



# 第六届全国玉米生物学学术研讨会

**The 6<sup>th</sup> Maize Biology Conference of China**

**June 23-26, 2023**

**Harbin • China**

## ***Program & Abstracts***

**主 办**

中国作物学会

中国作物学会玉米专业委员会

**承 办**

东北农业大学

寒地粮食作物种质创新与生理生态教育部重点实验室

**协 办**

北大荒垦丰种业股份有限公司

**Endorsed by**

The Crop Science Society of China

The Maize Committee of Crop Science Society of China

**Organized by**

Northeast Agricultural University

Key Laboratory of Germplasm Enhancement, Physiology and Ecology of Food Crops in Cold  
Region of Ministry of Education

**Co-organized by**

Beidahuang Kenfeng Seed Co., Ltd



## 会务信息

欢迎您来到哈尔滨参加第六届全国玉米生物学学术研讨会。本次会议于 2023 年 6 月 23 日-26 日在黑龙江省哈尔滨华旗饭店召开。为了保证会议的顺利进行，请认真阅读以下信息：

### ◆ 报到注册

报到注册时间为 6 月 23 日 8:00-22:00，注册地点位于哈尔滨华旗饭店国际会议中心 1 楼大厅。

### ◆ 报告

所有会议报告均在国际会议中心 101 环球剧场进行。会议日程和摘要详见内页。

### ◆ 会议墙报

墙报环节在 101 环球剧场廊厅进行，欢迎与会代表积极参与交流讨论。6 月 23 日 14:00 开始张贴墙报。

### ◆ 住宿

本次会议住宿地点为黑龙江省哈尔滨华旗饭店（哈尔滨市南岗区红旗大街 301 号）。

### ◆ 餐饮

华旗饭店 5 楼花园西餐厅为本次会议提供午餐、晚餐，请凭餐券按时用餐。早餐由入住酒店提供。在墙报交流期间，会务组提供茶点和饮料。

### ◆ 参展须知

#### 一、证件

参展商必须佩戴组委会制发的“参展证”。严禁将参展证件转借他人，违者一经查出，取消参展资格。

#### 二、知识产权

参展商必须保证自己的展品、包装和其他宣传材料等，不损害第三方的利益，包括知识产权（商标、版权、设计、名字、专利权）等，参展期间如被投诉，此间由于侵犯第三方知识产权而造成的一切损失，应由被投诉的参展商负责承担。

#### 三、参展不得出售产品

参展期间一律不得出售产品；展会结束后，如已与参会买家达成意向，可自行对接。

#### 四、资料的散发

参展商可在展台内散发资料，但不能在通道或展厅其他公共部位散发资料。

## 五、其他注意事项

1、进展及撤展符合酒店管理规定，大型展示器具请配合酒店走进货通道，撤展时请将展示内容撤走，保持酒店干净整洁。

2、请勿在人行通道、出入口、消防设施、非展位内摆、挂、贴和钉展览样品宣传品或其他标志；禁制在酒店柱面、墙面、地面等部位粘贴书画和不易清除物（包括双面胶）。

3、展位内展板画面请用可移除材质进行粘贴及展示，请勿直接粘贴（建议美纹纸上附泡沫胶打点粘贴/KT板夹子固定）。

4、参展人员严禁携带易燃、易爆、有毒危险品进入展区。严禁在参展区内吸烟，吸烟请到指定的区域或者场外。

5、参展商在展期内注意保管展位物品和随身携带物品的安全，谨防遗失。

## ◆ 温馨提示

一、请与会领导和代表佩戴参会证，按照会议日程安排准时参加。

二、为保证会议效果，在会议进行时请您将手机置于静音、振动或关闭状态。

三、大会报告具体会议地点，请留意会议日程安排，报告人请遵守报告时间。

四、会议期间出行，请您小心慢行，妥善保管私人物品；并随身携带房卡及酒店名片，以便司机送您。

五、酒店退房时间 13:00 前，14:00-18:00 之间退房加收半日房费，18:00 后退房收取整日房费。

六、会议期间，在饮食、住宿等方面如有特殊要求，请及时与会务组联系，我们将全力做好服务工作。

## ◆ 联系信息

如有任何疑问或需要帮助，请联系华旗饭店的大会工作人员。



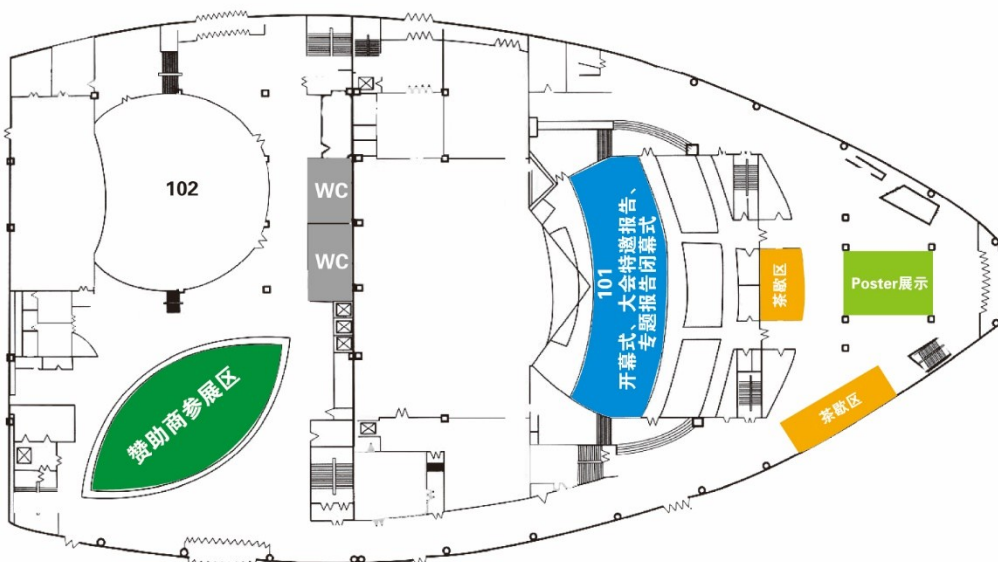
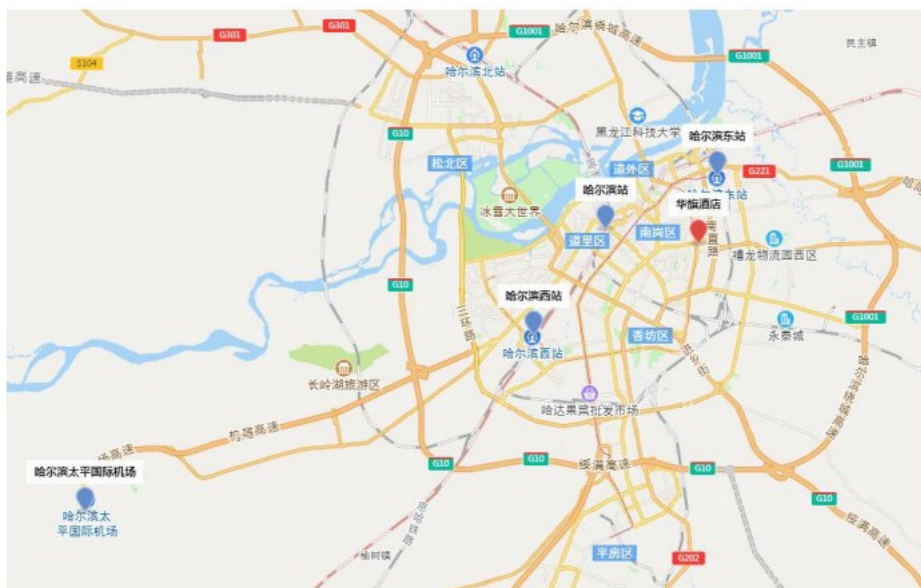
## 会议地址和地图

### (Hotel Information and Maps)

**会议地址：**黑龙江省哈尔滨华旗饭店（哈尔滨市南岗区红旗大街 301 号）

Address: Huaqi Hotel of Harbin in Heilongjiang (No. 301, Hongqi Street, Nangang District, Harbin)

电 话 (Tel):0451-81868888



国际会议中心一楼平面图

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# 第六届全国玉米生物学学术研讨会 组委会名单 (Organizing Committee)

**主办单位 (Sponsoring Organization):** 中国作物学会

中国作物学会玉米专业委员会

**承办单位 (Sponsoring Units):** 东北农业大学

寒地粮食作物种质创新与生理生态教育部重点实验室

**协办单位 (Co-sponsoring Units):** 北大荒垦丰种业股份有限公司

**组委会主席 (Chair):**

卢艳丽 (四川农业大学, SICAU; yanli.lu82@hotmail.com)

**组委会秘书长 (Secretary-general):**

王振华 (东北农业大学, NEAU; zhenhuawang\_2006@163.com)

**组委会委员 (Members):**

陈化榜 (中国科学院遗传与发育生物学研究所, IGDB·CAS;  
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**执行委员会主席 (Chair):**

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**执行委员会秘书长 (Secretary-general):**

王振华 (东北农业大学, NEAU; zhenhuawang\_2006@163.com)

**执行委员会委员 (Members):**

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(按姓氏首字母排序)

**地方组委会 (Local Organizing Committee):**

王振华、邸宏、张林、周羽、董玲、曾兴、刘显君、顾万荣、李晶、王玉波、李昊、刘金磊

**摘要编辑 (Abstract Editors):**

周羽，徐楚臻，卢晴，张嘉月，宋祎莹，贾悦

**大会秘书处 (Secretariat of the Conference):**

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注册签到负责人:	邸 宏	13945179332
	董 玲	15645199257
交通接待负责人:	曾 兴	15245089539
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媒体宣传负责人:	吕志超	18946116438

# 第六届全国玉米生物学学术研讨会日程表

## The 6<sup>th</sup> Maize Biology Conference of China

### Schedule of Events (June 23-26)

日期	时间	会议安排	地点
6.23	08:00-22:00	会议报到注册	国际会议中心 1 楼大厅
	17:30-19:30	自助晚餐	5 楼花园西餐厅
	20:00-21:30	特邀报告	101 环球剧场
	14:00-22:00	墙报张贴	101 环球剧场廊厅
6.24	08:30-08:45	开幕式、校长致辞	101 环球剧场
	08:45-09:30	特邀报告	101 环球剧场
	09:30-10:00	茶歇、合影留念	廊厅茶歇、室外合影
	10:00-10:45	特邀报告	101 环球剧场
	10:45-12:00	专题报告 1: 复杂农艺性状遗传与发育 I	101 环球剧场
	12:00-13:30	自助午餐	按餐券位置就餐
	14:00-15:25	专题报告 2: 基因组与表观遗传 I	101 环球剧场
	15:25-15:45	茶歇	101 环球剧场廊厅
	15:45-16:30	特邀报告	101 环球剧场
	16:30-17:10	专题报告 3: 复杂农艺性状遗传与发育 II	101 环球剧场
	17:10-21:30	墙报自由交流时间	101 环球剧场廊厅
	18:00-19:30	自助晚餐	5 楼花园西餐厅
	19:30-20:30	企业推介	101 环球剧场
	20:30-21:00	期刊推介	101 环球剧场
	20:00-22:00	组委会会议	国际会议中心 504 会议室
6.25	08:30-09:15	特邀报告	101 环球剧场
	09:15-10:10	专题报告 4: 基因组与表观遗传 II	101 环球剧场
	10:10-10:30	茶歇	101 环球剧场廊厅
	10:30-12:05	专题报告 5: 分子育种理论与方法技术	101 环球剧场
	12:05-13:30	自助午餐	按餐券位置就餐
	14:00-14:45	特邀报告	101 环球剧场
	14:45-15:55	专题报告 6: 应答生物与非生物胁迫 I	101 环球剧场
	15:55-16:15	茶歇	101 环球剧场廊厅
	16:15-16:55	专题报告 7: 应答生物与非生物胁迫 II	101 环球剧场
	16:55-17:30	颁奖及闭幕式	101 环球剧场
	17:30-19:00	自助晚餐	5 楼花园西餐厅
6.26	08:30-12:00	离会	

# 报告日程

## Conference Schedule

2023 年 6 月 23 日 星期五	
20:00-21:30 特邀报告	主持人：巫永睿 (中国科学院分子植物科学卓越创新中心)
20:00-20:45 PT1 韩斌 (中国科学院分子植物科学卓越创新中心)	水稻杂种优势遗传基础的量化和预测分析
20:45-21:30 PT2 李新海 (中国农业科学院生物技术研究所)	玉米种质改良的基础研究
14:00-22:00 墙报张贴	
2023 年 6 月 24 日 星期六	
08:30-08:45 开幕式、校长致辞	主持人：卢艳丽 (四川农业大学) 东北农业大学校长致开幕词
08:45-09:30 特邀报告	
08:45-09:30 PT3 David Jackson (Cold Spring Harbor Laboratory/ Huazhong Agricultural University)	Maize stem cells in ear development-Single cell analysis and heat stress responses
09:30-10:00 茶歇、合影留念	
10:00-12:00 特邀报告/专题报告 1: 复杂农艺性状遗传与发育 I	主持人：邸宏 (东北农业大学)
10:00-10:45 PT4 赖锦盛 (中国农业大学)	玉米基因组学研究 with 未来育种方向
10:45-11:05 IT1 苟明月 (河南农业大学)	The copine protein controls plant growth via regulating brassinosteroid signaling in maize
11:05-11:25 IT2 李刚 (山东农业大学)	玉米茎叶夹角形态建成的分子机理解析
11:25-11:45 IT3 王勇 (山东大学)	玉米中结构特殊的 PPR 蛋白参与细胞器 RNA 编辑机制的研究
11:45-12:00 ST1 郑智刚 (华南农业大学)	Local auxin biosynthesis regulates brace root angle and lodging resistance in maize
14:00-15:25 专题报告 2: 基因组与表观遗传 I	主持人：李林 (华中农业大学)
14:00-14:20 IT4 秦峰 (中国农业大学)	Genetic dissection and gene cloning from a prominent drought-resistant maize germplasm CIMBL55
14:20-14:40 IT5 周绍群 (中国农业科学院深圳农业基因组研究所)	基于多组学分析的玉米生化防御功能基因研究
14:40-14:55 ST2 陈川 (西北农林科技大学)	A leucine rich repeat receptor kinase gene confers quantitative susceptibility to maize southern leaf blight
14:55-15:10 ST3 徐庆御	Excavation of loci related to low temperature

(东北农业大学)	tolerance in maize germination and identification of key genes
15:10-15:25 <b>ST4</b> 康妍 (四川农业大学)	<i>ZmGII</i> 调控玉米开花的机制探究
<b>15:25-15:45 茶歇</b>	
<b>15:45-17:10 特邀报告/专题报告 3:</b> <b>复杂农艺性状遗传与发育 II</b>	<b>主持人: 田丰 (中国农业大学)</b>
15:45-16:30 <b>PT5</b> 张宪省 (山东农业大学)	植物干细胞与遗传转化
16:30-16:50 <b>IT6</b> 李林 (华中农业大学)	基于生物大数据的玉米重要农艺性状解析与智能育种初探
16:50-17:10 <b>IT7</b> 马泽阳 (中国农业大学)	玉米分化期胚乳的单细胞转录图谱和基因调控网络
<b>17:10-21:30 墙报自由交流时间</b>	
<b>19:30-20:30 企业推介</b>	<b>主持人: 顾万荣 (东北农业大学)</b>
19:30-19:45 何弦 (南京诺唯赞生物科技股份有限公司)	Vazyme 表观遗传产品助力植物深度研究
19:45-20:00 王楠楠 (赛默飞世尔科技(中国)有限公司)	多维度农业基因组学解决方案—助力种业创新
20:00-20:10 吴笑女 (石家庄博瑞迪生物技术有限公司)	靶向测序基因型分型技术在玉米育种中的应用
20:10-20:30 孙树民 (北大荒垦丰种业股份有限公司)	使命担当北大荒 佑护中华大粮仓
<b>20:30-21:00 期刊推介</b>	<b>主持人: 李林 (华中农业大学)</b>
20:30-20:45 王文佳 (Molecular Plant+Plant Communications)	Publishing with Molecular Plant & Plant Communications for Advancing Global Plant Sciences
20:45-21:00 余文静 (Genome Biology)	Publishing with Genome Biology
<b>20:00-22:00 组委会会议</b>	<b>主持人: 卢艳丽 (四川农业大学)</b>
<b>2023 年 6 月 25 日 星期日</b>	
<b>8:30-10:10 特邀报告/专题报告 4:</b> <b>基因组与表观遗传 II</b>	<b>主持人: 王海洋 (华南农业大学)</b>
8:30-9:15 <b>PT6</b> 严建兵 (华中农业大学)	玉米基因组育种的理论与实践
9:15-9:35 <b>IT8</b> 赵天永 (西北农林科技大学)	耐旱耐热转基因玉米品种研发进展
9:35-9:55 <b>IT9</b> 龙金成 (中国科学院分子植物科学卓越创新中心)	The nucleosome remodeler DDM1 shapes R-loop formation by interacting with DDX23 helicase in maize
9:55-10:10 <b>ST5</b> 李朝霞 (青岛农业大学)	SNAC1 亚家族基因通过调控 SGs 的水平提高玉米耐盐性
<b>10:10-10:30 茶歇</b>	



<b>10:30-12:05 专题报告 5: 分子育种理论与方法技术</b>	<b>主持人: 高世斌 (四川农业大学)</b>
10:30-10:50 <b>IT10</b> 徐辰武 (扬州大学)	作物杂交种精准选择方法与应用研究
10:50-11:10 <b>IT11</b> 赵涵 (江苏省农业科学院 种质资源与生物技术研究所)	玉米氮素吸收利用遗传机制解析
11:10-11:30 <b>IT12</b> 张学才 (CIMMYT-中国特用玉米研究中心/ 上海市农业科学院)	玉米全基因组选择育种研究进展与展望
11:30-11:50 <b>IT13</b> 祁显涛 (中国农业科学院作物科学研究所)	CRISPR/dCas 介导玉米靶向基因转录激活技术及其卵细胞基因表达操纵
11:50-12:05 <b>ST6</b> 孟冰 (中国农业科学院作物科学研究所)	利用靶向测序-液相芯片 (GBTS-LC) 进行玉米穗腐病 QTL 定位
<b>14:00-15:55 特邀报告/专题报告 6: 应答生物与非生物胁迫 I</b>	<b>主持人: 杨琴 (西北农林科技大学)</b>
14:00-14:45 <b>PT7</b> 杨淑华 (中国农业大学)	玉米耐冷性的遗传与分子机制
14:45-15:05 <b>IT14</b> 徐洁 (四川农业大学)	Mutation of RNA N <sup>6</sup> -methyladenosine methyltransferase confers enhanced drought tolerance in maize
15:05-15:25 <b>IT15</b> 周羽 (东北农业大学)	Analysis of the molecular mechanisms underlying resistance to maize rough dwarf disease
15:25-15:40 <b>ST7</b> 宋楠楠 (安徽农业大学)	ZmHSE42B is critical for heat stress responses in maize
15:40-15:55 <b>ST8</b> 魏小童 (吉林农业大学)	Molecular Mechanism of miR166e-ZmATHB-14 Module in Regulating Maize Root Response to Drought Stress
<b>15:55-16:15 茶歇</b>	
<b>16:15-16:55 专题报告 7: 应答生物与非生物胁迫 II</b>	<b>主持人: 王官峰 (山东大学)</b>
16:15-16:35 <b>IT16</b> 陈露 (浙江大学)	玉蜀黍属的遗传多样性与环境适应性演化
16:35-16:55 <b>IT17</b> 杨俊 (安徽农业大学)	玉米 rRNA 加工因子 RCL1 在籽粒中的分子功能研究
<b>16:55-17:30 颁奖及闭幕式</b>	<b>主持人: 卢艳丽 (四川农业大学)</b>
<b>2023 年 6 月 26 日 星期一</b>	
<b>离会</b>	

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## Plenary Talk

### Plenary Talk 1

#### 水稻杂种优势遗传基础的量化和预测分析

韩斌

中国科学院分子植物科学卓越创新中心



**韩斌**院士，中国科学院分子植物科学卓越创新中心主任，

中国科学院国家基因研究中心主任。曾任中国科学院上

海生命科学研究院副院长、植物生理生态研究所所长、

中国科学院北京基因组研究所副所长。主要从事水稻基

因组学和水稻遗传学研究，专注于水稻基因组测序、水

稻复杂性状的全基因组关联分析、栽培稻的起源驯化、水稻杂种优势分子遗传机制和水稻基

因组设计育种研究。完成了水稻第四号染色体精确测序及分析，揭示了亚洲栽培稻的扩散路

径，阐明了水稻杂种优势的主要遗传机制是基于父母本材料优良基因的聚合和不完全显性互

补效应。近年来，合作开展了高效的水稻基因组辅助设计育种工作。

## *Plenary Talk 2*

### 玉米种质改良的基础研究

李新海

中国农业科学院生物技术研究所



**李新海研究员**，1996 年获得东北农业大学博士学位，1996-1998 年在华中农业大学从事博士后研究，1999 和 2005 年分别到国际玉米小麦改良中心和美国艾奥瓦州立大学进修。现任中国农业科学院生物技术研究所所长，国家玉米产业技术体系首席科学家，中国作物学会常务理事，中国作物学会玉米专业委员会副主任委员、国家百

千万人才工程人选。一直从事玉米遗传改良与分子育种研究，揭示玉米抗粗缩病、灰斑病和开花期耐旱性遗传机制，阐明我国玉米种质基础。研发玉米杂种优势群划分技术、抗病分子标记选择技术、抗虫耐除草剂转基因育种技术，创制耐密抗病优质育种新材料 128 份和高配合力自交系 10 个；育成中单 111、中单 153 等高产抗逆宜机收玉米新品种 9 个。发表论文 126 篇，获发明专利 10 项，获国家科技进步二等奖 2 项。先后组织国家 863 计划重点课题、国家自然科学基金项目、国家科技重大专项“转基因生物新品种培育”重大课题实施，提出我国玉米育种发展重点任务和路径建议，积极促进我国玉米遗传育种发展。

### ***Plenary Talk 3***

## **Maize stem cells in ear development-Single cell analysis and heat stress responses**

David Jackson

Cold Spring Harbor Laboratory/Huazhong Agricultural University



**David Jackson** is a Professor of Cold Spring Harbor Laboratory (CSHL), NY, USA. He obtained his PhD at the John Innes Institute, UK, and after a post doc at the Plant Gene Expression Center, Berkeley, CA, he set up his own lab at CSHL in 1997. His lab studies genes that regulate plant growth and architecture, and has discovered novel

regulators of stem cell proliferation in plants, including a heterotrimeric G protein subunit that interacts with a completely different class of receptors than in animals, and helps to explain how signaling from diverse receptors is achieved in plants. His work has also demonstrated that weak mutations in developmental genes can enhance seed production in maize, leading to potential crop yield increases. His lab is also developing maize genomics tools and characterizing gene expression networks that have revealed new hypotheses in control of inflorescence development. Since 2015, he has also served as a visiting professor in Huazhong Agricultural University. Further information is available at <http://jacksonlab.labsites.cshl.edu/>.



## Plenary Talk 4

### 玉米基因组育种的理论与实践

严建兵

华中农业大学

基因组研究的迅速发展，让我们拥有了作物遗传改良新的和有力的工具。本报告将从群体遗传学的角度，以玉米为研究对象，探讨组学大数据如何帮助关键基因的克隆、复杂数量性状的遗传结构系统解析和育种应用。在此基础上，探讨未来玉米基因组育种的实现路径。



**严建兵**，华中农业大学教授、副校长。教育部长江学者特聘教授（2016）和国家杰出青年基金获得者（2015）。1995-2003 年在华中农业大学完成本-硕-博的学习获得学士和博士学位，2003-2006 年在中国农业大学工作，2006-2008 年在国际玉米小麦改良中心和康奈尔大学从事博士后研究，2008-2009 年被聘为国际玉米小麦改良中心副科学家 (Associate Scientist)，2009-2011 年被聘为科学家 (Scientist)，2011 年起在华中农业大学工作，被聘为教授。目前主要从事玉米基因组学和分子育种

方面的研究。在 Science, Nature Genetics, Nature Communications, Genome Biology, Plant Cell, Molecular Plant 等期刊发表论文 100 余篇，申请专利多项，多项专利获得转化。担任 Genome Biology, Plant Journal, JIPB, Science China Life Science 等多个期刊编委。曾获得中国青年科技奖、全国创优争先奖、国家科技发明二等奖、日本国际青年农业科学家奖、杜邦青年教授奖、刘易斯·斯塔德勒中期职业生涯奖等国内外奖项。

## *Plenary Talk 5*

### 植物干细胞与遗传转化

张宪省

山东农业大学

植物干细胞是植物体几乎所有细胞的来源，主要位于分生组织，产生各种组织和器官，是植物体形态建成的细胞学基础。植物细胞具有强大的可塑性，在一定条件下可形成干细胞，再生出完整的植物体。植物干细胞技术可广泛应用于生物育种、营养快繁和无融合生殖，调控作物的产量和品质。



**张宪省教授**，山东农业大学博士生导师，小麦育种全国重点实验室主任。担任中国植物学会监事、中国遗传学会常务理事、中国作物学会常务理事。《Seed Biology》《Plant Cell Reports》副主编、《aBIOTEC》《植物学报》编委。为国家重大科学研究计划项目首席科学家，享受国务院特殊津贴，入选国家“百千万”人才工程第一、二层次，入选山东省“泰山学者攀登计划”。主要研究方向为植物细胞全能性，植物干细胞形成与维持及器官发生的分子机制，作物重要农艺性状形成的机制与遗传改良。研究成果在 Nature Plants、PNAS、Plant Cell、Nature Commun、Mol Plant、Trends in Plant Sciences 等学术期刊发表论文 150 余篇，获山东省自然科学一等奖二项。

## *Plenary Talk 6*

### 玉米基因组学研究 with 未来育种方向

赖锦盛

中国农业大学



**赖锦盛**，中国农业大学教授，博士生导师。2009 年获聘“973 项目”首席科学家，2011 年获得“十一五”国家科技计划执行突出贡献奖，2012 年获得“国家杰出青年科学基金”资助，2014 年任“国家自然科学基金委创新研究群体”负责人，2014 年获得国务院政府特殊津贴，2022 年荣获农业农村部神农领军人才，2023 年获得第三届全国创新争先奖。赖锦盛教授长期从事玉米基因组学和生物育种

相关研究并取得了一系列创新性研究成果，是国际上玉米基因组学和基因编辑领域具有重要影响力的科学家。先后承担国家自然科学基金委重大研究计划项目、转基因重大专项、国家重点研发计划等多项科研任务。近年来在 *Nature Genetics*、*PNAS*、*Genome Research*、*Plant Cell*、*Molecular Plant* 等国际著名期刊发表论文 70 多篇，申请国内外发明专利 33 项。目前担任玉米生物育种全国重点实验室主任，中国农业大学国家玉米改良中心主任，农业农村部玉米生物学与遗传育种重点实验室主任，农业农村部基因编辑创新利用重点实验室主任，中国作物学会常务理事。兼任 *Plant Cell*、*JIPB* 等国际知名期刊编委。

## 玉米耐冷性的遗传与分子机制

杨淑华

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**杨淑华**, 中国农业大学植物科学系教授, 教育部长江学者特聘教授。1991 年和 1994 年获南开大学理学学士和硕士学位, 2002 年获新加坡国立大学博士学位, 2002—2004 年在美国康奈尔大学植物生物系从事博士后研究。先后获国家杰出青年科学基金资助, 北京市三八红旗奖章称号, 第三届国家“特支计划”科技创新领军人才, 第三届全国创新争先奖。在 *Mol Cell*、*Dev Cell*、*Nat Plants*、*Plant Cell*、*PNAS*、*EMBO J* 等国际期刊上发表 SCI 论文 70 余篇, 2020-2022 连续三年入选 Clarivate 全球高被引科学家。兼任 *Plant Cell*、*New Phytol*、*JIPB*、*Stress Biol* 编委, *J Plant Physiol* 资深编委和《植物学报》副主编。主要从事植物响应低温胁迫的分子机制研究, 以拟南芥和玉米为研究对象, 开展植物低温信号转导通路, 植物激素信号、光信号调控植物低温适应性的分子机制等研究工作。

Maize (*Zea mays* L.) is a cold-susceptible species that often faces cold stress, which has an adverse impact on growth, development, and yield in maize. We identified several genes that play important roles in regulating cold tolerance in maize using multiple approaches. Firstly, we demonstrated that natural variations in a type-A *Response Regulator 1* (*ZmRR1*) gene lead to divergence in chilling tolerance across maize inbred lines. The *ZmRR1* variant cannot be phosphorylated by *ZmMPK8* in the cytoplasm, resulting in enhanced protein stability of *ZmRR1*, which positively regulates cold tolerance in maize. Secondly, we identified *ZmbZIP68* transcription factor as a negative regulator of maize cold tolerance, and its stability is modulated by *ZmMPK8*-mediated phosphorylation. The *ZmbZIP68* locus underwent selection early in maize domestication. A 358-bp insertion/deletion polymorphism in the *ZmbZIP68* promoter resulted in the differential expression of *ZmbZIP68* between maize and its wild ancestor teosinte. Thirdly, a natural variation in the *ZmICE1* promoter modulates the amino acid homeostasis and cold tolerance in maize inbred lines. The detailed regulatory mechanisms will be presented.

