.3661	0.8464**	1	
Put	−0.8283**	−0.4556	−0.8162**	−0.9486**	1

\*Significant at the 0.05 probability level ( $n = 12$ ). \*\*Significant at the 0.01 probability level ( $n = 12$ ). GW, the final grain weight; MGR, mean grain-filling rates; AGP, active grain-filling period; Spd, spermidine; Put, putrescine. The  $n$  represents the treatment number; for the correlation analysis, there are six cultivars and two grain types, so it had 12 treatments.

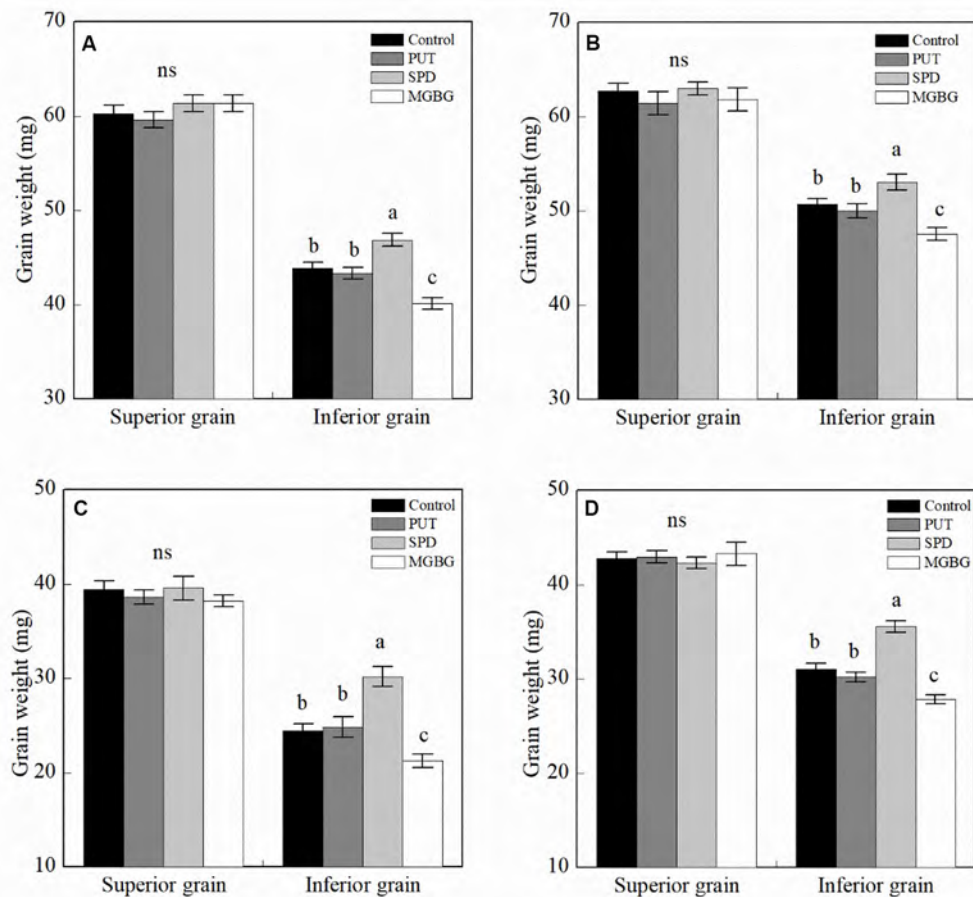


**FIGURE 5 |** The changes of polyamine (PA) biosynthetic enzyme activity in wheat grains during grain filling period. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ,  $n$  represents the biological replicates). Values for the same day followed by different letters are significantly different ( $P < 0.05$ ). SD 1, FG 1, ZM 22, XY 6, XY 22, and XN 538 are the cultivars Shuangda 1, Fugao 1, Zhengmai 22, Xiaoyan 6, Xiaoyan 22, and Xinong 538, respectively. (A, C, and E) represent the superior grain; (B, D, and F) represent the inferior grain.

that in the superior grain. However, the sucrose content in the inferior grain was significantly greater than that in the superior grain at 16–24 days post-anthesis. In contrast to sucrose, the starch content in the inferior grain was significantly lower than that in

the superior grain during the grain-filling period. Exogenous Spd and MGBG significantly altered the sucrose and starch contents in the inferior grain but had no significant effect on the sucrose and starch contents in the superior grain. Exogenous Spd significantly





**FIGURE 6 |** Effect of external PA on the grain weight of wheat. **(A and B)** Shuangda 1 at 2014–2015 and 2015–2016, respectively; **(C and D)** Xinong 538 at 2014–2015 and 2015–2016, respectively. PUT, SPD, MGBG, and control represent external applied Put, Spd, methylglyoxal-bis(guanylhydrazine) (MGBG), and water, respectively, to spikelets at anthesis stage. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ,  $n$  represents the biological replicates). Values within the same grain type followed by different letters are significantly different ( $P < 0.05$ ); ns represents there being no significant difference among all of the treatments for the same grain type.

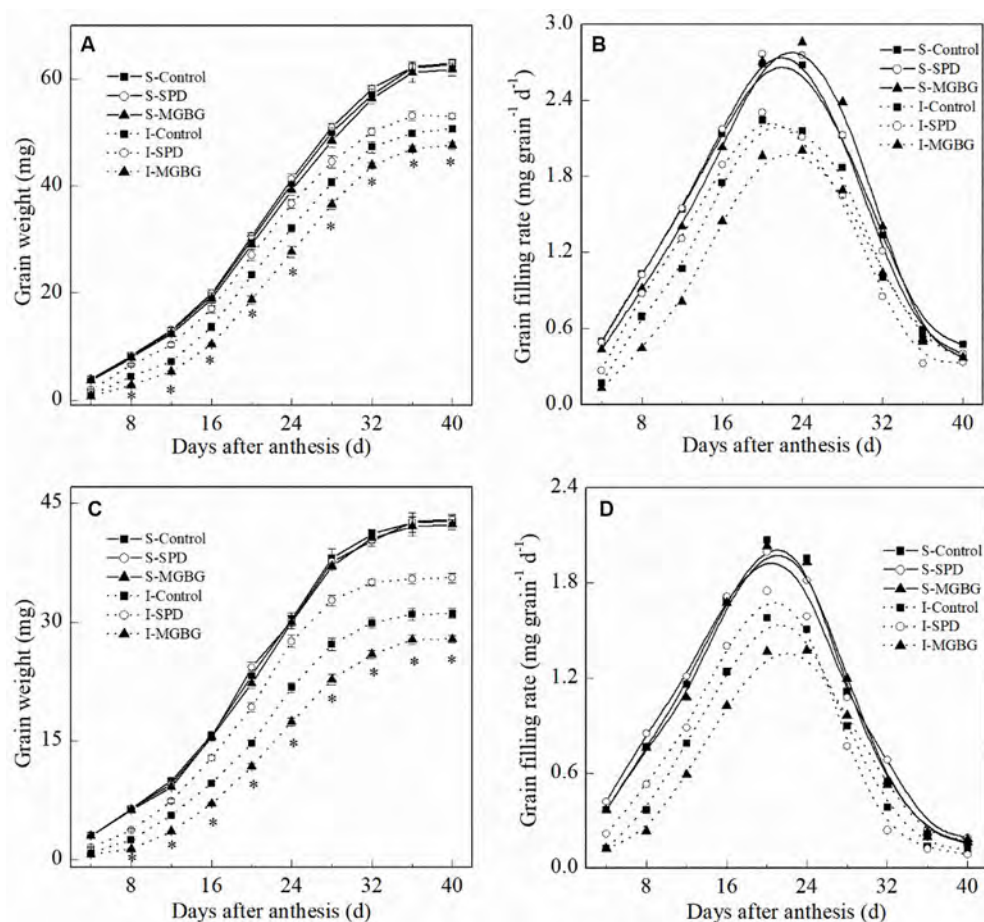
promoted an increase in sucrose content in the inferior grain at 4 and 8 days post-anthesis and an increase in starch content in the inferior grain at 16 and 40 days post-anthesis; exogenous MGBG had the opposite effect. In addition, at 4 and 8 days post-anthesis, exogenous Spd significantly promoted SS and AI activities in the inferior grain but had no significant effect on the SS and AI activities in the superior grain (**Figures 10E–H**); exogenous MGBG had the opposite effect.

## Hormones

Exogenous Spd significantly affected the Z+ZR, ABA, and ETH levels in the inferior grain but had no significant effect on those in the superior grain (**Figure 11**). Exogenous Spd significantly promoted an increase in Z+ZR and ABA levels in the inferior grain at 8 and 16 days post-anthesis. In contrast, the ETH evolution rate in the inferior grain in the SPD treatment was significantly lower than that in the CK treatment at 8 and 16 days post-anthesis; exogenous MGBG had the opposite effect.

## DISCUSSION

How to improve the poor grain-filling ability of inferior grain in cereals such as rice and wheat is important for promoting wheat thousand grain weight and yield (Yang and Zhang, 2010). Previous studies have suggested that PAs are notably involved in the grain filling of rice and wheat (Wang et al., 2012; Liu et al., 2013). Yang et al. (2008) suggested that a high Spd level in grain is one of the reasons why superior grain had a higher grain-filling rate than did inferior grain. In addition, exogenous Spd notably released the inhibitory effect of drought on wheat grain filling (Yang et al., 2014; Liu et al., 2016). In the present study, the superior grain had a higher Spd level than did the inferior grain, and the Spd levels in grain of the high-grain-weight cultivars were notably higher than those of the low-grain-weight cultivars. In addition, correlation analysis revealed that the Spd level in the grain was positively and significantly correlated with the grain-filling rate and thousand grain weight. Exogenous Spd significantly increased grain filling. These results indicate that



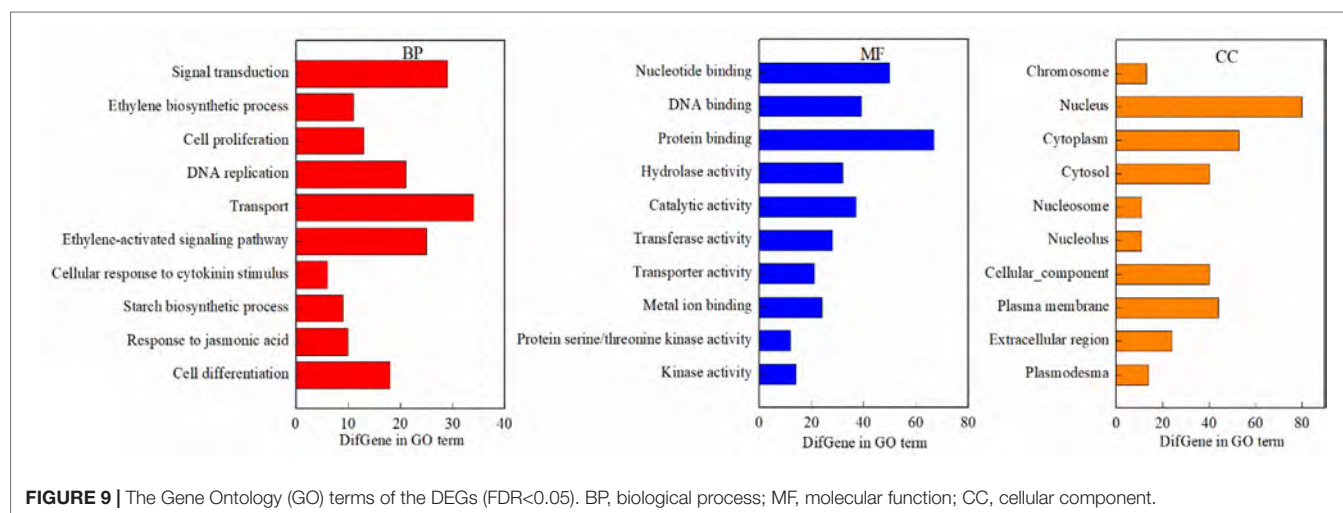
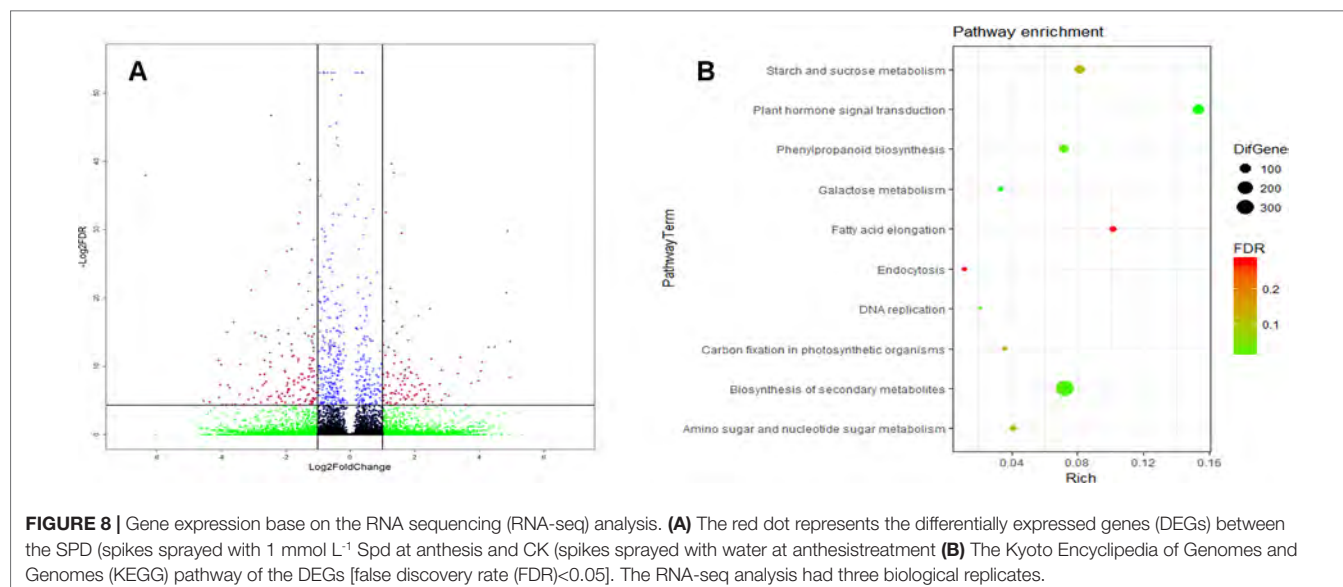
**FIGURE 7 |** Effect of external PA on the changes of grain weight and grain-filling rate of Shuangda 1 (A and B) and Xinong 538 (C and D) during the grain-filling period. SPD, MGBG, and control represent external applied Spd, MGBG, and water, respectively, to spikelets at anthesis stage. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ,  $n$  represents the biological replicates). \* represents there being significant difference among the treatments for the same grain type ( $F$  value  $> F_{0.05}$ ). S, superior grain; I, inferior grain.

**TABLE 2 |** Effect of external PA on the grain-filling characteristics of wheat.

Cultivar	Spikelet categories	Treatment	MGR	AGP	GW
			mg grain <sup>-1</sup> d <sup>-1</sup>	d	mg
Shuangda 1	S	CK	1.82a	35.94a	65.41a
		SPD	1.81a	35.85a	64.89a
		MGBG	1.80a	35.88a	64.58a
	I	CK	1.68b	31.27a	52.53b
		SPD	1.76a	31.56a	55.55a
		MGBG	1.56c	31.60a	49.30c
Xinong 538	S	CK	1.35a	32.20a	43.47a
		SPD	1.33a	33.04a	43.94a
		MGBG	1.33a	32.29a	42.95a
	I	CK	1.13b	27.93a	31.56b
		SPD	1.34a	26.84a	35.97a
		MGBG	0.96c	28.18a	27.05c

Values within a column and for the same cultivar and same grain type followed by different letters are significantly different ( $P < 0.05$ ). GW, the final grain weight; MGR, mean grain-filling rates; AGP, active grain-filling period; S, superior grain; I, inferior grain. SPD, MGBG, and CK represent external applied Spd, MGBG, and water, respectively, to spikelets at anthesis stage. The data list in the table is the average of the three biological replicates.

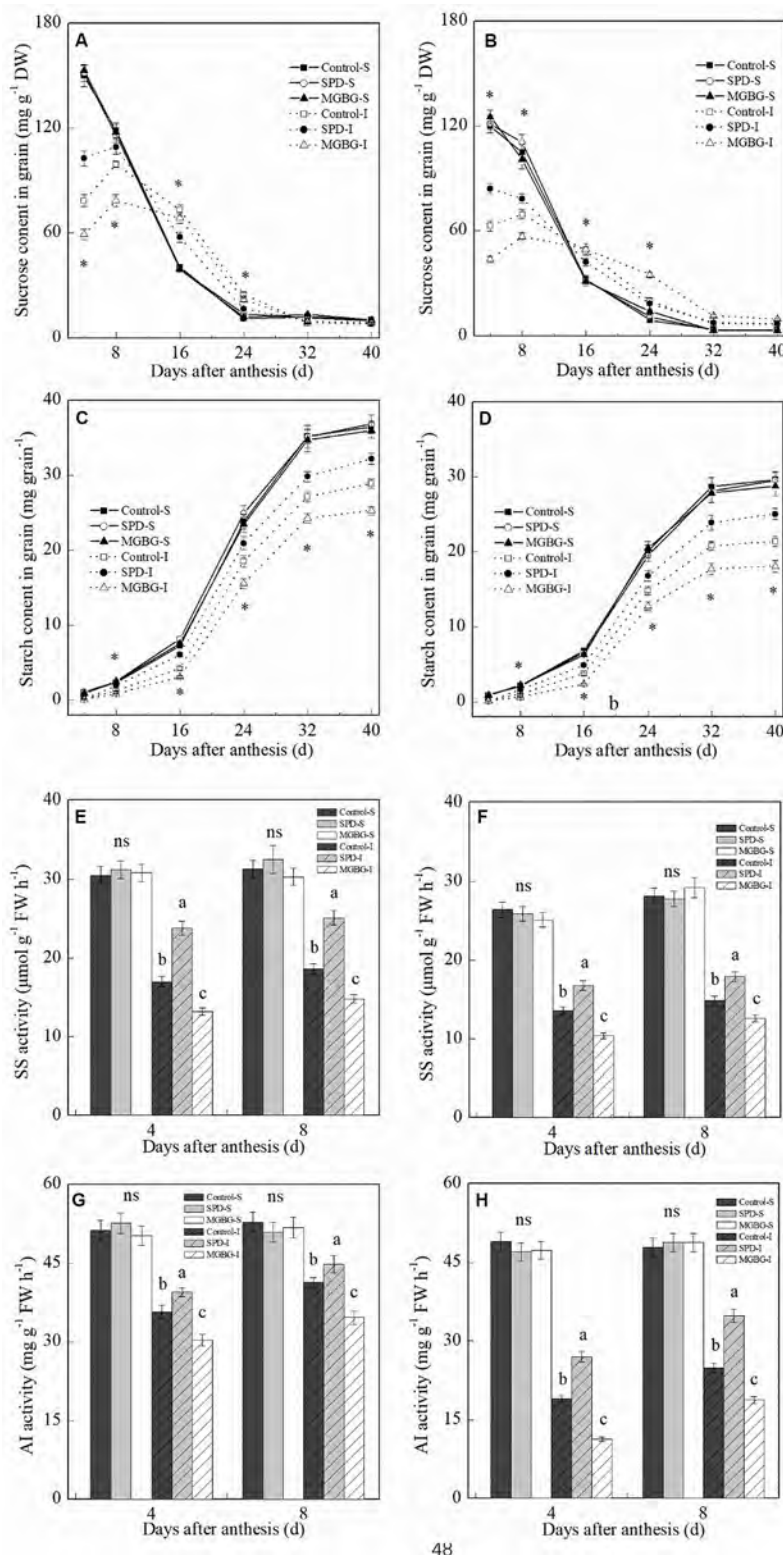




increased Spd levels in grain can notably promote increased grain filling in wheat. In contrast to the Spd content, the Put content was significantly greater in the inferior grain than in the superior grain, and the Put level in the grain was negatively and significantly correlated with the grain-filling rate and thousand grain weight. However, exogenous Put had no significant effect on the wheat grain filling, which means that Put may not be the key factor that regulates grain filling in wheat.