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## Invited Talk

### Invited Talk 1

#### The copine protein controls plant growth via regulating brassinosteroid signaling in maize

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**Abstract:** Copine proteins are highly conserved and ubiquitously found in eukaryotes. The conserved nature of copines in different organisms suggest that they play important roles in common biological pathways, which is supported by emerging studies demonstrating their roles in plant growth, defense and stress responses. However, the mechanism underlying plant growth regulation by copine proteins remains unclear. Specially, copine's function in the agronomically important crop plant maize awaits exploration. To study the function of the copine protein (ZmBON1) in maize, we generated its knockout mutant by CRISPR/Cas9 gene editing technology, and the biological functions of copine proteins were analyzed by protein-protein interaction and phosphorylation assay. We show here that loss-of-function mutants of *ZmBON1* in maize exhibit a dwarf phenotype similar to that of the Arabidopsis mutant *bon1-1*. However, unlike the autoimmune phenotype of *bon1-1*, the *Zmbon1-1* mutants did not show constitutive defense responses. In addition, the leaves of *Zmbon1-1* were disordered and twisted. The leaf angle of *Zmbon1-1* had a larger variation among different leaves compared to that of the wild type, mimicking the brassinosteroid (BR)-deficient mutants. Consistently, *Zmbon1-1* mutants are less sensitive to BR. Knockout of copine members also attenuated BR sensitivity in Arabidopsis, indicating a conserved function for copines in BR signaling in the two species. Further studies indicate that BON1 associate with BR receptor complex. In addition, the interaction between BRI1 and SERK upon BR perception was attenuated and the reciprocal phosphorylation of BRI1 and SERKs was largely compromised in the knockout mutant. These data collectively indicate that copine proteins are critical regulatory components of BR signaling in maize and Arabidopsis. This study advances the knowledge on BR signaling and provides an important target for optimizing valuable agronomic traits in maize, it also opens a way to study steroid hormone signaling and copine proteins of eukaryotes in a broader perspective.

**Key words:** Maize; Copine; Plant growth; Brassinosteroid; Protein-protein interaction; Phosphorylation

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苟明月, 教授, 博士生导师。2009 年在中国农业大学获得博士学位, 2009-2018 年在美国康奈尔大学和布鲁海文国家实验室从事植物抗病和细胞壁合成机制研究, 2017 年入职河南农业大学农学院。现任国家玉米改良(郑州)分中心副主任、河南农业大学作物基因组学与分子育种中心主任, 主要从事玉米抗病(逆)与生长发育调控机制研究及分子育种工作。在 Nature Plants、Plant Cell、Plant Physiology、Plant Journal、Journal of Experimental Botany 等期刊发表论文 30 余篇, 文章被引用 1800 余次, 申请国家发明专利 4 项(获批 2 项), 担任 Frontiers in Plant Science 期刊副主编及 Plant Cell、New Phytologist 等期刊审稿人。获河南省特聘教授、“中原千人计划”-青年拔尖人才、河南省杰青、河南省高层次人才等荣誉。

## Invited Talk 2

### 玉米茎叶夹角形态建成的分子机理解析

李 刚

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**摘要:**玉米茎叶夹角是一个复杂的数量性状,受到多种内部发育信号和外部环境信号的严格调控。在过去的几十年里,人们已经鉴定到多个调控叶夹角形成的关键因子,但茎叶夹角建成的分子调控网络尚不清楚。在前期研究中,我们发现叶枕区域的叶耳发育在茎叶夹角建成中至关重要。在后续研究中,通过对玉米和多种禾本科植物的茎叶夹角表型进行比较分析,揭示了玉米叶枕近轴侧细胞的纵向伸长和远轴侧厚壁细胞的增厚在茎叶夹角建成的分子调控机制。通过比较转录组和单细胞转录组分析鉴定可能参与调控叶枕发育的关键因子,并利用遗传学和分子实验验证ZmbHLH30和ZmbHLH155在叶夹角建成调控网络中的作用。在低密度种植条件下,光受体phyB通过感知环境中的high R:FR信号并与茎叶夹角建成中的关键转录因子LG1相互作用,促进其蛋白稳定导致茎叶夹角变大。在高密度种植或叶片相互遮蔽的条件下,转录因子LG1蛋白含量降低,解除对下游调控基因HB类转录因子的转录抑制,促进叶夹角的变小从而提高光能利用率。



**李刚**, 山东农业大学生命科学学院教授, 博士生导师。2006年毕业于中科院上海生命科学研究院获博士学位; 2007年2月至2011年8月在美国康奈尔大学、布思汤普森植物研究所、以及耶鲁大学从事博士后研究; 2011年9月至今受聘山东农业大学生命科学学院教授职位。近年来以模式植物拟南芥和重要农作物玉米为研究材料, 主要从事光信号调控植物避荫反应和茎叶夹角形态建成的机理研究。



### Invited Talk 3

## Maize PPR-E proteins mediate RNA C-to-U editing in mitochondria by recruiting *trans* deaminase PCW1

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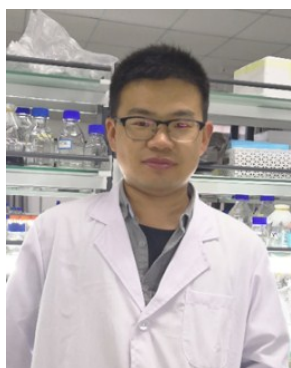
**Abstract:** RNA C-to-U editing in plant organelles involves proteins from diverse families. The PPR family proteins function in specifying the target site and providing the cytidine deaminase activity. PPR-E+ proteins lack the deaminase domain appear to recruit DYW2 as the *trans* cytidine deaminase. However, how PPR-E proteins carry out the C-to-U editing is unclear. We isolated the *bccp1-1* and *bccp1-2* mutants from the UniformMu maize population and the maize EMS mutant database and created the *pcw1-1* and *pcw1-2* mutants by using the CRISPR/Cas9-based genome editing. The STS-PCRseq and directly sequenced the amplicons were used to analysis the editing efficiency in wild type and the *bccp1* and *pcw1* mutants. In addition, we used the Y2H, BiFC and LCI assay were used to test the protein interactions. Loss-of-function of either bCCP1 or PCW1 severely arrests seed development, resulting in empty pericarp kernels in maize. bCCP1 and PCW1 are targeted to mitochondria where bCCP1 and PCW1 is required for the editing at 66 and 102 sites, respectively. Surprisingly, the 66 sites by bCCP1 are completely included in the 102 sites by PCW1. Analysis of known sites reveals that the PCW1 mediated editing sites appear to be exclusively associated with the PPR-E proteins, rather than the PPR-E+ or PPR-DYW proteins. A test using the PPR-E protein EMP7 shows that bCCP1 interacts with PCW1 and EMP7. ZmMORF1 and ZmMORF8 interact with PCW1, EMP7 and bCCP1, while ZmMORF8 may enhance the interaction between EMP7 and PCW1 in Y3H assay. EMP7, bCCP1 and PCW1 are required for the C-to-U editing at the *ccmF<sub>N</sub>-1553* site in maize. Taken together, these results suggest that PPR-E proteins probably recruit PCW1 as the *trans* deaminase where bCCP1 and MORFs assist the recruitment through protein-protein interactions with PCW1 and the PPR-E proteins.

**Key words:** Maize; Seed development; RNA editing; bCCP1; PCW1; PPR-E

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**王勇** 副研究员，山东大学生命科学学院，长安大学学士，山东大学硕士、博士和博士后。主要从事玉米种子发育关键基因克隆和功能解析以及植物细胞器 RNA 编辑机制解析的工作。以第一作者（或共同第一作者）分别在 Plant Cell、PLoS Genet.、Plant J.和 Front. Plant Sci.等学术期刊上发表论文 5 篇。以共同作者分别在 PNAS、Plant Physiol.、JIPB 和 J. Exp. Bot.等刊物发表论文 9 篇。申请获得国家自然科学基金（青年项目），作为主要参与人申请获得国家自然科学基金（重点项目）等。先后获得山东大学优秀博士后、重点资助类博士后和山东省遗传学年会报告一等奖（研究生论坛）等。

## *Invited Talk 4*

### **Genetic dissection and gene cloning from a prominent drought-resistant maize germplasm CIMBL55**

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**Abstract:** In the context of climate change, drought is one of the most limiting factors that influence crop production. Maize, as a major crop, is highly vulnerable to water deficit, which causes significant yield loss. Thus, identification and utilization of drought-resistant germplasm are crucial for the genetic improvement of the trait. We report on a high-quality genome assembly of a prominent drought-resistant genotype, CIMBL55. Genomic and genetic variation analyses revealed that 65 favorable alleles of 108 previously identified drought-resistant candidate genes were found in CIMBL55, which may constitute the genetic basis for its excellent drought resistance. Notably, ZmRtn16, encoding a reticulon-like protein, was found to contribute to drought resistance by facilitating the vacuole H<sup>+</sup>-ATPase activity, which highlights the role of vacuole proton pumps in maize drought resistance. In addition, another drought-resistant gene ZmSRO1d was identified in CIMBL55. Three non-synonymous variants in a drought-resistant allele, ZmSRO1d-R, enhanced mono-ADP-ribosyltransferase activity of ZmSRO1d toward ZmRBOHC on plasma membrane, which increased reactive oxygen species levels in guard cells and promoted stomatal closure. ZmSRO1d-R enhanced plant drought resilience and protected grain yields under drought conditions, but it led to yield drag under favorable conditions. In contrast, loss-of-function mutants of zmrbohc showed remarkably increased yields under well-watered conditions, whereas they showed compromised drought resistance, indicating the trade-off between drought-resistance and grain yield.

**Key words:** Drought resistance, Gene cloning, Genome assembly, Maize



**秦峰**，中国农业大学生物学院教授。主要从事玉米抗旱基因的克隆与功能研究。在玉米抗旱性这一复杂数量性状的解析和基因克隆研究中取得了具有国际影响的研究成果，为农作物抗旱性的分子设计育种提供了重要的基因资源和关键技术。研究成果发表在Nature Genetics、Nature Communications、Plant Cell、Genome Biology等国际学术期刊，获美国授权专利1项、中国发明专利6项、申请国际专利（PCT）3项。2014年获“杜邦青年教授奖”；2015年获“第一届中国作物学会青年科技奖”；2016年获得国家自然科学基金委“杰出青年”基金；2018年获“卫志明青年创新奖”；2019年入选“万人计划”领军人才。



## Invited Talk 5

### 基于多组学分析的玉米生化防御功能基因研究

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**摘要：**提升玉米品种的遗传抗性是对病虫害可持续防治策略中的重要一环。基于抗性表型的图位克隆是目前最为有效的抗性位点鉴定手段，但其仍存在表型不稳定，时间周期长等局限性。特异化代谢产物是玉米抵御病虫害侵害的重要手段，已有的抗性遗传机制研究中也报道了多个特异化代谢合成调控基因。因此，解析调控玉米特异化代谢的遗传机制可能是一条挖掘玉米抗性遗传位点的新途径。本课题组利用非靶向代谢组学分析挖掘了玉米苗枯病抗性相关的代谢生物标记，鉴定了与这一生物标记连锁的遗传位点，进而综合利用代谢与基因表达数据明确了关联位点中的候选基因。在此基础上，我们通过对玉米非靶向代谢组数据进行全基因组关联分析鉴定了多个 mGWAS“热点”，为后续玉米代谢遗传学与病虫害抗性研究提供了明确的候选基因。综上所述，在合理的实验设计前提下综合利用多组学数据分析可以有效助力抗性相关遗传位点与候选基因的快速精准鉴定，为高抗玉米品系培育提供了新思路。

**基金支持：**国家自然科学基金委员会，National Science Foundation （美国）



**周绍群**，博士，研究员，博士生导师，深圳市海外高层次人才。先后于美国华盛顿大学（2012）与康奈尔大学（2018）获生物学学士与植物生物学博士学位。2018-2019 于美国 Elo Life System 公司开展作物代谢工程方向的博士后工作。自 2019 年起就职于中国农业科学院深圳农业基因组研究所至今，任课题组长。课题组通过结合多组学与作物遗传学方法聚焦于作物代谢遗传学与病虫害抗性机制研究，在 Plant Cell, New Phytologist 等 SCI 期刊发表论文 20 余篇。

## Invited Talk 6

### 基于生物大数据的玉米重要农艺性状解析与智能育种初探

李林及玉米种质资源与系统生物学实验室全体同学

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**摘要:** 生物研究已经进入大数据时代, 育种也正迈向4.0智能时代。如何利用生物大数据进行作物重要性状的系统解析以及基于此进行高效精准智能育种仍然挑战巨大。本课题组聚焦玉米密植高产的株型建成机制解析, 构建多套玉米高通量群体, 定位了800多个株型相关QTL, 并对玉米株型的遗传基础进行了解析, 提出了稀有等位基因是玉米株型建成的决定因子的观点; 进而, 开发了基于基因组大数据快速精细定位克隆数量性状基因QTG的新策略QTG-seq与DeepBSA, 并提出了基于生物大数据系统挖掘基因功能的新方法; 构建了玉米第一代生物网络大数据图谱并开发了人工智能挖掘工具, 系统解析了玉米株型建成分子基础, 对玉米株型、产量、开花期等分子网络进行了系统鉴定, 为智能设计育种打下了基础; 最后, 本课题组整合玉米多维生物大数据和高通量遗传群体数据, 对玉米重要性状株高、开花期、以及穗部性状的智能育种初步实践。



**李林**, 华中农业大学植物科学技术学院教授, 智慧农业系系主任, 国家优秀青年基金获得者、湖北省海外高层次人才获得者。2001 年至 2010 年, 中国农业大学本硕博连读, 2010 年获农学博士学位, 同年去美国明尼苏达大学从事博士后及研究助理工作。于 2016 年 7 月正式回国建立实验室, 利用生物大数据进行玉米株型建成分子机制研究, 回国后, 以通讯作者(含共同)在 Nature Genetics, Nature Biotechnology, Genome Biology, Molecular Plant (三篇)等国际主流期刊上发表研究论文多篇, 总引用超过 3800 次。主持国家自然科学基金优秀青年基金项目、重大研究计划集成项目、国际合作项目、以及面上项目等。

## *Invited Talk 7*

### 玉米分化期胚乳的单细胞转录图谱和基因调控网络

马泽阳

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**摘要:** 玉米胚乳是淀粉和蛋白等营养物质的储藏组织, 包含五种不同细胞类型。解析胚乳细胞的分化发育及基因调控网络将为提高玉米产量和改善品质奠定基础。我们利用单细胞转录组测序技术绘制了玉米分化期胚乳细胞的转录图谱。共捕获了22,880个高质量的胚乳分化期细胞: 分析发现五种不同类型的胚乳细胞对应着12个具有特异表达特征的细胞亚群, 进一步揭示了胚乳细胞转录水平异质性的复杂程度; 通过连续时间点的实时表达分析, 描绘了胚乳细胞在授粉后6至8天这一发育关键转折期中单细胞水平的基因动态变化模式; 此外, 还通过整合转录因子在全基因组上的DNA结合图谱, 构建了高可靠度的胚乳转录调控网络, 预测出不同细胞亚群中的重要调控节点, 并进行了验证。本研究解析了单细胞分辨率的玉米胚乳转录图谱, 构建了高可靠度的调控网络, 为胚乳发育和相关研究提供了宝贵资源。



**马泽阳**, 男, 中国农业大学, 副教授。2010年毕业于中科院遗传发育所, 获博士学位; 2011年至2019年分别在北京生命科学研究所以和美国德州农工大学从事博士后研究, 主要方向为植物表观遗传调控; 2019年加入中国农业大学农学院, 研究方向是玉米籽粒发育的调控网络和玉米蛋白品质的分子遗传学研究。近年来在 *Dev Cell*, *PNAS* 和 *Curr Opin Plant Biol* 等国际杂志上发表了多篇高水平论文。

## *Invited Talk 8*

### 耐旱耐热转基因玉米品种研发进展

赵天永

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## ***Invited Talk 9***

### **The nucleosome remodeler DDM1 shapes R-loop formation by interacting with DDX23 helicase in maize**

龙金成

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**摘要:** 染色质重塑蛋白 DDM1 能够为 DNA 甲基转移酶提供染色质可及性, 从而维持基因组 DNA 甲基化的稳定。然而, 除 DNA 甲基化之外, DDM1 对其它染色质活动的调控依旧未知。通过免疫沉淀结合质谱分析, 我们发现 R-loop 解旋酶 DDX23 存在于玉米 DDM1 免疫沉淀产物当中。酵母双杂, 免疫共沉淀以及双分子荧光证实了 DDM1 与 DDX23 存在直接相互作用。体外实验结合遗传学证据表明 DDX23 在玉米中具有保守的 R-loop 解旋酶活性。在发育过程的胚中, 大多数 R-loop 与 DDM1 占据的染色质位置重合, 并且当 DDM1 突变之后, DDX23 与染色体结合能力下降, R-loop 整体水平上升。综上所述, 以上研究揭示了玉米 DDM1 可能通过调控染色质的开放程度, 帮助 DDX23 接近染色质从而完成 R-loop 解旋过程。

**基金支持:** 国家优秀青年 (海外) 项目



**龙金成**, 中科院分子植物科学卓越创新中心研究员, 博士生导师。2012 年毕业于中国农业大学, 获农学学士学位。2018 年毕业于中国农业大学国家玉米改良中心, 获作物遗传育种专业博士学位。2018 年至 2022 年在英国约翰英纳斯研究中心从事博士后研究。2022 年 6 月加入中科院分子植物科学卓越创新中心。以第一作者在 *Science*, *Plant Cell*, *JIPB* 等高影响力期刊发表研究论文数篇。目前承担国家优秀青年 (海外) 项目。课题组以玉米作为模式植物, 主要探究以下科学问题: 1) 玉米雄性生殖细胞中表观遗传重编程模式及其分子机制; 2) 玉米生殖发育时期应答热胁迫的遗传以及表观遗传调控机制。

## 作物杂交种精准选择方法与应用研究

徐 扬 王 欣 徐辰武

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**摘要:** 全基因组选择 (GS) 育种, 是根据训练群体全基因组上的分子标记基因型和表型之间的关联构建遗传模型, 进而对基因型已知的待选群体进行育种值估计或表型预测, 以实现育种群体高效和精确地选择。相比于常用的分子标记辅助选择 (MAS) 育种, GS 育种无需进行标记显著性测验, 特别适用于微效多基因控制的数量性状, 并可缩短育种周期, 现已成为动、植物育种领域的一项前沿技术。然而, 对受环境影响较大的产量等数量性状而言, 仍面临着基因组预测准确性难以提升的瓶颈问题。为此, 本实验室开展了作物 GS 育种相关的方法和应用研究, 以提高 GS 选择的精确度。(1) **GS 方法的比较研究** 以计算机模拟数据和作物实例数据, 研究性状遗传力、遗传结构、标记密度、训练群体大小和建模方法等对 GS 预测能力的影响。(2) **多变量 GS 方法研究** 探索利用 GBLUP 方法开展多性状和多环境数据联合 GS 分析, 以提高目标性状的预测能力。此外, 研究如何利用与目标性状相关的多个性状构建选择指数进行辅助 GS, 以进一步提高目标性状 GS 的预测能力。(3) **多组学 GS 方法研究** 研究利用双亲的多组学数据对杂种表型进行预测的多组学模型, 并研究如何整合双亲的表型以提高杂种表型预测的精确度。(4) **GS 方法的应用研究** 将 GS 方法与玉米亲本一般配合力 (GCA) 估计和杂种表型预测相结合, 探讨稀疏部分双列杂交 (SPDC) 设计对 GCA 估计和杂交种表型预测的可行性。研究结果表明, 不同建模方法适用于具有不同遗传结构的数量性状; 相比于传统 GS 方法, 多性状联合或多环境联合 GS 可显著提高目标性状的预测能力; 利用与目标性状相关的多个性状构建选择指数进行辅助 GS, 可以进一步提高目标性状 GS 的预测能力; 相比于单一组学预测, 多组学预测可进一步提高杂种表型预测的精确度, 将双亲已有表型数据整合进 GS 预测模型或多组学预测模型, 均可有效提高杂种表型的预测能力; 利用 SPDC 设计, 可以一次实现对数百个玉米亲本的 GCA 精确估计和杂交种的预测。以玉米杂种穗重为目标性状, 将 GS 预测的最好的 100 个杂种和最差的 100 个杂种进行田间组配和表型鉴定, 验证了 GS 的有效性。此外, 为了使 GS 技术有效应用于作物育种实践, 研发出“基因组选择 GBLUP 模型预测系统”等系列分析软件 5 个。

**关键词:** 作物育种; 全基因组选择; 预测模型; 多性状联合分析; 多组学联合分析

**基金项目:** 国家重点研发计划课题 (2016YFD0100303、2022YFD1201804); 国家自然科学基金项目 (32061143030, 32170636); 江苏省种业振兴揭榜挂帅项目 (JBGS[2021]009)。

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## 玉米氮素吸收利用遗传机制解析

赵涵

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**摘要:** 氮肥使用量决定玉米产量品质的形成。为解析玉米对氮素吸收和利用的遗传机制, 我们做了以下研究: 1、通过构建玉米氮响应基因共表达网络, 鉴定到ZmNLP5是氮代谢调控网络的重要节点基因。将ZmNLP5突变后, 突变体植株的穗位叶和种子氮含量显著降低。而过表达ZmNLP5可提升玉米氮吸收能力及籽粒氮含量, 说明ZmNLP5在玉米氮响应和氮同化过程发挥重要作用。2、利用氮响应差异表达模块启动子富集的基序进行表面等离子共振-质谱联用分析, 获得25个氮响应节点转录因子。通过ChinaMu突变体库中相关突变材料进行验证, 发现有11个转录因子可以显著影响玉米氮素吸收率。3、通过分析玉米籽粒氮碳代谢差异表达基因启动子序列发现其富含PBF1结合基序P-box。将PBF1突变后, 胚乳中淀粉和蛋白质含量均发生显著变化。然而PBF1在足氮和缺氮环境下表达水平及部位没有显著差异, 但是PBF1在两种氮素环境下结合靶标基因(尤其是碳氮化合物积累相关基因)存在显著差异, 说明PBF1在两种氮素环境下可能通过改变对靶标基因结合方式来调控玉米胚乳碳氮化合物的积累。

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## 玉米全基因组选择育种研究进展与展望

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**摘要:** 全基因组选择 (Genomic Selection, GS) 是现代生物育种中的前沿关键颠覆性技术, 采用全基因组分子标记信息预测未测试育种材料的基因组估计育种值, 用它代替表型育种值进行选择。与常规表型育种相比, GS 具有节约育种成本、缩短育种周期、加速遗传增益、提升育种效率等优点。目前, 玉米 GS 研究热点主要集中在预测模型开发、规模化实施途径的设计与优化、GS 育种有效性及预测精度准确性的评估等领域。预测模型是 GS 育种的核心, 主流预测模型是基于 BLUP 和 Bayes 方法的改进, 非加性效应模型、多变量模型和多组学数据整合模型是未来 GS 应用的重要方向, 人工智能和深度学习算法为解决预测准确性和计算效率等难题提供了新的契机。玉米育种规模化实施 GS 的途径主要是: 在早期测配阶段预测和选择最优未测试自交系; 预测未测试杂交种表现和未测试自交系的一般配合力表现, 选择最优杂交种和最优亲本; 加速轮回选择流程, 实施群体改良, 拓宽种质资源。影响 GS 育种有效性和预测精度准确性的主要影响因素有预测模型、设计合理的育种实施方案、建模群体大小与遗传多样性、建模群体与预测群体的亲缘关系、建模群体优化、预测目标性状的复杂程度及遗传力、分子标记密度和费用、主效基因贡献、基因型与环境互作程度、育种数据信息化程度等。GS 育种已在国际上规模化应用, 亟需结合我国玉米育种产业现状, 探索一套符合我国实际的规模化玉米全基因组育种发展方案。



**张学才**, 男, 国际玉米小麦改良中心 (CIMMYT) 高级科学家, 全球玉米项目组拉丁美洲玉米分子育种实验室主任, CIMMYT-中国特用玉米研究中心 (CCSMRC) 客座研究员。研究方向为热带玉米遗传育种与种质资源创新; 玉米复杂数量性状遗传解析; 玉米全基因组选择育种技术应用与拓展等。已在国际学术期刊上发表科研论文 50 多篇, 其中玉米全基因组选择育种相关论文 27 篇。现任《Frontiers in Plant Science》期刊副主编、《Journal of Integrative Agriculture》和《中国农业科学》的期刊编委。



## CRISPR/dCas 介导玉米靶向基因转录激活技术 及其卵细胞基因表达操纵

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**摘要:** 新一代CRISPR/Cas基因编辑技术可以对基因靶向定点精确突变, 包括敲除、碱基编辑、删除、敲入、表观修饰与转录表达调控等, 该技术颠覆了传统生物育种方法, 具备操作便捷、靶向精准、修饰灵活、产出高效、适应性广等突出优势, 应用前景广阔, 将对全球种业技术迭代与产业格局产生革命性影响。有性繁殖植物的卵细胞是个体发育的单细胞起点。因此, 通过CRISPR/Cas介导靶向卵细胞基因表达操纵具有重要的基础生物学研究与生物育种应用潜力与价值。本研究采用无核酸内切酶活性的CRISPR/dCas9作为靶向目标DNA的工具, 融合了ERF2m、VP64以及NF- $\kappa$ B反式激活亚基p65三组转录激活效应元件, 通过元件间组合构建了6组CRISPR基因激活载体CRISPR (a1-a6)。采用玉米叶肉原生质体检测体系对CRISPRa工具的活性及其特征进行了评估。结果表明, CRISPRa5基因激活工具 (sgRNA2.0引导dCas9-VP64::MS2-P65-HSF) 具有最高的转录激活水平, 可提高ZmDPS1内源基因转录水平11.3倍。在此基础之上, 本实验进一步建立了玉米卵细胞离体分离操作方法, 并基于前期已构建的玉米LTP2pro::DsRed2稳定转化株系, 分离得到卵细胞原生质体, 在离体细胞水平验证了卵细胞内激活胚乳糊粉层特异表达LTP2pro::DsRed2, 确定了CRISPRa5卵细胞定点基因转录激活活性。基于开发的玉米高效卵细胞基因调控技术体系, 在玉米卵细胞中针对玉米内源的ZmBBM2基因进行了精准的异位激活。实验结果表明, 玉米卵细胞内精准调控ZmBBM2基因异位激活可以诱发玉米孤雌生殖, 通过该方法获得了多胚体与约达3.55%孤雌生殖单倍体。该研究基于CRISPR建立了高效的玉米基因表达调控技术体系与卵细胞分离技术, 并实现活体水平转录激活工具对玉米卵细胞靶基因转录表达操纵, 开发的系列技术具有重要的基础理论与育种实践价值。

**关键词:** 玉米; CRISPR/dCas; 基因转录激活; 卵细胞; 孤雌单倍体

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**祁显涛**, 中国农业科学院作物科学研究所, 博士, 副研究员。研究方向为玉米等作物基因编辑技术研发与育种应用, 主要取得如下业绩: (1) 系统建立了高效的玉米基因编辑技术体系。鉴定的元件与构建的技术体系被冷泉港实验室 (CSHL) 与中国农业大学等 70 余家国际国内科研机构协议使用; (2) 研制出基于基因编辑技术赋能的第三代作物杂交种制种新技术, 该技术一步法解决了创制细胞核雄性不育与配套保持系分选元件问题, 被美国科学促进会 and 欧洲种子协会誉为是“利用基因编辑技术赋能解决重大农业产业问题的范例”; (3) 基于 CRISPR/Cas 的 DNA 识别与结合活性, 构建了与之融合的激活转录表达盒, 从而建立了可高效定向基因转录激活调控的技术工具, 为基因功能鉴定及其应用提供了新的技术手段; (4) 利用基因编辑技术, 创制了单倍体诱导、糯性、甜糯、株型等重要农艺性状育种技术, 系列技术产业应用前景广阔。部分工作已形成论文与专利成果, 已授权发明专利 4 项、申请国际 PCT 专利 1 项; 以第一作者或共同第一作者在《分子植物 (Molecular Plant)》和《植物生物技术杂志 (Plant Biotechnology Journal)》等主流期刊发表学术论文 5 篇。目前主持国家自然科学基金 1 项、博士后面基金 1 项, 获选中国农业科学院“优农计划”特别资助项目。

## ***Invited Talk 14***

### **Mutation of RNA N<sup>6</sup>-methyladenosine methyltransferase confers enhanced drought tolerance in maize**

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**Abstract:** RNA N<sup>6</sup>-methyladenosine (m<sup>6</sup>A) modification plays an important role in plant development and environmental adaptations. In our previous studies, we have found that the AdoMet MTases superfamily gene SAM-dependent methyltransferase (METTL) is one of the candidate genes for maize yield under drought stress using an integrated approach of QTL mapping and genome-wide associated analysis. The mutants of METTL generated through CRISPR/Cas9 exhibit well-developed roots and limited yield loss under drought conditions, coupled with a significant reduction of m<sup>6</sup>A modification on U6 snRNA in maize roots compared with the wild type plant KN5585. The activity and substrate of METTL methyltransferase were revealed through vitro activity assays. The decreased level of m<sup>6</sup>A modification on U6 snRNA in the mutants was confirmed using SELECT-qPCR and the transcription inhibition assays using actinomycin D revealed the decreased m<sup>6</sup>A probably promotes the RNA stabilization, resulting in the increased level of U6 snRNA. Over-expression of U6 snRNA in the maize inbred line B73 also exhibited a stronger root system and enhanced drought tolerance at seedling stage. In addition, the Dnase I-treated terminal deoxynucleotidyl transferase dUTP nick end labeling assays on the roots found a notable increase in chromatin openness in mutants, which was also confirmed by the changes of histone modifications H3K27me3 and H3K4me3. These results demonstrate that METTL-mediated m<sup>6</sup>A modifications on U6 snRNA play an important role in regulating maize drought tolerance.

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## Invited Talk 15

### Analysis of the molecular mechanisms underlying resistance to maize rough dwarf disease

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**Abstract:** Maize rough dwarf disease (MRDD) is a viral infection of maize (*Zea mays* L.) that results in extensive yield losses throughout the world. In the Yellow–Huai River summer maize-growing region and the Northern spring maize-growing region in China, MRDD is caused by rice black-streaked dwarf virus (RBSDV). We here analyzed molecular variations, evolutionary parameters, conserved regions, and other genomic properties of RBSDV isolates from maize and rice in nine geographic locations in China. During RBSDV infection, the maize protein first recognizes, then interacts with pathogenesis-related protein to initiate the disease resistance response. This module regulates MRDD resistance mechanisms, such as production of reactive oxygen species, jasmonic acid, and gibberellin. Analysis of several maize cultivars showed that *miRNA* expression enhanced MRDD resistance, whereas its target gene played a negative regulatory role. In the early stages of infection, this module participated in regulation of biosynthesis pathway, enhancing plant resistance. This study revealed a novel disease regulatory module in maize, providing new breeding targets to ultimately promote disease resistance and increase food security.

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## 玉蜀黍属的遗传多样性与环境适应性演化

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**摘要:** 玉米不仅是世界上种植最广泛的作物, 也是基础研究的模式物种之一。玉米起源于墨西哥西南部, 其野生同族物种统称为大刍草。野生大刍草虽然仅在墨西哥和中美洲地区分布, 但存在适应多种不同环境的亚种, 如可适应炎热、潮湿的中美洲环境的 *Zea nicaraguensis*, 可适应寒冷、干旱的墨西哥中部高原环境的 *Zea mays* subsp. *mexicana*。玉蜀黍属的这些特性使其可作为研究适应性进化的理想模式物种, 通过对玉蜀黍属适应性进化的遗传机制进行剖析, 将为利用野生资源培育可适应未来剧烈气候变化作物提供新的契机。但是, 目前我们对玉蜀黍属的遗传变异程度及其可能的应用还知之甚少。在本研究中, 我们对现代玉米及玉蜀黍属的所有野生分类群中的物种, 共计 744 份材料进行了高通量基因组重测序, 构建了玉蜀黍属的高密度基因组变异图谱。基于这个遗传变异图谱, 我们利用多物种合并模型, 重构了玉蜀黍属的进化历史。研究显示, 玉蜀黍属的不同亚种在距今约 120,000 年前开始分化, 并在距今 68,000 年左右, 快速分化成为现在的 7 个亚种, 并进一步驯化成现代玉米。在玉蜀黍属的进化过程中, 不同亚种积累了大量特有的遗传变异, 包括大量的转座子变异与倒位。在本研究中, 我们重点研究了高原大刍草和温带玉米的适应性等位基因, 发现了开花时间相关途径在其适应中的关键作用。为了证实这些变异资源在适应性位点发掘中的作用, 我们对两个与开花期相关的候选基因进行了功能验证。这项工作不仅提供了一个全面的玉蜀黍属多样性样本, 解决了玉蜀黍属的进化问题, 也确定了可直接用于现代育种的适应性变异。

**关键词:** 玉蜀黍属; 遗传变异; 适应性演化



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## Invited Talk 17

### 玉米 rRNA 加工因子 RCL1 在籽粒中的分子功能研究

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**摘要:** 核糖体RNA (rRNA) 是蛋白质翻译机器核糖体的骨架和主要组成部分。在真核生物中, rRNA组成和加工过程都比较保守。相对于酵母和动物, 植物的rRNA加工也在多个位点进行, 且同样需要大量加工因子参与, 但具体作用机制的研究要落后很多; 玉米中核糖体加工因子的报道更是极少。我们筛选到一个籽粒干瘪, 胚分化缺陷的籽粒突变体, 进一步的观察显示该突变体的糊粉层和基底转运层细胞异常。图位克隆发现突变体中RNA末端环化酶类似蛋白编码基因RCL1中存在转座子插入。RCL1定位于核仁, 参与到核糖体小亚基的18S rRNA前后三个位点的剪切加工, *rcl1*突变中成熟18S rRNA、40S小亚基、80S单体和多聚核糖体均显著下降。我们发现胚乳醇溶蛋白及其核心调控因子O2, 淀粉合成关键酶的翻译过程受到影响, 阐明了一个保守的核糖体加工因子在籽粒中通过翻译途径发挥作用的机制。我们正在尝试挖掘与RCL1共同发挥作用的蛋白和18S rRNA其它位点的加工因子, 发现酵母NOB1的玉米同源基因在籽粒发育中也起关键作用。

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## Short Talk

### *Short Talk 1*

#### **Local auxin biosynthesis regulates brace root angle and lodging resistance in maize**

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**Abstract:** Root lodging poses a major threat to maize production, resulting in reduced grain yield and quality, and increased harvest costs. Here, we combined expressional, genetic, and cytological studies to demonstrate a role of *ZmYUC2* and *ZmYUC4* in regulating gravitropic response of the brace root and lodging resistance in maize. We show that both *ZmYUC2* and *ZmYUC4* are preferentially expressed in root tips with partially overlapping expression patterns, and the protein products of *ZmYUC2* and *ZmYUC4* are localized in the cytoplasm and endoplasmic reticulum, respectively. The *Zmyuc4* single mutant and *Zmyuc2/4* double mutant exhibit enlarged brace root angle compared with the wild-type plants, with larger brace root angle being observed in the *Zmyuc2/4* double mutant. Consistently, the brace root tips of the *Zmyuc4* single mutant and *Zmyuc2/4* double mutant accumulate less auxin and are defective in proper reallocation of auxin in response to gravi-stimuli. Furthermore, we show that the *Zmyuc4* single mutant and the *Zmyuc2/4* double mutant display obviously enhanced root lodging resistance. Our combined results demonstrate that *ZmYUC2*- and *ZmYUC4*-mediated local auxin biosynthesis is required for normal gravity response of the brace roots and provide effective targets for breeding root lodging resistant maize cultivars.

**Funding:** National Key Research and Development Program of China, National Natural Science Foundation of China and Hainan Yazhou Bay Seed Lab.

## Short Talk 2

### A leucine rich repeat receptor kinase gene confers quantitative susceptibility to maize southern leaf blight

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**Abstract:** Southern leaf blight (SLB), caused by the necrotrophic fungal pathogen *Cochliobolus heterostrophus*, is a major foliar disease which causes significant yield losses in maize worldwide. A major quantitative trait locus, *qSLB<sub>3.04</sub>*, conferring recessive resistance to SLB was previously mapped on maize chromosome 3. Using a combination of map-based cloning, association analysis, ethyl methanesulfonate (EMS) and transposon mutagenesis and CRISPR-Cas9 editing, we demonstrate that a leucine-rich repeat receptor-like kinase gene which we have called *ChSK1* (*Cochliobolus heterostrophus* Susceptibility Kinase 1) at *qSLB<sub>3.04</sub>* causes increased susceptibility to SLB. Resistant parental lines Mo17 and NC292 have a 2344-bp Harbinger-like transposon insertion in the first exon of *ChSK1*, which is predicted to make the gene non-functional. Other resistance *ChSK1* alleles were identified in several NAM parents. It should be noted that the 2344-bp element found in the Mo17 and NC292 was not present in any of the NAM parents, implying an independent evolution of resistance alleles in these lines. Three independent mutations and three independent edits in *ChSK1* were all associated with increased SLB resistance at the adult or juvenile stage or both. We present evidence that ChSK1 may be associated with suppression of the basal immune response. These findings contribute to our understanding of plant disease susceptibility genes and the potential to use them for engineering durable disease resistance.

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### ***Short Talk 3***

## **Excavation of loci related to low temperature tolerance in maize germination and identification of key genes**

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**Abstract:** Maize is affected by low temperatures throughout its growth process, especially during germination. Therefore, it is important to identify more loci or genes associated with germination under low temperature conditions. QTL mapping of the 365 RILs which obtained from crossing Qi319 and Ye478. Twenty QTLs were detected and the consistent QTL *LtQTL1-1* related to tolerance of low temperatures at the germination stage was detected on bin1.06-1.07 of chromosome 1, at a confidence interval of between 200,400,148 and 201,775,619 bp. We contributed to the understanding of the genetic control of low-temperature tolerance in maize at the germination stage by the use of a panel of 222 maize inbred lines. By integrating the results of GWAS and DEG analysis of low temperature tolerance during germination in maize, we were able to identify a total of 30 SNPs and 82 related candidate genes, including 10 DEGs, two of which were involved in the response to tolerance to low temperature. We mapped 28 QTLs from the maize IBM Syn10 population, all of which were associated with low temperature tolerance traits during the germination stage. A total of 14 overlapping QTLs made up six QTL clusters.

**Key words:** maize; low temperature tolerance; germination; GWAS; QTL; genes

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## Short Talk 4

### *ZmGII*调控玉米开花的机制探究

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**摘要:** *GI* (*GIGANTEA*) 是植物特有的核蛋白基因, 参与调控植物生长发育的多个遗传途径。*GI* 为节律输出的通道基因, 在拟南芥中参与成花诱导。对 *ZmGIGANTEA1* (*ZmGII*) 的生物学功能及其分子调控机制进行深入研究将有助于改善玉米光周期敏感性, 丰富种质资源。*ZmGIs* 是否以及如何通过光周期途径调控玉米开花仍然未知。本研究利用候选基因关联分析, 过量表达以及 CRISPR/Cas9 基因敲除技术, 旨在探究 *ZmGII* 在玉米中调控开花途径中的基因功能。通过蛋白互作验证及发掘下游基因为进一步解析 *ZmGII* 在遗传途径中的功能机制提供基础。本研究采用候选基因关联分析方法挖掘其优异等位变异, 利用光周期敏感性极端材料分析 *ZmGII* 的表达模式, 并初步鉴定候选基因功能。利用 CRISPR/Cas9 和过量表达的遗传转化实验研究 *ZmGII* 的生物学功能。同时结合分子生物学技术: 酵母双杂交筛库、split-LUC、Co-IP、EMSA 等筛选并鉴定与其互作及下游的基因, 服务于后续分子调控机制研究。表达模式分析发现 *ZmGII* 具有典型节律性表达模式, 且在玉米各个组织中均有表达。长日照条件下, *zmgi1* 突变体花期显著推迟且 *ZmGII* 过表达株系显著提前。短日照条件下, *ZmGII* 的过表达株系花期变化不明显, 但 *zmgi1* 突变体依旧显著推迟。利用酵母双杂交实验验证了蓝光受体蛋白 ZmFKF1a 与 *ZmGII* 存在互作。RNA-seq 转录组分析发现 *ZmMADS4* 是 *zmgi1* 突变体中下调且 *ZmGII*-OE 上调的 DEG 之一, 双荧光素酶报告系统和凝胶阻滞实验证明 *ZmGII* 蛋白与 *ZmMADS4* 启动子 G-box 元件结合并激活其表达。本研究结果表明, 玉米节律基因 *ZmGII* 通过激活 *ZmMADS4* 的表达, 正向调控玉米开花, 为进一步解析 *ZmGII* 参与调控玉米开花奠定了数据基础。

**关键词:** 玉米; 光周期; 开花时间; 生物节律

## Short Talk 5

# SNAC1 亚家族基因通过调控 SGs 的水平提高玉米耐盐性

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**摘要:** 我国有9913万公顷盐碱地, 盐胁迫是制约我国粮食产量和品质的重要环境因素。玉米是一种盐敏感性作物, 随着人口增长及粮食供应压力的增大, 研究和改良玉米耐盐性已成为植物学家和育种家面临的迫切任务。我们之前的研究表明TsNAC1在盐芥干旱、盐胁迫响应和根系的发育中起重要作用, 其在玉米中的同源基因是SNAC1亚家族基因, 深入研究该家族基因与玉米耐盐性之间的关系有重要意义。本研究通过生物信息学检索和表达模式分析确定了玉米ZmSNAC1、ZmNAC23和ZmNAC20是TsNAC1在玉米中的同源基因。利用这三个基因的玉米Mu插入缺失突变体和转基因过表达株系进行植株的生长发育和耐盐性分析; 利用DAPseq、ChIP-Seq、转录组分析和细胞学观察等手段解析它们各自的下游调控网络及生物学效应。玉米ZmSNAC1、ZmNAC23和ZmNAC20是TsNAC1在玉米中的同源基因。表型分析表明突变体*snac1*、*nac20*和*nac23-1*植株明显大于其对照自交系W22, 而转ZmSNAC1、ZmNAC23或ZmNAC20的过表达株系植株则显著小于受体自交系Qi319。在盐胁迫处理中, ZmNACs过表达植物的生长速度受抑程度小, 抗性强; 而突变体植株表现出生长变缓、叶色变黄等盐敏感症状。比较转录组、DAPseq、ChIP-Seq和蛋白互作结果表明三个NACs基因都在胁迫应答中起重要调控作用, 但调控的靶基因和互作的转录复合体蛋白又不完全相同。鉴定了一个它们共有的靶基因编码ZmUBP1c, 该蛋白参与胁迫应激颗粒(SGs)的形成与解聚, 使mRNA翻译过程减缓、暂停和恢复。细胞学比较实验发现, 盐胁迫处理时, SGs在突变体*snac1*、*nac20*和*nac23-1*中的积累水平显著高于对照材料和未处理组; 而ZmSNAC1、ZmNAC23和ZmNAC20的过表达材料则呈现降低的SGs水平。ZmSNAC1、ZmNAC23和ZmNAC20在玉米盐胁迫响应中有重要的调控作用, 是遗传改良的理想靶点。利用ChIP-Seq等方法确定了这3个NACs结合的顺时作用元件的核心序列, 下游靶基因GO富集显示出这3个NACs的候选靶基因主要富集在非生物刺激响应、转录调控和细胞生长等生物学过程。在遭遇盐胁迫时, 细胞通过调控SGs的形成与解聚继而调控细胞的翻译水平, 调整细胞代谢状态。SNAC1亚家族蛋白能结合ZmUBP1c的启动子并抑制其转录从而降低盐胁迫诱发的SGs水平, 在翻译水平上调控玉米盐胁迫响应, 进而提高玉米耐盐性。

**关键词:** 玉米; 盐胁迫; SNAC1 亚家族转录因子; 转录调控; 细胞应激颗粒 (SGs); 翻译调控

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## Short Talk 6

# 利用靶向测序-液相芯片（GBTS-LC）进行玉米穗腐病 QTL 定位

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**摘要:** 目前, 穗腐病在玉米主产区有高发趋势, 本试验通过定位与穗腐病相关的QTL并开发KASP标记, 用于抗穗腐病分子标记辅助选择育种。利用穗腐病感病材料B73和抗病材料CXS161构建含有159个家系的DH群体, 分别于2021年种植在海南三亚, 2022年种植在吉林公主岭、北京顺义和河南新乡, 并用籽粒注射法进行接种, 成熟后进行表型调查。通过5K中密度靶向测序-液相芯片获得基因型信息。于2022-2023年冬在三亚种植回交群体进行KASP标记的验证。四个环境下玉米穗腐病的广义遗传率分别为0.81、0.61、0.50和0.49, 综合广义遗传率为0.55。在SNP构建的图谱中定位到了分布于7条染色体的11个QTL, 每个QTL解释了3.13% -5.89%的表型变异, 其中一个QTL在两个环境中被定位到。利用靶向测序-液相芯片（GBTS-LC）技术产生的18511个mSNP构建遗传图谱, 定位到了分布于7条染色体的10个QTL, 每个QTL解释了3.47% -19.22%的表型变异。上述在两个环境中被同时定位到的QTL, 同样, 利用该图谱在两个环境下检测到。通过单标记分析法成功开发两个KASP标记, 其中一个标记可以有效检测玉米穗腐病的抗性。除了 $qMG22-2-1$ 外, 其余QTL解释的表型变异较少, 证明玉米穗腐病抗性受主效和微效QTL共同作用。另外, 相似的QTL结果以及解释更大的表型变异表明, 由靶向测序-液相芯片所产生的mSNP可以作为一种新的标记用于QTL作图。开发的KASP标记可以有效检测穗腐病抗性。本试验为精细定位和克隆相关基因提供了参考。

**关键词:** 玉米穗腐病; QTL; 靶向测序-液相芯片（GBTS-LC）; KASP 标记

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## Short Talk 7

### ***ZmHSFA2B* is critical for heat stress responses in maize**

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**Abstract:** The growth and development of maize (*Zea mays* L.) are significantly impeded by prolonged exposure to high temperatures. Understanding the physiological responses of plants to heat stress, as well as their adaptation mechanisms, is crucial in identifying pathways that can enhance plant heat tolerance and genes associated with heat tolerance. The objective of this investigation is to examine the regulatory pattern of gene expression and function of *ZmHSFA2B*. The present investigation employs molecular biology, molecular genetics, and biochemistry methodologies to explore the function and regulatory mechanism of *ZmHSFA2B*. We present genetic evidence to show that *ZmHSFA2B* is required for heat tolerance in maize. *ZmHSFA2B* has two splicing variants: *ZmHSFA2B-I* and *ZmHSFA2B-II*. *ZmHSFA2B-I* encodes the full-size *ZmHSFA2B* (*ZmHSFA2B-I*) and the *ZmHSFA2B-II* encodes a truncated *ZmHSFA2B* (*ZmHSFA2B-II*). Overexpression of *ZmHSFA2B-I* leads to a significant improvement in heat tolerance in both maize and *Arabidopsis thaliana* by inducing the expression of heat-responsive genes. Combining RNA-seq and ChIP-Seq analyses, we identified *ZmMBR1* as one of the putative targets of *ZmHSFA2B-I*. Overexpression of *ZmMBR1* in *Arabidopsis* enhances heat tolerance. We found that *ZmHSFA2B-II* is primarily synthesized in response to heat stress. *ZmHSFA2B-II* interacts directly with *ZmHSFA2B-I* and competitively forms heterodimers with *ZmHSFA2B-I*, thereby reducing the DNA binding activity of *ZmHSFA2B-I* homodimers to the promoter of *ZmMBR1*. Taken together, we propose that alternative splicing of *ZmHSFA2B* generates a self-regulatory loop fine-tune heat stress-responsive genes and heat tolerance in maize.

**Key words:** maize; heat stress; HSFs; alternative splicing

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## ***Short Talk 8***

# **Molecular Mechanism of miR166e-ZmATHB-14 Module in Regulating Maize Root Response to Drought Stress**

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**Abstract:** Drought is the main abiotic stress factor affecting the yield and quality of maize. Analyzing the molecular mechanism of drought response of maize is the premise of cultivating new drought-tolerant maize varieties. miRNAs can participate in the regulation of post-transcriptional gene expression by mediating the expression of target genes, and play a vital role in plant abiotic stress response and growth and development. In the early stage of this study, the small RNA, transcriptome and metabolome of the root system of maize inbred line H8186 under drought treatment were analyzed to screen the target gene miR166e and its target gene *ZmATHB14*. On this basis, 5'RACE and luciferase were used to complementarily verify the cleavage sites and interaction between the two; Yeast binary hybridization and bimolecular fluorescence were used to complementarily screen the interaction proteins of *ZmATHB14*; Yeast one-hybrid and luciferase were used to complementarily explore the downstream regulatory genes of *ZmATHB14*; The expression regulation mechanism of key genes in the miR166e-ZmATHB14 regulatory network in the drought response of maize at seedling stage was analyzed to identify and screen drought-tolerant maize mutants. This study provides a theoretical basis for the innovation of drought-resistant germplasm and the cultivation of new varieties of maize.

**Key words:** Maize; drought; miR166e; ATHB-14

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# Abstract

## *Abstract 1*

### **A New Allelic Variation and Developing Its Molecular Markers of *wx* Gene in Waxy Corn**

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**Abstract:** **【Objective】** Waxy corn germplasm resources are abundant, and some scarce *wx* gene allelic variations are easily ignored and lost in the process of waxy corn germplasm breeding. Exploring new allelic variations of the *wx* gene and developing its molecular markers provide a material basis for breeding new varieties of special corn. **【Method】** The *wx* gene was amplified and sequenced by PCR in this study and analyzed using bioinformatics methods. **【Result】** According to the sequence of exons 1 to 3, the *wx* genes of 18 core waxy corn inbred lines were divided into 9 haplotypes. The *wx*-301 inbred line had a 12 bp insertion at the nucleotide position of exon 301 in the *wx* gene, with the insertion sequence ACGTCCTCGGCG, which was a new *wx* gene allele variation. Waxy corn *wx*-301 is an inbred line derived from natural mutations in common corn during the breeding process. The *wx* gene is located on the short arm of chromosome 9, with a total length of 3130bp. Further PCR amplification and sequencing comparison of *wx*-301 and its wild-type, only a 12 bp difference was found in exon 2, further proving that the insertion of 12 bp was the reason why corn inbred line *wx*-301 becomes waxy. A pair of InDel markers have been developed based on difference of 301 loci. This PCR functional molecular marker could distinguish maize materials carrying *wx*-301 type homozygous allelic variation, heterozygous, and wild *Wx* genes. **【Conclusion】** This result suggests that the molecular markers developed in this study are effective to be used in selection of waxy corn varieties. The research results can promote the application of molecular marker assisted breeding in waxy corn.

**Key words:** Waxy corn; *wx* gene; Allelic variation; Molecular markers

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## Abstract 2

### *ZmSPLn* 调控玉米氮素利用的分子机制

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**摘要：**【目的】玉米是我国也是全球第一大作物，是重要的粮食、饲料和工业加工原料，近年随着畜牧业的发展，我国玉米供需缺口逐渐增大，在耕地有限情况下，大幅提高玉米单产，是保障我国粮食安全的首要任务。密植是提高单产的有效措施之一，但高密度种植会造成对养分的竞争，减少单株玉米可利用的氮素含量，因此提高玉米氮素利用效率对提高密植条件下的玉米产量极为重要。前期研究发现，SBP家族转录因子*ZmSPLn*过表达株系能在密植条件下产量保持单株产量不降低，但生物学机制尚不清楚。【方法】本研究首先使用含有不同浓度硝酸盐的营养液对苗期玉米进行处理，确定了*ZmSPLn*基因能够在苗期玉米的叶片中响应硝酸盐信号。并且*ZmSPLn*过表达株系在苗期经过低氮处理后地上部分含氮量显著高于野生型玉米。随后本研究通过转录组数据对*ZmSPLn*过表达株系及*ZmSPLn*敲除突变体#KO1与对照材料两叶一心时期根系差异表达基因进行分析，结合DAP-seq分析结果推测出*ZmSPLn*可能结合的氮素吸收利用相关下游基因，并通过酵母单杂交技术和烟草叶片瞬时表达技术进行验证。【结果】最后本研究确定了*ZmSPLn*能够直接结合并促进硝酸盐转运蛋白*ZmNPF6.3*和谷氨酰胺合成酶*ZmFGS1*的表达。同时，田间NUE试验表明将*ZmSPLn*基因过表达片段通过回交转育渗入到其他的现代玉米自交系中后极大地增加了整个植物在低氮环境的含氮量，其中*ZmSPLn*///Jing92过表达材料在低氮条件下的产量与正常条件下的产量没有显著差异。【结论】因此，*ZmSPLn*可能通过调节硝酸盐转运及谷氨酰胺合成途径来调控玉米对氮素的吸收利用。我们将进一步验证*ZmSPLn*与硝酸盐转运及谷氨酰胺合成途径的关系，探究其对玉米氮素吸收利用的调控作用，从而解析玉米氮素吸收利用的分子机理，对提高玉米在密植条件下氮利用效率有重要意义。

**关键词：**转录因子；SBP；氮素利用效率；硝酸盐转运蛋白

### *Abstract 3*

## **Analysis of Molecular Mechanism Regulating Maize Rough Dwarf Disease Resistance**

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**Abstract: 【Objective】** Maize rough dwarf disease (MRDD) is one of the most serious viral diseases in the world, which affects the yield of maize seriously, the main pathogen of maize in China is Rice Black Streaked Dwarf Virus (RBSDV), analyzing the regulatory molecular mechanisms from the perspective of the interaction between pathogens and host maize can provide theoretical support for the analysis of genetic mechanisms and the cultivation of disease resistant varieties. **【Method】** In this study, the gene was used as the target, The stable T<sub>2</sub> generation overexpressing and editing gene lines were artificially vaccinated to identify MRDD, and the transgenic lines with clear functions were screened; the reactive oxygenspecies (ROS), jasmonic acid (JA) content in leaves were measured; the network and molecular mechanism of *ZmHIR* gene involved in the regulation of MRDD resistance were regulated by binding transcription levels. **【Result】** 13 overexpression lines significantly increased resistance by one-level ( $P < 0.05$ ); The three gene-edited lines were significantly lower resistant than recipient control ( $P < 0.05$ ), the disease grade was decreased by one grade compared to the recipient control. It were able to burst out the ROS; JA content was significantly increased in overexpressed lines, but significantly decreased in gene-edited lines. After 2d inoculation, the expression of gene in the leaves of overexpressed strains was significantly up-regulated ( $P < 0.01$ ), while the expression of target gene in the gene-edited strains was significantly down-regulated ( $P < 0.05$ ). Differentially expressed genes (DEGs) are mainly involved in the regulation of signaling pathways such as plant hormones and  $\text{Ca}^{2+}$ . **【Conclusion】** After being infected with RBSDV, the gene caused ROS burst, its promoted the generation of JA, the series of physiological and biochemical reactions such as  $\text{Ca}^{2+}$  binding were activated to activate the HR and form the defense mechanism of MRDD.

**Key words:** Maize; Rough dwarf disease; Molecular mechanism

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## ***Abstract 4***

### **The small PPR protein SPR2 interacts with PPR–SMR1 to facilitate the splicing of introns in maize mitochondria**

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**Abstract:** Splicing of plant mitochondrial introns is facilitated by numerous nucleus-encoded protein factors. Although some splicing factors have been identified in plants, the mechanism underlying mitochondrial intron splicing remains largely unclear. In this study, we identified a small P-type pentatricopeptide repeat (PPR) protein containing merely four PPR repeats, small PPR protein 2 (SPR2), which is required for the splicing of more than half of the introns in maize (*Zea mays*) mitochondria. Null mutations of *Spr2* severely impair the splicing of 15 out of the 22 mitochondrial Group II introns, resulting in substantially decreased mature transcripts, which abolished the assembly and activity of mitochondrial complex I. Consequently, embryogenesis and endosperm development were arrested in the *spr2* mutants. Yeast two-hybrid, luciferase complementation imaging, bimolecular fluorescence complementation, and semi-in vivo pull-down analyses indicated that SPR2 interacts with small MutS-related domain protein PPR-SMR1, both of which are required for the splicing of 13 introns. In addition, SPR2 and/or PPR-SMR1 interact with other splicing factors, including PPR proteins EMPTY PERICARP16, PPR14, and chloroplast RNA splicing and ribosome maturation (CRM) protein Zm-mCSF1, which participate in the splicing of specific intron(s) of the 13 introns. These results prompt us to propose that SPR2/PPR-SMR1 serves as the core component of a splicing complex and possibly exerts the splicing function through a dynamic interaction with specific substrate recognizing PPR proteins in mitochondria.