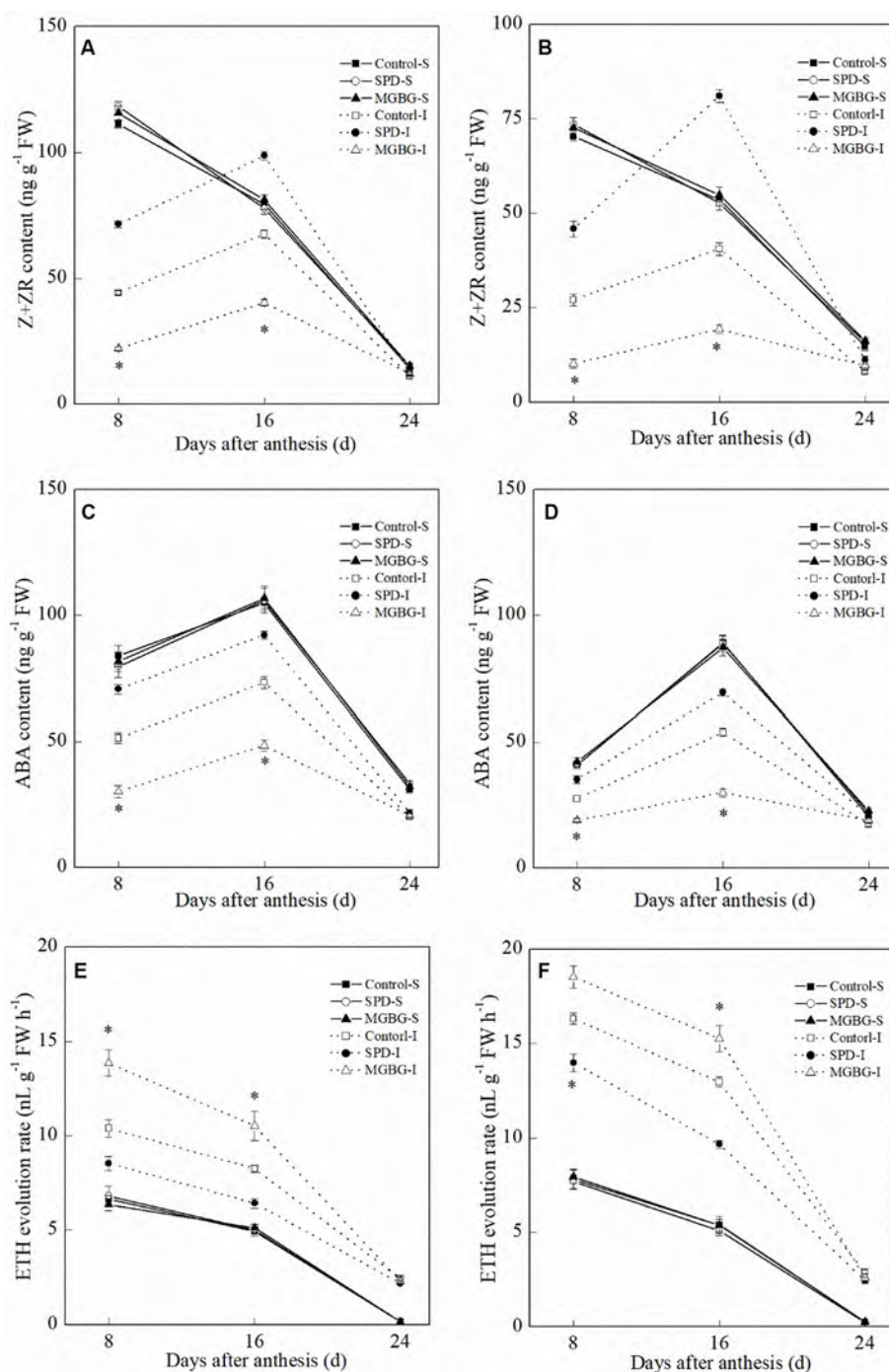
ADC, SAMDC, and SpdSy are key enzymes that regulate PA synthesis and are significantly involved in the regulation of grain filling in rice and wheat (Yang et al., 2008; Liu et al., 2016). ADC regulates Put synthesis, and SAMDC and SpdSy are involved in the pathway in which Spd is synthesized from Put (Chen et al., 2013). In the present study, the ADC activity in the grain did not significantly differ between the high-weight cultivars and the low-weight cultivars. However, the SAMDC and SpdSy activities

in the grain of the high-grain-weight cultivars were significantly higher than those of the low-grain-weight cultivars, and the superior grain had higher SAMDC and SpdSy activities than did the inferior grain for the same cultivar. Correlation analysis suggested that SAMDC and SpdSy activities were positively and significantly correlated with Spd levels in the grain but were negatively and significantly correlated with Put levels (data not shown). However, ADC activity was not significantly correlated with PAs levels in the grain. This finding means that the synthesis of Put may not be significantly related to wheat thousand grain weight. In contrast, compared with the low-grain-weight cultivars and inferior grain, the high-grain-weight cultivars and superior grain had a more direct Put-to-Spd biosynthesis pathway. These results suggest that the direct synthetic pathway from Put to Spd within grain may be in favor of the grain filling and promoting the thousand grain weight in wheat.



**FIGURE 10 |** Effect of external PA on the sucrose and starch contents and sucrose synthase (SS) and acid invertase (AI) activities in grains of Shuangda 1 (**A, C, E, and G**) and Xinong 538 (**B, D, F, and H**). SPD, MGBG, and control represent external applied Spd, MGBG, and water, respectively, to spikelets at anthesis stage. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ,  $n$  represents the biological replicates). S, superior grain; I, inferior grain. E–H, Values within the same day and same grain type followed by different letters are significantly different ( $P < 0.05$ ). ns represents there being no significant difference among all of the treatments for the same grain type. \* represents there being significant difference among the treatments for the same grain type ( $F$  value  $> F_{0.05}$ ).





**FIGURE 11** | Effect of external PA on the hormonal changes in grains of Shuangda 1 (A, C, and E) and Xinong 538 (B, D, and F). SPD, MGBG, and CK represent external applied Spd, MGBG, and water, respectively, to spikelets at anthesis stage. Vertical bars represent  $\pm$  the standard error of the mean ( $n = 3$ ,  $n$  represents the biological replicates). \* represents there being significant difference among the treatments for the same grain type ( $F$  value  $> F_{0.05}$ ). S, superior grain; I, inferior grain.

The grain filling in wheat is governed by the grain-filling rate and the active grain-filling period. In the present study, compared with the superior grain, the significant low grain weight of inferior grain for these six cultivars seems due to the lower level of grain-filling rate (Figure 1 and Table 1). In addition, the Put

and Spd levels in the grain were not significantly correlated with the active grain-filling period. Exogenous Spd had no significant effect on the active grain-filling period, although it notably increased the wheat grain-filling rate and thousand grain weight. In addition, our results showed that exogenous Spd affected the

grain-filling rate mainly during the early grain-filling period. The combination of the results in which exogenous Spd and MGBG significantly affected only the grain filling of inferior grain and not the grain filling of superior grain suggests that PAs regulated the grain-filling rate of the inferior grain mainly during the early grain-filling period, affecting the grain filling and thousand grain weight of wheat. Therefore, in the present study, we selected the inferior grain during the early grain-filling period (10 days post-anthesis) to research the regulatory mechanism of Spd in wheat grain filling.

Fu et al. (2011) suggested that sink strength is an important factor in regulating grain filling in rice. Low sink strength is one of the main reasons for the poor grain filling of inferior rice and wheat grain, and increased sink strength notably improves the poor grain filling of inferior cereal grain (Kato, 2004). With respect to the synthesis of starch from sucrose in grain, endogenous hormone levels are important indices of sink strength in cereals (Ishimaru et al., 2005; Wang et al., 2008). In the present study, exogenous Spd significantly promoted an increase in sucrose content in the inferior grain during the early grain-filling period, and MGBG had the opposite effect. In addition, the SS and AI activities in the inferior grain in the Spd treatment were significantly higher than those in the control treatment, and MGBG notably reduced the activities of both enzymes. The previous study suggested that the high SS activity in grain promotes the synthetic from sucrose to starch, and this effect was in favor to the sucrose transport from stem to grain (Jiang et al., 2003; Yang et al., 2004). Beside this, the AI was related to the sucrose unloading in grain, and the nitrogen application notably promoted the AI activity in wheat grain and increased the sugar content in grains (Zhang et al., 2014). SS and AI activities notably promote increased sink activity, and the activity of these two enzymes is positively and significantly correlated with the grain-filling rate in rice and wheat (Verma et al., 2018). These results indicate that Spd notably promoted the unloading of sucrose, which was then transported from source tissue, in the sink tissue of inferior grain. Zhang et al. (2009) suggested that one of the reasons for the poor grain filling of inferior rice grain is the “energy barrier” of inferior grain, which has difficulty receiving sucrose transported from stem source tissue. On the basis of these findings, we suggested that the promotive effect of Spd on the unloading of sucrose in inferior grain may significantly increase the reception ability of inferior grain for sucrose, thus promoting the transport of sucrose from the stem to inferior grain.

Previous studies have suggested that multiple plant hormones are involved in the regulation of grain filling in cereals. CTKs represent an important factor that is significantly related to grain filling in cereals. CTK levels in roots were positively correlated with photosynthesis in the flag leaves of rice during the grain-filling period (Yang et al., 2002). Moreover, grain-filling patterns were significantly related to Z+ZR contents in both the grain and the roots during the early and middle grain-filling periods (Yang et al., 2000). The results of our previous study suggested that drought stress significantly reduced Z+ZR contents in grain and inhibited the grain filling of wheat (Liu et al., 2013b). In the present study, exogenous Spd significantly promoted an increase in Z+ZR contents in the inferior grain at 8 and 16 days

post-anthesis; exogenous MGBG had the opposite effect. Previous studies suggested that the effects of PA on rice grain filling were notably related to Z+ZR contents in the grain (Yang et al., 2008). In addition, the results of our previous study suggested that Spd notably relieves the inhibition effect of drought on grain filling of wheat, and this may relate to the significant increasing of the Z+ZR concentrations in the grains. However, the external Put had no significant effect on the Z+ZR concentrations in grains, and it cannot relieve the inhibition effect of drought on grain filling of wheat (Liu et al., 2016). These findings mean that the promotive effect of Spd on the filling of inferior wheat grain is notably related to the Z+ZR content. GO analysis in the present study revealed that exogenous Spd significantly affected the BPs involving cellular responses to CTK stimulus and cell differentiation (Figure 6D). The number and division rate of endosperm cells determine the sink size in cereals such as wheat and rice (Chen et al., 2013). Zhang et al. (2010) suggested that CTKs significantly increase endosperm cell division and promote the sink strength of rice grain. In addition, CTK levels notably increased during the early development of the seeds of both pea and bean (Michael and Seiler-Kelbitsch 1972; Saha et al., 1986; Morris et al., 1993; Dietrich et al., 1995). Beside this, CTK was notably related to the sucrose metabolism. Yang et al. (2002) suggested that ABA and CTK are involved in controlling plant senescence and enhanced carbon remobilization when wheat is subjected to water stress. Lee and Huang (2013) suggested that CTK affects sucrose metabolism conducting to *de novo* shoot organogenesis in rice callus. Wang et al. (2016) suggested that lovastatin, a CTK inhibitor, inhibited the invertase activity and metabolism and transport of glucose, fructose, and sucrose of *tasg1*, a wheat stay-green mutant; however, the activity of invertase was partially recovered in *tasg1* when treated with 6-benzylaminopurine, and this means that CTK might regulate the stay-green phenotype of *tasg1* by regulating the invertase activity involved in sucrose remobilization. Our previous study suggested that the Z+ZR contents in grain were significantly and positively correlated with the SS activities in wheat grain and that a high Z+ZR content in grain promoted the synthesis of starch, which promoted the grain filling of wheat (Liang et al., 2017). These mean that CTK significantly regulated the sucrose metabolism and was involved in the synthesis from sucrose to starch during wheat grain filling. These results suggested that Spd increased the Z+ZR contents in the inferior grain of wheat and promoted endosperm cell division, thereby increasing the sink size of the inferior grain. These phenomena caused the inferior grain to accommodate increased amounts of carbohydrates transported from the stem. In addition, exogenous Spd also promoted SS and AI activities and sucrose unloading in the inferior grain, which means that Spd may affect CTK levels in the grain to promote both increased sink size and sink strength in the inferior grain and the transport of sucrose from the stem to that grain. These phenomena may constitute the main reason why Spd promoted increased filling of the inferior wheat grain.

In addition to CTKs, ETH and ABA are also involved in the regulation of grain filling of cereals. Yang et al. (2006) suggested that high ratio of ABA/ETH under soil drought stress notably promoted the grain-filling rate of wheat. Lv et al. (2017) suggested



that foliar applications of potassium significantly promoted the ABA concentration and reduced ETH evolution rates in inferior grain and increased the grain-filling rate of inferior grain in wheat. PAs and ETH share the same synthetic precursor, and their metabolism is affected by each other (Bisbis et al., 2000). Wang et al. (2012) suggested that the interaction between PAs and ETH notably regulated rice grain filling. Liu et al. (2013) suggested that the promoting effect of external Spd on grain filling of wheat was significantly related to the increasing of ABA concentration in grains. In the present study, exogenous Spd significantly reduced the ETH evolution rate and promoted the ABA concentration in the inferior grain, whereas MGBG had the opposite effect. These results are similar to those of previous studies (Chen et al., 2013; Liu et al., 2013). In addition, previous studies suggested that ETH inhibits wheat endosperm cell division (Yang et al., 2017). The ABA increased the sink strength of the inferior grain and promoted the carbohydrate transport to inferior grain of rice (Yang and Zhang, 2010). These results mean that Spd maybe inhibited ETH synthesis and promoted the ABA concentration to promote the endosperm cell division and sink strength of wheat grain.

## CONCLUSION

PAs significantly affected wheat grain filling and thousand grain weight. The direct synthetic pathway of Spd from Put in the grain was a key factor in promoting increased grain filling and thousand grain weight in wheat. Spd regulates the grain-filling rate of inferior grain mainly during the early grain-filling period, affecting wheat grain filling and thousand grain weight. The promotive effect of Spd on the grain filling of the inferior grain of wheat was notably related to carbohydrate transport from the stem to that grain. Spd significantly increased the Z+ZR contents

but reduced the ETH evolution rate in the inferior grain. In addition, Spd significantly increased the SS and AI activities in the inferior grain. These effects of Spd led to increased sucrose content in the inferior grain. Together, these findings may be some of the main reasons why Spd significantly promoted the increased filling and weight of the inferior wheat grain.

## DATA AVAILABILITY STATEMENT

The RNA-seq data were deposited in the Sequence Read Archive of NCBI; the accession number is SRP217735.

## AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: YaL. Performed the experiments: JL, BW. Analyzed the data: JH, BW. Wrote the paper: YaL, JL, YuL.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2019.01309/full#supplementary-material>

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## 外源多胺对小麦小花退化的调控机制

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**摘 要:** 小麦穗粒数与小花退化密切相关, 多胺是调控小花发育的一种重要植物生长调节剂。本研究利用小麦品种(系)双大 1 号(大穗型)和西农 538 (小穗型), 于小花退化阶段在穗部施用腐胺(Put)、亚精胺(Spd)和精胺(Spm), 分析外源多胺对小麦小花退化的影响及其与内源激素、植株碳氮的关系。结果表明, 外源 Spd 和 Spm 显著抑制小花退化、提高了可孕小花数目, 而 Put 加具有显著的负效应; 并且多胺的调控具有明显的位置效应, 对小穗上部弱势小花退化的调控效应显著大于下部强势小花。施用外源 Spd 和 Spm 后, 弱势小花中 Spd、Spm 显著增加, 同时玉米素+玉米素核苷(Z+ZR)含量及其与脱落酸(ABA)的比值也显著升高, 而内源乙烯的释放速率降低, 并且弱势小花中可溶性总糖和可溶性蛋白质含量显著提高。外源 Put 对弱势小花的调控效应与此相反, 施用后弱势小花中 Put、ABA 含量以及内源 ETH 释放速率显著提高, 而(Z+ZR)与 ABA 比值和可溶性总糖含量降低。因此认为, 多胺参与了对小麦小花退化的调控, 其对小麦小花退化的调控与内源激素、植株碳氮代谢密切相关。

**关键词:** 多胺; 小麦; 小花退化; 激素; 可溶性总糖; 可溶性蛋白

## Effect of Exogenous Polyamines on Mechanism of Floret Degeneration in Wheat

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**Abstract:** Kernel number per spike has a close relationship with floret degeneration in wheat, which is regulated greatly by polyamines (PAs), one of important plant growth regulators. In this study, we applied exogenous spermine (Spm), spermidine (Spd) and putrescine (Put) to the young spikes of cultivars Shuangda 1 (large-spike type) and Xinong 538 (small-spike type) to investigate the effects of PAs on floret degeneration, endogenous hormones, and the carbon and nitrogen changes in wheat floret. The results indicated that exogenous Spd and Spm had similar effects on floret degeneration whereas exogenous Put was in function. In the treatments with external Spd and Spm applied, the floret degeneration was inhibited and the number of fertile florets increased significantly. In the external Put treatment, floret degeneration aggravated and the fertile floret number decreased significantly. Such influence by external PAs was different in upper and lower florets of a spike and the upper florets (inferior florets) showed greater effects than the lower ones (superior florets). The concentrations of endogenous Spd and Spm, zeatin (Z) + zeatin riboside (ZR), and the ratio of Z+ZR-to-abscisic acid (ABA) in inferior florets increased after applying exogenous Spd and Spm. However, the evolution rate of endogenous ethylene (ETH) in inferior floret decreased. As a result, the total soluble sugar and protein contents increased significantly in inferior floret. In contrast, exogenous Put showed reverse effects compared to Spd and Spm. Our results indicate that PAs were involved in the regulation of floret degeneration by changing the endogenous hormone concentrations and the carbon and nitrogen metabolism in wheat plants.