**Key words:** PPR protein; maize seed development; mitochondria; intron splicing; splicing complex

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## *Abstract 5*

# **Distinct phenotypic plasticity for maize inbreds and hybrids determined by different sets of environmental and genomic variants**

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**Abstract: 【Objective】** Understanding phenotypic plasticity under natural conditions is critical for breeding crops that adapt to climate change. Recent studies have revealed that maize inbreds and hybrids exhibit substantial differences in the phenotypic plasticity; however, the underlying drivers behind the differences are unclear. Here, we investigated the environmental and genomic variants behind the differential phenotypic plasticity between inbreds and hybrids. **【Method】** We planted 456 inbreds and their corresponding 912 F1 hybrids (inbreds crossed with two elite testers) in multiple environments, and obtained genotype information as well as phenotypic data of three traits, days to anthesis (DTA), plant height (PH), and ear weight (EW). Using CERIS algorithm, we assessed the responses of inbreds and hybrids to 25 environmental factors. GWAS was conducted on the varied responses in inbred and hybrid populations, separately, for detecting the genetic basis of phenotypic plasticity. In addition, using CERIS-JGAR method we predicted inbreds and hybrids trait performance in new environments. **【Result】** We detected a weak plasticity correlation between inbred and hybrid populations. And the plasticity variation was greater among inbreds than among hybrids (e.g., the coefficient of variation for DTA was 19.98% in inbreds, but 6.03% in hybrids). To the same individual environmental factors, inbreds and hybrids showed differential responses in multiple ways, such as changes of direction, degree, or consistency. For instance, as DTR (diurnal temperature range) increased, flowering time decreased in inbreds while increased in hybrids, showing a direction change between inbreds and hybrids. We next identified different sets of genetic factors that explained the differential responses between inbreds and hybrids, as shown by genetic loci on chromosomes 1, 5, and 7 for hybrids while a locus on chromosome 2 for inbreds when responding to PAR (photosynthetically active radiation). Additionally, multiple environmental factors improved genomic prediction accuracy when predicting trait performance in new environments. **【Conclusion】** We revealed the environmental and genetic bases underlying the differential phenotypic plasticity between inbreds and hybrids. These results would enhance our understanding of phenotypic plasticity meanwhile help hybrid breeding in a changing climate.

**Key words:** phenotypic plasticity; gene and environment interaction; environmental factor; genome-wide association studies; phenotype prediction; inbreds and hybrids

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## Abstract 6

### Genome-wide association studies reveal the genetic basis of maize leaf and kernel ionome

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**Abstract: 【Objective】** Maize (*Zea mays* L.) is the most widely grown crop, globally, for food, feed, and fuel. Whilst the plant mineral nutrient and trace element composition are known to be essential for both plant growth and human health, the genetic basis underlying maize ionome remains largely unknown. **【Method】** Here, we profiled the ionome contents of maize leaf and kernel in a Complete-diallel design plus Unbalanced Breeding-like Inter-Cross (CUBIC) population and found that maize ionome varied widely across genotypes, tissues, and locations.

**【Result】** We identified 602 loci associated with natural ionic variations through genome-wide association studies, especially 328 for kernels, providing new insights into the genetic basis of ionic diversity in maize kernels. Among them, we first validated that *ZmMOT1* is the causal gene for the natural variation of molybdenum (Mo) content in both leaf and kernel. Moreover, we identified a nicotianamine (NA) efflux transporter named *ZmNAT1* that controls the natural variation of nickel (Ni) concentration in maize kernel and provided the first genetic evidence that Ni is irreplaceable for seed germination and yield traits. **【Conclusion】** As such our study provides a solid genetic basis for the natural variation of maize ionome and represents a treasure chest for molecular breeding of yield-secure and nutritional maize.

**Key words:** Maize; Ionome; GWAS; Ni; Mo

## Abstract 7

# Molecular mechanism of heat stress mediated by *ZmMKK1* in maize

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**Abstract: 【Objective】** High temperature is a major environmental stress that affects plant growth and crop yield. The mitogen-activated protein kinase kinase (MAPKK) is widely involved in plant responses to various environmental stresses, and plays key roles in plant growth and stress signaling, but their involvement in the High temperature stress response is poorly understood.

**【Method】** In our previous research, mutant seeds of eight genes in maize MAPKK family were obtained by querying the EMS mutant library, the high temperature sensitive mutant *zmmkk1* was screened. To further elucidate *ZmMKK1*'s function, we created knockout mutant of *ZmMKK1* using CRISPR/Cas9 system. **【Result】** knockout of *ZmMKK1* in maize was also found to be sensitive to high temperature. RT-qPCR analysis showed that *ZmMKK1* was expressed in all surveyed tissues and organs. In root, *ZmMKK1* showed the highest expression level. Subcellular localization analysis showed that *ZmMKK1* was localized in chloroplast in maize protoplasts. We performed a yeast two-hybrid screen and further demonstrated that catalase *ZmCAT2* could interact with *ZmMKK1* in vitro and in vivo, suggesting that *ZmMKK1* may regulate maize response to high temperature stress through *ZmCAT2*. **【Conclusion】** This project is not only helpful to our understanding for the mechanisms how MAPKK is involved in high temperature and in plant response to stress, but also provide an important basis for improving maize tolerance.

**Key words:** *ZmMKK1*; Maize; High temperature

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## Abstract 8

# Genome-wide association study presents insights into the genetic architecture of drought tolerance in maize seedlings under field water-deficit conditions

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**Abstract: 【Objective】** Drought stress is one of the most serious abiotic stresses leading to crop yield reduction. Due to the wide range of planting areas, the production of maize is particularly affected by global drought stress. The cultivation of drought-resistant maize varieties can achieve relatively high, stable yield in arid and semi-arid zones and in the erratic rainfall or occasional drought areas. Therefore, to a great degree, the adverse impact of drought on maize yield can be mitigated by developing drought-resistant or -tolerant varieties. However, the efficacy of traditional breeding solely relying on phenotypic selection is not adequate for the need of maize drought-resistant varieties. Revealing the genetic basis enables to guide the genetic improvement of maize drought tolerance. **【Method】** We utilized a maize association panel of 379 inbred lines with tropical, subtropical and temperate backgrounds to analyze the genetic structure of maize drought tolerance at seedling stage. We obtained the high quality 7837 SNPs from DArT's and 91,003 SNPs from GBS, and a resultant combination of 97,862 SNPs of GBS with DArT's. The maize population presented the lower heritabilities of the seedling emergence rate (ER), seedling plant height (SPH) and grain yield (GY) under field drought conditions. **【Result】** GWAS analysis by MLM and BLINK models with the phenotypic data and 97,862 SNPs revealed 15 variants that were significantly independent related to drought-resistant traits at the seedling stage above the threshold of  $P < 1.02 \times 10^{-5}$ . We found 15 candidate genes for drought resistance at the seedling stage that may involve in (1) metabolism (*Zm00001d012176*, *Zm00001d012101*, *Zm00001d009488*); (2) programmed cell death (*Zm00001d053952*); (3) transcriptional regulation (*Zm00001d037771*, *Zm00001d053859*, *Zm00001d031861*, *Zm00001d038930*, *Zm00001d049400*, *Zm00001d045128* and *Zm00001d043036*); (4) autophagy (*Zm00001d028417*); and (5) cell growth and development (*Zm00001d017495*). The most of them in B73 maize line were shown to change the expression pattern in response to drought stress. These results provide useful information for understanding the genetic basis of drought stress tolerance of maize at seedling stage.

**Key words:** maize (*Zea mays* L.); genome-wide association study; seedling stage; field drought tolerance; SNPs

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## Abstract 9

### Discovery of Kernel Row Number2 that enhances grain yield and its application in maize breeding

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**Abstract: 【Objective】** Increasing maize yield per unit area plays an important role in food demands and security. As a key component of maize yield, kernel row number (KRN) has a direct effect on improving maize yield per unit area. Therefore, understanding the genetic basis of KRN and cloning the gene controlling KRN are of great significance for molecular breeding of new maize varieties with high yield. **【Method】** Following a recombinant-derived progeny testing strategy, we performed positional cloning of *qKRN2* for KRN identified in a maize recombinant inbred line (RIL) population developed from a cross between an inbred line, B73, and an introgression line, MT-6, of which ~25% of its genome is derived from that of teosinte, the wild ancestor of maize. **【Result】** We delimited *qKRN2* to a 5799–base pair (bp) region that contained only one candidate gene, which we named *KRN2* (Kernel Row Number2). Selection in the noncoding upstream regions resulted in a reduction of *KRN2* expression and an increased grain number through an increase in kernel rows. *KRN2* encodes a WD40 protein and function synergistically with a gene of unknown function, DUF1644, by protein-protein interaction. Field tests show that knockout of *KRN2* in maize increased grain yield by ~10%, with no apparent trade-off in other agronomic traits. To further test whether the genetic effect of *KRN2* on KRN is affected by the genetic background, we used two strategies for molecular breeding—CRISPR/Cas9-mediated gene editing and marker assisted selection (MAS)—to improve a set of maize inbred lines. Compared with the corresponding wild-types, most of the improved lines showed an increased KRN by ~0.8 to 2.1 rows. Subsequently, we will develop the improved hybrids with null allele of *KRN2* and estimate the genetic effect in maize hybrids. **【Conclusion】** In summary, we identified *KRN2* that enhanced grain yield in maize and had potential application in maize breeding. These findings provide important theoretical basis and germplasm resource to breed new maize varieties with high yield.

**Key words:** Maize; *KRN2*; Grain yield; Gene editing; Molecular breeding

## Abstract 10

# WRKY63 调控 *ZmTPS2* 基因表达增加 DMNT 和 TMTT 的释放提高玉米抗虫性

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**摘要:** 【目的】萜类合成酶 (terpene synthase, TPS) 基因 *ZmTPS2* 是玉米重要的虫害防御基因, 但目前对于其表达调控和抗虫机制等方面缺乏了解。因此系统深入的研究 *ZmTPS2* 的表达调控和抗虫机制将为玉米抗虫分子育种及创建生态防控系统提供理论基础和技术支撑。

【方法】本研究采用实时荧光定量PCR方法检测目的基因的表达, GUS组织化学染色及酶活分析检测 *ZmTPS2* 启动子活性, 转录组分析差异表达基因, 酵母单杂交、原生质体瞬时表达分析和凝胶阻滞实验筛选并确定与 *ZmTPS2* 启动子互作的转录因子, 转基因和CRISPR技术获得 *ZmTPS2*-OE和 *ZmTPS2*-KO玉米材料, GC-MS和昆虫行为选择实验检测玉米挥发物及其对昆虫的行为影响。【结果】*ZmTPS2* 基因受虫害和JA诱导表达, 其起始密码子上游227bp具有全长启动子诱导活性。酵母单杂交筛选与 *ZmTPS2* 共表达的转录因子, 获得6个转录因子能够与 *ZmTPS2*P互作 (其中3个WRKY转录因子, 2个NAC转录因子, 1个EREB转录因子)。玉米原生质体瞬时表达分析和凝胶阻滞实验确定WRKY63能够结合 *ZmTPS2*P的-195至-180区段激活下游基因表达。*ZmTPS2*-OE转基因玉米材料中DMNT和TMTT的释放量与对照相比显著升高, 分别达到 $14.50 \pm 0.79$  ng/g h和 $88.73 \pm 7.87$  ng/g h, 而 *ZmTPS2*-KO材料中DMNT未检测到, TMTT的含量为 $6.68 \pm 3.08$  ng/g h, 与对照材料相当。昆虫行为选择实验结果表明与 *ZmTPS2*-KO材料相比, *ZmTPS2*-OE转基因玉米对玉米螟雌蛾和寄生蜂具有显著的吸引效果, 对玉米螟幼虫具有显著的驱避效果。【结论】玉米WRKY63转录因子通过与 *ZmTPS2* 启动子中的-195至-180序列结合激活 *ZmTPS2* 基因的表达及DMNT和TMTT的释放, 增强玉米对寄生蜂的吸引及对玉米螟幼虫的驱避效果, 提高了玉米的抗虫性。

**关键词:** 玉米; WRKY63; TPS2; DMNT; TMTT; 抗虫

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## ***Abstract 11***

### **The molecular mechanism of *KRN2* regulating kernel row number in maize**

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**Abstract:** Kernel row number (KRN) is an important component factor of maize yield. We previously found that a WD40 protein, KENEL ROW NUMBER2 (*KRN2*), interacted with a gene of unknown function, DUF1644, to negatively regulate KRN in maize. However, the regulatory mechanism of *KRN2* remains unknown. In this study, we conducted one-to-one verification by split firefly LUC complementation assays in tobacco on 41 potential interaction proteins previously identified from a yeast two-hybrid (Y2H) screen. Eight interactors of *KRN2* were confirmed, including zinc knuckle (CCHC-type) family protein, pentatricopeptide repeat-containing protein, transcript variant X1, and etc. Expression profiles of these interactors showed that all of them were expressed in ear. Subsequently, we will generate their single mutant by CRISPR/cas9 technology to test whether they influence KRN. Besides, we will conduct one-to-one verification by yeast two-hybrid system and CO-IP to further confirm their interactions with *KRN2*. Based on the structure of WD40 proteins and the interaction function of *KRN2*, we speculate that it may act as a scaffold protein for protein-protein interaction with many other proteins involved in diverse biological processes to regulate KRN. Thus, we will explore how *KRN2* interacts with these proteins by double mutants, even triple or quadruple mutants, and etc. In addition, we will identify the differentially expressed genes (DEGs) between wild-type plants and single and multiple mutants via single cell sequencing, and select some key DEGs to verify their functions.

**Keywords:** KRN; *KRN2*; WD40 protein; molecular mechanism



## Abstract 12

# Map-Based Cloning and genetic dissection of the major effect QTL-qRSL8 associated with lodging resistance in maize

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**Abstract: 【Objective】** Lodging resistance is one of the main target traits selected in maize breeding. According to statistics, the annual yield loss caused by lodging is up to 5-20%, the loss is nearly 1 million tons, and for every 1% increase in lodging rate, the ear fall rate increases 0.12%-0.59%, which seriously affects the efficiency of mechanized harvesting. However, due to its complex genetic dissection, only a few anti-lodging-related QTL genes have been cloned by map-based cloning. **【Method】** In this study, the F<sub>2</sub> population constructed by ZH422 inbred line with high lodging resistance and CC093 inbred line with lodging-susceptible was used as experimental material to analyze the genetic characteristics was conducted on two traits, namely, the rind penetrometer resistance(RPR) with the third internode on the ground and the third internode under the main spike. QTL mapping was performed by using the interval mapping method in R/qtl, and fine mapping was performed by using the method based on the recombinant-derived progeny-testing strategy. Candidate genes were targeted and validated by CRISPR knockout and overexpression of *qRSL8*. Then ChIP-seq and RNA-seq were used to screen downstream genes for experimental verification. **【Result】** Two major QTLs were co-located in two traits, which were named *qRSL8* and *qRSL9*. After three consecutive seasons of fine mapping, *qRSL8* was located in a 7.6kb interval. Bioinformatics analysis found that there was only one gene in this interval, encoding a MYB transcription factor, and expression trend analysis showed that the expression of this gene in the stalk of ZH422 was significantly higher than that of CC093, indicating that this gene was involved in the development of early maize stalks. **【Conclusion】** In conclusion, a MYB transcription factor was identified to regulate maize stalk strength by fine mapping, and the expression of *qRSL8*<sup>ZH422</sup> was higher than that of *qRSL8*<sup>CC093</sup> at the elongation stage, which may play a role in the early stage of maize stalk development.

**Key words:** Maize; stalk lodging resistance; RPR; MYB transcription factor

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## 时空转录和染色质可及性图谱揭示玉米成花转变的 调控机制

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**摘要：**【目的】玉米从营养生长向生殖生长的转换过程称为成花转变，该过程深度影响玉米的开花期和株型建成等方面，并主要受茎尖分生组织发育分化进程的影响。因此，为了解析玉米成花转变的调控机制，【方法】本研究对播种后8天（DAP8）至DAP28的茎尖分生组织进行8个时间点的连续性取材，并获得了高质量的RNA-seq和ATAC-seq数据。【结果】分析发现DAP8向DAP12茎尖分生组织的分化过程发生了较为剧烈的基因表达变化，其中成花抑制因子*ZmRAP2.7*和*ZmCOL3*显著下调表达。有趣的是，DAP14到DAP22相邻时间点分生组织间差异表达基因（DEGs）数目变化不大，但*ZMM4*、*ZAP1*和*DLF1*等成花促进因子显著在DAP16上调表达且表现持续升高水平。此外，DAP22和DAP26间DEGs数目也相对较多，其中成花促进因子*ZFL1*、*ZFL2*，花序分枝促进因子*UB2*、*UB3*、*LG1*、*LG2*在DAP26显著上调表达，说明其在成花转变后期以及花序形态建成初期起到重要调控作用。最后我们发现染色质重编程与分生组织发育分化过程的基因表达动态相关，基因调控区可及性的改变塑造了不同的转录因子调控模式，从而影响分生组织发育分化进程。【结论】以上结果，说明玉米中成花转变是多个关键因子在不同时间节点协同调控的持续过程，加深了我们对玉米成花转变形态建成生物学过程的理解。

**关键词：**茎尖分生组织；成花转变；染色质可及性

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## 玉米群体非编码区的遗传调控机制解析

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**摘要:** 【目的】在玉米群体水平鉴定苗期叶片lincRNA并分析其分子特征, 比较其与蛋白质编码基因调控模式的异同, 解析lincRNA基因如何通过调控蛋白质编码基因从而影响表型变异, 同时挖掘可能影响表型变异的候选基因。【方法】本研究利用234份关联群体苗期叶片的RNA-seq数据集对转录本进行组装, 基于基因位置、长度和蛋白质编码能力在全基因组鉴定lincRNA基因, 比较lincRNA基因与蛋白质编码基因的分子特征的差异, 基于eQTL分析比较调控模式的差异, 利用共定位解析lincRNA基因如何调控蛋白质编码基因, 探究lincRNA基因如何影响表型。【结果】全基因组鉴定了位于基因间区的293,298个候选lincRNA基因。这些lincRNA基因在染色体上广泛分布, 与邻近蛋白质编码基因的距离主要分布在25kb附近, 在基因组的覆盖度约为蛋白质编码基因的3倍。结合这些lincRNA在群体中的表达情况, 认为有59,996个lincRNA基因具有高置信度。这些高置信度的lincRNA与蛋白质编码基因在基因的长度、外显子数目、外显子长度、转录本长度、具有同样外显子数目的转录本长度和CG含量等特征下均有显著差异。lincRNA基因具有eQTL的比例更低, 具有的cis-eQTL的数目更少,  $R^2$ 更低。与基因最近的eQTL区间相比, lincRNA更靠近蛋白质编码基因, 17.6%的蛋白质编码基因的eQTL区间与lincRNA重叠。【结论】与蛋白质编码基因相比, lincRNA的表达水平更低, 但变异更大。与蛋白质编码基因相比, lincRNA基因具有相对较短基因序列, 外显子数目更少、以单外显子为主、具有相对较长的外显子等, 二者在多类分子特征上具有显著差异, 说明其发挥功能的方式存在不同。lincRNA基因受到的调控更少, 调控模式更简单。lincRNA可能通过调控蛋白质编码基因影响表型变异。

**关键词:** 玉米; 长链非编码 RNA; 基因表达; 表型变异

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## Abstract 15

### ***ZmBRCB5* regulates the branch RATIO of carotenoid biosynthesis in maize kernels**

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**Abstract: 【Objective】** Vitamin A deficiency (VAD) is a widespread nutritional deficiency that affects a large number of people globally. Supplementation of vitamin A is effective in reducing the risk of blindness among preschool children and the occurrence of age-related macular degeneration in the elderly population. **【Method】** In the carotenoid metabolism pathway, RATIO depends on the total carotenoid contents of  $\alpha$ - and  $\beta$ -branches. In this study, we performed resequencing analysis, constructed CRISPR/Cas9 and overexpressing materials of *ZmBRCB5*, subcellular localization by Immuno-gold electron microscopy and immunofluorescence, molecular chaperone experiment and a split firefly LUC complementation assay to identify the function of *ZmBRCB5* that influence the branch RATIO in maize kernels. **【Result】** We previously identified *ZmBRCB5* significantly associated with the branch RATIO of carotenoid biosynthesis in maize kernels via a genome-wide association study in a set of 487 diverse yellow inbred lines, resequencing results of *ZmBRCB5* once again confirmed this conclusion. Next, we confirmed that *ZmBRCB5* positively affected the branch RATIO of carotenoid biosynthesis via overexpression and knockout of *ZmBRCB5*. *ZmBRCB5* has a conservative C2-C2 type zinc finger domain in many species via sequence alignment analysis. *ZmBRCB5* encodes a putative chaperone protein and is located in the endosperm starch granule. Furthermore, a split firefly LUC complementation assay in tobacco confirmed the interaction between *ZmBRCB5* and crtRB1 and LCYE, suggesting that *ZmBRCB5* regulates RATIO by interacting with crtRB1 and LCYE. **【Conclusion】** These results enrich our understanding of the molecular mechanism of carotenoid synthesis in maize kernels.

**Key words:** association analysis; carotenoids; *ZmBRCB5*; chaperone; maize

## 玉米萌发期耐低温相关基因功能的初步解析

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**摘要:** 【目的】低温是我国北方春玉米区重要的非生物逆境之一, 严重影响玉米的产量和品质。挖掘玉米耐低温相关基因, 进而选育耐低温新品种是解决这一问题最经济有效的措施。【方法】本研究以本课题组前期基于转录组测序挖掘到的玉米萌发期耐低温相关关键基因为目标, 明确该基因的时空表达模式、亚细胞定位和启动子活性并且利用过表达该基因的拟南芥进行耐低温功能验证明确其功能。【结果】主要在玉米自交系的萌发期的胚、芽期的芽和苗期的叶中有所表达, 且均在低温处理 6h 后响应低温胁迫具极显著的差异 ( $P<0.01$ ); 亚细胞定位表明, 转化空载体的对照组样品的绿色荧光信号存在于玉米原生质体内, 而融合蛋白的绿色荧光信号则出现在原生质体的细胞质区域内; 启动子活性分析结果表明, 过表达 *ZmPHLD1* 基因的拟南芥在萌发期低温处理下的相对发芽率显著高于受体对照 ( $P<0.05$ ), 在苗期低温处理下的相对根长、相对根表面积、相对叶长、相对叶表面积和相对叶平均直径也显著高于受体对照 ( $P<0.05$ )。【结论】以上结果表明, 在高耐低温玉米自交系早 8-3 萌发期的胚、芽期的芽、苗期的叶和根中的相对表达量在大部分时间点显著高于高度敏感玉米自交系吉 853 ( $P<0.05$ ); 转基因拟南芥在低温处理下萌发期和苗期的表型性状显著优于受体对照, 说明该基因过表达可以提高拟南芥萌发期和苗期的耐低温能力。

**关键词:** 玉米; 耐低温; 萌发期; 功能分析

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## PEPCK 途径增强玉米光合作用

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**摘要:** 【目的】解析玉米光合作用中 PEPCK 途径的生物学功能。【方法】利用转基因技术获得 PEPCK 途径被显著抑制或增强的 RNAi 或过表达玉米株系, 观察植株在阴影及全日照下的表型, 对阴影及全日照条件下的叶片样本进行生理生化分析, 利用转录组学、蛋白质组学、代谢组学、脂质组学等多组学复合分析玉米 PEPCK 途径受抑制叶片中的基因表达、蛋白丰度及物质变化。【结果】玉米 PEPCK 途径被抑制后, 在全日照条件下生长的 RNAi 植株比野生型生长延迟, 叶片浅绿, 叶片中的叶绿素含量显著降低; 而在阴影条件下生长的植株与野生型无显著差异。全日照条件下, RNAi 株系叶片中含氮类物质, 如氨基酸、核苷酸等含量显著降低, 可溶性总蛋白含量显著降低, 氮同化关键酶、氨基酸代谢过程关键酶及含氮物质转运蛋白在基因、蛋白或翻译后修饰水平发生显著变化, 暗示玉米 PEPCK 途径被抑制导致叶片氮代谢过程被抑制; 淀粉含量显著增加, 酮戊二酸含量显著增加; 光呼吸产物丝氨酸和苏氨酸含量增加, 4 个光呼吸过程关键酶的蛋白丰度显著提高, 暗示光呼吸过程增强; 大量转录组学、蛋白质组学及磷酸化修饰组学结果中显著差异的基因或蛋白定位于膜系统, 脂质组学结果表明大量磷脂, 尤其是含多个不饱和键的磷脂, 含量显著降低, 而三酰甘油含量显著增加, 暗示 PEPCK 途径被抑制后影响膜脂代谢及膜稳定性; PEPCK 途径被增强 (过表达株系) 后, 叶片中可溶的总蛋白含量及淀粉含量显著增加。【结论】玉米 PEPCK 途径驱动叶片中高光下的氮同化过程, 与 NADP-ME 途径协同作用, 实现玉米叶片碳、氮同化耦合, 使玉米在宽泛的光谱下保持高光合效率。

**关键词:** C4 PEPCK;  $\text{NO}_3^-$  同化; 光呼吸; 氮利用效率

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## 玉米 *ZmARF1* 响应缺磷胁迫的功能研究

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**摘要:**【研究背景】磷是作物正常生长发育所必需的大量营养元素之一。生长素在植物的主要生长发育过程中起着重要的调控作用,其作用主要是由生长素响应因子(ARFs)介导的。本研究利用 356 个玉米自交系进行候选基因关联分析,以确定 *ZmARF1* 多态性与玉米低磷性状之间的显著关联。构建了过量表达 *ZmARF1* 和 *zmarf1* 玉米突变体的转基因玉米和拟南芥品系,探究了 *ZmARF1* 在生长发育和响应磷胁迫中的功能缺失和获得,并揭示了其调控功能的分子机制。【材料与方法】本研究利用拟南芥 *Col-0* 和玉米 KN5585 进行遗传转化的生物学功能验证,结合转录组测序、Y1H、Y2H 和 BiFC 等实验筛选并验证 *ZmARF1* 的靶基因及互作蛋白。【结果与分析】与野生型相比,过表达的玉米和拟南芥品系有更好的生长表现和根的发育,而 *zmarf1* 玉米突变体表现为生长和根系发育减少。转基因玉米和拟南芥在缺磷处理下 *ZmARF1* 蛋白的积累更高, Pi 转运相关基因 *PHR1*、*PHT1*; 2 和 *PHO1*; 2 的转录水平显著提升, *ZmARF1* 的过表达明显提高了对磷胁迫的耐受性。转录组结果发现, *ZmLBD1* 是 *zmarf1* 突变体中下调的差异化表达的基因之一, Y1H 和 EMSA 试验证实 *ZmARF1* 和 *ZmLBD1* 的启动子之间的相互作用。【结论】*ZmARF1* 调控植物的生长发育,提高了植物对低磷胁迫的耐受性。*ZmARF1* 调控根发育的分子机制,转录组分析显示 *ZmLBD1* 是 *ZmARF1* 潜在的下游靶点。Y2H 和 BiFC 实验表明, *ZmARF1* 蛋白与 *ZmILR4* 相互作用,可合成并水解共轭 IAA,导致生长素永久失活或暂时储存,促进根的发育。