**Keywords:** Polyamine; Wheat; Floret degeneration; Hormone; Total soluble sugar; Soluble protein

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小麦单位面积产量由单位面积穗数、每穗粒数和粒重构成,其中穗粒数对环境及栽培措施最为敏感,在保证穗数的基础上提高穗粒数是小麦高产的主要调控目标。小麦穗粒数是小花分化、发育、退化和结实等一系列生理过程的最终体现<sup>[1]</sup>。研究表明,小麦所分化的小花数比较稳定,但最终仅有20%~30%的小花能够发育成籽粒,70%~80%的小花先后退化而成为无效小花<sup>[2]</sup>。小麦穗生成籽粒的潜在能力远远超过实际的结实粒数<sup>[3-4]</sup>。因此,减少小花退化、增加可孕小花数量,从而提高小花结实率是实现小麦高产的关键问题。

多胺是植物体内一类具有生物活性的低分子量脂肪族含氮碱的总称,被认为是生长调节物质或激素的第二信使<sup>[5-6]</sup>。植物体内常见的多胺有腐胺(Put)、亚精胺(Spd)、精胺(Spm)等,其具有刺激细胞分裂、促进生长、延缓衰老、提高抗性、调节开花、提高种子活力、促进根系吸收等作用,能显著调节植物的生长发育及其对逆境的响应<sup>[7]</sup>。多胺参与了对禾谷类作物颖花发育的调控,在水稻上的研究发现,保持系幼穗中Spd和Spm含量显著高于不育系,外源施用MGBG(Spd和Spm合成抑制剂)显著降低幼穗中Spd和Spm含量,进而抑制雄蕊发育、降低花粉育性;氯化钴能够提高水稻幼穗中多胺含量,促进雄蕊及花粉发育<sup>[8-11]</sup>。Ma等<sup>[12]</sup>研究发现,*OsARG*基因通过调控多胺合成途径关键酶精氨酸合成酶影响水稻幼穗中多胺含量,而该基因的缺失会降低幼穗中多胺含量,进而导致穗型变小、结实率显著下降。在小麦上的研究也表明外源施用Spd能够显著促进小麦幼穗生长,提高小麦穗粒数<sup>[13]</sup>。这些研究结果表明,多胺显著影响禾谷类作物颖花发育。但是,关于多胺对小麦小花退化的影响,还缺乏相关研究。

激素在调控禾谷类作物小花发育过程中起着重要作用,乙烯(ETH)显著提高小麦、玉米及水稻颖花退化率、降低可孕花数目和结实率进而减低穗粒数<sup>[14-16]</sup>。外源脱落酸(ABA)显著提高了水稻、小麦的不结实籽粒比例,降低了结实小花率<sup>[17]</sup>。外源玉米素(Z)显著提高小麦小花数目,较高的内源细胞分裂素(CTK)有利于小麦小花发育<sup>[2,4]</sup>,细胞分裂素氧化酶基因显著影响小麦和水稻等作物小花发育及穗粒数形成<sup>[18-19]</sup>。除激素外,植物营养也显著影响小花发育及退化,有研究认为,小麦小花退化的主要原因是对植株营养的竞争,由于开花前茎、穗之间对有限营养资源的

竞争,造成穗部生长营养供应不足引起小花退化<sup>[2-3]</sup>。Serrago等<sup>[3]</sup>发现,小麦可孕小花数目与碳水化合物含量呈正相关,在药隔期至四分体期,保证氮素营养可减少退化小花数,增加结实粒数。

外源多胺能显著提高植株内源生长素(IAA)和ABA含量<sup>[20-21]</sup>,Spd和ETH的合成代谢间存在相互抑制的关系<sup>[22]</sup>,外源Spd和Spm能显著促进水稻弱勢粒淀粉合成<sup>[22]</sup>。这表明多胺代谢与激素、植株营养之间关系密切。但是关于多胺对小麦小花退化的影响及其与激素、植株营养的关系,目前还缺乏深入了解。本研究采用外源多胺处理,在小麦小花两极分化及退化阶段外源向穗部位注射多胺,分析了外源多胺对小麦小花退化的影响及其与内源激素、植株碳氮的关系,旨在探讨多胺对冬小麦小花退化的影响及其生理机制,为穗粒数形成提供理论依据。

## 1 材料与方法

### 1.1 试验材料 with 处理

2012—2013和2013—2014年度,在西北农林科技大学标本园(陕西杨凌,34°20' N, 108°04' E)进行田间试验。供试土壤为壤土,耕层(0~20 cm)土壤含有机质12.07 g kg<sup>-1</sup>、全氮1.42 g kg<sup>-1</sup>、速效氮55.56 mg kg<sup>-1</sup>、速效磷18.33 mg kg<sup>-1</sup>、速效钾112.67 mg kg<sup>-1</sup>,pH 7.11,容重1.31 g cm<sup>-3</sup>。供试材料为双大1号(大穗型品系)和西农538(小穗型品种),2012年和2013年均于10月14日播种,播量为150 kg hm<sup>-2</sup>,行距0.20 m。播种前基施375 kg hm<sup>-2</sup>尿素和375 kg hm<sup>-2</sup>磷酸二胺。小麦生长期进行常规大田管理。

参考王兆龙等<sup>[1,4]</sup>和倪英丽<sup>[2]</sup>关于小麦小花发育的研究,并结合本课题组前期预备试验结果,每品种设4个处理,在倒二叶完全抽出(旗叶露尖,此期处于开花前20 d,小花分化基本结束,小花开始两级分化)时选择长势一致的植株进行挂牌标记,每个处理在倒二叶完全抽出期、四分体时期(叶龄余数为0.5)、小花退化期(叶龄余数为0.2)向幼穗与叶鞘的空隙中分别注入1 mmol L<sup>-1</sup> Spd (S1)、1 mmol L<sup>-1</sup> Spm (S2)、2 mmol L<sup>-1</sup> Put (P1)和清水,每株注入500 μL。每个注射时期均连续2 d,于每天17:00—18:00实施。小区面积3 m × 3 m,随机区组排列,3次重复。

### 1.2 测定项目与方法

1.2.1 小花发育 从小麦穗分化开始定期取样观察并记载穗分化进程,记载分化小穗数、小花数以及穗分化各阶段的特征。从开花前18 d开始至开花

期每隔4 d选发育一致的主茎35个左右,以穗中部第7~14小穗基部小花为强势小花(双大1号第1~3位小花、西农538第1~2位小花),上部小花为弱势小花(双大1号第4~6位小花、西农538第3~5位小花),将两部分小花在冰面上进行剥取、分装、称鲜重,其后液氮速冻后放入-40℃冰箱中保存。于抽穗期取样,采用XTS2022电子光学显微镜(北京泰克)观察可孕小花分化数目,以具有完整绿色花药和羽状柱头的小花作为可孕小花判断标准<sup>[1]</sup>。以各处理总小花数和可孕小花数相对于对照的增加率来表示各处理对小麦小花退化的影响,增加率 = (处理测定值 - 对照测定值) / 对照测定值 × 100。

**1.2.2 内源游离态多胺测定** 参照刘俊等<sup>[23]</sup>和Yang等<sup>[22]</sup>的方法测定游离态Spd、Spm和Put含量,5%三氯乙酸提取后采用高效液相色谱法,用1525二元梯度泵-2489紫外检测器(Waters)测定。色谱柱为Waters Symmetry C18柱(7.5 cm × 4.6 mm × 3.5 μm),流动相为甲醇-水(59:41),流速0.7 mL min<sup>-1</sup>,柱温30℃,检测波长230 nm。

**1.2.3 内源激素测定** 采用酶联免疫法测定Z+ZR、ABA含量。用80%甲醇(含1 mmol L<sup>-1</sup> BHT)提取内源激素,试剂盒购自中国农业大学,具体步骤参照试剂盒说明书进行。使用酶标仪测定激素含量<sup>[24]</sup>,采用气相色谱法测定乙烯含量<sup>[14]</sup>。

**1.2.4 小花可溶性总糖和可溶性蛋白测定** 采用80%乙醇提取可溶性总糖,用蒽酮比色法测定其含量<sup>[25]</sup>。采用Tris-HCl (pH 7.0)缓冲液提取可溶性蛋白,用考马斯亮蓝法测定其含量<sup>[26]</sup>。

### 1.3 数据分析

采用Microsoft Excel 2010进行数据整理,用SPSS16.0软件进行强势小花与弱势小花的*t*测验和处理间多重比较(LSD 0.05),Origin8.0软件绘图。2年试验数据规律基本一致,文中所列内源多胺、激素、可溶性总糖、可溶性蛋白含量为2013—2014年度测定值,其他指标均采用两年数据。

## 2 结果与分析

### 2.1 外源多胺对小麦小花发育的影响

外源施用多胺对总小花数无显著影响,但是其显著影响小麦可孕小花数目。与对照相比,S1和S2处理显著提高了可孕小花比例,但是P1处理可孕小花比例显著低于对照(图1)。这表明外源Spd和Spm抑制了小麦小花退化,而外源Put促进了小麦小花

退化(图1)。外源Spd和Spm显著提高了小麦穗粒数,而外源Put显著降低了小麦穗粒数,2个品种之间并未表现出明显的多胺作用差异(图2),表明无论是大穗型还是小穗型品种,多胺均能显著调控其小花退化及穗粒数形成。

多胺对不同小花位小花的调控效果不同。外源Spd、Spm和Put对着生在小穗下部的强势小花发育无显著影响,但是其显著影响着生在小穗上部的弱势小花发育,外源Spd和Spm显著提高了两品种弱势小花的可孕小花比率,而Put显著降低了弱势小花的可孕小花比率(图3)。

### 2.2 外源多胺对小麦小花内源多胺含量的影响

从开花前18 d到开花期,小花中Spd、Spm和Put含量呈明显的下降趋势(图4)。双大1号小花中Spd和Spm含量显著高于同期西农538,而双大1号小花中Put含量显著低于同期西农538。对于同一个品种,强势下小花中Spd和Spm含量显著高于弱势小花,而强势下小花中Put含量显著低于弱势小花。外源Put和Spm分别显著提高了小花中内源Put和Spm含量,外源Spd显著提高了小花中内源Spd和Spm含量。

### 2.3 外源多胺对小麦小花内源激素含量的影响

在小花两级分化及退化阶段,小花中Z+ZR含量呈先升高后下降的趋势,在开花前18 d至开花前10 d,小花中Z+ZR含量无显著变化,且强势小花和弱势小花之间无显著差异(图5)。从开花前10 d开始,小花内源Z+ZR含量迅速提高,至开花前2 d达到峰值,其后快速下降,此期强势小花内源Z+ZR上升幅度较弱势小花大,在开花前6 d和2 d,强势小花中Z+ZR含量显著高于弱势小花。在整个小花两级分化及退化阶段,双大1号小花内源Z+ZR含量显著高于西农538。外源Spd和Spm显著提高了小花内源Z+ZR含量,但是外源Put对小花内源Z+ZR含量无显著影响。

小麦强势小花和弱势小花内源ABA含量在小花两级分化及退化阶段的变化存在显著不同,强势小花内源ABA含量呈现先降低后升高的趋势,从开花前18 d至开花前6 d强势小花内源ABA含量呈显著下降趋势,其后又显著上升。与此相反,弱势小花内源ABA含量在小花两级分化及退化阶段呈现上升趋势。外源Spd和Spm对小花中ABA含量无显著影响,但是外源Put显著提高了小花中ABA含量。双大1号小花ABA含量显著高于西农538。



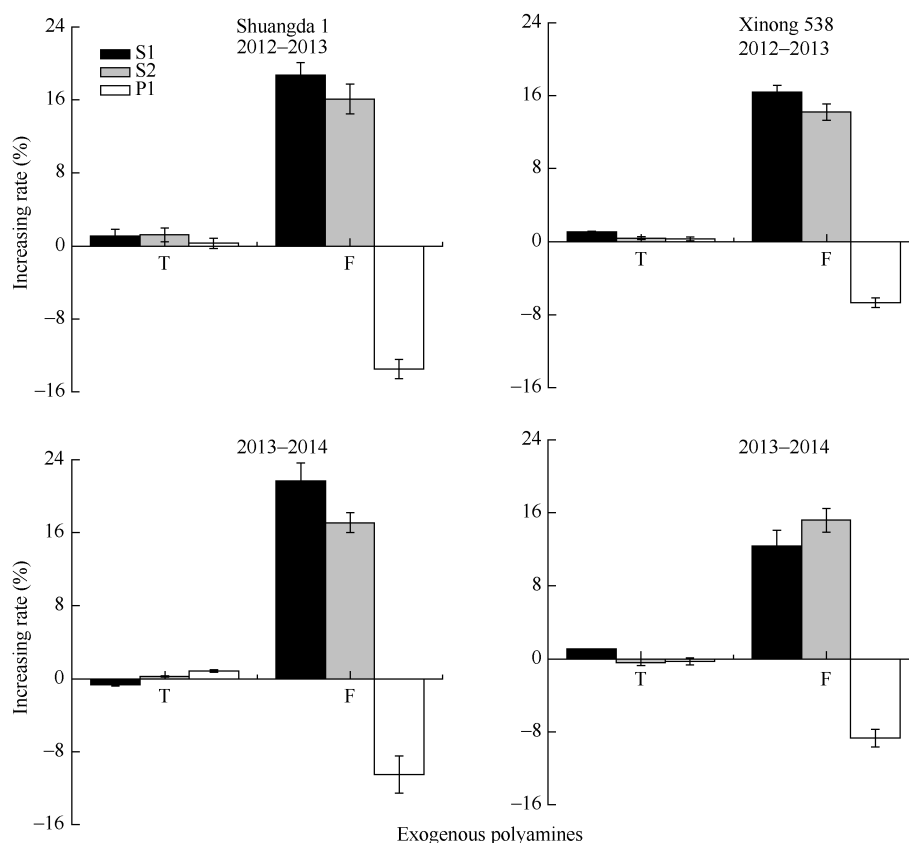


图 1 外源多胺对小麦总小花数和可孕小花增加率的影响

Fig. 1 Effects of exogenous polyamines on increased rates of total and fertile florets of wheat

S1: 施用 1 mmol L<sup>-1</sup> Spd; S2: 施用 1 mmol L<sup>-1</sup> Spm; P1: 施用 2 mmol L<sup>-1</sup> Put. T: 总小花数; F: 可孕小花数。

S1: exogenous 1 mmol L<sup>-1</sup> Spd applied; S2: exogenous 1 mmol L<sup>-1</sup> Spm applied; P1: exogenous 2 mmol L<sup>-1</sup> Put applied. T: number of total floret; F: number of fertile floret.

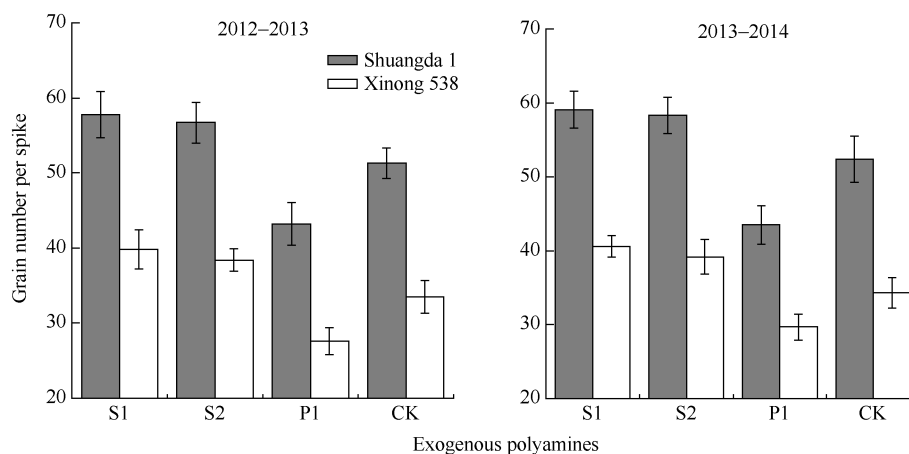


图 2 外源多胺对小麦穗粒数的影响

Fig. 2 Effects of exogenous polyamines on the kernel number per spike of wheat

S1: 施用 1 mmol L<sup>-1</sup> Spd; S2: 施用 1 mmol L<sup>-1</sup> Spm; P1: 施用 2 mmol L<sup>-1</sup> Put; CK: 对照(清水)。

S1: 1 mmol L<sup>-1</sup> exogenous Spd applied; S2: 1 mmol L<sup>-1</sup> exogenous Spm applied; P1: 2 mmol L<sup>-1</sup> exogenous Put applied; CK: control.