**关键词:** 玉米; 磷; 胁迫; *ZmARF1*; 转录因子

## 气候与组学数据共同解析玉米开花期适应性的遗传基础

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**摘要:** 【目的】气候变化严重威胁玉米生长和产量提升, 但鲜有研究从多组学角度探索玉米气候适应性的遗传基础。开花期是玉米区域适应的关键性状, 阐明影响开花期的关键环境和遗传因素, 有助于解释玉米适应环境的模式与遗传基础。本研究的目标有以下三点: ①挖掘影响开花期的关键环境和遗传因素; ②从基因表达的层面鉴定与开花期相关的基因; ③探索如何利用气候与组学数据, 解释开花期表型变异。【方法】在这项研究中, 我们将一份关联群体 (包含温带和热带材料, 一共484份自交系) 跨纬度梯度种植在了6个环境, 测定了每个材料的散粉期, 并且针对成熟叶和幼叶组织进行取样, 测定了每份样品的RNA-seq。同时, 我们从NASA Power数据库下载了覆盖玉米全生育阶段, 与光温水气土壤相关的25种环境数据。【结果】首先, 我们以开花期为研究对象, 评估了开花期的表型可塑性, 发现热带材料比温带材料的可塑性更高。通过将25种环境因子与开花期建立关联, 我们鉴定到多个环境指数, 如APAR<sub>56-67</sub> (种植后第56到67天的光合有效辐射), 能完美解释种植环境间的开花期变异 ( $R^2=0.992$ )。用APAR<sub>56-67</sub>与开花期建立线性模型, 结果表明种植后第56-67天的APAR每升高1个单位, 整个群体推迟开花0.75天。将开花期可塑性作为表型, GWAS分析鉴定到一个结构变异与可塑性显著关联, 该结构变异位于ZmCCT10上游2.3Kb, 前人报道是一个转座子插入。其次, 利用成熟叶和幼叶组织的表达数据进行全转录组关联分析 (TWAS), 我们鉴定到很多基因的表达与开花期显著关联, 其中包含了已知的开花期基因, 比如: ZmCCT10、ZCN8、MADS69、ZmELF3.1等。最后, 我们联合GWAS与TWAS解释开花期表型变异。我们发现TWAS解释开花期表型变异的综合效力强于GWAS, 联合GWAS与TWAS, 能够解释开花期表型变异的63.3~83.0%。【结论】我们的研究从环境和遗传的角度阐述了玉米开花期适应性的遗传基础, 期望该研究为未来培育具有气候韧性的育种材料提供理论基础。

**关键词:** 玉米; 气候适应性; 转录组; 开花期; 表型可塑性

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## ZmIAA15 基因在烟草中的功能研究

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**摘要:** 【目的】生长素受Aux/IAA基因家族的调控, 具有调节植物的生长发育, 提高植物的抗盐抗旱性的功能。因此探究生长素相关基因*ZmIAA15*在烟草中根系发育及对干旱和盐胁迫的响应, 可为后续*ZmIAA15*基因在玉米中的功能研究及其抗旱耐盐机理的解析, 抗旱耐盐品种的遗传改良提供一定的理论基础。【方法】本文以玉米自交系昌7-2为材料克隆了*ZmIAA15*基因, 构建了pCambia1300-*ZmIAA15*的双元表达载体, 通过农杆菌介导, 烟草叶盘转化法转到烟草中, 获得了*ZmIAA15*基因过表达的T<sub>3</sub>烟草株系, 对其功能进行了表型和分析。构建了亚细胞定位载体, 对烟草叶片进行了瞬时转化。【结果】*ZmIAA15*在玉米不同组织器官中均有表达, 其中根中的表达量最高, 其次为茎和叶。*ZmIAA15*的表达受到了盐胁迫和干旱胁迫的诱导。*ZmIAA15*基因所编码的开放阅读框序列长度为663 bp, 编码220个氨基酸, 编码的蛋白质相对分子量为23.48 kDa, 理论等电点为7.77, 且在细胞核发挥重要作用。*ZmIAA15*基因在本生烟烟草中过表达后, 其10 d龄根长均显著大于野生型本生烟; 在一月龄的转基因本生烟中, 其株高、茎粗、地上鲜重和地下鲜重均显著增加。在转基因本生烟中, 与植物生长发育相关基因ROT3和AN3的表达量显著高于野生型。在200 mmol/L甘露醇和120 mmol/L NaCl两种胁迫处理下, 过表达*ZmIAA15*基因本生烟萌发率均显著高于野生型; 利用350 mmol/L NaCl和20% PEG6000处理T<sub>3</sub>转基因本生烟和野生型本生烟幼苗, 发现经盐和干旱两种逆境胁迫处理后, 野生型烟草相较于转基因烟草叶片萎蔫更加明显, 茎粗、地上鲜重、叶面积、地下鲜重和根长等表型性状也显著低于过表达株系; 转基因株系中叶绿素含量显著增加, 保护酶POD、SOD的活性显著提高, 而MDA含量显著降低。【结论】*ZmIAA15*基因在烟草中的过表达可以促进烟草的生长发育, 并响应干旱和盐的逆境胁迫, 增强了烟草植株的抗旱耐盐能力。

**关键词:** 烟草; 玉米 *ZmIAA15*; 抗旱性; 耐盐性

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## ***ZmKOB1* 调控玉米雌穗发育与产量的遗传机理研究**

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**摘要:** 玉米是我国最大的粮食作物之一, 由于人口增加但耕地面积有限, 提高玉米单产仍是当下保障国家粮食安全的重要任务。玉米单产主要由单位面积种植密度、穗粒数与穗粒重关键三要素决定, 其中穗粒数由行粒数和穗行数决定, 而行粒数又由穗长等关键性状决定。在本研究前期, 通过对中美近几十年不同育种时期广泛使用的350份玉米自交系的GWAS分析, 我们发现了一个在育种过程中受到选择的基因, 与拟南芥*ABI8/KOB1*基因同源性较高, 故命名为*ZmKOB1*。该基因编码蛋白含有保守糖基转移酶结构域, 且在玉米父母本杂优群中受到选择分化。我们创制了该基因的基因编辑敲除突变体与超表达材料, 发现其突变体V9期雌穗IM组织长度显著缩短, 超表达植株IM组织长度显著增长。进一步统计相关表型, 我们发现突变体的行粒数、穗长与穗粒重明显减少, 而超表达株系则明显增多。有趣的是, 我们将超表达材料与对照材料, 分别与6个优良自交系(郑58、昌7-2、伟科702F、PH4CV、京92和B73)杂交, 构建了12个F<sub>1</sub>杂交组合。结果表明, 与对照材料相比, 与超表达材料杂交的F<sub>1</sub>后代产量相关指数显著提高。因此, *ZmKOB1*还能提高杂交种产量。综上所述, 我们发现了一种编码糖基转移酶的基因*ZmKOB1*, 它是玉米果穗长度、行粒数、穗重和穗粒重等关键产量性状的正向调控因子, 具有巨大的育种利用价值, 但其调控机制尚不清楚。未来我们将进一步通过相关生化、分子生物学与遗传学实验, 构建*ZmKOB1*的糖基化修饰与基因调控网络, 并研究其在杂交育种中的生产应用。

**关键词:** 玉米; 雌穗发育; 产量

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## 丝轴黑粉菌侵染玉米雄穗症状差异生理机制分析

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**摘要：**【目的】玉米丝黑穗病是由丝轴黑粉菌（*Sporisorium reilianum*）引起的真菌性土传病害。目前国内外关于该病的研究主要集中于抗性基因的机制解析，并明确了该基因的抗性机制。关于该病病症的发生及病原与寄主的互作机制前人也进行了初步解析，但对感病材料间显症症状差异的发生机理研究尚未见报道。【方法】本试验选择 55 份含四平头血缘的玉米自交系为材料验证其接种后雄穗症状，对 3 个侵染丝轴黑粉菌后雄穗不同症状典型玉米自交系黄早四、京七、昌 7-2 酶活性、激素及差异表达蛋白分析，初步探索雄穗不同症状形成机制。【结果】酶活性研究发现，抗病对照自交系 Mo17 酶活性变化主要发生在 VE 期，后期酶活性变化不显著。3 个典型玉米自交系在接种后 POD、SOD、PAL 活性均上升，材料间差异达到极显著水平。其中 POD、SOD 活性变化趋势相似，与雄穗不同显症症状形成关系密切，增长幅度昌 7-2 最高而黄早四最低，且均在 V12 期增幅达到高峰。对 V4、V8、V12、VT 期生长点或雄穗 GA<sub>3</sub>、IAA、ABA 含量变化进行分析，抗病对照自交系 Mo17 接种后激素含量变化各时期均不显著，3 个典型玉米自交系在接种后激素含量均发生改变，GA<sub>3</sub>、IAA 含量的变化与侵染玉米丝轴黑粉菌后雄穗不同显症症状密切相关。黄早四与京七、黄早四与昌 7-2、京七与昌 7-2 间分别存在 44、259、238 个差异表达蛋白，3 个自交系间共同差异表达蛋白 23 个。比较发现差异表达蛋白主要分布在膜系统与细胞质中，参与次生代谢产物合成等代谢通路，A5H8G4、P09233、Q8VXG7 等差异表达蛋白与 POD、SOD、PAL 含量变化相关，P49353、P13689、P10979 等差异表达蛋白与 GA<sub>3</sub>、IAA、ABA 含量变化相关。【结论】V12 期可能不仅是侵染丝轴黑粉菌后玉米雄穗不同显症症状形成的关键时期，也是雄穗不同症状典型自交系间激素含量产生差异的关键转折点，ROS（活性氧）与 NO 信号的爆发引起膜结构破坏与次生代谢产物的合成可能是玉米侵染丝轴黑粉菌后造成雄穗症状差异的主要原因。

**关键词：**玉米；丝轴黑粉菌；显症症状；机制

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## Abstract 23

### Development and validation of molecular markers targeting the *ZmWRKY48* gene

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**Abstract:****【Objective】**To investigate the relationship between sequence variations of *ZmWRKY48*, an important gene related to cold tolerance during maize germination, and resistance, to develop molecular markers targeting key loci, and to verify its feasibility, providing theoretical and technical support for the breeding of new maize varieties with cold tolerance during germination.

**【Methods】** By amplifying the CDS region of the *ZmWRKY48* gene from 40 maize inbred lines with different levels of cold tolerance, we performed sequence variation analysis, and identified key SNPs for cold tolerance phenotype, which were used to develop molecular markers. **【Results】** Phylogenetic analysis revealed that the function of *ZmWRKY48* is mainly associated with biotic and abiotic stresses. Genetic variation analysis was performed on the CDS region of *ZmWRKY48* gene in 40 maize inbred lines with different levels of cold tolerance, and the genotype data was correlated with the cold tolerance phenotype data. HAP2 was identified as the excellent haplotype. One SNP site was found in the CDS region which was highly significantly correlated ( $P<0.01$ ) with the germination vigor index of maize, and its contribution to the phenotype was the highest. This SNP site was converted into a dCAPS marker based on PCR and named DNCAPS603.

**【Conclusion】** The DNCAPS603 marker can be used for screening maize inbred lines with high cold tolerance, which can provide assistance for breeding cold-tolerant maize varieties and screening high-quality germplasm resources.

**Key words:** Maize; Low-temperature resistance; *ZmWRKY48* gene; Molecular marker

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## Abstract 24

# Identification and cloning of *zb10* controlling zebra leaf in maize

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**Abstract: 【Objective】** Leaf color deficient mutants are important for the study of photosynthesis mechanism, chlorophyll synthesis, chloroplast development and genetic regulation mechanism.

**【Method】** In this study, the mutant *zebra leaf 10*(*zb10*) was screened in mutant lines generated by Ion Beam irradiation-treatment of inbred “Lhc”. The *zb10* were carried out research of phenotypic identification, genetic analysis, mapping and cloning and function study. **【Result】** The *zb10* showed zebra or variegated leaf at the seedling stage under planting in the field. After the 5th-leaf stage, the defect phenotype of *zb10* was gradually suppressed, and after the 9th-leaf stage, the new leaves were completely green. The contents of photosynthetic pigments in white section of *zb10* were extremely significantly lower than its the green section and WT at the 4th-leaf stage. Transmission electron microscope observation of *zb10* white section leaves showed that the chloroplast thylakoid membrane structure was abnormally developed and no thylakoid and grana at the 4th-leaf stage. Genetic analysis showed that the leaf color deficient phenotype of *zb10* was controlled by a recessive nuclear genes. The mutant gene was mapped in the 422 kb interval of the short arm end of chromosome 2 by Mutmap and map-based cloning strategies. Sequencing found that the first exon of *ZB10* was inserted 7 bases, leading to premature termination of protein translation. Allelism tests and overexpression analysis showed that the mutation of *ZB10* gene resulted in zebra leaves at seedling stage in maize. Bioinformatics analysis revealed that *ZB10* contains an DOX domain, which belongs to the ferritin-like superfamily of diiron-containing four-helix-bundle proteins. This protein has iron atom binding motifs composed of four glutamate and two histidine (Glu<sup>132</sup>-Glu<sup>171</sup>-His<sup>174</sup>-Glu<sup>223</sup>-Glu<sup>303</sup>-His<sup>307</sup>). Subcellular localization results showed that *ZB10* protein functions in chloroplasts. **【Conclusion】** These results confirmed that *ZB10* plays a key role in chloroplast development and photosynthetic pigment synthesis at seedling stage, and provided insight into the regulation of photosynthesis in maize.

**Key words:** Maize; Zebra leaf; Photosynthesis; Chloroplast development

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## Abstract 25

### **Maize *developmentally delayed kernel 1* encoding an Importin-4 $\beta$ protein regulates seed development and grain filling by mediating nuclear exporting of eIF1A**

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**Abstract: 【Objective】** Nuclear-cytoplasmic trafficking is critical for protein synthesis in eukaryotic cells, because transcription and translation are spatially separated by the nuclear envelope. However, the mechanism and factors remain largely unknown in plants. **【Method】** Here, we isolated a maize (*Zea mays*) mutant, *developmentally delayed kernel 1* (*ddk1*), that displays delayed seed development and slower grain filling. **【Result】** *Ddk1* encodes a plant-specific Importin-4  $\beta$  protein localizing to the cytoplasm and nuclei. Mutation in *Ddk1* resulted in the up-regulation of ribosome biogenesis-related genes but repressed protein synthesis. We screened and confirmed eIF1A family proteins as DDK1-specific cargos. DDK1 strongly interacted with eIF1A proteins in vivo but had low affinity without RanGTP in vitro. eIF1A mainly localized to the cytoplasm in the wild type, but a significant portion of eIF1A was retained in *ddk1* nuclei. **【Conclusion】** Together, we first reported a mechanism by which Importin function as an Exportin in the plants that cooperates with RanGTP to mediate eIF1A nuclear exporting, thus regulating endosperm development and filling at the translational level.

**Key words:** Maize; endosperm; grain filling; Importin; eIF1A; ribosome; protein translation

## Abstract 26

### ***THP9* enhances seed protein content and nitrogen-use efficiency in maize**

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**Abstract: 【Objective】** Teosinte, the wild ancestor of maize (*Zea mays* subsp. *mays*), has three times the seed protein content of most modern inbreds and hybrids, but the mechanisms that are responsible for this trait are unknown. **【Method】** Here we use trio binning to create a contiguous haplotype DNA sequence of a teosinte (*Zea mays* subsp. *parviglumis*) and, through map-based cloning, identify a major high-protein quantitative trait locus, *TEOSINTE HIGH PROTEIN 9* (*THP9*), on chromosome 9. **【Result】** *THP9* encodes an asparagine synthetase 4 enzyme that is highly expressed in teosinte, but not in the B73 inbred, in which a deletion in the tenth intron of *THP9-B73* causes incorrect splicing of *THP9-B73* transcripts. Transgenic expression of *THP9-teosinte* in B73 significantly increased the seed protein content. Introgression of *THP9-teosinte* into modern maize inbreds and hybrids greatly enhanced the accumulation of free amino acids, especially asparagine, throughout the plant, and increased seed protein content without affecting yield. **【Conclusion】** *THP9-teosinte* seems to increase nitrogen-use efficiency, which is important for promoting a high yield under low-nitrogen conditions.

**Key words:** Maize; Quality; Seed; High protein content; Nitrogen-use efficiency

## ***Abstract 27***

### **ABA-inducible *ZmDRO1* improves adaptability of maize to water deficiency**

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**Abstract:** Maize is a common agricultural crop with large water requirement. Drought has become a major limiting factor in maize production. It is therefore imperative to study the response mechanism of maize to drought stress and elucidate the molecular mechanisms governing such responses. Identify drought-related genes and use them in breeding research to improve maize stress resistance and yield. The rice *OsDRO1* gene has previously been reported to confer drought stress in rice by controlling root growth Angle and promoting root growth to deeper soil. In this study, we found a homolog of *OsDRO1* in maize, *ZmDRO1*, through amino acid sequence alignment. Teosinte is the natural ancestor of maize and has a good performance in resisting abiotic stress. Our result revealed a difference in response to ABA and drought between maize cultivar B73 and *Zea mays ssp. Mexicana*, this was attributed to variation in drought avoidance ability. *ZmDRO1* was more significantly induced by ABA in *Zea mays ssp. mexicana*, Making it portray superior stronger drought avoidance ability. We generated transgenic lines of maize expressing *ZmDRO1* driven by the strong ABA inducible promoter. These transgenic lines, compared to the wild type, displayed superior growth and yield potentials under drought stress, but were at par under normal conditions.

**Key words:** maize; drought avoidance; root Angle; Root system architecture

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## Abstract 28

### Genome-wide Association Analysis and Candidate Gene Prediction of Tassel Related Traits in Maize

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**Abstract: 【Objective】** The tassel is an important reproductive organ of maize, which is directly related to the yield. The study of its related genetic mechanism has important guiding significance for maize breeding. Previous studies showed that the tassel-related traits of maize were quantitative traits controlled by multiple genes, and related QTLs loci were evenly distributed on 10 maize chromosomes. At present, most of the studies focuses on the genetic study of single tassel-related traits, and it is necessary to strengthen the joint analysis of multiple traits. **【Method】** In this study, under the Genome-wide association (GWAS) analysis method, 214 maize inbred lines with a broad genetic basis were used as test materials to investigate maize tassel-related traits to establish a phenotype database, and combined with SNP chip genotype data to conduct association analysis, then the SNP loci that were significantly associated with the traits were mined, and predict related candidate genes. **【Result】** In 214 maize inbred lines tested, 10 tassel related traits were investigated. Combined with the genotype data of 41101 SNP markers evenly distributed on the chromosome and the phenotype data of 10 tassel-related traits, the whole genome was analyzed. A total of 19 significantly associated SNP loci were detected on chromosomes 1, 2, 4, 7, 9 and 10. A total of 9 maize task-related candidate genes were predicted based on 19 significantly associated SNP sites, and using the genome sequence of the sequenced selfed line B73 as the reference genome, 7 of them had annotation function, mainly associated with ABCC15 transporter protein, eukaryotic translation initiation factor eIF-3B3, 18.9 kDa heat shock protein, bHLH transcription factor, among others. Gene *GRMZM2G017349* was predicted to be associated with the number of spikelet per spike based on the SNP locus with the highest phenotypic contribution. **【Conclusion】** In addition, bioinformatics analysis showed the gene *GRMZM2G017349* belongs to the bHLH\_MYC class of transcription factors and is a basic protein with some hydrophilic capacity. It contains a conserved bHLH domain associated with a photosensitive pigment and a bHLH Myc functional site; the promoter region contains multiple hormone response elements and light response elements. The results are helpful to deepen the understanding of the genetic basis of maize tassel-related traits, which is of great significance for the improvement of maize yield.

**Key words:** maize; tassel related traits; genome-wide association analysis; candidate gene prediction

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## ***Abstract 29***

### **A leaf rolling mutant3 (*lrn3*) affects plant height in maize**

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**Abstract: 【Objective】** Maize is an important food crop and fodder source, and plant height traits have important effects on desirable plant size and yield of maize. **【Method】** Fine-mapping, Scanning electron microscope, CRISPR, Y1H. **【Result】** The group performed EMS mutagenesis on the maize selfing line G4675Z and found a dwarf, leaf involution mutant *lrn3* in the M2 generation mutant population, which was confirmed as a single gene recessive mutation by chi-square test. The mutant showed a significant reduction in plant height compared to the wild type, shorter internodes, and impaired growth and development. We crossed the *lrn3* mutant with B73 to construct a segregating population and used Indel molecular markers to localize the gene to maize chromosome X. Sequencing revealed an early termination mutation in the *Lrn3* gene, and CRISPR/Cas9 knockdown of the gene also revealed a dwarf phenotype. In order to elucidate the mechanism of this gene involved in plant height regulation, this project, based on previous studies, analyzed the physiological, biochemical and cytological functions of this gene involved in plant height, analyzed differentially expressed genes and differential metabolites using transcriptomics and metabolomics, and also studied the relationship between this gene and the main gibberellin response to elucidate the signaling pathway of *Lrn3* gene mediating plant height regulation, and provided genetic resources and theoretical support for the regulation of plant height in maize. **【Conclusion】** This study will provide genetic resources and theoretical support for the regulation of maize plant height.

**Key words:** maize; secondary cell wall; plant height; leaf rolling mutant3 (*lrn3*)

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## **A pan-sequence map associated with accessible chromatin from a diverse set of maize genomes**

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**Abstract:** **【Objective】** Previous studies have extensively investigated the pan-genome and pan-gene in maize (*Zea mays* ssp. *mays*) and other species, but to date there has been no report on the sequence diversity associated with accessible chromatin, especially those from non-coding regions. **【Method】** We utilized the ATAC-seq (Assay for Transposase-Accessible Chromatin with high throughput sequencing) data and the genome assemblies of 26 NAM (Nested Association Mapping) founders to identify accessible chromatin sequences for each founder by aligning their own ATAC-seq data to their respective genome. **【Result】** The pan-ACR sequence set we constructed has a total length of approximately 48 Mb, which is 2 to 4 times of the accessible chromatin sequence in a single NAM founder. The saturation predicted by Michaelis-Menten equation was around 90%, indicating that our pan-ACR set has captured most of the sequence diversity of accessible chromatin in maize. We further divided the pan-ACRs into specific ACRs (present only in one founder) (39.3%), dispensable ACRs (present in 2-10 founders) (47.6%), and common ACRs (present in 11-26 founders) (13.0%). Compared with previous Pan-gene studies, we found that the non-coding regulatory regions on the genome are more dynamic and less conserved among individuals. Our pan-ACR sequence set has higher representation of distal (more than 2 kb away from the nearest TSS) ACR (39.3%) and lower representation of genic (overlapping with gene regions) ACR (20.4%), indicating the higher polymorphism of the distal non-coding regulatory that likely originated from the activity of transposable elements. **【Conclusion】** Collectively, this study presents, for the first time, a non-redundant pan-ACR sequence set in maize. Our data may open a new avenue for pan-genome analysis and help to understand and utilize the diversity of functional regulatory elements in maize.

**Key words:** Pan-genome; accessible chromatin; Transposable elements; transcriptional regulation

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## **Unraveling mechanisms underlying primary metabolite accumulation enhances crop genetic improvement**

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**Abstract:【Objective】**Primary metabolites lay the foundation for maize growth and development. However, the genetic mechanism of metabolome contribution to crop field performance or yield still remain fragmentary. **【Method】** Here we explored the complex network of maize primary metabolites based on a Complete-diallel plus Unbalanced Breeding-derived Inter-Cross (CUBIC) population containing 1404 progenies with genomic, transcriptomic and multiple complex traits.

**【Result】** The combined results of multi-omics data revealed that the regulation of the primary metabolome is considerably more complex than previously thought and a handful of novel genes were nominated, which laid the basis of metabolite associated complex traits. Transgenic and molecular studies of the novel gene encoding an amino acid transporter - *ZmAVT1A-1* validated its role in amino acid accumulation in leaf, and revealed that the controlling of amino acid remobilization is the target of improving maize nitrogen use efficiency. Furtherly, a comprehensive map guiding the genetic-breeding of complex traits was thereafter established in which the metabolites served as linkers. In this map, three candidate genes responsible for different metabolites were proved to regulate kernel filling through variate metabolic regulation.

**【Conclusion】** The present study has thereby updated the current genetic resource for primary metabolites regulation and provides new horizons for crop genetic improvement.

**Key words:** Metabolome; Genetic architecture; Nitrogen; Yield; Network

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## 玉米氮代谢优异基因挖掘与功能解析

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**摘要:**【目的】玉米是世界上最重要的农作物之一, 施用化学氮肥已成为世界范围内提高玉米产量的有效途径。在过去的三十年间, 我国的玉米单产水平只达到美国的 60%, 然而氮肥施用量却是美国的 2.2 倍 (FAO; <http://faostat.fao.org/>)。因此, 在减少氮肥施用的同时保持玉米产量是一项严峻的挑战。理想的解决方法是提高玉米的氮素利用效率 (Nitrogen use efficiency, NUE)。然而玉米的 NUE 涉及氮素吸收、转运、同化以及再利用等多个生物学过程, 是一个复杂的农艺性状。且不同的谷物以及不同的品种之间具有广泛的遗传变异, 通过传统的育种方式难以剖析其遗传机理。【方法】随着测序技术的发展, 生命科学已进入了生物大数据时代, 使用先进的生物和遗传技术发现数以千计的高质量基因组序列和许多基因型-表型关联的方式寻找关键基因逐渐成为主流方式。全基因组关联分析 (Genome wide association study, GWAS) 成为理解复杂性状变异遗传基础的有力工具。本研究使用来自中美两国的 350 份玉米优异自交系为试验材料。在玉米苗期采用氯酸盐 (Chlorate) 替代硝酸盐 (Nitrate) 进行培养。氯酸盐是一种有毒的硝酸盐类似物, 植物对其和硝酸盐的吸收无选择性。用氯酸盐处理玉米幼苗后, 根据表型的变化可以反映出玉米对氮素的敏感程度, 氯酸盐敏感的玉米材料具有更高的硝酸盐吸收效率。氯酸盐敏感的玉米材料出现株高降低和叶片萎蔫变黄的表型。我们收集了反应玉米生长状态的三个表型数据, 生物量 (Biomass)、株高 (Height) 和叶绿素含量 (SPAD)。将收集的数据进行最佳线性无偏估计值 (BLUE) 拟合后使用 GEMMA 软件进行 GWAS 分析。为缩小候选基因范围, 本研究使用 Camoco (Co-analysis of molecular components) 算法, 通过整合 GWAS 结果和共表达网络来筛选高优先级重叠 (high-priority overlap, HPO) 基因。【结果】结果发现, 我国不同年代的玉米品种在苗期氯酸盐胁迫试验中受到胁迫的程度逐渐加强, 说明我国玉米品种的氮素吸收能力在育种过程中受到选择, 这可能是由于我国长期过量施用氮肥引起的。结合 GWAS 分析结果和 Camoco 算法, 通过 snp-gene 作图筛选到 36 个可能参与玉米氮素代谢的候选基因。【结论】本研究通过苗期氯酸盐筛选试验, 通过 GWAS 分析和 Camoco 算法成功定位到 36 个可能参与氮素吸收的关键候选基因, 后续研究将围绕这些基因进行功能研究, 解析其分子调控机制, 为提高我国玉米 NUE 提供优良基因资源。

**关键词:** 玉米; NUE; GWAS; 硝酸盐

## Abstract 33

# UB2/UB3/TSH4-anchored Transcriptional Networks Regulate Early Maize Inflorescence Development in Response to Simulated Shade

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**Abstract: 【Objective】** To explore the regulatory networks of maize inflorescence development in response to high-density planting. **【Method】** The end-of-day far-red light (EOD-FR) and white light supplemented with far-red light (WL+FR) treatments were used to mimic shade and perform a comprehensive transcriptome analysis of consecutive development stages of maize tassels and ears. **【Result】** In this study, we showed that shade-mimicking treatments cause precocious development of the tassels and ears. Comparative transcriptome profiling analyses revealed the enrichment of phytohormone-related genes and transcriptional regulators among the genes co-regulated by developmental progression and simulated shade. Network analysis showed that three homologous Squamosa promoter binding protein (SBP)-like (SPL) transcription factors, Unbranched2 (UB2), Unbranched3 (UB3), and Tasselsheath4 (TSH4), individually exhibited connectivity to over 2,400 genes across the V3-to-V9 stages of tassel development. In addition, we showed that the *ub2 ub3* double mutant and *tsh4* single mutant were almost insensitive to simulated shade treatments. Moreover, we demonstrated that UB2/UB3/TSH4 could directly regulate the expression of *Barren inflorescence2 (BIF2)* and *Zea mays teosinte branched1/cycloidea/proliferating cell factor30 (ZmTCP30)*. Furthermore, we functionally verified a role of *ZmTCP30* in regulating tassel branching and ear development. **【Conclusion】** Our results reveal a UB2/UB3/TSH4-anchored transcriptional regulatory network of maize inflorescence development, and provide valuable targets for breeding shade-tolerant maize cultivars.