## 2.4 外源多胺对小麦小花内源激素含量的影响

在小花两级分化及退化阶段, 小花中 Z+ZR 含量呈先升高后下降的趋势, 在开花前 18 d 至开花前 10 d, 小花中 Z+ZR 含量无显著变化, 且强势小花和

弱势小花之间无显著差异(图 5)。从开花前 10 d 开始, 小花内源 Z+ZR 含量迅速提高, 至开花前 2 d 达到峰值, 其后快速下降, 此期强势小花内源 Z+ZR 上升幅度较弱势小花大, 在开花前 6 d 和 2 d, 强势小花

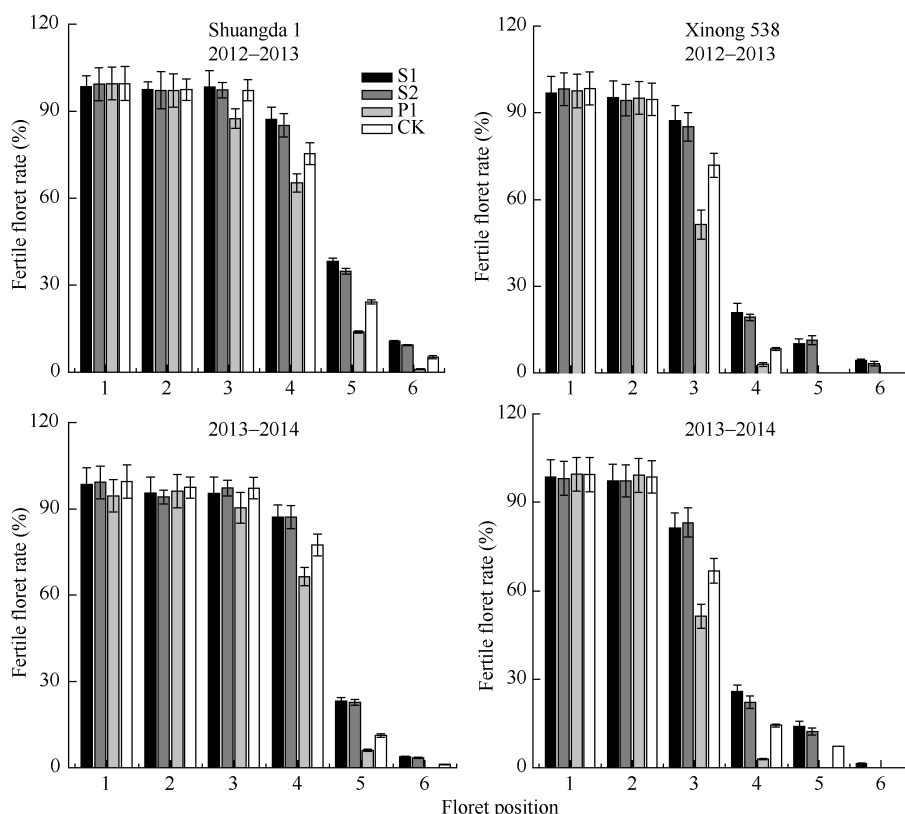


图3 外源多胺对不同花位可孕小花率的影响

Fig. 3 Effect of exogenous polyamines on the fertile floret number ratio of different floret set of wheat

S1: 施用 1 mmol L<sup>-1</sup> Spd; S2: 施用 1 mmol L<sup>-1</sup> Spm; P1: 施用 2 mmol L<sup>-1</sup> Put; CK: 对照(清水)。

S1: 1 mmol L<sup>-1</sup> exogenous Spd applied; S2: 1 mmol L<sup>-1</sup> exogenous Spm applied; P1: 2 mmol L<sup>-1</sup> exogenous Put applied; CK: control.

中 Z+ZR 含量显著高于弱势小花。在整个小花两级分化及退化阶段, 双大 1 号小花内源 Z+ZR 含量显著高于西农 538。外源 Spd 和 Spm 显著提高了小花内源 Z+ZR 含量, 但是外源 Put 对小花内源 Z+ZR 含量无显著影响。

小麦强势小花和弱势小花内源 ABA 含量在小花两级分化及退化阶段的变化存在显著不同, 强势小花内源 ABA 含量呈现先降低后升高的趋势, 从开花前 18 d 至开花前 6 d 强势小花内源 ABA 含量呈显著下降趋势, 其后又显著上升。与此相反, 弱势小花内源 ABA 含量在小花两级分化及退化阶段呈现上升趋势。外源 Spd 和 Spm 对小花中 ABA 含量无显著影响, 但是外源 Put 显著提高了小花中 ABA 含量。双大 1 号小花 ABA 含量显著高于西农 538。

在小花两级分化及退化过程中, 小花中 ETH 释放量呈降低趋势。弱势小花 ETH 释放量显著强势小花, 且西农 538 小花 ETH 释放量显著高于双大 1 号。外源 Spd 和 Spm 显著降低了弱势小花中 ETH 释放量, 而外源 Put 则显著提升了弱势小花中 ETH 释放量。但是外源多胺对强势小花中 ETH 释放量无显著

影响。

## 2.5 外源多胺对小麦小花可溶性糖和蛋白含量的影响

在小麦小花两级分化及退化过程中, 强势小花中可溶性总糖和蛋白含量显著高于弱势小花, 小花中可溶性总糖含量表现出升高-下降-升高-下降的趋势, 在花前 14 d 和 6 d 达到峰值, 而可溶性蛋白含量表现为升高-下降-升高的趋势, 在花前 6 d 达到峰值(图 6)。双大 1 号小花中可溶性总糖和蛋白含量高于同期西农 538。外源 Spd、Spm 和 Put 对强势小花内源可溶性总糖和蛋白含量均无显著影响。除此之外, 外源 Put 对弱势小花内源可溶性总糖和蛋白含量也无显著影响, 但是外源 Spd 和 Spm 显著提高了弱势小花中可溶性总糖和蛋白含量。

## 3 讨论

### 3.1 多胺对小麦小花退化的影响

多胺是植物体内一类具有生物活性的低分子量脂肪族含氮碱的总称, 被认为是生长调节物质或激素的第二信使, 也有学者将其归为植物激素的一类<sup>[5-6]</sup>。



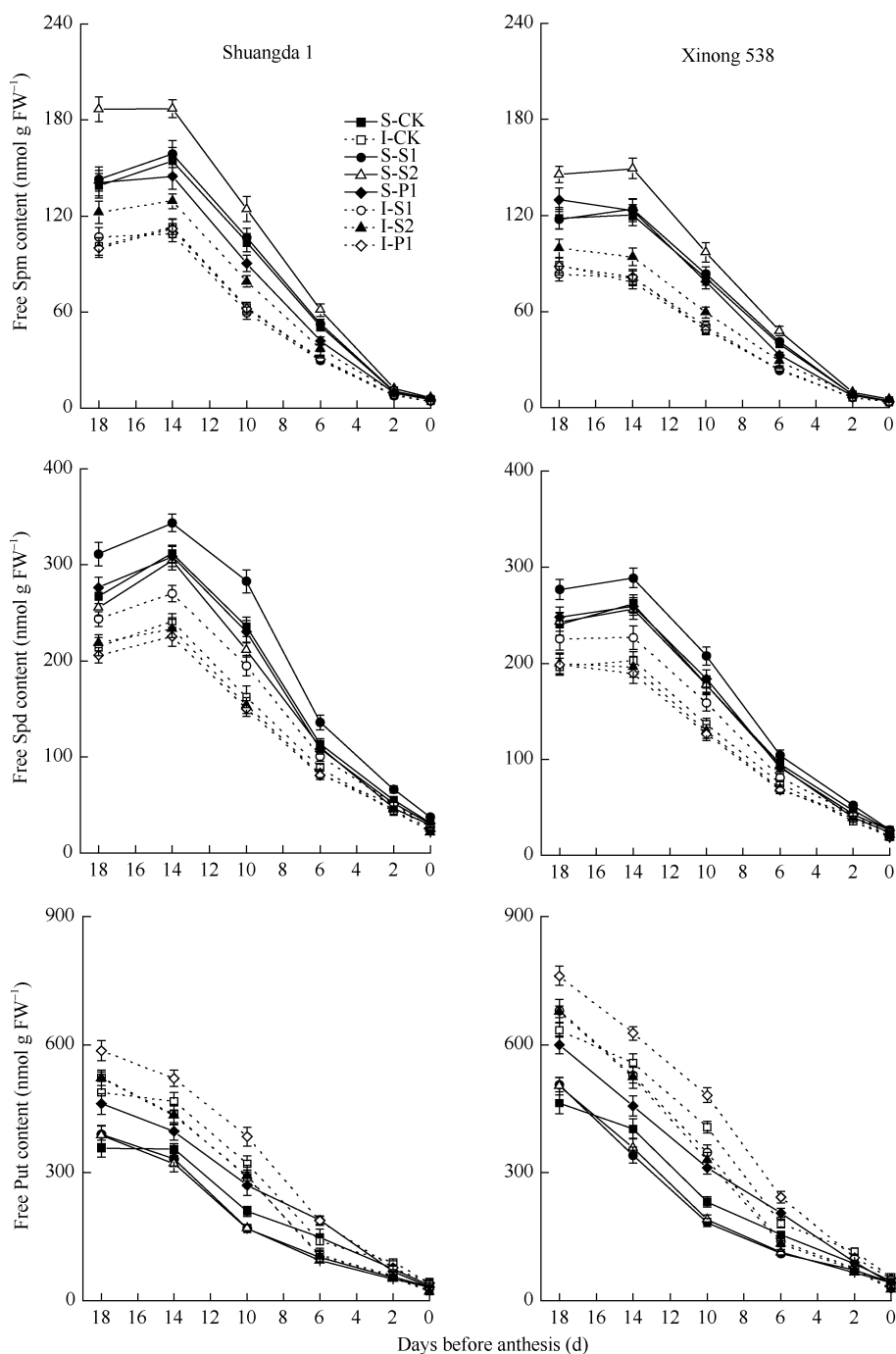


图 4 外源多胺对小麦小花内源多胺含量的影响(2013–2014)

Fig. 4 Effect of exogenous polyamines on endogenous PA contents in wheat floret (2013–2014)

S: 强势小花; I: 弱势小花; S1: 施用  $1 \text{ mmol L}^{-1}$  Spd; S2: 施用  $1 \text{ mmol L}^{-1}$  Spm; P1: 施用  $2 \text{ mmol L}^{-1}$  Put; CK: 对照(清水)。  
S: superior floret; I: inferior floret; S1:  $1 \text{ mmol L}^{-1}$  exogenous Spd applied; S2:  $1 \text{ mmol L}^{-1}$  exogenous Spm applied; P1:  $2 \text{ mmol L}^{-1}$  exogenous Put applied; CK: control.

研究表明, 多胺参与了对禾谷类作物颖花发育的调控<sup>[8-13]</sup>。外源施用 MGBG 显著降低了水稻幼穗中 Spd 和 Spm 含量, 进而抑制雄蕊发育、降低花粉育性; 提高水稻幼穗中多胺含量能够促进雄蕊及花粉发育; 降低水稻幼穗中多胺含量会导致穗型变小、结实率显著下降<sup>[8-11]</sup>。外源施用 Spd 能够显著促进小麦幼穗

生长, 提高小麦穗粒数<sup>[13]</sup>。在本研究中, 小麦强势小花内源 Spd 和 Spm 含量显著高于弱势小花, 同时外源 Spd 和 Spm 显著提高了可孕小花数目。与此不同, 小麦强势小花内源 Put 含量显著高于弱势小花, 而外源 Put 显著降低了可孕小花数目。这表明 3 种多胺对小麦小花退化的调控效果不同, Spd 和 Spm 能

够促进小花发育, 显著提高可孕小花比率, 而 Put 可能参与了对小花发育的抑制。同时本研究发现 Spd 和 Spm 对小麦小花发育的调控存在明显的位置效应, 其主要调控小穗上部的弱势小花发育, 但其对强势小花发育无显著影响。Put 是 Spd 和 Spm 的合成前体, 从本试验结果推测, 强势小花和弱势小花间 Put

与 Spd、Spm 含量相反的现象可能与两者在 Put 向 Spd、Spm 转化、合成上的不同有关, 弱势小花可能 Put 向 Spd、Spm 转化能力较弱, 造成了小花中大量 Put 积累, 造成对其生长的不利影响, 此时外源施用 Put 加剧了这一过程, 造成弱势小花大量退化, 可孕小花比率显著下降。

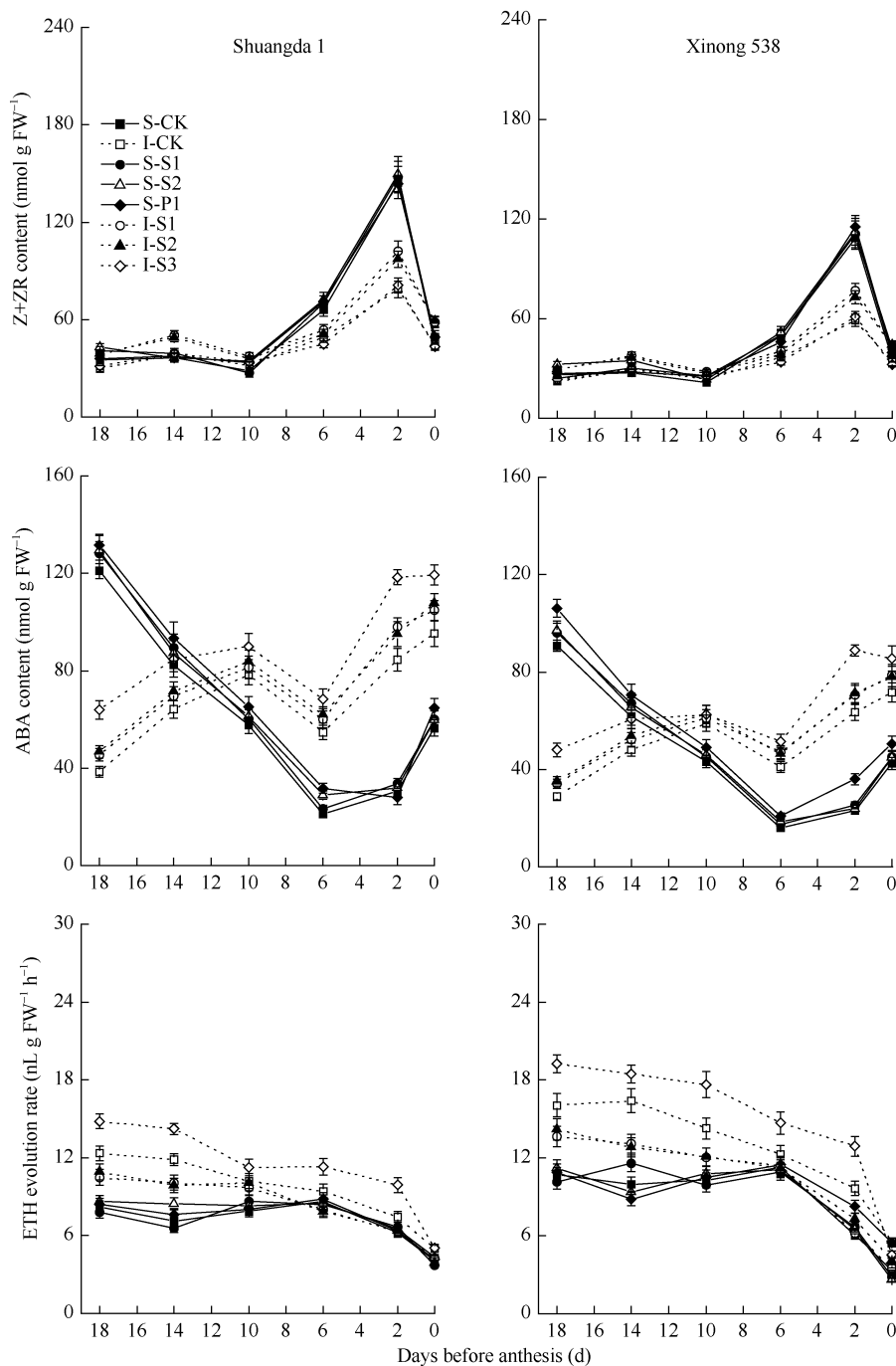


图5 外源多胺对小麦小花内源激素含量的影响(2013–2014)

Fig. 5 Effect of exogenous polyamines on endogenous hormone contents in wheat floret (2013–2014)

S: 强势小花; I: 弱势小花; S1: 施用  $1 \text{ mmol L}^{-1}$  Spd; S2: 施用  $1 \text{ mmol L}^{-1}$  Spm; P1: 施用  $2 \text{ mmol L}^{-1}$  Put; CK: 对照(清水).  
S: superior floret; I: inferior floret; S1:  $1 \text{ mmol L}^{-1}$  exogenous Spd applied; S2:  $1 \text{ mmol L}^{-1}$  exogenous Spm applied; P1:  $2 \text{ mmol L}^{-1}$  exogenous Put applied; CK: control.



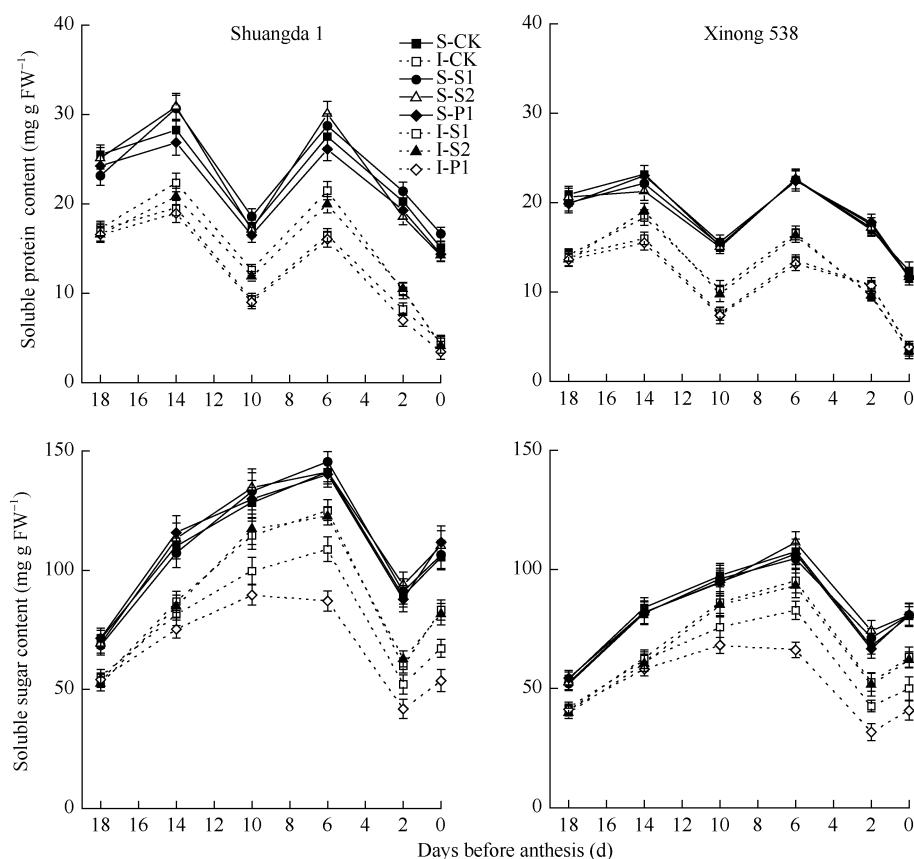


图 6 外源多胺对小麦小花可溶性总糖和蛋白含量的影响(2013–2014)

Fig. 6 Effects of exogenous polyamines on soluble sugar and protein contents in wheat floret (2013–2014)

S: 强势小花; I: 弱势小花; S1: 施用  $1 \text{ mmol L}^{-1}$  Spd; S2: 施用  $1 \text{ mmol L}^{-1}$  Spm; P1: 施用  $2 \text{ mmol L}^{-1}$  Put; CK: 对照(清水).  
S: superior floret; I: inferior floret; S1:  $1 \text{ mmol L}^{-1}$  exogenous Spd applied; S2:  $1 \text{ mmol L}^{-1}$  exogenous Spm applied; P1:  $2 \text{ mmol L}^{-1}$  exogenous Put applied; CK: control.

### 3.2 多胺对小麦小花退化的调控与激素的关系

多胺的合成前体为蛋氨酸, 与 ETH 相同, 这暗示多胺的代谢可能与 ETH 存在密切联系。外源施用 Spd 能抑制番茄或李果实中 ETH 的产生, 使用 ACC 合成酶抑制剂处理水稻植株能促进多胺合成<sup>[27]</sup>。本研究发现外源 Spd 和 Spm 显著降低了弱势小花中 ETH 释放量, 而外源 Put 显著提高了弱势小花中 ETH 释放量, 这与本课题组在小麦籽粒灌浆中的研究结果<sup>[28]</sup>类似。ETH 显著抑制禾谷类作物小花发育, 在小麦、玉米及水稻的研究中均发现 ETH 显著降低可孕花数目和穗粒数<sup>[14–16]</sup>。这表明外源 Spd 和 Spm 对小麦弱势小花退化的抑制作用可能与其对 ETH 释放量的抑制作用有关, 而外源 Put 可能通过促进小花中 ETH 释放量加剧了小麦弱势小花退化。

ABA 和 CTK 参与了对水稻、小麦等禾谷类作物小花退化的调控<sup>[2,4,14]</sup>。外源多胺可以提高内源 ABA 浓度<sup>[3]</sup>; 外源 Spd 和 Spm 可以显著提高水稻弱

势籽粒 Z+ZR 含量, 而 Spd 和 Spm 合成抑制剂 MGBG 能显著降低水稻弱势籽粒 Z+ZR 含量<sup>[22]</sup>。这表明多胺与 ABA、CTK 代谢间关系密切。本研究发现外源 Spd、Spm 和 Put 对强势小花中 ABA 和 Z+ZR 含量无显著影响, 但是 Spd 和 Spm 显著提高了弱势小花中 Z+ZR 和 ABA 含量, 而 Put 对弱势小花中 Z+ZR 含量无显著影响, 但是其显著提高了弱势小花中 ABA 含量, 且 P1 处理小花中 ABA 含量显著高于 S1 和 S2 处理。这表明, 多胺对弱势小花发育的调控可能与内源 Z+ZR 和 ABA 关系密切, 但是并不是仅与某一种激素有关。通过将 ABA 和 Z+ZR 做比值发现, 外源 Spd 和 Spm 显著提高了弱势小花 (Z+ZR)/ABA 值, 而外源 Put 则显著降低了弱势小花 (Z+ZR)/ABA 值(图 7), 这表明外源多胺对弱势小花退化的调控可能与其对 ABA、Z+ZR 之间比值的调控有关, 其通过调控 ABA、Z+ZR 的平衡关系调控小麦小花退化。

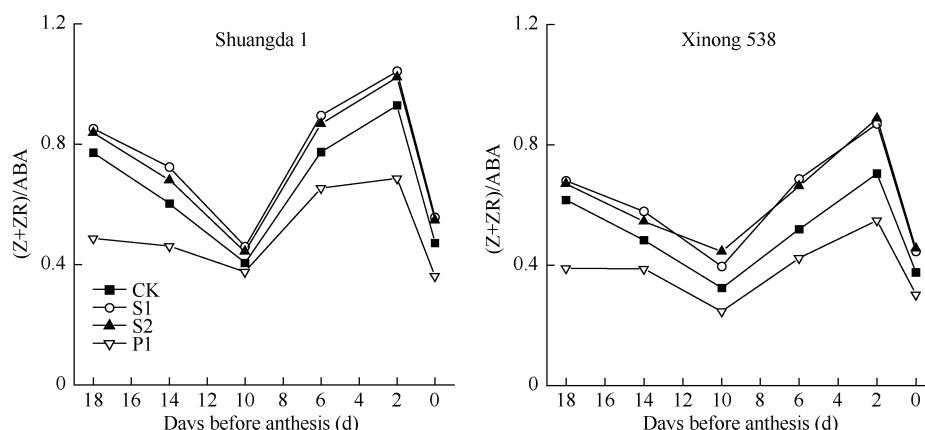


图7 外源多胺对小麦弱势小花 Z+ZR 与 ABA 比值的影响(2013–2014)

Fig. 7 Effects of exogenous polyamines on the ratio of Z+ZR to ABA in inferior floret of wheat (2013–2014)

S1: 施用 1 mmol L<sup>-1</sup> Spd; S2: 施用 1 mmol L<sup>-1</sup> Spm; P1: 施用 2 mmol L<sup>-1</sup> Put; CK: 对照(清水)。

S1: 1 mmol L<sup>-1</sup> exogenous Spd applied; S2: 1 mmol L<sup>-1</sup> exogenous Spm applied; P1: 2 mmol L<sup>-1</sup> exogenous Put applied; CK: control.

植物生长发育过程很多是受到多种激素平衡的影响,而不是受到某一种特定激素的调控<sup>[29]</sup>。本研究结果显示,多胺对小麦小花退化的调控与 ETH、ABA 和 Z+ZR 存在密切关系,可能是多胺与这些激素间的平衡调控着小麦小花的退化,这些激素或生长调节物质间的相互关系,还需要进行进一步的深入研究。

### 3.3 多胺对小麦小花退化的调控与碳氮代谢的关系

谈桂露等<sup>[30]</sup>推测内源多胺可能是通过调节籽粒蔗糖-淀粉代谢途径关键酶活性实现对籽粒灌浆的调节, Yang 等<sup>[22]</sup>报道 Spm 和 Spd 能够显著提高水稻弱势粒蔗糖合成酶等碳代谢相关酶活性从而促进弱势粒淀粉合成。本课题组研究表明,在小麦籽粒灌浆过程中,外源 Spd 和 Spm 能显著提高小麦叶绿素含量及净光合速率,影响植株碳氮代谢<sup>[31]</sup>。在本研究中,外源 Spd、Spm 和 Put 对强势小花可溶性总糖和可溶性蛋白含量均无显著影响,但是,外源 Spd 和 Spm 显著提高了弱势小花中可溶性总糖和可溶性蛋白含量,而外源 Put 显著降低了弱势小花中可溶性总糖含量。有研究认为,小麦小花退化的主要原因是对植株营养的竞争,由于开花前茎、穗之间对有限营养资源的竞争,造成穗部生长营养供应不足引起小花退化,而弱势小花正因为对营养竞争的劣势造成其大量退化<sup>[1,3]</sup>。其中,碳氮营养的供应最为重要。Serrago 等<sup>[3]</sup>发现小麦可孕小花数目与碳水化合物含量呈正相关;也有研究认为,小花的大量退化并不受碳水化合物供给总量的限制,而更多的与同化物在营养器官与穗中的分配比例有关<sup>[2]</sup>。氮肥

可以延长穗分化的时间,增加小穗小花的数目,特别是药隔期至四分体期,保证氮素营养可减少退化小花数,增加结实粒数<sup>[2]</sup>。从这些研究我们推测,外源多胺对小花退化的调控与其对小花碳氮营养的调控关系密切。外源 Spd 和 Spm 显著提高了弱势小花中碳氮含量,保证了弱势小花发育过程中有比较充足的碳氮营养,从而减少了弱势小花的退化,而外源 Put 显著降低了弱势小花中可溶性总糖含量,这会加剧小花发育过程中营养的不足,从而加剧小花退化。

多胺对小麦退化的调控与激素、植株碳氮营养均存在密切联系,且多胺与激素、多胺与植株碳氮营养、激素与植株碳氮影响之间均关系密切,表明多胺可能通过多种途径参与了对小麦小花退化的调控,其可能是小花退化的调控因子网络中的重要一环。但是关于多胺对小麦小花退化的调控机理以及各种调控途径之间的相互关系,还需进一步研究。

## 4 结论

外源多胺显著影响小麦小花退化, Spd 和 Spm 作用相似,而 Put 作用相反,其效应与小花穗位有关,对小穗上部弱势小花的影响明显,而对小穗下部强势小花无显著影响。施用外源 Spd 和 Spm 后,小麦小花退化明显被抑制,提高了可孕小花数;小花内源激素含量,如 Spd、Spm、Z+ZR,以及 Z+ZR 与 ABA 比值显著升高,同时降低了内源 ETH 释放速率;弱势小花中可溶性总糖以及可溶性蛋白质含量显著提高。Spd 和 Spm 通过提高弱势小花内源 Z+ZR 与 ABA 的比值、改善弱势小花碳氮营养促进了小麦弱



势小花发育,抑制了弱势小花的退化从而提高了可孕小花数及穗粒数。

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# 保护性耕作对西北旱区土壤微生物空间分布 及土壤理化性质的影响

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**摘要:**【目的】保护性耕作在中国西北旱区已得到了广泛应用, 是农业生产中重要的技术措施。探析保护性耕作对土壤肥力和土壤微生物群落结构的影响, 有助于农业生产的可持续发展。本试验从土壤理化性质和微生物相结合的角度, 探讨保护性耕作对土壤微生物空间结构的影响, 以及旱作麦田微生物群落丰度和土壤理化性质的相关性, 为推广保护性耕作措施提供理论依据和实践支持。【方法】以中国西北旱区土壤为研究对象, 常规耕作翻耕 (PT) 为对照, 设计深松耕 (CPT) 和免耕 (ZT) 两种保护性耕作方式, 采用实时荧光定量 PCR 技术, 测定土壤微生物群落丰度和土壤理化性质指标, 并分析微生物群落空间分布与土壤理化性质和保护性耕作之间的关系。【结果】长期应用保护性耕作已对旱作麦田的环境产生显著影响, 不同的耕作方式对土壤真菌和细菌群落丰度有不同的影响, 两者对 3 种耕作方式均有不同程度的响应; 在不同的耕作方式下, 土壤微生物空间分布不均, 连续性较差, 空间变异程度较高, 表现出强烈的空间聚集分布。耕作方式对土壤理化性质和酶活性也有显著的影响, 与传统翻耕相比, 深松耕和免耕方式能显著提高土壤黏粒、水分、全氮、铵态氮含量和脲酶、蔗糖酶活性。典范主分量分析 (CPCA) 结果表明, 土壤微生物群落丰度和理化性质变化主要受到耕作方式的影响, 并且土壤微生物群落丰度与理化性质密切相关, 在免耕方式下, 土壤黏粒、水分和铵态氮含量等显著影响土壤细菌群落分布; 在深松耕方式下, 土壤可溶性碳含量和过氧化氢酶活性等显著影响土壤真菌群落分布。【结论】旱作麦田采用保护性耕作, 可以影响土壤微生物群落丰度和空间分布, 并且显著影响土壤理化性质, 进而影响土壤微生物空间结构。同时, 土壤水分和碳氮含量分别显著影响土壤细菌和真菌丰度。

**关键词:** 保护性耕作; 土壤微生物; 空间分布; 群落丰度; 土壤理化性质; 西北旱区

## Effect of Conservation Tillage Practices on Soil Microbial Spatial Distribution and Soil Physico-Chemical Properties of the Northwest Dryland

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**Abstract:** 【Objective】Conservation tillage has been applied widely in the arid region of Northwest China, it is an important technical measure of agricultural production, exploring the effect of conservation tillage on soil fertility and soil microbial community structure and will help the sustainable development of agricultural production. This experiment from the angle of the combination of soil physico-chemical properties and microorganism to investigate the effect of conservation tillage on soil microbial spatial structure, and the relevance of abundance of microbial communities and soil physico-chemical properties of dryland wheat field, providing a theoretical basis and practical support measures for the promotion of conservation tillage. 【Method】In this

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experiment, the soil of arid region in Northwest China was used as the research object, compared to conservational plow tillage (PT), two kinds of conservation tillage methods, including chisel plow tillage (CPT) and zero tillage (ZT) were designed, the indicators of soil physico-chemical properties and the abundance of microbial communities were measured by using real-time PCR, and the relations between the spatial distribution of microbial communities and soil physico-chemical properties and conservation tillage were analyzed. **【Result】** Long-term conservation tillage had a significant effect on the environment of dryland wheat field, the abundance of soil fungi and bacterial communities had different effects with different tillage methods, they showed different levels of response to the three tillages. In different tillage methods, the spatial distribution of soil microbe was uneven, continuity was poor, higher spatial variability, and showed a strong spatial aggregated distribution. There were also significant effects of tillage method on soil physico-chemical properties and enzyme activities, compared with traditional tillage, chisel plow tillage and zero tillage could significantly improve soil clay, water, total nitrogen, ammonium nitrogen content and urease and invertase activities. The results of canonical principal component analysis (CPCA) showed that the changes of soil microbial community abundance and physico-chemical properties were mainly affected by tillage methods, and the soil microbial community abundance is closely related to physico-chemical properties, under the zero tillage mode, the content of soil clay, water and ammonium nitrogen significantly affected the distribution of soil bacterial community, and under chisel plow tillage mode, the content of soil soluble carbon and the activity of catalase significantly affected the distribution of soil fungal communities. **【Conclusion】** Using conservation tillage in dryland wheat field could affect the abundance and the spatial distribution of soil microbial communities, and significantly affected soil physico-chemical properties, and then affecting the spatial structure of soil microbes. Simultaneously, soil moisture and the contents of carbon and nitrogen were respectively significantly affected soil bacteria and fungi community abundance.

**Key words:** conservation tillage; soil microbial; spatial distribution; community abundance; soil physico-chemical properties; Northwest Dryland

## 0 引言

**【研究意义】**中国西北旱作农田面积为 7 391.98 万  $\text{hm}^2$ , 约占耕地总面积的 56.84%<sup>[1]</sup>。西北干旱地区约有 30 517 万  $\text{hm}^2$  的土壤遭到侵蚀<sup>[2]</sup>, 土壤肥力大幅度下降, 严重损害耕地土壤可持续生产能力<sup>[3]</sup>。西北地区多采用铧式犁作为耕地工具进行翻耕, 加大耕地土壤的裸露面积, 进一步加剧土壤侵蚀<sup>[4]</sup>, 土壤被翻动的频率高、程度强, 加速土壤的呼吸作用, 降低土壤有机质含量<sup>[5]</sup>, 破坏土壤结构等<sup>[6]</sup>。耕作措施对土壤的扰动, 可以影响土壤微生物的生命活动过程, 改变土壤理化性质和酶活性。保护性耕作是指在采用秸秆覆盖的基础上减少对土壤过度扰动的一种保土增肥的耕作方式<sup>[7]</sup>。相对于翻耕方式, 保护性耕作能提高土壤有机质, 增强土壤水分保持能力, 提高土壤酶活性<sup>[8]</sup>, 增加土壤微生物数量和土壤微生物多样性<sup>[9]</sup>。土壤是丰富的土壤微生物资源库, 微生物对土壤有机质降解及养分循环起到重要作用<sup>[10]</sup>, 对外界环境的变化反应敏感, 常被用作土壤健康灵敏性指标<sup>[11]</sup>。因此, 研究保护性耕作下土壤微生物群落空间结构及理化性质的变化趋势, 对中国西北旱区农作制度和农田土壤的可持续发展具有重要意义。**【前人研究进展】**旱作麦田耕作采用保护性耕作可以改善土壤理化因素, 使作物的生长发育趋于良好, 有利于农业生产可持续发展。

李玉洁等<sup>[12]</sup>发现旱作麦田耕作长期采用不合理的耕作方式会导致土壤中水分含量下降, 对土壤生物化学活动有不良影响。不同的耕作方式对土壤有机碳也有不同的影响<sup>[13]</sup>, 保护性耕作较传统耕作土壤总有机碳含量高<sup>[14]</sup>。有研究表明, 保护性耕作能够改良土壤理化性质<sup>[15]</sup>, 提高土壤的碳贮藏<sup>[16]</sup>和肥料利用能力<sup>[17]</sup>, 显著影响土壤微生物群落结构<sup>[15]</sup>, 增加土壤微生物群落的丰富度和多样性, 增强土壤微生物群落的稳定性<sup>[18]</sup>, 是平衡农业生产和土壤生态保护关系的有效措施。**【本研究切入点】**目前有关保护性耕作对土壤微生物和土壤理化性质影响的研究较多, 而本研究从两者相结合的角度出发, 探讨保护性耕作对旱作麦田微生物群落丰度和空间结构的影响。**【拟解决的关键问题】**本研究以保护性耕作土壤为研究对象, 采用网格取样法, 运用半方差函数和地统计学方法揭示土壤微生物的空间变异特征, 了解保护性耕作对农田生产的重要作用, 为西北旱区科学推广保护性耕作措施、微生物地理学的发展提供理论依据和实践支持。

## 1 材料与方法

### 1.1 试验地介绍

试验在陕西省杨凌区西北农林科技大学北校区西区试验地 (34°21'N, 108°10'E) 进行, 该区属于渭北旱塬, 暖温带季风气候, 无霜期 210 d, 年均日照



时数 2 196 h, 年均降雨量 500—600 mm, 试验区土壤属瘠土。该试验为长期定位试验, 于 2010 年进行, 试验设两种保护性耕作: 秸秆还田深松耕 (简称深松耕, CPT)、秸秆还田免耕 (简称免耕, ZT) 及常规耕作: 秸秆还田常规耕 (简称翻耕, PT), 共 3 种耕作方式处理。3 个小区, 每个小区面积 375 m<sup>2</sup> (15 m×25 m)。

## 1.2 试验材料和取样方法

供试品种为冬小麦陕麦 139。将试验地各处理划分为 40 个 2.5 m×3.75 m 网格 (图 1), 在冬小麦扬花期取土样 (2014 年 5 月), 取 0—20 cm 的耕层土壤, 取样时在每个网格内采用 5 点取样法, 采取 5 个样本, 混匀后代表该样方土样, 每个处理取 20 个样本, 共计 60 个样本。样本过 2 mm 筛, 去除土壤中的可见杂质, 混匀后置于 4℃冰盒保存, 运回实验室于 -80℃冰箱长期保存。

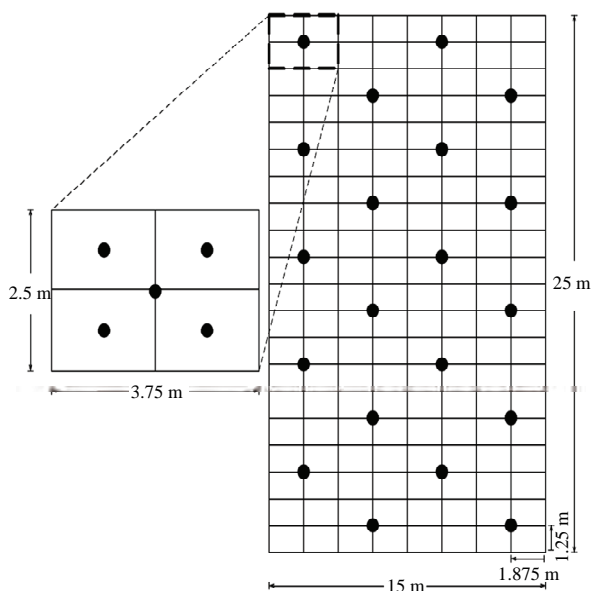


图 1 取样设置图

Fig.1 Sampling set grid

## 1.3 土壤理化性质和微生物群落测定方法

**1.3.1 土壤理化性质测定** 土壤理化性质参照 WANG 等<sup>[9]</sup>、ZHAO 等<sup>[19]</sup>和 BERTHRONG 等<sup>[20]</sup>的方法。土壤含水量用烘干法测定; 土壤粉粒、黏粒和砂粒采用激光粒度仪测定; 土壤 pH 采用电位法测定, 水土比例为 5 : 1; 土壤总有机碳含量用重铬酸钾外加热法测定; 全氮含量用半微量凯氏定氮法测定; 硝铵