**Key words:** Maize; inflorescence development; shade avoidance syndrome; UB2/UB3/TSH4; *ZmTCP30*

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## Abstract 34

# Chromosomal-scale, well-phased assemblies of plant haplotype-resolved genomes and their applications using graph strategy

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**Abstract: 【Objective】** Hybrid plants are universally existed in wild and often exhibit greater performance of complex traits than their parents and other selfing plants, a phenomenon known as heterosis that has been applied in plant breeding for decades. Despite their prevalence and significance, the process of decoding their genome sequences has seriously lagged due to the difficulties in assembling hybrid genomes and the lack of proper methods to further represent and analyze them. **【Method & Results】** Based on the PacBio High Fidelity (HiFi) sequencing and chromatin conformation capture sequencing data, combined with a graphical genome construction strategy, here we report the assembly and analysis of a highly heterozygous maize genome and provide evidence that our haplotypic assemblies are well-phased at chromosomal level and can successfully resolve the complex loci with extensive parental structural variations (SVs). We further demonstrate the feasibility of integrating this assembly into a genome graph to facilitate downstream short-reads based SV calling and allele-specific gene expression analysis. **【Conclusion】** Our work provides an entire workflow that could promote the deciphering of the large numbers of hybrid plant genomes, and ultimately help to understand heterosis.

**Key words:** Genome assembly; Graph genome; Structural variation; Heterosis

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## **Enhancing Nitrogen Utilization Efficiency in Maize Germplasm through Gene Editing of Growth-Regulating Factors**

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**Abstract:【Objective】**Growth regulating factors (GRFs) are unique transcription factors in plants that play a crucial role in regulating cell volume, leaf size, chloroplast proliferation, leaf senescence, and photosynthesis. This study aimed to explore the response of maize different GRFs-edited to miR396 regulation and the influence on the expression of downstream target genes. **【Method】** By using CRISPR/Cas9, we eliminated *ZmGRFs* activity or removed regulation of *miR396* to *ZmGRFs*, which changed plant height, tassel development, flowering time, disease resistance, ear length, and hundred-grain weight. **【Result】** We obtained 18 GRFs knockout (GRFs-KO) and GRFs base editing (GRFs-BE) lines. One line (GRFs-BE1) showed an increase in ear length and row number, and the yield per plant increased under normal nitrogen conditions (up to 10%). Two lines (GRFs-BE2 and GRFs-KO1) showed an increase in ear length and seed weight, and the yield per plant increased under low nitrogen (up to 15%) and normal nitrogen (up to 20%).

**【Conclusion】** Our findings suggest that further research on the *ZmGRFs* can provide theoretical guidance and industrial application for nitrogen utilization efficiency (NUE) and yield increasing of maize. Moving forward, we will further analyze the regulatory effects of maize *miR396* to *ZmGRFs*, clarify the molecular mechanism of GRF-mediated pathway, dig out the major genes in maize that are regulated by GRF and related to nitrogen utilization. This will enable us to overcome metabolic bottleneck, improve nitrogen utilization efficiency, cultivate environment-friendly maize varieties requiring less nitrogen fertilizer, create high-yield germplasm, and lay the technical and material foundation of research on molecular design and breeding.

**Key words:** *miR396*; GRF; maize; NUE; ear developmen



## Abstract 37

### Mechanistic dissection of accelerated cell death and multiple disease resistance in a maize *lethal leaf spot 1* allele

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**Abstract: 【Objective】** Multiple disease resistance (MDR) has attracted increasing attention in maize. However, the interplay between cell death and metabolite changes and their contributions to MDR remains elusive in maize. **【Method】** In this study, we identified a disease *lesion mimic 30* (*les30*) mutant that showed suicidal lesion formation and enhanced resistance to *Curvularia lunata* (Wakker) Boed. **【Result】** By map-based cloning, a gene encoding the pheophorbide *a* oxidase (PAO/LLS1) known to be involved in chlorophyll degradation and MDR was found to be the causal gene. *LLS1* was found induced by both biotic and abiotic stresses. Based on transcriptomics analysis, genes involved in defense response and secondary metabolite biosynthesis were mildly activated in *les30* leaves without lesion, while those processes were dramatically amplified in lesioned *les30* leaves. Moreover, the defense-associated phytohormones including jasmonic acid and salicylic acid, and phytoalexins including phenylpropanoids, lignin and flavonoids were over-accumulated in the lesioned *les30* leaves, suggesting the activation of defense-associated phytohormone and metabolite biosynthesis in a lesion-dependent manner. **【Conclusion】** In general, this study implies the existence of an interactive amplification loop of interrupted chlorophyll degradation, cell death, defense-related gene expressions, and metabolite changes resulting in suicidal lesion formation and MDR, which allows potential genetic manipulation to improve maize disease resistance.

**Key words:** cell death; *LLS1*; maize; metabolomics and transcriptomics; multiple disease resistance; JA and SA

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## Abstract 38

### Genome-wide association study of *Aspergillus flavus* resistance in maize

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**Abstract: 【Objective】** Maize is a critical crop for ensuring food security in China. However, *Aspergillus flavus*, a fungus that can invade maize kernels during maturity, threatens its yield and quality. due to various factors, such as climate and environmental conditions, which affects maize yield and quality. Despite its importance, the genetic mechanism underlying maize kernel resistance to *A. flavus* remains unclear. **【Method】** Therefore, in this study, integrating with approximately 10.77 million SNPs covering the entire maize genome, we conducted a genome-wide association study (GWAS) to investigate the genetic basis of maize kernels' resistance to *A. flavus*. **【Result】** The results showed that the *A. flavus* resistance trait followed a normal distribution and was a typical quantitative trait. Significant differences in *A. flavus* resistance were observed among different types of maize inbred lines, with temperate inbred lines having stronger resistance than tropical/subtropical inbred lines. Our GWAS analysis identified 12 significant SNPs ( $P \leq 2.03 \times 10^{-6}$ ), which corresponded to eight non-redundant quantitative trait loci (QTLs), named ZmAFR1 ~ ZmAFR8. Each QTL explained 8.44% to 10.79% of the phenotypic variation, and a total of 62 genes were involved, including seven genes on chromosome 2, consistent with previous reports. We also detected seven more significant SNPs on chromosomes 1, 3, 4, 5, 7, and 9, which indicates that increasing marker density could improve the statistical power of GWAS. Through GO enrichment and KEGG pathway analysis of 62 genes, it was found that gene B located on ZmAFR1 played an important role in plant hormone signal transduction, which could activate the ethylene signal pathway in maize kernels and promote cell metabolism, indicating that maize kernels might resist the negative effects of *Aspergillus flavus* infection by promoting respiratory metabolism and accelerating seed germination. In addition, gene D located on ZmAFR7 was involved in the formation of intracellular membranes and had  $\alpha$ -L-glucosidase activity, which could promote glycoside metabolism and play an important role in the polysaccharide degradation pathway. Haplotype analysis found that genes A, B, C, and D haplotypes had significant differences in resistance to *Aspergillus flavus*. In addition, it was also found that materials carrying the Hap1 haplotype combination showed stronger resistance to *Aspergillus flavus*. **【Conclusion】** These results not only enriched and expanded the genetic basis of maize kernel resistance to *Aspergillus flavus*, but also provided a theoretical reference for improving existing germplasm and breeding new maize varieties with better resistance to *Aspergillus flavus*.

**Key words:** Maize; *Aspergillus flavus* resistance; Genome-wide association analysis; Statistical power

## 玉米 *ZmARF4* 响应缺磷胁迫的功能研究

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**摘要:** 【研究背景】植物的生长发育与磷酸盐 (Pi) 和生长素密切相关。磷是植物生长发育所必需的大量营养元素之一。生长素响应因子 (Auxin response factors, ARFs) 在植物生长发育和逆境胁迫响应中发挥重要作用。然而, 玉米中关于生长素响应因子 (ARFs) 对磷饥饿响应的研究数据有限。本研究分离鉴定了一个玉米 *ARF* 基因 *ZmARF4*, 并解析了其响应磷胁迫的生物学功能, 阐明了其发挥作用的分子机制。【材料与方法】本研究利用玉米耐低磷自交系178和低磷敏感自交系9782进行 *ZmARF4* 响应缺磷胁迫的表达模式分析。利用拟南芥 *Col-0*、双突变体 *arf7 arf19* 和玉米 KN5585 进行遗传转化的生物学功能验证。利用酵母单杂交、双荧光素酶报告系统、免疫沉淀和质谱联用、凝胶阻滞实验等技术, 筛选 *ZmARF4* 上下游关键调控基因和互作蛋白。【结果与分析】玉米耐低磷自交系178和低磷敏感自交系9782中 *ZmARF4* 的启动子活性存在显著差异, 且均响应缺磷胁迫。 *ZmARF4* 在玉米耐低磷自交系178和低磷敏感自交系9782根系中响应缺磷胁迫, 表达模式差异显著。 *ZmARF4* 具有促进植物根系生长发育的生物学功能, 促进了玉米株高和产量的提高。 *ZmARF4* 在低磷条件下促进磷的吸收, 并增强了对盐和渗透胁迫的耐受性。 *ZmARF4* 蛋白定位于叶肉原生质体的细胞核和细胞质中, 分别与介导侧根起始和防御反应的 *Zm ILL4* 和 *Zm Chc5* 互作。【结论】玉米 *ZmARF4* 主要在根系中表达, 在极端材料中响应缺磷胁迫的表达模式存在差异。 *ZmARF4* 在玉米原生质体中定位在细胞质和细胞核。酵母双杂交文库筛选检测到 *ZmARF4* 与调控植物生长发育、抗病抗逆的候选基因 *Zm Chc5* 互作。拟南芥遗传转化实验表明 *ZmARF4* 具有促进根系生长发育的生物学功能。

**关键词:** 玉米; 生长素应答因子; 根系; 缺磷胁迫

## *Abstract 40*

### **iBP-seq: an efficient and low-cost multiplex targeted genotyping and epigenotyping system**

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**Abstract: 【Objective】** Inter- and intra-specific variations are common and can be associated with genomic mutations as well as epigenomic variation. Profiling both genomic and epigenomic variants is the core of dissecting phenotypic variation. However, an efficient targeted genotyping and epigenotyping system is still currently lacking. **【Method】** Here, by combining next-generation sequencing with multiplex PCR, we devised a new multiplex targeted genotyping and epigenotyping system that we named improved bulked-PCR sequencing (iBP-seq). Multiple barcode sequences and unique molecular identifiers (UMIs) were implemented into iBP-seq to discriminate different samples and minimize the sequence bias caused by PCR duplication.

**【Result】** Here, we present the novel method iBP-seq for targeted genotyping and DNA methylation profiling by combining next-generation sequencing with multiplex PCR. We also established a user-friendly bioinformatics platform that enables the automated analysis of raw reads and the visualization of these targeted genotyping and epigenotyping results. iBP-seq eliminates PCR redundancy by introducing UMI sequences. In addition, iBP-seq can discriminate hundreds of samples for tens of target genomic regions with the use of a barcode primer for indexing. iBP-seq can be employed for fine-mapping, the construction of genetic maps, and for genotyping CRISPR-edited individuals as well as the profiling of methylation levels at target genomic regions at a low cost. Besides, iBP-seq can also be used for a wide range of species.

**【Conclusion】** Overall, iBP-seq shows efficient performance for genotyping and epigenotyping among various populations because of the advantages of high efficiency, and low cost, and huge potential for application of modern crop breeding process.

**Key words:** Breeding 4.0; Genotyping; Epigenotyping; iBP-seq; CRISPR editing

## ***Abstract 41***

### **Dynamic patterns of gene expression and regulatory variation in the maize seed coat**

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**Abstract: Background** Seed size is an important factor contributing to maize yield, but its molecular mechanism remains unclear. The seed coat, which serves as one of the three components of the maize grain, determines seed size to a certain extent. The seed coat also shares the maternal genotype and is an ideal material for studying heterosis.

**Results** In this study, the self-pollinated seeds of the maize hybrid Yudan888 and its parental lines were continuously collected from 0 day after pollination (DAP) to 15 DAP for phenotyping, cytological observation and RNA-seq. The phenotypic data showed that 3 DAP and 8 DAP are the best time points to study maize seed coat heterosis. Cytological observations indicated that maize seed coat heterosis might be the result of the coordination between cell number and cell size. Furthermore, the RNA-seq results showed that the nonadditive genes changed significantly between 3 and 8 DAP. However, the number of genes expressed additively was not significantly different. Our findings suggest that seed coat heterosis in hybrid is the result of nonadditive expression caused by dynamic changes in genes at different time points during seed expansion and seed coat development. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment indicated that genes related to DNA replication, cell cycle regulation, circadian rhythms and metabolite accumulation contributed significantly to hybrid seed coat heterosis.

**Conclusion** Maize seed coat phenotyping allowed us to infer that 3 DAP and 8 DAP are important time points in the study of seed coat heterosis. Our findings provide evidence for genes involved in DNA replication, cell cycle regulation, circadian rhythms and metabolite accumulation in

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hybrid with high or low parental expression as major contributors to hybrid seed coat heterosis.

**Keywords** Maize; Seed coat; Heterosis; RNA-seq

## Abstract 42

# Mechanism of natural variation of ZmPF5 in regulating pollen fertility in maize (*Zea mays*)

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**Abstract:** **【Objective】**Maize (*Zea mays*) is one of the most important food crops in the world. The fertility of maize pollen is a major yield-related agronomical trait, well-fertile and healthy pollen is crucial to ensure the yield, and meanwhile in breeding, the application of male sterility reduces the cost of a large amount of labor. Therefore, it is advantageous to identify the key genes involved in pollen fertility and study the related mechanisms. **【Method】** With this aim, we observed pollen characters in a large number of maize inbred lines and found that the natural ecotype L403 possessed a distinct pollen abortion phenotype compared with B73. **【Result】** We cloned the causative gene *PF5* for pollen fertility by the BSAsseq method and confirmed its function in the study of maize *pf5* mutant. Importantly, we identified there was only one A-C SNP site in the promoter region of *PF5* of L403 versus B73 and the promoter activity of L403 was weaker than that of B73. Consistent with this, the near-isogenic lines obtained from the cross of L403 to the recurrent parent B73 showed similar pollen abortion phenotype as L403. By JASPAR prediction, we found that *PA4* can bind the SNP site of *PF5* and repressive its expression, and transcriptional activation experiments showed that *PA4* can bind the promoters of L403 and B73 and repressive *PF5* differently, which were validated by EMSA experiments. **【Conclusion】** This work will pave a new pathway that may regulate pollen fertility in maize and the natural variation at *PF5* promoter may be applied to maize breeding in the future.

**Key words:** Maize; Pollen fertility; Natural variation; SNP

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## Abstract 43

### Multi-omics analyses reveal the flavor code of sweet corn

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**Abstract: 【Objective】** Sweet corn is an important vegetable crop which is consumed worldwide. However, little is known about the differentiation and selection of field and sweet corn, and indeed how the breeding process changed the metabolite composition and flavor of sweet corn.

**【Method】** Here, we *de novo* assembled a typical sweet corn genome and re-sequenced 295 further diverse inbred lines of sweet corn. A total of 507 representative inbred lines of field corn with high density SNP was also collected. We subsequently extensively examine the genetic architecture of sweet corn kernel quality by combining genetic, metabolite and expression profiling methodologies and a subset of this panel was, furthermore, evaluated in consumer panels. **【Result】** Using these multi-omics analyses, we were able to dissect the genetic discrepancy between sweet and field corn. Key genes and metabolites associated with flavor and consumer liking were identified with important target flavor metabolites including sugars, acids and volatiles. In total, 59.0% of the flavor score variation could be explained by combining variation at the gene, transcript and metabolite level. **【Conclusion】** These results thus provide valuable information for future genetic breeding of sweet corn and indeed more generally for the diversification of our major crops.

**Key words:** Sweet corn; Genome assembly; Flavor; Multi-omics; GWAS



## **Optimizing and application of haploid inducer-mediated genome-editing system in maize**

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**Abstract: 【Objective】** Haploid-inducer mediated genome editing system (IMGE or HI edit) overcomes the genotype dependency of transformation and produces stable homozygous lines mutated at precise loci within two generations, which has the broad application for crop genetics and breeding. So far, proof of concept for haploid inducer mediates genome editing has been proposed for three years. However, in practical, the efficiency of several stages needs to be further optimized to meet the requirements of breeding practice. **【Method】** In this study, we first used CRISPR-Cas9 to target knockout the haploid induction-related genes (*ZmPLA1/ZmMTL/NLD* and *ZmDMP*) to produce a high-efficiency haploid inducer for maize. Meanwhile, we developed an efficient haploid screening markers based on the specific expression of red and green fluorescent proteins in endosperm or embryo. The CRISPR-Cas9 gene editing component targeting *ZmSH2* and *ZmWx* genes were stacking to create the gene-editing haploid inducer Edit<sup>Sh</sup>, Edit<sup>Wx</sup>, Edit<sup>Sh&Wx</sup>. **【Result】** The gene-editing haploid inducers generated in this study achieved a haploid induction rate of 6.32% - 22.28%. The haploid identification system used in this study enabled discriminate haploid 100% accurately at several stages, including early embryo development (as early as 10 days after pollination), mature seed and germination to emergence of coleoptile stage. The developed Edit<sup>Sh</sup>, Edit<sup>Wx</sup>, Edit<sup>Sh&Wx</sup> gene-editing haploid inducer lines were successfully applied to the rapid improvement of sweet and waxy traits in several maize inbred lines, and the editing efficiency of haploid target genes was 0.62% -1.72%. Furthermore, the application of Edit<sup>Sh & Wx</sup> demonstrated for the first time that using multi-targets can improve the efficiency of obtaining target haploids for genetic improvement. **【Conclusion】** In this study, we developed several gene-editing haploid inducers and successfully achieved sweet and waxy trait improvement in maize. Although the low target editing efficiency of haploid remains a main constraint of this technique, our study provides a foundation for further improving the editing efficiency of haploid for massive applications in crop breeding.

**Key words:** Maize; Gene editing; Haploid induction; Precision breeding

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## ***Abstract 45***

### **Two maize *TCP* genes redundantly regulate inflorescence development and floral sex determination**

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**Abstract:** Inflorescence architecture is a key determinant of grain yield in maize. Among the major plant growth regulators, TEOSINTE BRANCHED1/CINCINNATA/PROLIFERATING (TCP) proteins control multiple growth-related processes and plant architecture traits, including circadian rhythm, leaf development, branching, floral organ morphogenesis, and hormone signaling. However, whether specific members of TCPs proteins orchestrate inflorescence development and sex determination remains poorly understood in maize. Here, we report that a pair of *TCP* genes play a redundant role in these developmental processes as each single mutants are indistinguishable from the wild-type plants, whereas the double mutants displayed distinct developmental phenotype including a reduced inflorescence meristem size and feminization of male florets. Expression analysis supports redundancy between the TCP genes, with the two, *TCPX* and *TCPY* having largely overlapping expression patterns. The strong developmental phenotype of double mutants was associated with impaired jasmonic acid (JA) biosynthesis. Taken together, we provide new insights in maize inflorescence development and floral sex determination by identifying two novel TCP proteins that function redundantly via a JA-dependent pathway.

**Key words:** maize; inflorescence; meristem; JA; TCP

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## ***Abstract 46***

# **Functional gene cloning and mechanism analysis of One *MADS* transcription factor in maize**

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**Abstract:** Ear related traits were the important compounds of grain yield in maize. And MADS-box transcription factors play a key role in regulating the ear formation and differentiation. Through combing selection analysis and genome-wide association analysis, we identified a MADS transcription factor as one candidate gene, which was located in the selected region and associated with ear length and fruit length. In this study, 25 polymorphism sites were associated with ear length, ear row number and kernel number per row by candidate association mapping in a new association population. After observing ear morphology of the immature ears (~5mm) by SEM, it showed that the ear row number was ambiguous in over-expression plants compared with that in wild-type plants. Also, there were always more than 3 ears harvested per plant in over-expressed plants with short tassel. RNA sequencing using 7-10-mm immature ears from over-expressed, crisper-edited and wild-type plants showed that differentially expressed genes were significantly enriched in the processes of photosynthesis, plant hormone signal transduction, starch and sucrose metabolism. All these results showed that this gene was indeed associated with ear development, and more works should be done to dissect the function and the regulation mechanism.

**Key words:** Ear development; MADS-box; Candidate association mapping; Functional analysis

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## ***Abstract 47***

### **Cloning and functional validation of *qHO1-2* for oil content in maize kernels**

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**Abstract:** Oil content and composition are important determinants of maize kernel quality. Maize oil is rich in high - energy lipids in the form of triacylglycerols (TAGs), which include unsaturated fatty acids, making maize oil a valuable resource for human food, animal feed, and bio - energy. The kernel oil content in commercial high-oil maize hybrids averages ~8%, which is far lower than that in developed high-oil maize lines (as high as 20%). Thus, exploring excellent alleles in high-oil maize inbred lines lays a foundation for cultivating high-oil maize. We detected a quantitative trait locus (QTL), *qHO1*, controlling oil content in the B73/BY804 recombinant inbred line (RIL) population. *qHO1* was split into two QTLs, of which *qHO1-2* was narrowed down to a around 3-Mb interval by using 7 markers and 4069 individuals. Furthermore, a genome-wide association study based on six RIL populations detected four candidate genes in the 3-Mb interval. Through further fine mapping and functional verification of four candidate genes by gene editing or EMS mutant materials, *G4* was the causal gene underlying *qHO1-2*, named *HO1-2*. *HO1-2* encodes a putative C-terminal binding protein, which is constitutively expressed. Further experiments will be performed to elucidate the molecular mechanism of affecting the oil content in maize kernels.

**Key words:** oil content; QTL; fine mapping; functional validation

## Abstract 48

# Toward the Functions of *ZmNAC-D* genes in the regulation of stem viability and juiciness in maize

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**Abstract: 【Objective】** Alive stem at the harvest stage is a critical factor determining biomass production and composition in biomass crops (*i.e.*, maize, sorghum and sugarcane), which was recently found to be largely controlled by the ‘Dry’ gene encoding a NAC transcription factor in sorghum (namely *NAC-D*). While it is proposed that *NAC-D* regulates the programmed cell death of stem parenchyma cells after stem elongation, the underlying mechanisms of *NAC-D* on stem viability and juiciness remain unclear, as sugarcane species with contrasting stem juiciness showed differential expression of *NAC-D*. Our work aims to test the effects of *NAC-D* expression on stem viability and juiciness in maize and Arabidopsis. **【Method】** Phylogenetic analysis, expression analysis and genetic approaches were combined to study the functions of maize *NAC-D* orthologs. **【Result】** Phylogenetic analysis identified the orthologs of *SbNAC-D* (Sobic.006G147400) in maize and Arabidopsis, namely *ZmKIL2* (GRMZM2G043813), *ZmKIL1* (GRMZM2G081930) and *KIR1* (AT4G28530), respectively. Expression analysis showed that, except for the reported functions in silk viability, *ZmKIL1* and *ZmKIL2* were expressed in the stem at the elongation and maturation stages with different expression levels, suggesting possible functional divergence. By contrast, *AtKIR1* was barely expressed in the Arabidopsis stem. Mutant analysis of *ZmKIL2* suggested that the *ZmKIL2* single mutant does not induce the juicy-stem phenotype. To test if knock-down of both *ZmKIL1/2* could lead to the juicy-stem phenotype, RNAi transgenic lines targeting *ZmKIL1* and 2 were generated. In addition, transgenic plants of Arabidopsis were produced to induce ectopic expression of either *AtKIR1*, *ZmKIL1/2* or *SbNAC-D*, respectively, in the stem parenchyma cells in order to see if these orthologs are functionally conserved in the model species. **【Conclusion】** The current evidence suggests that *NAC-D*-mediated regulation of stem viability and juiciness may be evolved in monocots and *ZmKIL1* and *ZmKIL2* may be functionally redundant.

**Key words:** maize; stem; NAC transcription factor; pith parenchyma

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## Abstract 49

# DeepBSA: A deep-learning algorithm improves bulked segregant analysis for dissecting complex traits

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**Abstract: 【Objective】** Bulk segregant analysis (BSA) is a rapid, cost-effective method for mapping mutations and quantitative trait loci (QTLs) in animals and plants based on high-throughput sequencing. However, the algorithms currently used for BSA have not been systematically evaluated and are complex and fallible to operate. **【Method】** In this study, we developed a BSA method driven by deep learning—DeepBSA; to our knowledge, this is the first application of deep learning to BSA for QTL detection or functional gene cloning. DeepBSA uses two new algorithms—DL and K—and can be applied to different numbers (at least 2) of bulked pools for increased mapping accuracy. **【Result】** DeepBSA is compatible with a variable number of bulked pools and performed well with various simulated and real datasets in both animals and plants. DeepBSA outperformed all other algorithms when comparing absolute bias and signal-noise-ratio. Moreover, we applied DeepBSA to an F<sub>2</sub> segregating maize population of 7,160 individuals and uncovered five candidate QTLs, including three well-known plant-height genes. Finally, we developed a user-friendly graphical user interface (GUI) for DeepBSA, integrating five widely used BSA algorithms and our two newly developed algorithms. **【Conclusion】** The DeepBSA GUI is easy to operate, especially for researchers without sophisticated bioinformatics skills, and can quickly identify QTLs and functional genes. We believe that our newly developed deep-learning BSA algorithm and GUI software will greatly promote the detection of complex traits in both animals and plants. The DeepBSA software is publicly available at <http://zeasystemsbio.hzau.edu.cn/tools.html> or <https://github.com/lizhao007/DeepBSA>.

**Key words:** bulked segregant analysis (BSA); algorithm; deep learning (DL); GUI software; DeepBSA

## 基于杂交种群体的玉米产量及其配合力的全基因组 组关联分析

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**摘要:** 【目的】通过分析陕A群和陕B群选育自交系组配的杂交种产量, 评估自交系的配合力, 并开展以产量和配合力为目标性状的全基因组关联分析, 挖掘产量及其配合力的关联位点, 为陕A群和陕B群选育玉米自交系的改良及育种中的应用提供依据。【方法】基于NC II 遗传设计, 以陕A群和陕B群选育的85份优良玉米自交系为亲本, 构建包含246份F<sub>1</sub>的杂交种群体, 在3个环境下进行产量测试, 并评估产量的一般配合力和特殊配合力; 利用6H90K芯片进行亲本基因型检测, 获得63879个高质量SNP标记, 并进行群体遗传特征分析, 在杂交种群体推测出高质量SNP标记55951个, 采用加性模型和非加性模型对杂交种产量、一般配合力和特殊配合力开展了全基因组关联分析, 并基于B73参考基因组对显著关联SNPs内的基因进行挖掘和功能注释。【结果】3个环境下的产量表现符合正态分布且变异广泛, 产量广义遗传力为59.04%, 环境效应显著; 杂交种产量、一般配合力和特殊配合力三者之间均达到极显著相关性, 杂交种产量与特殊配合力的相关性 ( $r=0.95$ ) 大于与一般配合力的相关性 ( $r=0.62$ ); 陕A群与陕B群遗传特征具有一定差异, 陕A群具有较高的一般配合力。全基因组关联分析分别检测到7、5和9个SNP与杂交种产量、一般配合力和特殊配合力显著相关 ( $-\log_{10}(P)>3.86$ ), 其中4个SNP为杂交种产量和特殊配合力共定位, 最终锚定了17个关联SNP。对不同性状关联位点的优势等位基因型分析发现, 4个GCA关联SNP受加性效应控制, F<sub>1</sub>产量BLUE关联位点可分为4种表现形式, 以显性效应为主, 其杂合基因型为最优等位基因型或次优等位基因型。通过功能注释发现, 候选基因在玉米生长发育和籽粒建成中特异表达, 例如GRMZM2G165828、GRMZM2G057557均与玉米籽粒发育相关。【结论】一般配合力和特殊配合力共同影响杂交种的产量, 特殊配合力效应影响更大; 一般配合力和特殊配合力具有不同的遗传基础, 可通过有利等位基因聚集提高一般配合力。在F<sub>1</sub>杂交种群体采用全基因组关联分析策略可开展配合力相关遗传解析, 挖掘产量及其配合力相关遗传位点, 可加速关联位点在分子育种中的应用。

**关键词:** 玉米; 杂交种; 一般配合力; 特殊配合力; 全基因组关联分析

## *ZmDT1* 通过调节 ROS 水平参与玉米抗旱性调控

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**摘要：**【目的】玉米是重要的粮食作物、饲料和工业原材料，而干旱是威胁玉米产量的主要自然灾害。因此，鉴定参与玉米抗旱的关键基因，增强玉米的抗旱能力具有重大意义。【方法】前期通过 GWAS 已发现 *ZmDT1* 与玉米苗期抗旱性极显著相关。本研究鉴定了 *ZmDT1* 的功能变异位点，并通过分子生物学手段验证了该变异位点的功能。此外，本研究通过苗期干旱实验、转录组分析、互作蛋白的鉴定和田间实验等，解析了 *ZmDT1* 调控玉米抗旱性的分子机制。【结果】1. 发现 *ZmDT1* 基因启动子中，与抗旱性显著相关的 2 个变异位点能够被转录抑制因子 ZmMYBn 结合，干旱胁迫下 ZmMYBn 负调控 *ZmDT1* 基因表达量。2. 苗期干旱实验表明 *ZmDT1* 负调控玉米抗旱性。ZmMYBn 通过抑制 *ZmDT1* 基因表达从而正调控玉米抗旱性。3. *ZmDT1* 突变体转录组分析发现，与野生型相比干旱胁迫下大量调节细胞氧化还原状态的基因差异表达。4. *ZmDT1* 互作蛋白鉴定发现，*ZmDT1* 与叶绿体氧化还原相关酶 ZmM6 互作，并抑制其活性。5. 干旱下 *zmdt1-ko* 突变体中的 H<sub>2</sub>O<sub>2</sub> 含量低于野生型。6. 田间干旱实验也表明 *ZmMYBn* 的过表达材料和 *zmdt1-ko* 突变体材料更抗旱，产量显著高于野生型。【结论】*ZmDT1* 通过调节 ZmM6 的酶活性参与叶绿体内 ROS 水平调控，负调控玉米抗旱。*ZmMYBn* 识别 *ZmDT1* 启动子中的 2 个自然变异并抑制该基因在干旱胁迫下的表达，正调控玉米抗旱性。

**关键词：**玉米；抗旱性；关联分析；MYB 转录因子；活性氧

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## **Dissection of the rhizosphere microbiomes of maize germplasms differing in head smut resistance**

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**Abstract:** Head smut in maize is a soil-borne fungal disease caused by the causal pathogen *Sporisorium reilianum*, which poses a severe threat to maize production worldwide. The pathogen infects maize plants mainly at the vegetative stage and resistant germplasms are supposed to recruit beneficial microbiomes at the rhizosphere to resist *S. reilianum* infection. However, little is known about how the rhizosphere microbiome of resistant maize responds to the pathogen infection and contributes to disease resistance. The aim of this study is to investigate the time-series dynamics of the pathogen infection and the shifts of corresponding rhizosphere microbiomes of maize germplasms with different resistance against *S. reilianum*. In this study, four maize genotypes with different head smut resistance were challenged with *S. reilianum* or not in a field trial. A longitudinal dense sampling of their leaves during the vegetative stages was performed to examine the pathogen infection rate. The infection ratios of the four genotypes reached to peak at the V3 stage and decreased to stable levels at the V7 stage. The resistant genotypes displayed lower infection ratios than the susceptible genotypes. Moreover, rhizosphere soils of the four genotypes were collected to investigate their rhizosphere microbiomes using 16S rDNA and ITS amplicon sequencing. We found that under the pathogen challenge, the rhizosphere of resistant genotypes enriched fungi such as *Hamigera*, *Lecythophora* and *Vishniacozyma*, whereas the abundance of several pathogens including *Sporisorium*, *Fusarium*, and *Alternaria* significantly increased in the susceptible genotypes. Our preliminary results suggest that the rhizosphere microbiomes of resistant germplasms seem more robust to buffer pathogen infection than the susceptible ones. On this basis, the microbial taxa and functions in the rhizosphere of resistant germplasms will be investigated in more detail. This work brings new insights into the selection of rhizosphere microbiomes in resistant breeding.