态氮含量用流动分析仪测定; 可溶性碳和可溶性氮用元素分析仪测定; 蔗糖酶含量用 3-5 二硝基水杨酸比色法测定; 脲酶含量用苯酚钠比色法测定; 过氧化氢酶含量用高锰酸钾滴定法测定。

**1.3.2 土壤微生物群落丰度测定** 土壤微生物 DNA 的提取采用 FastDNA® Spin Kit for Soil 方法进行, 将提取的 DNA 样品用 Nanodrop 2000 分光光度计测浓度值, 采用实时荧光定量 PCR (Real-time PCR) 技术测定土壤微生物群落丰度。在荧光定量 PCR 反应体系中, 加入过量荧光染料, 荧光染料特异性地掺入 DNA 双链后, 发射出荧光信号, 可以根据荧光信号检测出 PCR 体系存在的双链 DNA 数量。试验以土壤微生物总 DNA 为模板, 将真菌 FR1 (5'-ANCCATTCAATCGGTANT-3') 和 FF390 (5'-CGAT AACGAACGAGACCT-3')<sup>[21]</sup>, 细菌 F515 (5'-GTGCCA GCMGCCGCGGTAA-3') 和 R806 (5'-GGACTACHVG GGTWTCTAAT-3')<sup>[20]</sup> 的特异性基因经 PCR 扩增的产物克隆到载体上, 挑选阳性克隆子培养并测定其 DNA 浓度, 根据摩尔常数计算成拷贝数, 制成标准品稀释后扩增, 计算出特异性基因片段拷贝数目和各微生物群落的丰度。

## 1.4 数据处理方法

**1.4.1 半方差函数分析** 半方差函数也称为半变异函数, 指两个观测点  $x$  和  $x+h$  的观测值  $Z(x)$  和  $Z(x+h)$  之间的变异, 用其增量平方的数学期望来表示, 计算公式如下:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [Z(x_i) - Z(x_i+h)]^2$$

式中,  $\gamma(h)$  为半方差函数;  $N(h)$  为距离  $h$  的样本对的个数;  $h$  为具有长度和方向特性的向量;  $Z(x_i)$  为在  $x_i$  的观测值;  $x_i$  为向量点。常用的半方差函数模型有基台模型、指数模型、球状模型和高斯模型等。半方差函数参数有块金值 ( $C_0$ )、基台值 ( $C_0+C$ )、变程 ( $R$ )、决定系数 ( $R^2$ ), 块金值表示区域化变量内部随机变异的大小, 基台值表示系统内的总变异的程度, 变程表示区域化变量影响范围的大小, 块金值和基台值的比值可以反映系统内变量的空间相关程度, 一般认为  $<0.25$  时, 空间变量为较弱的空间自相关, 介于  $0.25—0.75$  时, 为中等自相关,  $>0.75$  为较强的空间自相关<sup>[22]</sup>。

**1.4.2 地统计学方法分析** 采用 Kriging 最优内插值法, 在已知变量的半方差函数模型条件下, 用样点观测值对未知样点的区域化变量值进行最小误差估计, 任何一个样点的估计值通过其影响范围内的  $n$  个

样本值  $Z(x_i)$  的线性组合得到，计算公式为：

$$Z_V^* = \sum_{i=1}^n \lambda_i Z(x_i)$$

式中， $Z_V^*$  为估计值； $\lambda_i$  为加权系数，表示样本值  $Z(x_i)$  对  $Z_V^*$  的贡献<sup>[22]</sup>。

本试验的基础数据处理由 Excel 2010 和 R 语言完成，半方差函数分析和克里金插值模型由地统计学软件 GS+v9 完成。

2 结果

2.1 保护性耕作对土壤微生物群落丰度及空间分布的影响

土壤真菌丰度及空间分布如图 2 所示，免耕土壤真菌丰度范围在拷贝数  $1.73 \times 10^9$ — $1.88 \times 10^{10}$  cfu/g 干土；翻耕土壤真菌丰度范围在拷贝数  $3.29 \times 10^9$ —

$1.85 \times 10^{10}$  cfu/g 干土；深松耕土壤真菌丰度范围在拷贝数  $2.52 \times 10^9$ — $1.77 \times 10^{10}$  cfu/g 干土。通过相关关系分析 ( $\alpha=0.05$ ) 得出，3 种耕作方式下，土壤真菌丰度差异性不显著 ( $P=0.367$ )，总体服从正态分布，空间分布不均匀，空间连续性差，多呈点状空间聚集分布。免耕方式下，土壤真菌呈点状集中分布在西北和东南方向，并向中部递减，中部丰度值最低；翻耕方式下，土壤真菌多集中分布在西南和东北处，并向中部及两边递减；深松耕方式下，土壤真菌呈条带状集中分布在中央，并呈现出向南北方向递减的趋势。

细菌丰度及空间分布如图 3 所示，免耕土壤的细菌丰度范围在拷贝数  $1.46 \times 10^9$ — $7.52 \times 10^9$  cfu/g 干土；翻耕土壤的细菌丰度范围在拷贝数  $1.36 \times 10^9$ — $4.16 \times 10^9$  cfu/g 干土；深松耕土壤的细菌丰度范围在拷贝数  $2.27 \times 10^9$ — $5.89 \times 10^9$  cfu/g 干土。通过相关关

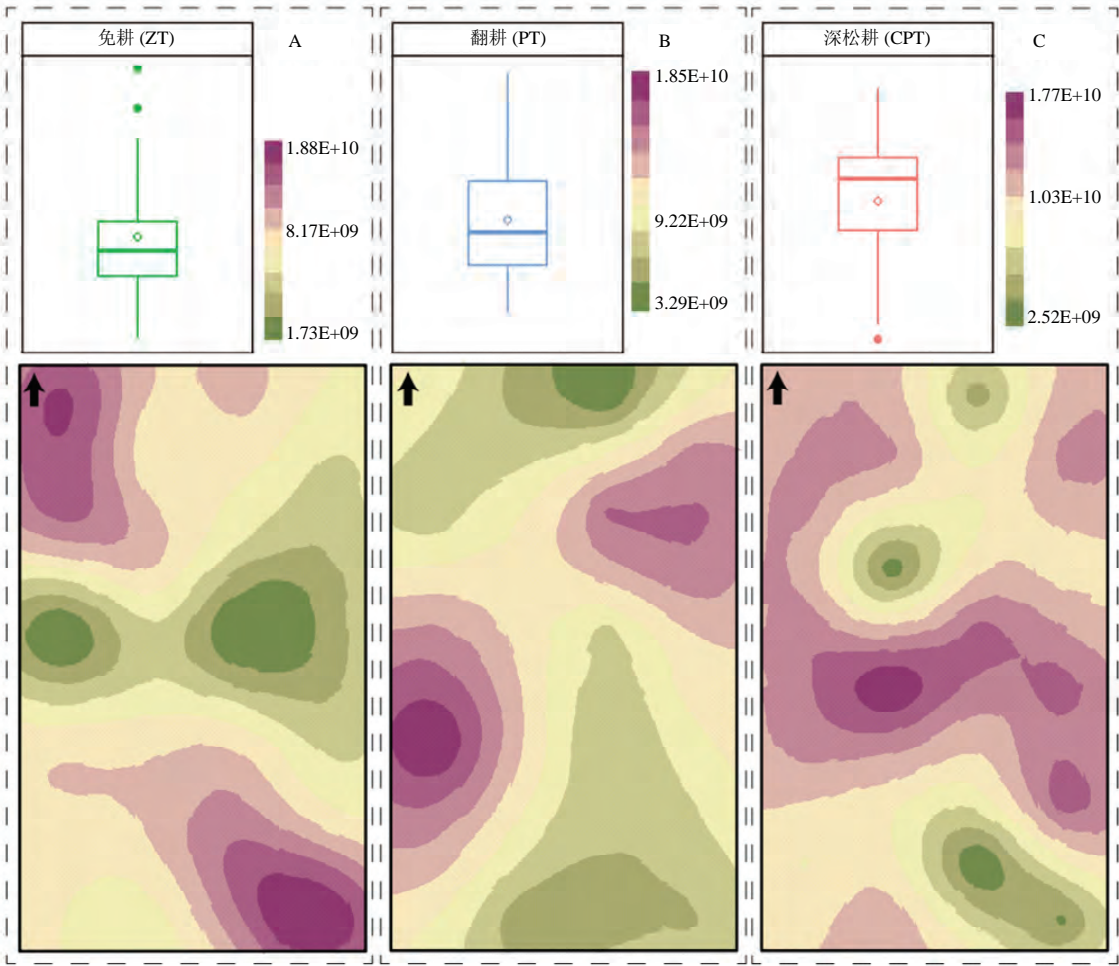


图 2 3 种耕作方式下土壤真菌群落丰度的空间插值图

Fig. 2 Kriging maps of the spatial interpolation of soil fungi community abundance with three tillages



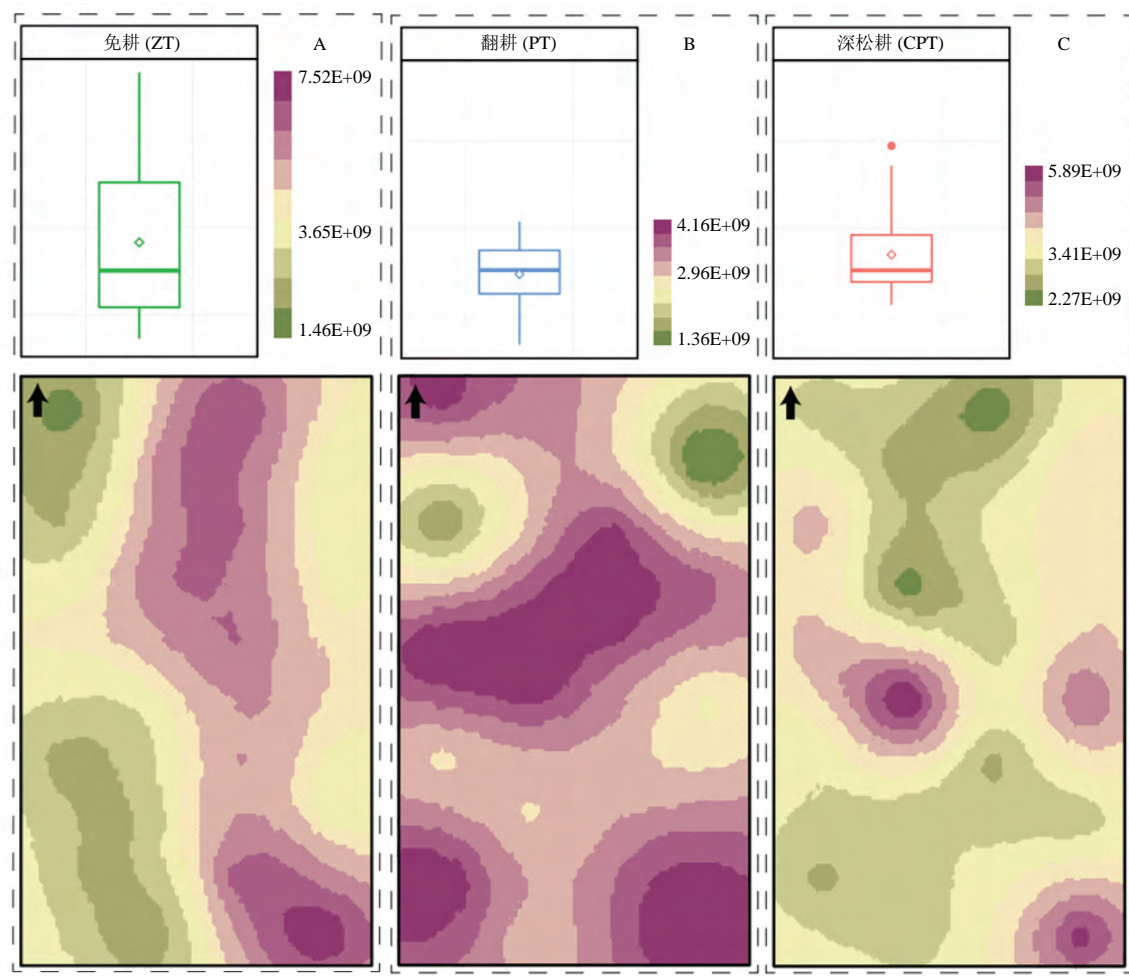


图 3 3 种耕作方式下土壤细菌群落丰度的空间插值图

Fig. 3 Kriging maps of the spatial interpolation of soil bacteria community abundance with three tillages

系分析 ( $\alpha=0.05$ ) 得出, 3 种耕作方式下, 土壤细菌丰度差异性不显著 ( $P=0.214$ ), 总体服从正态分布, 空间分布不均匀, 空间连续性差, 多呈点状空间聚集分布。免耕方式下, 土壤细菌呈现条带状集中分布在东部, 并向西部递减; 翻耕方式下, 土壤细菌多集中分布在中部, 呈现向四周递减到递增的趋势, 东北处丰度值最低; 深松耕方式下, 土壤细菌丰度较高处呈点状分布在中部和东南部, 呈现向四周递减的趋势, 北部丰度值最低。

采用克里金差值模型对土壤真菌和细菌的空间变异特征进行拟合<sup>[23]</sup> (表 1), 比较不同耕作方式下其空间变异程度。土壤真菌在免耕方式下的最佳拟合模型是高斯模型, 在翻耕和深松耕方式下是球状模型; 土壤细菌在不同耕作方式下的最佳拟合模型均是球状模型。由决定系数可以发现, 土壤真菌

和细菌均在免耕和翻耕方式下拟合效果很好, 在深松耕方式下拟合效果较差。根据块金值与基台值的比值可以看出, 土壤真菌和细菌群落分布在翻耕、免耕和深松耕下都表现出较弱的空间自相关, 说明其结构性的变异小, 随机性的变异大。

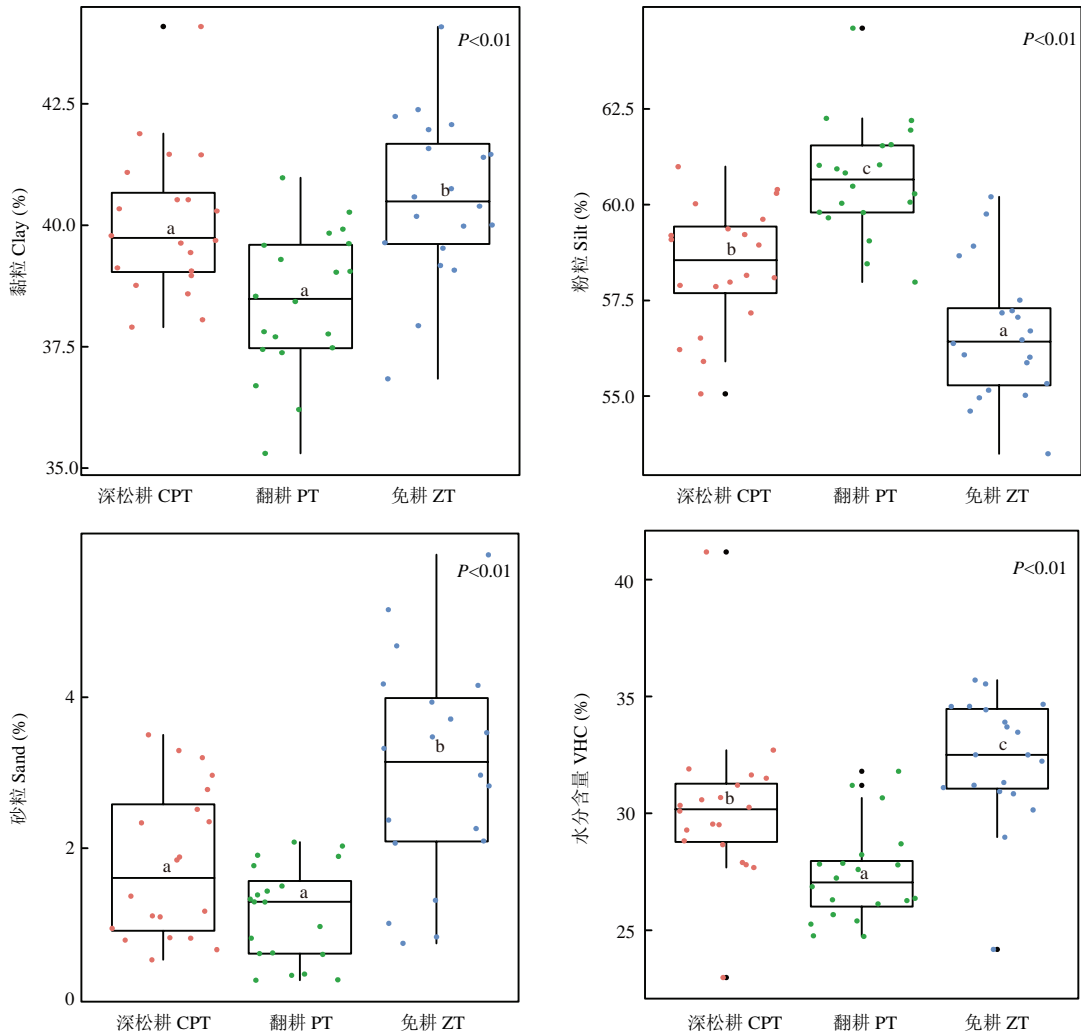
2.2 保护性耕作对土壤理化性质的影响

土壤物理性质如图 4 所示, 3 种耕作方式下, 免耕土壤中黏粒含量显著高于深松耕 (1.2%) 和翻耕 (5.5%), 深松耕和翻耕差异性不显著; 翻耕土壤中粉粒含量高于深松耕 (3.9%) 和免耕 (7.2%), 深松耕高于免耕 (3.1%), 三者之间差异性显著; 免耕土壤中砂粒含量显著高于深松耕 (77.4%) 和翻耕 (203.2%), 深松耕和翻耕差异性不显著; 免耕土壤中水分含量高于深松耕 (7.0%) 和翻耕 (18.2%), 深松耕高于翻耕 (10.5%), 三者之间差异性显著。

表 1 土壤微生物群落丰度半方差函数的模型类型及参数

Table 1 The semi-variance model type and parameters of soil microbial community abundance

耕作方式 Tillages	土壤微生物 Soil microbes	模型 Model	块金值 Nugget ( $C_0$ )	基台值 Sill ( $C+C_0$ )	变程 Range( $R$ )	块金值/基台值 $C/C_0+C_0$	决定系数 $R^2$
翻耕 PT	真菌 Fungi	球状 Spherical	4.60+E7	4.13+E8	9.85	0.113	0.999
免耕 ZT		高斯 Gaussian	1.98+E8	1.16+E9	17.74	0.170	1.000
深松耕 CPT		球状 Spherical	0.003	0.083	5.55	0.041	0.182
翻耕 PT	细菌 Bacterial	球状 Spherical	0.005	0.081	7.83	0.067	0.786
免耕 ZT		球状 Spherical	0.011	0.217	6.85	0.049	0.740
深松耕 CPT		球状 Spherical	0.005	0.074	5.27	0.063	0.204



不同小写字母表示  $P<0.01$  下差异显著。下同 Different small letters mean significantly different at  $P<0.01$ . The same as below

图 4 保护性耕作对土壤物理性质的影响

Fig. 4 Effect of conservation tillage on soil physical properties

土壤化学性质如图 5 所示，3 种耕作方式下，深松耕土壤中有有机碳含量高于翻耕（6.3%）和免耕（13.6%），翻耕高于免耕（6.8%），三者之间差异性显著；土壤可溶性碳含量差异性不显著；深松耕土壤中全氮含量高于免耕（9.2%）和翻耕（31.7%），免耕高于翻耕（20.6%），三者之间差异性显著；深



松耕土壤中可溶性氮含量最高，显著高于翻耕（18.6%）和免耕（5.3%）；深松耕土壤中硝态氮含量最高，显著高于翻耕（17.5%）和免耕（24.5%）。免耕土壤中铵态氮含量最高，显著高于深松耕（37.9%）和翻耕（71.7%）；土壤 pH 均呈弱碱性，且翻耕 pH 值高于深松耕（0.5%）和免耕（0.6%）。

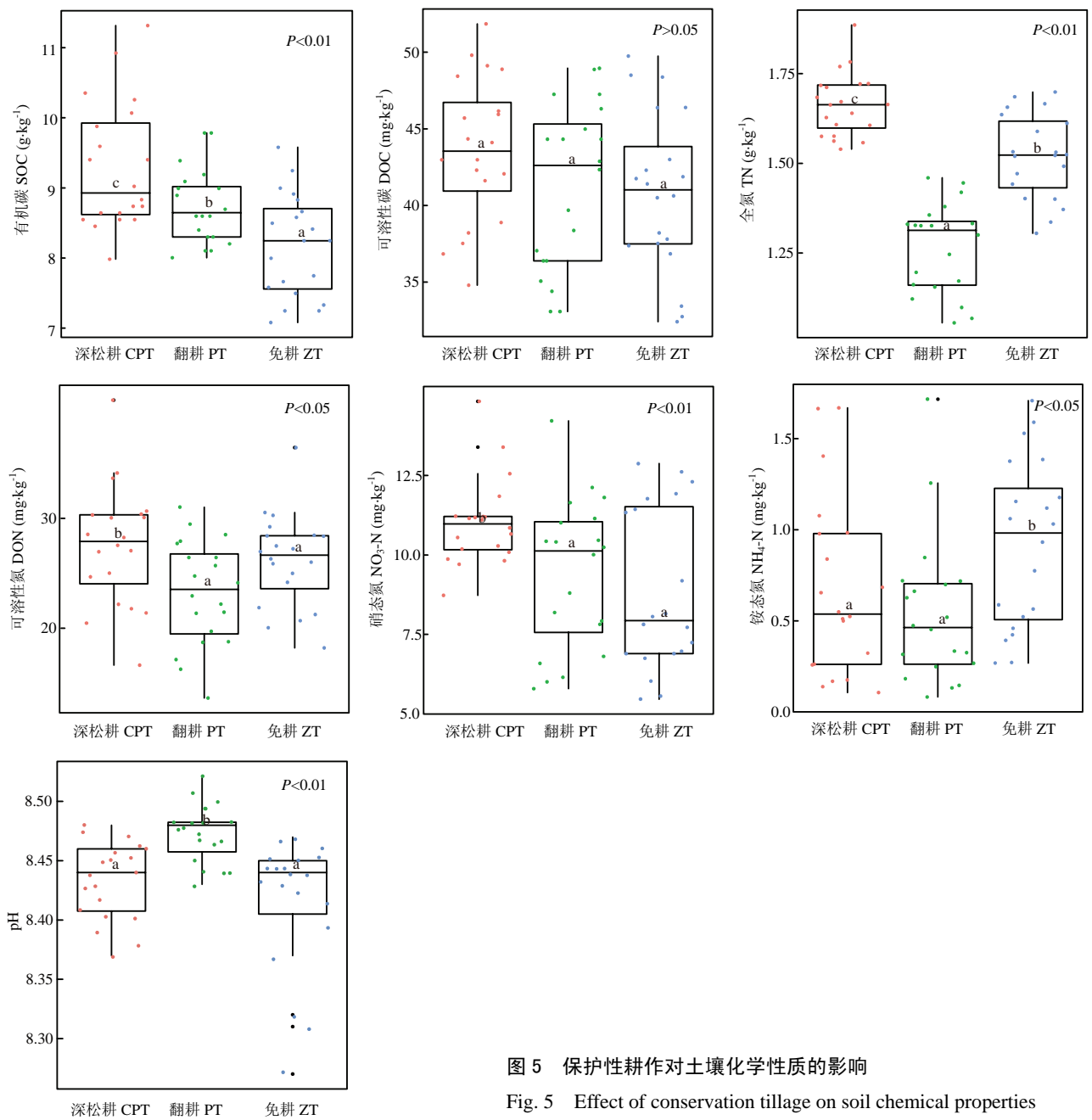


图5 保护性耕作对土壤化学性质的影响  
Fig. 5 Effect of conservation tillage on soil chemical properties

土壤酶活性如图 6 所示，3 种耕作方式下，深松耕土壤中过氧化氢酶活性最低，显著低于翻耕（1.5%）和免耕（1.1%），翻耕和免耕过氧化氢酶差异性不显著；深松耕土壤中脲酶活性高于免耕（39.4%）和翻耕（107.4%），免耕高于翻耕（48.7%），三者差异性显著。深松耕土壤中蔗糖酶活性最高，显著高于翻

耕（25.6%）与免耕（27.8%），翻耕与免耕差异性不显著。

2.3 不同耕作方式下，土壤微生物空间分布与理化性质的相关关系

在不同的耕作方式下，土壤理化因子对土壤真菌和细菌群落分布的影响不同（表 2）。由决定系数可

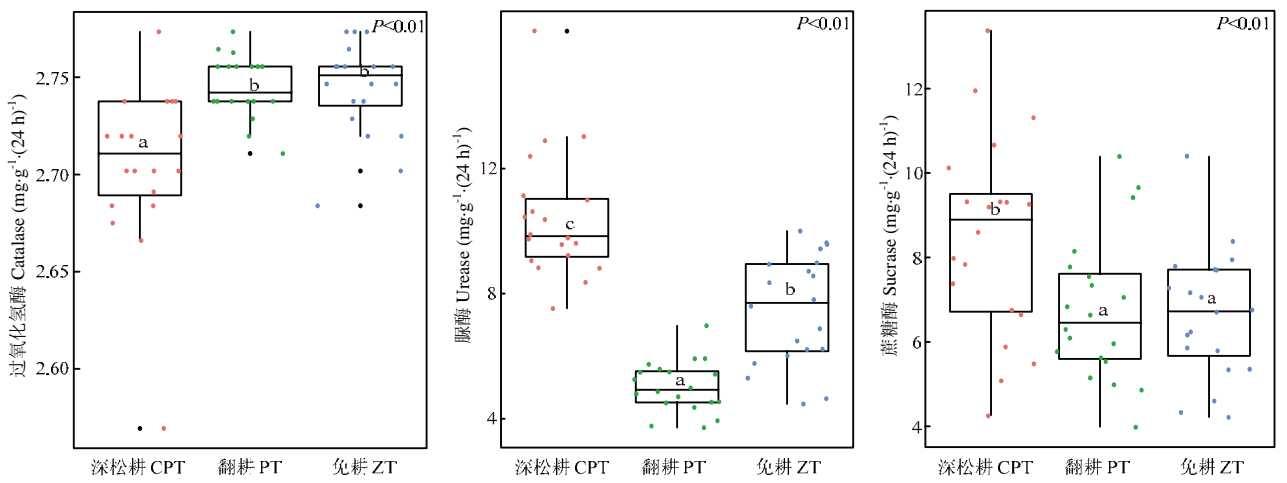


图 6 保护性耕作对土壤酶活性的影响

Fig. 6 Effect of conservation tillage on soil enzyme activities

表 2 不同耕作方式下影响细菌和真菌群落分布的理化因素

Table 2 Physico-chemical factors affecting the distribution of bacteria and fungi communities under different tillages

耕作方式 Tillage	重复 Repeat	细菌 Bacterial		真菌 Fungi	
		决定系数 $R^2$	土壤理化 Soil physico-chemical	决定系数 $R^2$	土壤理化 Soil physico-chemical
翻耕 PT	1	0.131	Clay	0.284	$\text{NH}_4\text{-N}$
	2	0.174	Clay, Sucrase	0.44	DOC, $\text{NH}_4\text{-N}$
	3	0.195	Clay, TN, Sucrase	0.41	DOC, $\text{NH}_4$ , Sucrase
免耕 ZT	1	0.318	VHC	0.249	Catalase
	2	0.32	VHC, $\text{NO}_3\text{-N}$	0.382	pH, Catalase
	3	0.368	Clay, Sucrase, Catalase	0.424	pH, DOC, Catalase
深松耕 CPT	1	0.255	$\text{NH}_4\text{-N}$	0.203	$\text{NO}_3\text{-N}$
	2	0.348	$\text{NH}_4\text{-N}$ , TN	0.264	DOC, DON
	3	0.347	$\text{NH}_4\text{-N}$ , DON, TN	0.286	DOC, $\text{NO}_3\text{-N}$ , DON

土壤黏粒 Clay; 土壤蔗糖酶 Sucrase; 土壤全氮 TN; 土壤含水量 VHC; 土壤硝态氮  $\text{NO}_3\text{-N}$ ; 土壤过氧化氢酶 Catalase; 土壤铵态氮  $\text{NH}_4\text{-N}$ ; 土壤可溶性碳 DOC; 土壤酸碱度 pH; 土壤可溶性氮 DON

以看出，影响土壤细菌群落分布的主要因子是土壤黏粒、水分和铵态氮含量等；影响土壤真菌群落分布的主要因子是可溶性碳含量和过氧化氢酶活性等。结合土壤微生物群落丰度、土壤理化性质与耕作方式之间的典范主分量分析结果（图 7）可以看出，耕作方式与 PC1 轴关系最密切，翻耕主要分布在 PC1 轴的正半轴，保护性耕作分布在负半轴，说明土壤微生物群落丰度和土壤理化性质变化主要受到耕作方式的影响。土壤物理性质、化学性质和酶活性等对排序有明显的作用，说明这些因素对土壤微生物群落丰度有显著的影响，而可溶性氮对土壤

微生物群落丰度没有显著相关性。深松耕方式下，土壤真菌丰度较高，并与土壤蔗糖酶、脲酶活性和可溶性碳、硝态氮等养分因子含量相关性显著；免耕处理下，土壤细菌丰度较高，并与土壤水分、黏粒和砂粒等含量相关性显著。

3 讨论

3.1 保护性耕作对土壤微生物空间分布的影响

耕作方式能显著影响土壤微生物群落<sup>[23]</sup>。本研究发现，土壤真菌和细菌对耕作方式的响应都极为敏感，空间分布极度不均匀，连续性差，表现出强烈的空间



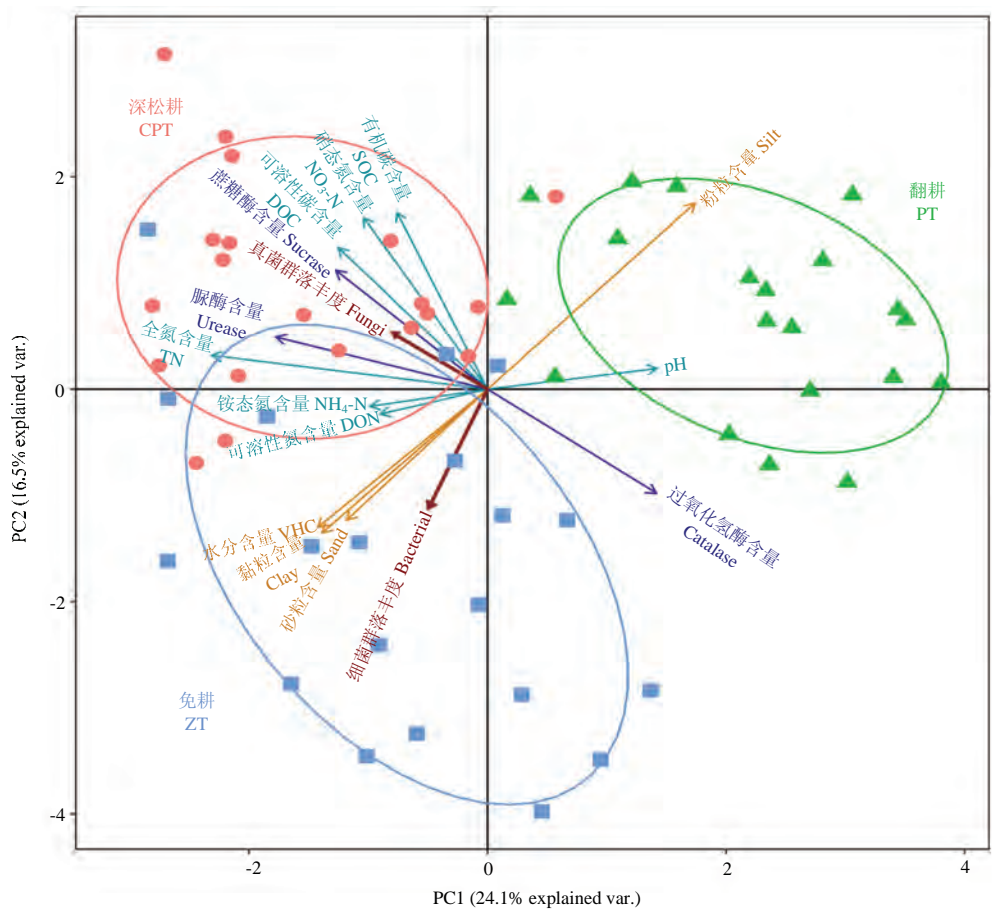


图 7 不同耕作方式下变量之间的 CPCA 排序图

Fig.7 CPCA ordination diagram of variables under different tillages

聚集分布，空间变异较高，表明耕作措施对土壤微生物群落空间分布有较大影响。土壤真菌和细菌丰度总体服从正态分布，通过半方差函数分析，在一定范围内土壤微生物存在较弱的空间自相关性，空间结构性变异小，随机性变异大<sup>[22]</sup>。与传统的翻耕方式相比，保护性耕作可以增加土壤微生物丰度，与陈蓓等<sup>[24]</sup>、沈宝明等<sup>[25]</sup>和李桂喜等<sup>[26]</sup>研究结果一致；而且保护性耕作可以改变土壤的生态过程，改善土壤质量，为微生物生存提供丰富的资源，影响土壤微生物丰度及群落结构<sup>[12]</sup>。

3.2 保护性耕作对土壤理化性质的影响

不同的耕作方式可以影响土壤理化性质。本研究表明，保护性耕作可以增加土壤水分含量，提高土壤黏粒含量，与 BAUMHARDT 等<sup>[27]</sup>和李友军等<sup>[28]</sup>研究结果一致。保护性耕作减小了对土壤扰动的程度，有利于形成良好的土壤结构，土壤孔隙连通性较好，较传统耕作有更好的储水和抗水蚀的能力<sup>[22]</sup>，且秸秆覆

盖可以减少农田土壤表面水分蒸发，有利于增加土壤水分含量。此外，保护性耕作相对于翻耕方式能显著增加土壤全氮含量，深松耕方式能提高土壤有机碳、碱解氮含量，与张洁等<sup>[29]</sup>通过对保护性耕作对坡耕地土壤微生物量碳、氮的影响研究结果一致；VARVEL 等<sup>[30]</sup>和 LI 等<sup>[13]</sup>研究认为保护性耕作有利于农田土壤积累较多的碳，提高土壤有机碳含量。

3.3 保护性耕作对土壤酶活性的影响

不同耕作方式对土壤酶活性影响不同。本研究发现，保护性耕作显著影响土壤酶活性，深松耕方式下显著增加土壤脲酶和蔗糖酶活性，与王芸等<sup>[31]</sup>、苗琳等<sup>[32]</sup>和路怡青等<sup>[33]</sup>研究结果一致；免耕方式下土壤过氧化氢酶活性有所提高，与 MADEJÓN 等<sup>[34]</sup>研究结果一致。而深松耕方式下过氧化氢酶活性较低，与张洁等<sup>[35]</sup>研究结果不一致，或许是本研究中保护性耕作时间较长，土壤过氧化氢酶活性趋于稳定。另外，土壤理化因子也显著影响土壤酶活性，土壤脲酶活性与全

氮含量呈显著正相关<sup>[36]</sup>,蔗糖酶活性与土壤有机质转化密切相关<sup>[37]</sup>。究其原因,保护性耕作改善土壤质量和结构,提高土壤微生物生物量和土壤碳氮含量,进而提高土壤酶活性<sup>[31]</sup>。

### 3.4 不同耕作方式下,土壤微生物群落丰度和理化因子的关系

不同耕作方式对土壤微生物和理化性质都有不同的影响,土壤微生物空间分布与土壤理化因子也紧密相关。本研究表明,保护性耕作显著影响土壤微生物群落丰度和理化性质,土壤水分和黏粒含量显著影响土壤细菌丰度,土壤矿质氮、过氧化氢酶和有机碳等含量与土壤真菌丰度有紧密联系,这与高玉峰等<sup>[38]</sup>和WANG等<sup>[39]</sup>的研究结果一致;WANG等<sup>[40]</sup>对真菌的研究也发现地上部生物量和碳氮比是决定真菌群落组成的重要因素。所以,土壤微生物群落丰度会受到耕作方式和土壤理化因子综合影响,并为分析微生物分布提供参考依据,便于研究理化因子变化影响微生物群落分布的机制。

## 4 结论

耕作方式对土壤微生物群落空间分布有较大影响,土壤真菌和细菌空间连续性差,表现出空间聚集分布;土壤真菌和细菌对耕作方式的响应都极为敏感。保护性耕作显著提高了土壤水分、黏粒和全氮含量,脲酶和蔗糖酶活性。土壤水分和黏粒含量显著影响土壤细菌群落丰度,土壤碳氮含量显著影响土壤真菌群落丰度。