**Key words:** Maize; Rhizosphere microbiome; Head smut; Disease resistance

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## *Abstract 53*

### **Zinc transporter ZmLAZ1-4 modulates zinc homeostasis on plasma, chloroplast and vacuolar membrane in maize**

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**Abstract: 【Objective】** Zinc is an essential micronutrient for plant growth and development, that functions as a cofactor for hundreds of transcription factors and enzymes and is involved in numerous biological processes. Zinc deficiency is common abiotic stress resulting in yield loss and quality deterioration of crops, but excess Zn may cause toxicity to biological systems. Therefore, zinc homeostasis in plants is tightly modulated by zinc transporters and binding compounds that uptake/extrude, transport, localize and store zinc, as well as their upstream regulators. The members of the zinc-iron permease (ZIP), the zinc-regulated transporter (ZRT), the natural resistance-associated macrophage protein (NRAMP), the heavy metal ATPase (HMA), the zinc-induced facilitator-1 (ZIF1), the metal tolerance protein (MTP), and the cation diffusion facilitator (CDF) families are documented to transport zinc ion across the plasma, chloroplast and vacuolar membrane, respectively. **【Method】** we bioinformatically predicted that a member (ZmLAZ1-4) of the ZmLAZ1 family transported zinc ion across both plasma, chloroplast and vacuolar membrane and was negatively regulated by the ZmBES1/BZR1-11 transcription factor, and verified by thermal shift assay, overexpression in the zinc-sensitive yeast mutant, wild type Arabidopsis and maize, subcellular localization in protoplasts of maize, scales of onion bulbs and leaves of *Nicotiana benthamiana*, yeast one-hybrid, and dual-luciferase assay, respectively. **【Result】** All these results indicated that the ZmLAZ1-4 protein was a novel zinc transporter on plasma, vacuolar and chloroplast membrane, and modulated zinc homeostasis under the negative regulation of the ZmBES1/BZR1-11 transcription factor. **【Conclusion】** The ZmLAZ1-4 protein is a zinc transporter distinctive from the previously documented Zn transporters. It transports zinc ions across the plasma, vacuolar and chloroplast membrane, and modulates zinc homeostasis under the negative regulation of the ZmBES1/BZR1-11 transcription factor.

**Key words:** Zea mays; LAZ1; Zinc transporter; Zinc homeostasis

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## 质外体和液泡双定位糖苷水解酶介导 玉米广谱病虫害抗性的遗传分析

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**摘要:** 【目的】玉米茎腐病是世界范围内最具破坏性的玉米病害之一, 腐霉是引起玉米茎腐病的主要病原之一, 目前还未有抗玉米腐霉菌基因被克隆。本研究拟通过玉米自交系群体定位新的玉米腐霉茎腐病抗性基因。【方法】本研究结合全基因组关联分析、分离群体连锁分析鉴定玉米腐霉茎腐病抗性基因, 明确抗性基因在群体中的遗传变异, 并通过细胞生物学和生化手段解析抗性基因贡献玉米病害抗性的分子机制。【结果】基于玉米自交系群体的全基因组关联分析鉴定到一个玉米瓜果腐霉菌侵染抗性新位点*ZmRPA2*, 单倍型分析和分离群体连锁分析表明*ZmBGLU17*是*ZmRPA2*的候选基因。接种实验表明抗病单倍型*ZmBGLU17*<sup>GEMS10</sup>能快速应病原菌侵染, 而感病单倍型*ZmBGLU17*<sup>TY4</sup>则在病原菌接种后快速下调。序列分析发现*ZmBGLU17*不同单倍型之间启动子区域具有丰富的自然变异, 包含一个240bp差异的结构变异。通过双荧光素酶报告系统确定了*ZmBGLU17*不同单倍型启动子启动效率差异的关键区域。同时, 在部分感病单倍型中发现*ZmBGLU17*第五内含子剪切位点处发生gtaa-gtat单碱基变异, 点突变分析发现该单碱基变异是造成*ZmBGLU17*可变剪切的关键位点, 且仅有正常剪切的转录本具有糖苷水解酶活性。基因沉默和过表达转基因玉米材料表型测试表明*ZmBGLU17*可以显著提高玉米对瓜果腐霉菌和亚洲玉米螟的广谱抗性。功能分析发现*ZmBGLU17*具有质外体和液泡的双重定位, 并且过表达*ZmBGLU17*可以显著提高玉米细胞壁木质素的累积和活性化合物DIMBOA的合成。最后, 田间试验表明过表达*ZmBGLU17*对玉米百粒重有微弱影响, 但是不影响单株产量。【结论】玉米糖苷水解酶*ZmBGLU17*的自然变异决定了不同单倍型在响应病原菌侵染和基因可变剪切中的差异, 赋予了玉米腐霉茎腐病抗性; *ZmBGLU17*通过其在质外体和液泡中的双重定位, 正向调节玉米细胞壁木质素的累积和DIMBOA的合成, 进而提高玉米对病害和虫害的广谱抗性。

**关键词:** 玉米; 糖苷水解酶; 木质素; DIMBOA; 广谱抗

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## MT-gMLP：基于深度学习的玉米单性状和多性状联合基因组预测模型

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**摘要：**【目的】开发准确的面向不同作物复杂表型性状的基因组预测模型是目前基因组智能育种中的一个关键问题。【方法】通过充分提取SNP位点间的局部和全局特征，提出了基于gMLP的单性状基因组预测模型。为了有效提取相关性状间的特征以及提高目标性状的预测准确性，通过计算表型性状之间的相关性确定辅助性状的数量，提出了一种多性状联合基因组预测深度学习模型MT-gMLP，大幅度提高产量等目标性状的预测准确率。利用深度学习可解释性方法挖掘到一些影响玉米特定性状的关键位点。【结果】多个物种（玉米、水稻、小麦、番茄）的预测结果显示gMLP模型预测的准确率优于现有的13种GS方法。MT-gMLP模型在以产量为代表的复杂性状预测方面，不仅高于单性状预测模型，而且高于现有多性状预测模型。【结论】提出的基于深度学习的单性状和多性状联合基因组预测模型有助于提升玉米、水稻等作物的基因组预测效果，加快作物智能设计育种。

**关键词：**基因组预测；深度学习；多性状；智能育种

## ***Abstract 56***

### **A genome-wide association study dissects the genetic architecture of the metaxylem vessel number in maize brace roots**

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**Abstract: 【Objective】** Metaxylem vessels in maize brace roots are key tissue, and their number (MVN) affects plant water and inorganic salt transportation and lodging resistance. Dissecting the genetic basis of MVN in maize brace roots can help guide the genetic improvement of maize drought resistance and lodging resistance during late developmental stages. **【Method】** In this study, we used 508 inbred lines with tropical, subtropical and temperate backgrounds to analyze the genetic architecture of MVN in maize brace roots. **【Result】** The phenotypic variation in MVN in brace roots was evaluated in three environments, which revealed broad natural variation and relative low levels of heritability ( $h^2 = 0.42$ ). Stiff-stalk lines with a temperate background tended to have higher MVNs than plants in other genetic backgrounds. MVN was significantly positively correlated with plant height, tassel maximum axis length, ear length and kernel number per row, which indicates that MVN may affect plant morphological development and yield. In addition, MVN was extremely significantly negatively correlated with brace root radius, but significantly positively correlated with brace root angle, diameter, and number, thus suggesting that the morphological function of some brace root traits may be essentially determined by MVN. Association analysis of MVN in brace roots combined 1,253,814 single nucleotide polymorphisms (SNPs) using FarmCPU revealed a total of nine SNPs significantly associated with MVN at  $P < 7.96 \times 10^{-7}$ . Five candidate genes for MVN that may participate in secondary wall formation (*GRMZM2G168365*, *GRMZM2G470499*, and *GRMZM2G028982*) and regulate flowering time (*GRMZM2G381691* and *GRMZM2G449165*). **【Conclusion】** These results provide useful information for understanding the genetic basis of MVN in brace root development. Further functional studies of identified candidate genes should help elucidate the molecular pathways that regulate MVN in maize brace roots.

**Key words:** Maize (*Zea mays* L.); brace root; metaxylem vessel number; GWAS; candidate gene

## **Genetics of Anthesis to Silking Interval in maize**

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**Abstract:** Drought stress at flowering stage often causes delay of silk emergence, which greatly affect maize grain yield. Selection for reduced anthesis to silking interval (ASI) has shown to be effective to improve genetic gain for yield under drought. An elite maize inbred KB3020 showed short ASI, with silks emerging before pollen shed beginning. To dissect genetic architecture of ASI, we generated an  $F_{2:3}$  population consisting of 286 families crossed with KB3020 and HZ4. Five QTL located on chromosomes 2, 3, 5, 7, and 10 were identified. Two major QTL,  $qASI_{2.01}$  and  $qASI_{5.05}$ , which could explain 16% and 14.3% of the phenotypic variation respectively, were selected for allele effects validation.  $qASI_{2.01}$  was a partial dominant ASI QTL, while  $qASI_{5.05}$  was a recessive one. Alleles from KB3020 significantly reduced ASI at both loci. To fine map the two QTL, a large  $BC_2F_2$  population was generated and screened for recombinants. The progeny testing strategy will be used to narrow down the QTL intervals. The findings of this study will enhance our understanding of ASI genetic basis and facilitate breeding for reduced ASI, which are important for improving grain yield under drought conditions.

**Key words:** maize; anthesis to silking interval; QTL

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## Abstract 58

### Decreased DNA methylation level during meiosis of pollen mother cells in maize *argonaute 5* mutant results in male sterility

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**Abstract:** **【Objective】** Maize is one of the world's most important food crops and pollen development is essential for successful reproduction. **【Method】** In this study, an ethyl methanesulfonate (EMS)-induced *argonaute 5* mutant was obtained from the maize B73 to investigate its potential in regulating male sterility. **【Result】** Phenotypic and physiological analysis revealed that homozygous *argonaute 5* mutants could not shed pollen and exhibited complete male sterility, while the heterozygous mutants showed normal pollen shedding and fully fertile. Further cytological examination coupled with histochemical staining revealed that the abnormal lipid metabolic pathway in the anthers of homozygous mutant plants led to an aberrant disintegration of the anther tapetum. This abnormality hindered the microspores in the anther chamber from absorbing nutrients and developing into physiologically active pollen grains. In addition, significant differences in the quantity of 21nt phasiRNAs and 24nt phasiRNAs in mutant anthers at the meiosis stage were observed compared to the wild type plants. Anther DNA bisulfite sequencing revealed that the DNA methylation level was significantly lower compared to wild-type plants and the DNA methylation differential region (DMR) in the mutants was predominantly localized in mitochondria. **【Conclusion】** In summary, our findings provide valuable insights into the functional mechanisms of DNA demethylation in *argonaute 5* mutants during pollen mother cell (PMC) meiosis, which ultimately lead to male sterility in maize.

**Key words:** maize; genic male sterility; tapetum; ARGONAUTE

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## ***SI* 调节玉米根系构型及抗旱性的分子遗传机制**

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**摘要:**【目的】玉米是全球粮食、饲料和工业淀粉的来源。干旱胁迫严重限制全球玉米生产，了解植物根系构型（RSA）发育分子机理，对于开发抗旱品种至关重要。【方法】研究通过筛选玉米 EMS 突变体库，寻找玉米根系构型突变体，并进行根系表型及抗旱性分析，以研究玉米 RSA 发育分子机理。本研究筛选到了一个玉米浅根构型突变体 *sl-1*，对突变体测序分析定位到目的基因，并命名为 *SI*。研究发现 *SI* 特异在根尖中表达且定位于细胞核中。与野生型植物相比，*sl* 表现出根系角度增大、侧根数量增多，而根系总生物量无显著差异。根系向重性实验表明 *sl* 向重性存在缺陷，表明 *sl* 可能在响应重力刺激时生长素的重新分配方面存在缺陷。RNA-seq 分析表明 *SI* 可能参与调控生长素相关途径。干旱条件下，*sl* 表现为干旱敏感及产量降低。【结论】研究结果表明 *SI* 可能通过介导调控生长素的合成与运输，调控根的向重反应和侧根发生，从而影响根系构型。*SI* 基因可能为玉米根系构型和抗旱性的遗传改良提供重要靶点。

**关键词:** 根系构型；向重性；生长素

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## ***Abstract 60***

### **A pan-tissue map of accessible chromatin reveals subgenome concerted regulomes in allotetraploid maize**

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**Abstract: 【Objective】** Maize originated from the combination of two diploid ancestors about 5 to 12 million years ago (Mya) after their split with sorghum about 12 Mya. However, there is still a lack of clarity regarding the regulatory divergence and cooperative interactions between subgenomes, as well as their association with the variation of complex traits. **【Method】** Using ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing), we profiled the accessible chromatin landscape for 11 tissues with 15 samples of cultivar B73, covering approximately 0.67%-1.1% of the maize genome. **【Result】** We found that there were subtle differences in both the number and length of accessible chromatin regions (ACRs) between two subgenomes, despite the enhanced dominance of subgenome one (M1) in highly conserved regions with subgenome two (M2). Additionally, the proportion of syntenic ACRs (~7%) was much lower than that of protein-coding genes (~20%), suggesting rapid evolution of regulatory elements than coding sequences. Interestingly, further analysis revealed a highly convergent pattern of TF binding sites among these rapidly divergent regions, implying that core ACRs are highly conserved between two subgenomes. Meanwhile, there was still a certain amount of subgenome-specific TF contributing to subgenome regulatory diversity. Additionally, we discovered significant enrichment of GWAS loci associated with agronomic traits in ACRs, and the subgenome contribution varied among different traits. **【Conclusion】** Our findings provided numerous resources and generated a pan-tissue map of accessible chromatin which reveals subgenome concerted regulomes in allotetraploid maize. This could lead to novel targets and approaches for improving maize breeding and research.

**Key words:** Maize; Chromatin accessibility; Subgenome; Regulatory elements

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## ***ZmZFP* 调控玉米与 AM 真菌共生的功能研究**

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**摘要：**【目的】AM真菌与植物互利共生的关系需要大量的基因协同作用，其中转录因子调控网络发挥着重要作用。C2H2型锌指蛋白家族作为一个庞大的转录因子家族，与AM真菌共生调控的研究甚少。*LjZFP*基因已经被发现调控百脉根与AM真菌共生，因此本研究旨在探究*ZmZFP*在玉米中调节菌根共生信号过程中的作用。【方法】在玉米全基因组上筛选出玉米中可能参与共生途径的*ZmZFP*，通过对其基因结构、组织表达模式、亚细胞定位等方式进行分析，利用百脉根毛状根转化体系及玉米突变体材料，对其功能进行深入研究，此外，结合转录组分析，对*ZmZFP*调控的下游靶基因进行了筛选和鉴定分析。【结果】*ZmZFP*是一个典型的C2H2型锌指蛋白。在进化分析上与共生相关的蛋白聚于一支。定位在细胞核中，不具有自激活活性。*ZmZFP*主要在成熟的根中表达，*ZmZFP*受AM真菌诱导，在共生的中后期显著表达，主要影响AM真菌与玉米共生过程中丛枝的形成发育与降解，在共生中期调控丛枝的形成发育，共生的后期调控丛枝的降解。在转录水平上，*ZmZFP*突变体的丛枝发育相关基因 *ZmSWEET3a*、*ZmPIP2:4*及*ZmMATH*的表达量显著低于野生型 WT；而在后期可以显著提高丛枝降解相关基因*ZmMBY*、*ZmCP3*、*ZmCHITase*的表达量。【结论】*ZmZFP*作为C2H2型锌指蛋白家族中的一员，在与AM真菌的共生中发挥重要作用。*ZmZFP*主要影响AM真菌与玉米共生过程中丛枝的形成发育与降解，在共生中期调控丛枝的形成发育，共生的后期调控丛枝的降解。

**关键词：**玉米；丛枝菌根真菌；共生；C2H2 型锌指蛋白

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## Abstract 62

# *ZmTPK2* 介导硫胺素焦磷酸精细调控玉米穗长和产量的机制解析

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**摘要:** 【目的】穗长是重要的产量构成因子。一般而言, 穗长越长、行粒数越多, 为籽粒发育提供的可能性空间越大、进而增产潜力越大。目前, 在玉米中已鉴定到数百个穗长 QTL, 但仅少数玉米穗长基因被克隆。因此, 挖掘玉米穗长新基因, 不仅能丰富其遗传基础及调控机制, 对培育高产玉米新品种也具有重要意义。【方法】图位克隆、表达分析、遗传转化、亚细胞定位、体内硫胺素焦磷酸 (thiamine pyrophosphate, TPP) 含量测定、蛋白酶活测定、转录组、能量代谢组、单倍型分析、分子标记开发及 *ZmTPK2* 育种效应评估。【结果】我们图位克隆了一个新的调控玉米穗长 QTL-*qKB6.2* 的功能基因, *ZmTPK2*; 该基因编码硫胺素焦磷酸激酶, 定位于细胞质中, 将硫胺素磷酸化成 TPP 进而影响玉米的生长发育。相比于野生型, *ZmTPK2* 敲除系内源 TPP 含量更低、植株更矮小、雌穗花序分生组织更短、每行小花数更少、穗长更短、产量更低; 同时 *ZmTPK2* 敲除系发育叶片及幼穗中与三羧酸循环、磷酸戊糖、卡尔文循环等途径相关的代谢物含量显著下降, 表明 *ZmTPK2* 通过影响内源 TPP 含量和植物的能量代谢进而调控玉米植株发育和产量。有意思的是, 在 *ZmTPK2* 不同转录水平的过表达材料中, 随着 *ZmTPK2* 表达量不断增加, 玉米内源 TPP 含量和穗长呈现先增加后降低的现象, 表明 *ZmTPK2* 转录水平与内源 TPP 含量及穗长存在一定剂量效应, 维持合适的 *ZmTPK2* 表达水平是保证最优穗长的关键。另外, *ZmTPK2* 优良等位基因型 NIL<sup>*qKB6.2*</sup> 的频率在关联群体中只有 0.4%, 为稀有等位基因型; 且与 NIL<sup>*qKB6.2*</sup> 构建的杂交组合相比, Chang7-2、Jing724 和 PH6WC 与 NIL<sup>*qKB6.2*</sup> 构建的杂交组合的穗长分别增加 7.1%、6.3% 和 17.7%, 产量分别提高 5.5%、6.6% 和 17.4%, 表明 *ZmTPK2* 优良单倍型可以用于改良杂交种的穗长, 进而提高杂交种的籽粒产量。【结论】*ZmTPK2* 是一个新的参与 TPP 代谢途径精细调控玉米穗长和产量的功能基因, 其优良等位基因在玉米关联群体中十分稀有, 导入其优良基因可以使杂交种的穗长及籽粒产量增加。*ZmTPK2* 的克隆不仅加深了我们对基础代谢物 TPP 精细调控玉米穗长遗传基础及分子机制的认识, 也为玉米穗长及产量遗传改良提供了新的靶基因和新的思路。

**关键词:** 玉米; 穗长; 硫胺素焦磷酸 (thiamine pyrophosphate, TPP); 精细调控; 产量

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## Abstract 63

### HomeoBox genes regulatory network assists the dissection of genetic variation of plant height in maize

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**Abstract: 【Objective】** Plant height is an important trait for ideal plant type breeding. Reducing moderately plant height can achieve higher planting density, thereby achieving the goal of increasing yield. Maize plant height is a complex quantitative trait controlled by multiple genes, and the genetic regulation mechanism is complex. **【Method】** Here, we developed a transient and simplified CUT&Tag (tsCUT&Tag) that combines transient expression of transcription factor proteins in protoplasts with a simplified CUT&Tag without nucleus extraction. **【Result】** First used tsCUT&Tag technology system to systematically identify the target genes of the HomeoBox genes from HomeoBox family that were significantly related to plant height development. Based on the potential functional genes of plant height, a molecular regulatory network of 32 transcription factors was constructed, and the functional interaction and regulatory relationship between genes were systematically analyzed. The mutant of *wox13a* were dwarfed, increased number of tassel branches and early flowering by up-regulating *DLF1*, indicating the molecular regulatory network of *WOX13A* in maize. **【Conclusion】** HomeoBox genes regulatory network constructed using tsCUT&Tag assists the dissection of genetic variation of plant height, and reveal the molecular relationship between HomeoBox genes and known functional genes.

**Key words:** tsCUT&Tag; HomeoBox Genes; Plant Height

## Abstract 64

# High-resolution mapping reveals a *Ht3*-like locus against northern corn leaf blight

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**Abstract: 【Objective】** Maize (*Zea mays* L.) is the most widely grown crop around the world and is one of the most important crops for providing calorie and biofuel to human. Northern corn leaf blight (NCLB), caused by the fungal pathogen *Exserohilum turcicum*, poses a grave threat to maize production worldwide. Maize grown in areas with high humidity and moderate temperatures is more prone to NCLB outbreaks. Maize resistance to NCLB can be qualitative determined by race-specific *Ht* genes with major effects or quantitative controlled by multiple genes with minor effects. The resistance gene in A619*Ht3*, discovered decades ago, is an important genetic resource for NCLB control. **【Method】** To identify the causative locus resistance to NCLB in A619*Ht3*, we conducted near-isogenic lines (NILs) analysis for A619 and A619*Ht3*, together with the bulked-segregant analysis (BSA) for resistant and susceptible bulks derived from the cross of A619*Ht3* and L3162 lines. We used a sequential fine-mapping strategy based on recombinant-derived progeny to narrow down the location of the *Ht3*-like (*Ht3L*) locus. **【Result】** We initially detected the *Ht3L* locus in bin 8.06 that was closely associated with NCLB resistance. We then performed five rounds of sequential fine-mapping, which ultimately delimited the *Ht3L* locus to a 577-kb interval flanked by SNP markers KA002081 and KA002084. Plants homozygous for the *Ht3L/Ht3L* genotype exhibited an average reduction in diseased leaf area (DLA) by 16.5% compared to plants lacking *Ht3L* locus. The *Ht3L* locus, encompassing 15 annotated genes based on the reference genome from the line B73, showed extensive variation in genomic architecture among different maize lines and did not appear to contain any genes encoding canonical cell wall-associated kinases against NCLB. Moreover, the *Ht3L* locus was located ~2.7 Mb away from the known *Htn1* locus. **【Conclusion】** We speculate that the *Ht3L* locus may contain a bona fide *Ht3* gene or a novel NCLB resistance gene closely linked to *Ht3*. In practice, the *Ht3L* locus is a valuable resource for improving maize resistance to NCLB. Our results will facilitate the cloning of the causative gene underlying the *Ht3L* locus and accelerate application of *Ht3L* in the breeding of NCLB-resistant maize varieties.

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**Key words:** *Ht3*; northern corn leaf blight (NCLB); maize (*Zea mays* L.); maize disease; fine-mapping

## *ZmSPLn* 协同调控玉米株高和耐盐性的分子机制研究

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**摘要:** 提高我国玉米生产能力, 尽快摆脱进口玉米的依赖性, 是保障我国粮食安全的首要任务。在耕地有限的情况下, 有效利用中低产田, 是缓解我国玉米危机的有效途径。盐碱地是我国极为重要的后备耕地资源, 培育耐盐碱玉米新品种, 合理利用盐碱地是增加我国玉米产量的有效措施。但由于植物响应逆境的遗传网络非常复杂, 目前可用、具有我国自主知识产权的关键耐盐基因和调控元件还很少。因此, 克隆玉米中协同调控植株生长发育和耐盐性的关键基因, 解析其耐盐分子机制, 为耐盐玉米新品种提供优良基因源和理论依据具有重要意义。赤霉素在植物耐盐调控方面发挥着重要作用。研究表明, 植物能够通过调节体内赤霉素信号通路来适应高盐环境。本实验室前期发现一个转录因子 *ZmSPLn* 直接靶向和抑制赤霉素合成相关基因 *D1* 的表达, 进而影响株高和抗倒伏性。对 *ZmSPLn* 突变体及 *ZmSPLn* 过表达株系的耐盐性进行系统研究, 结果表明, *ZmSPLn* 突变体对盐敏感, 叶片中  $\text{Na}^+$ 、 $\text{Cl}^-$  含量升高,  $\text{K}^+$ 、 $\text{NO}_3^-$  含量降低, ROS 含量增加, 过表达材料则出现相反表型, 暗示 *ZmSPLn* 可能通过调控离子平衡, 清除过量 ROS 参与了玉米耐盐。*ZmSPLn* 主要在根中柱表达, 并且根中 *ZmSPLn* 能够快速响应高盐信号上调表达, 预示其可能是连接盐信号和赤霉素信号通路的关键因子, 并通过对赤霉素途径的调控影响玉米的耐盐性。进一步盐碱地田间实验分析表明, *ZmSPLn* 超量表达能明显提高玉米的出籽率; 常规地田间实验则发现, *ZmSPLn* 超量表达能显著提高密植条件下玉米产量, 预示着该基因有巨大的生产应用潜力。接下来我们将重点剖析 *ZmSPLn* 通过赤霉素信号途径协同调控玉米株高和耐盐性, 或直接调控玉米耐盐性的分子机理, 进而挖掘 *ZmSPLn* 的优异自然变异和单倍型, 开发其功能性分子标记, 为培育耐盐高产玉米新品种提供理论基础和基因资源。

**关键词:** 粮食作物; 玉米; 耐盐; 分子机理; 赤霉素

## Abstract 66

# Genetic variation in *ZmWAX2* confers resistance to *Fusarium verticillioides* in maize

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**Abstract:** **【Objective】** *Fusarium verticillioides* (*F. verticillioides*) is a widely distributed phytopathogen and incites multiple destructive diseases in maize, posing a grave threat to corn yields and quality worldwide. However, there are few reports of resistance genes to *F. verticillioides*. **【Method】** Here we identified a gene of *ZmWAX2* associated with quantitative resistant variations to *F. verticillioides* through a genome-wide association study in maize. **【Result】** A lack of *ZmWAX2* compromises maize resistance to *F. verticillioides*-caused seed rot, seedling blight, and stalk rot by reducing cuticular waxes deposition, while the transgenic plants overexpressing *ZmWAX2* show significantly increased immunity to *F. verticillioides*. A natural occurrence of two 7-bp deletions within the promoter increases *ZmWAX2* transcription, thus enhancing maize resistance to *F. verticillioides*. Upon *Fusarium* stalk rot, *ZmWAX2* greatly promotes the yield and grain quality of maize. **【Conclusion】** Our studies demonstrate that *ZmWAX2* confers a broad-spectrum resistance to *F. verticillioides*-caused diseases and can serve as an important gene target for the development of *F. verticillioides*-resistant maize varieties.