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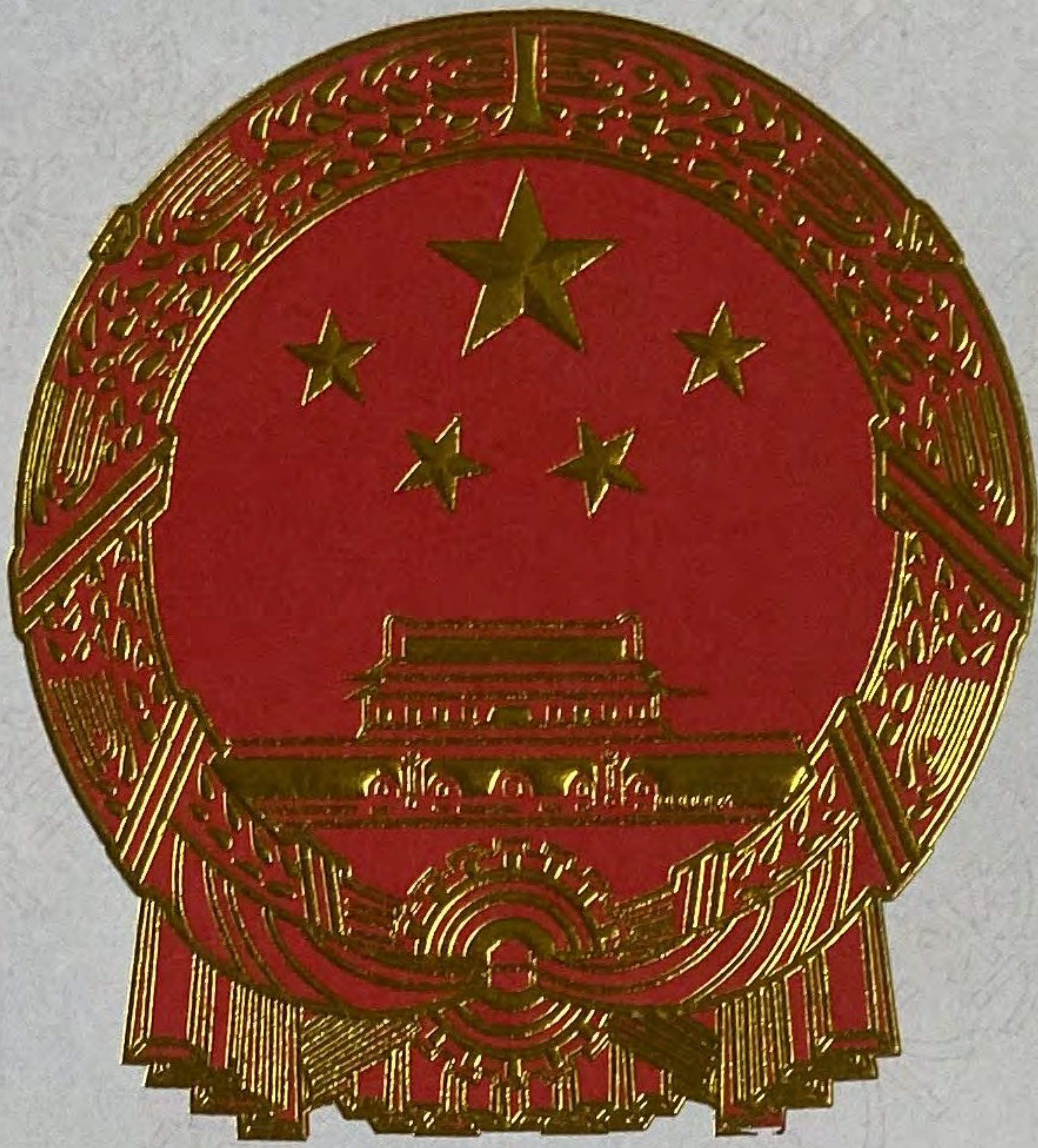


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# 陕西省科学技术奖 证书

为表彰陕西省科学技术奖获得者，  
特颁发此证书。

项目名称：陕西省农业温室气体减排技术及其应用

奖励等级：贰等

获奖者：刘杨



证书号：2017-2-004-R5



# 杨凌示范区科学技术奖

## 证书

为表彰杨凌示范区科学技术奖  
获得者，特颁发此证书。

项目名称：陕西省农业温室气体减排技术及其应用

奖励等级：一等

获奖者：刘 杨



证书号：16-1-01-R5



证书号第9552479号



## 实用新型专利证书

实用新型名称：一种旱作农田起垄覆膜播种机

发 明 人：刘杨；廖允成；吴伟；温晓霞；韩娟；李彤

专 利 号：ZL 2019 2 0166418.8

专利申请日：2019年01月30日

专 利 权 人：西北农林科技大学

地 址：712100 陕西省咸阳市杨凌邠城路3号

授权公告日：2019年11月01日

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# DB6111

## 杨凌农业高新技术产业示范区地方标准

DB 6111/T 128—2018

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### 西农 979水稻-小麦两熟区 优质高产栽培技术规程

Technical Specification for High-quality and High-yield cultivation of the Wheat  
Variety XiNong 979 in Area of Rice-wheat Rotation

2018 - 12 - 26发布

2019 - 01 - 01实施

杨凌示范区质量技术监督局      发 布





## 前 言

本规程依据 GB/T 1.1-2009 给出的规则起草。

本规程由杨凌农业标准化专业技术委员会提出并归口。

本规程起草单位：西北农林科技大学、杨凌现代农业产业标准化研究推广服务中心。

本规程主要起草人：刘 杨、郭东伟、闵东红、李学军、孙道杰、张玲丽、冯 毅、王辉、杨 静、孙亚莉。

本规程首次发布。





# 西农 979水稻-小麦两熟区优质高产栽培技术规程

## 1 范围

本规程规定了水稻-小麦两熟区西农 979小麦优质高产栽培的术语与定义、产地环境、播前准备、播种、田间管理、收获贮藏。

本规程适用于杨凌示范区西农 979小麦优质高产栽培。陕西关中麦区、黄淮南部麦区等同类生态区的杨凌示范基地可参考执行。

## 2 规范性引用文件

下列文件对于本文件的应用是必不可少的。凡是注日期的引用文件，仅所注日期的版本适用于本文件。凡是不注日期的引用文件，其最新版本（包括所有的修改单）适用于本文件。

GB 4285-1989 农药安全使用标准

GB 4404.1-2008 粮食作物种子 第 1 部分：禾谷类

GB/T 17320-2013 小麦品种品质分类

NY/T 496-2010 肥料合理使用准则 通则

NY/T 851-2004 小麦产地环境技术条件

NY/T 1276-2007 农药安全适用范围 总则

## 3 术语与定义

下列术语和定义适用于本文件。

### 3.1

优质

指小麦品质符合 GB/T 17320-2013 规定的强筋小麦要求：粗蛋白质含量（干基） 14.0%，湿面筋含量（14%水分基） 30.5%，吸水率 60%、稳定时间 10.0 min,最大拉伸阻力 Rm.E.U.（参考值） 450, 拉伸面积 100 m<sup>2</sup>。

### 3.2

高产

指目标产量为 450 kg/667m<sup>2</sup>~ 500 kg/667m<sup>2</sup>。

## 4 产地环境

产地环境选择应符合 NY/T 851-2004 小麦产地环境技术条件。



## 5 播前准备

### 5.1 基肥

轻施基肥，重施有机肥，氮、磷、钾结合（详见7.1）。

### 5.2 种子

符合GB 4404.1-2008良种指标的西农979小麦种子。

### 5.3 种子处理

#### 5.3.1 播前晒种

播种前将小麦种子以5 cm~7 cm厚度，晾晒1 d~2 d，每隔1 h~2 h翻动一次。注意不宜在水泥地、铁板、石板和沥青路面晒种。

#### 5.3.2 种子包衣与拌种

应结合当地病虫害种类，选用杀虫剂、杀菌剂的包衣剂进行种子拌种或包衣。小麦种子包衣剂种类杀虫剂和杀菌剂的使用应符合GB 4285和NY/T 1276-2006的规定。

——根腐病、纹枯病、黑穗病病区，用40%的辛硫磷100 mL或2%戊唑醇150 g，加水5 kg，拌种100 kg，闷种4 h~8 h，晾干后备播。

——全蚀病病区，在上述配方中另加12.5%的硅噻菌胺200 mL/667 m<sup>2</sup>。

——蝼蛄、蛴螬、金针虫等地下害虫多发区，可用40%辛硫磷乳油药剂拌种。

### 5.4 整地开沟

#### 5.4.1 整地

前茬作物收获后，根据农时和墒情，结合施用基肥进行耕翻整地。秸秆还田视实际情况适宜加大旋耕深度，秸秆还田量大时，基肥应增施尿素5 kg/667 m<sup>2</sup>~10 kg/667 m<sup>2</sup>。

#### 5.4.2 开沟

田间外三沟在前茬水稻收获后人工清理开挖，沟系配套，逐级加深，排水沟深100 cm以上。田内三沟（畦沟、腰沟、田边沟）于播种后机械或人工开挖，每2.5 m~3 m开挖一条竖沟，沟深别达到0.2 m~0.25 m~0.35 m左右。距田块两端横埂2 m~3 m，各挖一条横沟，沟深30 cm~40 cm，田块超过100 m的应加挖腰沟，沟深30 cm~40 cm，内外沟配套相同。

## 6 播种

### 6.1 播期与播量

播种期以10月15~25日为宜，播量12 kg/667 m<sup>2</sup>~15 kg/667 m<sup>2</sup>，基本苗宜14万株/667 m<sup>2</sup>~16万株/667 m<sup>2</sup>。抢茬早播，适迟播后每推迟1 d，播量增加0.25 kg/667 m<sup>2</sup>。

### 6.2 播种方式

免耕机条播，宽幅播种，一次完成旋耕、开沟、播种、覆土、镇压等工序。行距25 cm，播深3 cm~5 cm，播深适宜、深浅一致，出苗均匀、苗量合理。

出苗后要进行查缺补漏，可浸种催芽补苗。

## 7 田间管理

### 7.1 施肥

#### 7.1.1 基肥

施有机肥 1000 kg/667m<sup>2</sup>，纯氮 (N) 14 kg/667m<sup>2</sup>~ 16 kg/667m<sup>2</sup>，P<sub>2</sub>O<sub>5</sub> 6 kg/667m<sup>2</sup>~ 8 kg/667m<sup>2</sup>，K<sub>2</sub>O 8 kg/667m<sup>2</sup>~ 10 kg/667m<sup>2</sup>。N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O = 5: 3: 3。氮肥施用以基肥: 壮蘖肥: 拔节肥: 孕穗肥 = 5: 1: 2: 2 为宜 (纯氮)，结合整地底肥施用尿素 15-17kg/667m<sup>2</sup>，P<sub>2</sub>O<sub>5</sub> 6 kg/667m<sup>2</sup>~ 8 kg/667m<sup>2</sup>，K<sub>2</sub>O 8 kg/667m<sup>2</sup>~ 10 kg/667m<sup>2</sup>。

#### 7.1.2 壮蘖肥

越冬初期宜施尿素 3.0 kg/667m<sup>2</sup>~ 3.5 kg/667m<sup>2</sup>。

#### 7.1.3 拔节肥

小麦基部第一节间接近定长，宜施尿素 6.0 kg/667m<sup>2</sup>~ 7.0 kg/667m<sup>2</sup>、复合肥 7 kg/667m<sup>2</sup>~ 10 kg/667m<sup>2</sup>。

#### 7.1.4 孕穗肥

小麦叶龄余数为 0.5 时，施尿素 6 kg/667m<sup>2</sup>~ 7 kg/667m<sup>2</sup>。

### 7.2 灌水

#### 7.2.1 齐苗水

播后 1 d~ 2 d，耕层土壤相对含水量低于 60 % 应补灌齐苗水。

#### 7.2.2 越冬水

底墒不足 (耕层土壤相对含水量 50 %) 应及时冬灌，浇水后及时划锄，增温保墒。

#### 7.2.3 拔节孕穗水

结合拔节孕穗肥施用，在 3 月下旬，灌水 40 m<sup>3</sup>/667m<sup>2</sup>~ 50 m<sup>3</sup>/667m<sup>2</sup>。

#### 7.2.4 排水

冬春注意清沟理墒，保持沟系畅通，并及时培土。遇苗情干旱时及时镇压。

### 7.3 病虫草害防治

#### 7.3.1 防倒

为建立合理群体，防止倒伏。群体一般控制在 42 万穗 /667m<sup>2</sup>~ 45 万穗 /667m<sup>2</sup>。

#### 7.3.2 化学除草



根据草相、草龄、墒情等选择药剂，重点做好冬前除草。在播后苗前或者杂草 1~ 2 叶时用异丙隆 25 %可湿性粉剂 250 g/667m<sup>2</sup>~ 300 g/667m<sup>2</sup>喷雾。2 月中旬约返青期时，宜用精恶唑禾草灵 60ml /667m<sup>2</sup>~ 100 ml /667m<sup>2</sup>加 75 %苯磺隆 1.5 g, 兑水 50 kg 均匀喷雾。防治方法及使用药剂参见附录 A.2

### 7.3.3 地下害虫

播种前药剂拌种(详见 5.3.2)，土壤处理采用毒饵或毒土法，用炒香麦麸、豆饼、米糠等饵料 2 kg, 50%辛硫磷乳油 25 ml, 加适量水稀释农药制作毒饵，傍晚撒于田间幼苗根际附近，每隔一定距离一小堆，15 kg/667m<sup>2</sup>~ 20 kg, 或用 50 %辛硫磷 200ml /667m<sup>2</sup>拌细土 30 kg~ 40 kg, 耕翻时撒施；喷雾法，用 50 %辛硫磷乳油 250ml 稀释 1500 倍，顺麦垄喷施；或用 48 %毒死蜱乳油 100ml 稀释 1500 倍，顺麦垄喷施，喷药液 40 kg/667m<sup>2</sup>。防治方法及使用药剂参见附录 A.1

### 7.3.4 纹枯病

在小麦返青拔节时，当病株率达 20 %时，可用 5 %井冈霉素水剂 300 ml /667m<sup>2</sup>~ 400 ml /667m<sup>2</sup>，兑水 100 kg, 均匀喷雾。防治方法及使用药剂参见附录 A.3

### 7.3.5 白粉病、锈病

早春发病时用 15 %的三唑酮（粉锈宁）可湿性粉剂 20 g/667m<sup>2</sup>~ 30 g/667m<sup>2</sup>，兑水 50 kg 喷雾防治。防治方法及使用药剂参见附录 A.3

### 7.3.6 赤霉病

在扬花期宜施 50 %的多菌灵可湿性粉剂 100 g/667m<sup>2</sup>兑水 50 kg 均匀喷雾。防治方法及使用药剂参见附录 A.3

### 7.3.7 蚜虫

小麦扬花至灌浆初期，用吡虫啉可湿性粉剂 30 g/667m<sup>2</sup>，或者 50%抗蚜威粉剂 10 g/667m<sup>2</sup>~ 15 g/667m<sup>2</sup>兑水 50 kg 均匀喷雾。防治方法及使用药剂参见附录 A.3

结合病虫害防治进行叶面喷施磷酸二氢钾（100 kg/667m<sup>2</sup>）、尿素（浓度 1 %~ 1.5 %）或者过磷酸钙浸出液等以提高千粒重，预防早衰。

## 8 收获贮藏

小麦蜡熟末期及时收割，避开烂场雨，防治穗发芽。脱粒后及时晾晒 3~ 4 个晴天，使得籽粒含水量降至 13 %以下，贮藏于通风干燥处。

附 录 A  
(资料性附录)  
西农 979小麦病虫害防治方法

### A.1 小麦种衣剂种类及防治对象

小麦种衣剂种类及防治对象参见表 A.1

表 A.1 小麦种衣剂种类及防治对象

单位：mL/10 kg种子

药剂种类	防治对象	用 量
12.5 %硅噻菌胺	全蚀病	20
2.5 %咯菌腈	纹枯病、黑穗病、根腐病	20
15 %粉锈宁 50 g/667m <sup>2</sup> ~ 60 g/667m <sup>2</sup>	白粉病、锈病	4
40 %毒死蜱乳油	地下害虫、蚜虫	10
50 %辟硫磷	地下害虫	20

### A.2 小麦化学除草剂种类及防治对象

小麦化学除草剂种类及防治对象参见表 A.2

表 A.2 小麦化学除草剂种类及防治对象

单位：株 /m<sup>2</sup>

药剂种类	防治对象	防治时期	防治指标	喷施部位
唑草酮、苯磺隆、氯氟吡氧乙酸、 苄嘧磺隆、噻吩磺隆	播娘蒿、荠菜、猪殃殃、 野油菜和繁缕	秋苗期、返青期	30	叶面喷施
精恶唑禾草灵、炔草酸(炔草酸酯、 炔草酯)、甲基二磺隆	野燕麦、硬草、看麦娘	秋苗期、返青期	30	
氟唑磺隆、甲氧磺草胺、阔世玛， 或者精恶唑禾草灵与苯磺隆混用、炔草 酸与苯磺隆混用	禾本科杂草和阔叶杂草混 生	秋苗期、返青期	30	

### A.3 小麦病虫害防治常用药剂及防治对象

小麦病虫害防治常用药剂及防治对象参见表 A.3

表 A.3 小麦病虫害防治常用药剂及防治对象

药剂种类	防治对象	防治时期	防治指标
12.5 %烯唑醇可湿性粉剂、50 %多菌 灵可湿性粉剂	纹枯病、茎基 腐病	返青拔节期	病茎率 15 %



表 A.3 小麦病虫害防治常用药剂及防治对象 (续)

药剂种类	防治对象	防治时期	防治指标
15%三唑酮可湿性粉剂、烯唑醇(禾果利)可湿性粉剂、12.5%烯唑醇可湿性粉剂、25%环丙唑	锈病、白粉病、叶枯病	返青拔节期	病叶率 5%~10%或病情指数 15以上
15%哒螨灵乳油、1.8%阿维菌素	红蜘蛛		虫口密度达单行长虫量 600头/m
50%抗蚜威可湿性粉剂	蚜虫		虫口密度达到每株 5头以上
50%多菌灵可湿性粉剂、40%多菌灵胶悬剂、25%氰烯菌酯悬浮剂	赤霉病	抽穗至成熟期	遇多雨、连阴雨天气,小麦齐穗期喷药预防
10%吡虫啉可湿性粉剂、50%抗蚜威可湿性粉剂	粘虫、蚜虫 麦叶蜂		苗蚜百株 100头、穗蚜百茎 500头
虫螨克乳油、甲氰菊酯乳油、马拉硫磷乳油、阿维菌素乳油	麦蜘蛛		虫口密度达到单行长虫量 600头/m
吡虫啉可湿性粉剂、顺式氯氰菊酯、高效氯氰菊酯乳油	吸浆虫		每方(10m×10m×20cm) 5头,10网复次 10头以上成虫