**Key words:** maize (*Zea mays* L.); *Fusarium verticillioides*; disease resistance; *ZmWAX2*; cuticular wax

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## Abstract 67

### MicroRNA156 interplay with microRNA166 to remold maize brace root architecture

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**Abstract: 【Objective】** The increasing studies have proved microRNAs (miRNAs) to play crucial roles in plant development and stress response. miR156 and miR166, two conserved miRNA families in monocots and dicots, are important regulators in plant leaf shaping, developmental change, organ polarity, floral fertility, fruit growth, abiotic stress response, and lateral root development. Moreover, numbers of researches displayed the possible crosstalk between two miRNA families, which still need to be further studied. **【Method】** In previous study, we have obtained transgenic mutants, *STTM156* and *STTM166*, with miR156 and miR166 specifically knockdown, respectively. **【Result】** The *STTM156* plants displayed decreased brace root whorls, root number per whorl and root angle. The *STTM166* plants exhibited decreased brace root whorls, root number per whorl, root length, but enlarged root angle. Our sRNA-seq data revealed miR166 to up-regulated express in *STTM156* plants, which implies the putative crosstalk within miR156 and miR166 is also exist in maize. In further study, we aim to dissect the underlying mechanisms of the interaction between miR156 and miR166, and to uncover their regulatory roles in remodeling maize brace root architecture

**Key words:** maize; microRNAs; *STTM156*; *STTM166*; brace root

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## Abstract 68

# Dissecting the genetic architecture of important agronomic traits in maize using the PI186182×B73 RIL population

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**Abstract:** Maize (*Zea mays* ssp. *mays*) originated from its wild ancestor, teosinte (*Zea mays* ssp. *parviglumis*) in the Balsas River Valley in southwestern Mexico. During the long process of maize domestication and selection, the morphological structure and growth habits of maize underwent drastic changes. Meanwhile, due to the genetic bottleneck and artificial selection, the genetic diversity was significantly reduced in maize. Recently studies indicate that South America is the second domestication center of maize and the germplasm from here contains abundant genetic diversity. Here, we construct a recombinant inbred lines population derived from the cross between PI186182 (a landrace from Uruguay, South America) and B73 (a temperate inbred line from North America). Using 4,583 high-quality SNPs obtained from GBTS sequencing, a genetic map with a total length of 1568.1 cM was constructed. 54 QTLs regulating 12 agronomic traits were detected by R/qtl with the mqm model, with a range of 5.3% to 27.3% of phenotypic variation explained by single QTL. QTL mapping results indicated that the traits of the same category exhibited similar genetic architecture, which was consistent with the analysis results of phenotype correlation. By dissecting the genetic structure of important agronomic traits in this RIL population and fully exploiting and utilizing the excellent genetic variation of South American germplasm, it is of great significance to broaden the genetic base of Chinese maize germplasm and break the current bottleneck of maize breeding.

**Key words:** Maize; agronomic traits; QTL mapping; landraces in South America

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## 玉米单倍体育性自然恢复基因定位研究

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**摘要:** 【目的】玉米单倍体育种技术获得纯合自交系仅仅需要 2-3 代, 显著缩短育种周期, 为打破单倍体基因组加倍瓶颈, 促进单倍体育种技术广泛应用。快速定位育性恢复相关 QTL 位点, 初步探明自然加倍临界时期。【方法】研究以低自然加倍种质 B73、MO17、CO1 为母本, 高自然加倍种质 DX 为父本组配  $F_1$ , 利用孤雌生殖高频诱导系豫高诱 1 号 (YHI-1) 生物诱导产生不同遗传背景的单倍体群体。利用集团分离分析法 (BSA) 进行育性恢复性状 (露药) 初定位; 区间开发 Indel 标记验证初定位结果; 单倍体不同发育时期开展流式细胞仪倍性检测。【结果】结合 Indel 标记和群体极端类型最终在 1、3、9 号染色体上鉴定到 3 个育性自然恢复相关 QTL 位点; 不同时期不同组织流式细胞结果显示: 不同组织均检测到二倍化细胞, 早期存在 2C 细胞比例高于 C 细胞的单倍体植株; 体细胞和生殖细胞加倍为独立过程。【结论】单倍体育性恢复性状受多基因控制, 单倍体在早期和后期均可发生基因组加倍。单倍体育性自然恢复 QTL 位点可应用于辅助选择高自然加倍种质, 而且可通过聚合提高基础材料育性恢复水平, 从而促进单倍体育种技术广泛应用。

**关键词:** 玉米; 单倍体; 自然加倍; QTL 定位

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## **KRN5b functions as a positive regulator for maize kernel row number**

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**Abstract: 【Objective】** Maize (*Zea mays* L.) yield is a major goal in maize breeding. An essential goal for raising yield is increasing the number of kernels produced per plant. Thus, identifying new quantitative trait loci (QTLs) corresponding kernel row number (KRN) will accelerate the process of yield related traits. **【Result】** Here, we describe a new kernel number-related gene *KRN5b* underlying the *qKRN5b* for KRN, which encodes an inositol polyphosphate 5-phosphatase (5PTase). The KRN5b/5PTase has phosphatase activity towards PI(4,5)P<sub>2</sub>, PI(3,4,5)P<sub>3</sub> and Ins(1,4,5)P<sub>3</sub> *in vitro*. Knockout of *KRN5b* leads to a significant accumulation of PI(4,5)P<sub>2</sub> and Ins(1,4,5)P<sub>3</sub>, resulting in disordered kernel rows and fewer kernels as well as low branch number on the tassel. Further investigation shows that *KRN5b* regulates inflorescence development through the phosphatidylinositol mediated signal transduction system. Furthermore, introgression of NX531 allele to different inbred lines shows that *KRN5b* can increase the yield of inbreds and corresponding hybrids from 10.1% - 12.2% via enhancing the KRN, with no trade-offs in other agronomic traits. **【Conclusion】** These results indicate that *KRN5b* involves in spikelet pair meristem development via the metabolism of inositol phosphates and phosphatidylinositols, and provide an excellent target for improving maize yield efficiently.

**Key words:** maize; kernel row number; inositol polyphosphate 5-phosphatase (5PTase)

## 玉米染色体数目变异与基因调控

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**摘要:** 【目的】基因剂量效应是指结构基因或者调控序列在基因组上的拷贝数发生改变, 而引起基因表达水平改变或者表型变化的现象, 对作物的抗病性、株型和果实发育等性状有重大影响, 也在多倍体和杂种优势形成中具有重要作用。利用基因剂量效应实现对玉米重要功能基因表达的精准调控, 对于突破产量、品质与抗逆等重要性状的表型瓶颈具有重要科学意义, 然而相关分子机制尚不清晰。【方法】本研究对322份含不同染色体数目的非整倍体(部分染色体数目变异)以及单倍体、二倍体、三倍体和四倍体(染色体组数目变异)玉米材料进行了RNA-seq和sRNA-seq, 对基因剂量效应影响基因表达及其分子机制进行了深入研究。【结果】研究发现玉米非整倍体中部分染色体数目的变异不仅影响拷贝数变异区域的基因表达, 同时也影响拷贝数无变异区域基因的表达, 造成非整倍体玉米整体基因表达的失衡与转录因子调控网络的紊乱, 是基因剂量效应影响作物农艺性状的重要原因。相较而言, 单倍体和多倍体中的蛋白质复合体化学计量未发生变化, 基因组仍处于平衡状态。同时, 研究通过对microRNA(miRNA)靶基因的预测及降解组测序的数据分析, 阐明了miRNA参与非整倍体的基因调控, 其剂量变化导致靶基因的差异性表达。【结论】研究阐明了基因剂量效应对基因互作关系造成的不同影响是导致非整倍体和多倍体性状差异的重要原因, 揭示了基因和miRNA的剂量效应可用于实现重要功能基因的精准调控, 从而打破基因多效性造成的权衡效应, 为突破育种瓶颈、创制玉米新品种提供新的思路。

**关键词:** 玉米; 剂量效应; miRNA; 基因调控网络

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## Abstract 72

# Combined QTL mapping and RNA-Seq pro-filing reveal candidate genes related to low-temperature tolerance in maize

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**Abstract: 【Objective】** Maize is affected by low-temperatures throughout its growth process, especially during germination. Therefore, it is important to identify more QTLs or genes associated with germination under low-temperature conditions. **【Method】** QTL analysis for low-temperature related traits during germination using 213 maize inbred lines from the Syn10 DH population. Combining RNA sequencing and qRT-PCR techniques to identify key candidate genes related to low-temperature tolerance during germination in maize. **【Result】** Twenty-eight QTLs associated with eight phenotypic traits for maize germination at low-temperatures were detected, with phenotypic contributions ranging from 5.4% to 13.34%. 14 overlapping QTLs generated six QTL clusters on each chromosome, with three (*cQTL1-2*, *cQTL9-1* and *cQTL9-2*) all with phenotypic contribution rates greater than 10%, all associated with radicle traits. Combined with RNA sequencing analysis, these QTL clusters included six DEGs associated with low-temperature tolerance. qRT-PCR analysis showed that the *Zm00001d045568* gene in the LT\_BvsLT\_M and CK\_BvsCK\_M groups at four-time points expression trends were highly significantly different ( $P<0.01$ ), encoding the RING zinc finger protein. It was located on *qRTL9-2* and *qRSVI9-1* and was associated with total length and simple vigour index. **【Conclusion】** Six consistent QTLs related to low-temperature tolerance in maize germination were detected. Among them, the phenotypic contribution of *cQTL1-2*, *cQTL9-1*, and *cQTL9-2* exceeded >10%. A key DEG for low-temperature tolerance in maize germination, encodes a RING zinc finger protein and is located in *cQTL9-2* (*qRTL9-2* and *qRSVI9-1*). This study provides a genetic basis for molecular marker-assisted breeding, low-temperature tolerant seed germination, and future functional studies of growth and development.

**Key words:** Maize; Low-temperature; QTL; RNA-Seq; Candidate genes

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## ***Abstract 73***

### **Ectopic expression of *Zig* leads to the degradation of TPR1/2, resulting in fused organs above the ear in maize**

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**Abstract:** Delineation between distinct populations of cells is essential for organ development. Boundary formation is necessary for the maintenance of pluripotent meristematic cells in the shoot apical meristem and differentiation of developing organs. Boundary formation and maintenance are poorly understood processes. Here, we characterize the maize dominant mutant *Zig*, which exhibits fused organs and curved stems above the ear. Positional cloning analysis indicate *Zig* encodes serine carboxypeptidase-like protein. Ectopic expression of the *Zig* gene in the marginal domain of leaf primordium base in the *Zig* mutant shoot apex leads to a significant reduction in its interaction protein RELK2 and RELK3 abundance. *relk2/3* mutant likewise exhibits an organ fusion phenotype similar to *Zig* mutant. Moreover, overexpression of *Zig* gene in maize can reduce plant height by reducing the number of nodes under the ear. In summary, this study identified a novel family protein, ZIG, maintained organ morphogenesis by degrading RELK2/3 protein in maize and opened a new perspective for the studying of molecular mechanisms underlying organ morphogenesis.

**Key words:** organ boundary; serine carboxypeptidase-like protein; ZIG; RELK2; RELK3; maize

## ***Abstract 74***

### **Mechanism dissection of maize sRNA biogenesis based on genome-wide association study**

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**Abstract:** **【Objective】** sRNA is widely involved in plant growth and environmental adaptation progresses. As an important scientific question of plant RNA biology, dissection of plant sRNA biogenesis mechanisms are urgently needed. Maize is a model monocot plant, the mechanisms of maize sRNA biogenesis are largely unknown. **【Method】** In this study, a series expression traits were integrated from sRNA sequencing data of 338 maize inbreds by using a repeated “truncate-align” method. These expression traits were further used for identifying sRNA biogenesis related genes through genome-wide association studies (GWAS). **【Result】** Totally several hundred sRNA expression associated loci are identified, including dozens of genes such as RACK1, CPL1 and MOS1 whose orthologs take part in regulating Arabidopsis sRNA biogenesis. A candidate gene sRAG1 (sRNA Associated Gene 1), which encodes a deubiquitinating enzyme, localizes in both nucleus and cytoplasm. Y2H result indicates that sRAG1 can interact with multiple D-body (Dicing-body) proteins such as SMA1 and STV1, suggests a potential role of sRAG1 in regulating D-body and maize sRNA biogenesis. **【Conclusion】** In this study, we construct a series of expression traits which can be effectively used to characterize maize sRNA biogenesis, identify hundreds of candidate loci and genes associated with maize sRNA expression, and reveal a potential role of sRAG1 in maize sRNA biogenesis by combining GWAS and molecular experiments. Although the effects of sRAG1 on maize sRNA biogenesis regulation need further study, our study provides a feasible approach to dissect maize sRNA biogenesis progress.

**Key words:** maize; sRNA biogenesis; GWAS; D-body



## 时空转录和染色质可及性图谱揭示雌雄花序间的形态性别差异

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**摘要:** 【目的】玉米穗粒数产量性状与穗型建成有关, 而穗型建成决定于穗发育早期, 受花序发育相关基因调控, 另外玉米雌穗和雄穗是两种不同的花序类型, 具有相似的起始发育过程, 但会逐渐分化出各自独特的形态性别结构, 因此解析雌、雄花序发育分化的时空转录调控动态, 对穗型优化和穗粒数改良具有重要意义。【方法】本研究通过精细解剖雌、雄穗上6种不同类型/时期的花序分生组织, 然后获得了高质量的RNA-seq和ATAC-seq数据。【结果】分析发现雌、雄花序分生组织分化过程中, 差异表达基因数量均存在两个峰, 分别为顶端分生组织向侧生分生组织转换过程和早期小花分生组织分化产生花器官过程, 表明这两个分化过程分子调控更为剧烈。另外雄穗中的分生组织分化转换过程相对于雌穗涉及更多的生物学调控途径, 暗示雄穗具有更加复杂的调控模式。进一步对雌、雄相同分生组织间的基因表达动态分析表明随着分化进程的发展, 雌、雄分生组织间的差异表达基因呈逐渐增多的趋势, 特别当花器官产生后, 雄穗具有大量上调表达的基因, 这些基因与茉莉酸、水杨酸、乙烯、细胞程序性死亡和超敏反应途径有关, 可能决定了雄穗小花的形态和性别命运。最后我们发现染色质重编程与分生组织发育分化过程的基因表达动态相关, 基因调控区可及性的改变塑造了不同的转录因子调控模式, 从而影响分生组织发育分化进程。【结论】以上结果加深了我们对玉米雌、雄花序形态建成和性别决定生物学过程的理解, 并表明雄花序在该过程中具有更加复杂的调控机制。

**关键词:** 穗发育; 花序分生组织; 花器官; 性别决定; 染色质可及性

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## *Abstract 76*

# **Chromosome-level Genome Assembly of Maize Inbred Line LH244**

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**Abstract:****【Objective】**The maize LH244 is a superior inbred line for its good agronomic traits and high efficiency for genetic transformation. **【Method】** Here, we present a chromosome-level genome assembly of LH244 using long reads and Hi-C. **【Result】** The LH244 genome comprises 10 chromosomes (15 contigs), with a total size of 2.28Gb. Notably, the contig N50 reaches an impressive length of 206.11Mb. Moreover, the entire genome exhibits minimal fragmentation, as only 5 gaps were identified. Particularly noteworthy is the gapless assembly achieved at the T2T level for chromosomes 3, 5, 7, 8, 9, and 10. Through comparative analysis, we observed a significant similarity between LH244 and the B73 inbred line. A striking 99.96% of B73 genes were found within the LH244 genome, surpassing the core gene count of maize. Employing the AnchorWave software, we conducted a comprehensive comparison between the Mo17(T2T) and LH244 genomes. Remarkably, satellite sequences emerged as the major contributors to megabase-scale structural variations (SV) in both genomes, which correlates with the primary disparity in genome sizes. **【Conclusion】** Our study provides a high-quality reference-genome sequence and a basis for analyzing the relationship between genetic differences and morphological differences and for subsequent mining of potential genes with high genetic transformation efficiency of LH244.

**Key words:** LH244; chromosome-level genome assembly; reference-genome sequence

## miR164 调控玉米丝黑穗病抗性功能和机制初步解析

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**摘要:** 【目的】玉米丝黑穗病是由丝轴黑粉菌 (*Sporisorium reilianum*) 引起的世界性真菌土传病害, 在我国北方春玉米区尤为严重。因此, 开展miRNA参与调控玉米丝黑穗病的抗性功能和机制解析研究具有重要价值。【方法】本研究以Mo17和黄早四为材料, 混合交配型菌液分别采用浸泡胚根法和针刺中胚轴法进行玉米丝黑穗病人工接种, 分别在响应病原菌侵染和扩展时间点取玉米中胚轴, 进行小分子RNA和降解组测序, 并采用qRT-PCR的方法验证重要差异表达miRNA。构建关键miRNA过表达和抑制表达载体并转化玉米自交系B104, 对转基因后代株系进行玉米丝黑穗病室内和田间接种鉴定并明确其抵抗丝轴黑粉菌侵染的时空表达模式, 进而检测与植物抗病相关的POD、SOD、PAL酶活性和GA3、IAA、ABA、JA、SA等内源激素含量, 观察丝轴黑粉菌菌丝生长和玉米细胞结构变化情况, 从而初步明确miR164h调控玉米丝黑穗病抗性的机制。【结果】筛选并验证出玉米响应丝轴黑粉菌侵染和扩展的关键miRNA, 其启动子可驱动GUS基因在拟南芥种皮和叶片中表达。室内人工接种丝轴黑粉菌抗性鉴定发现, 与受体对照相比过表达转基因株系丝黑穗病抗性降低, 而抑制表达转基因株系则表现为抗病性提高。田间接种鉴定表明抑制表达株系的发病率降低16%-24%, 极显著低于受体对照 ( $P<0.01$ ), 而过表达株系的发病率增加18%-26%, 极显著高于受体对照 ( $P<0.01$ )。玉米根、中胚轴和叶片均参与miR164h调控的丝黑穗病抗性途径, 且受病原菌侵染12h后, miR164在根部先识别丝轴黑粉菌并表达。POD、SOD和PAL均参与miR164调控的丝黑穗病抗性途径, 在12h的中胚轴部POD和SOD首先参与miR164调控的丝黑穗病抗侵染途径, 到V2期仅POD参与调控, 而PAL则是在V10和VT期参与miR164调控的丝黑穗病抗性途径。ABA仅在早期参与miR164调控的丝黑穗病抗性途径, 而JA和SA主要在接种后6d的中胚轴参与miR164调控的丝黑穗病抗扩展途径。在过表达和抑制表达株系的抗侵染时间点12h, 丝轴黑粉菌的菌丝和玉米细胞开始发生变化, 且在VT期差异极显著。过表达株系中丝轴黑粉菌菌丝扩展广泛, 细胞结构完全破坏, 出现大量冬孢子, 而抑制表达株系中菌丝集中分布, 玉米细胞内部出现自噬现象, 但细胞壁较为完整。【结论】玉米中miR164负向调控玉米丝黑穗病抗性, 其在12h根部抵抗病原菌的侵染, 并调控中胚轴POD和SOD等酶的活性, 玉米细胞出现程序性细胞死亡现象并阻滞病原菌扩展, 导致JA和SA等激素含量下降, 到V10期PAL酶活性升高, 进而限制丝轴黑粉菌菌丝的扩展, 最终在VT期玉米细胞出现自噬现象, 将菌丝隔离在死细胞内, 从而提高其抗病性。

**关键词:** 玉米; 丝黑穗病; miR164; 抗性功能和机制; 初步解析

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## **Genetic basis of sexual conversion of the terminal lateral inflorescence during maize domestication**

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**Abstract:** The sexual conversion of the terminal lateral inflorescence from tassel (male) to ear (female) is a key event during the domestication of maize from its wild progenitor teosinte (*Zea mays* ssp. *parviglumis*). However, the underlying molecular mechanism is largely unknown. To dissect the genetic basis of sexual conversion of the terminal lateral inflorescence, we performed Quantitative trait locus (QTL) mapping a maize-teosinte population. A total of six QTL were identified, including three novel QTLs *STAM1.1*, *STAM1.2*, *STAM2.1*. Through positional cloning, we have narrowed *STAM2.1* down to a 600 kb region containing 11 genes. The goals of this study are to (1) Clone and verify the underlying gene of *STAM2.1* that has a large effect conditioned on teosinte *tb1* allele on the male into female conversion; (2) Fine map and clone a second QTL *STAM1.1* that has a similar effect; (3) Characterize the statistical and molecular interactions of *STAM1.1* and *STAM2.1* with *tb1*. Uncovering the molecular regulatory mechanism of QTLs that are responsible for the sexual conversion will enhance our understanding of maize inflorescence development.

**Key words:** Maize; Sexual conversion; QTL

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## **Analysis of the kernel characteristics of maize inbred lines with different dehydration types**

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**Abstract:** High moisture content of maize kernels at harvest not only limits mechanized harvesting. It also affects post-harvest quality and storage costs. Therefore, it is crucial to select and breed machine-harvested maize varieties. To less the noise of the different growth period, two pairs of maize inbred lines with different kernel moisture were sowed at the different period according to their maturity and growth period to keep the starting point of seed development. The physiological mechanism of kernel dehydration in terms of kernel water content, kernel starch structure and programmed cell death (PCD) was then investigated in different inbred maize lines. It was found that the two pairs of materials differed significantly in the rate of dehydration at least one week after physiological maturity: 35DAP-49 DAP for the mid-early maturity materials and 49 DAP-56 DAP for the mid-late maturity materials. With the process of kernel development, the range of PCD first extended from the middle of the endosperm to the top, and then extended to the base of the kernel. Meanwhile, the inbred lines with fast dehydration rates had faster PCD processes and reached maximum filling more quickly, with larger maximum and average filling rates. Further, it was found that the inbred lines with faster dehydration rates had more powdery starch, which was mostly spherical in structure and loosely arranged, and the proteins were dispersed in the cytoplasmic matrix; while the inbred lines with slower dehydration rates had more angular starch, which was mostly polyhedral and connected with the proteins to form a dense protein matrix network, in which the starch was mosaic. In conclusion, the characterization of starch in kernel will affect the dehydration and the PCD performed significantly different between inbred lines with fast dehydration rate.

**Key words:** after physiological maturation; dehydration; kernel characteristics; starch granules

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## **Cloning and functional analysis of *ppa1* controlling upper leaf angle in maize**

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**Abstract:** Dense planting is a crucial cultural technique to continuously increase maize grain yields. The size of leaf angle is the key factor determining whether maize plants can adapt to dense planting. The maize production has shown that the pyramid-like plant architecture with narrow leaf angle at upper canopy but flat leaf angle at lower canopy is more adapted to dense planting. However, most of known maize leaf angle genes affect leaf angle of all leaves in a plant. We previously identified an elite line with a pyramid-like plant architecture, named *pyramid-like plant architecture 1 (ppa1)*. A 273-bp insertion in the first exon of *ppa1* was found is the functional variant causing the decreased leaf angle through map-based cloning. The ligular region is the key anatomical tissue that establishes leaf angle in maize. The histological analyses of *ppa1* and wild-type plants indicate that *ppa1* exhibits smaller auricle size and curved auricle, thereby leading to the pyramid-like plant architecture. Meanwhile, the yeast one-hybrid, EMSA and the transient expression assays demonstrate that ZmRAVL1 could directly bind *ppa1* promoter and activate *ppa1* expression. The field trials of *ppa1* and wild-type showed that *ppa1* with upright leaf angle out-yielded wild-type under high planting density. We will further screen and pyramid favorable alleles at different leaf angle genes to create ideal plant architecture for dense planting, which will set important theoretical and practical basis for breeding high-yield varieties for adaptation to high-density planting.

**Key words:** Maize; leaf angle; *ppa1*

## **RNA sequencing of cleanly isolated early endosperms reveals coenocyte-to-cellularization transition features in maize**

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**Abstract:【Objective】**Early endosperm development in maize (*Zea mays*) is essential for creating a functional endosperm for filling, but its rapid and dynamic process remains largely unknown. The coenocytic stage is a particular stage with rapid nuclear division without cytokinesis. From 48-144 hours after pollination (HAP), endosperm mainly undergoes four cellular processes: coenocyte, cellularization, cell proliferation, and differentiation. Although the high temporal-resolution transcriptome data within 144 HAP of maize kernel development have been investigated, due to technical limitations, the samples contained the maternal nucellus and the embryo sac; as a consequence, many endosperm-specifically-expressed genes might be over-looked. **【Method】**In this study, we isolated early endosperms by free hand and laser-capture microdissection (LCM) and generated high-resolution transcriptome data from 48 to 144 HAP with an interval of 24 h. **【Result】**Through weighted gene co-expression network analysis (WGCNA), we identified nine distinct modules of co-expressed gene sets, of which Module 7 was composed of 5,555 genes that showed the highest expression levels at the coenocytic stage. In Module 7, 391 genes were not expressed in nucellus, and thus were named as the Coenocyte-Expressed (CE) Gene Set. These genes were involved in transcriptional regulation and auxin-activated signaling pathway. Consistent with the stage transition of early endosperm development, the co-expressed gene sets and enriched gene function modules were changed accordingly. We verified the reliability of the transcriptome data by in situ hybridization. **【Conclusion】**Our work provides a valuable gene resource for early endosperm development studies in the future.

**Key words:** maize; Transcriptome; early maize endosperm development; coenocyte

## 控制玉米香蕉穗性状的位点 *qBE1* 的精细定位与 候选基因预测

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**摘要:** 【目的】受全球气候变化影响, 玉米香蕉穗的发生率和发生区域逐年升高, 成为玉米减产的一个重要原因。香蕉穗表型出现的遗传基础是雌穗穗柄节间叶腋分生组织活性维持失衡打破休眠从而启动腋芽/次生穗的形态建成。本研究的目的是精细定位并克隆控制玉米穗柄节间叶腋分生组织活性维持的主效位点, 为解析雌穗穗柄节间分生组织活性维持的分子调控机理, 减少香蕉穗的发生打下基础。【方法】本研究收集到两个除香蕉穗性状外无差异的两个掖478自交系, 分别重命名为掖478<sup>S</sup>和掖478<sup>R</sup>。首先使用以掖478<sup>S</sup>为背景、齐319为供体构建的染色体片段代换系(CSSL; 部分株系渗入了掖478<sup>R</sup>的染色体片段)家系构建极端池进行BSA分析, 在1号染色体上定位到1个QTL位点 $qBE1$ ; 随后使用掖478<sup>R</sup>和无香蕉穗表型CSSL家系CL16分别与掖478<sup>S</sup>构建高代次级分离群体进行精细定位。结合定位区间内基因的时空表达信息和测序结果预测候选基因。【结果】芯片分析显示掖478<sup>S</sup>和掖478<sup>R</sup>间仅有3.84%的SNP差异。BSA分析在1号染色体检测到1个QTL位点 $qBE1$ 。随后利用构建的高代次级分离群体将该位点限定在约750 kb的物理区间内, 共包含11个基因。我们利用MaizeGDB网站的基因表达数据对候选基因的表达部位进行分析, 发现仅有 $gene11$ 在玉米幼穗和节间高表达, 我们对掖478<sup>S</sup>、掖478<sup>R</sup>和CL16的 $gene11$ 基因组序列进行了测序, 结果显示掖478<sup>R</sup>与CL16的序列一致, 与掖478<sup>S</sup>共有15处SNP和2处插入缺失差异, 导致蛋白序列出现4处氨基酸置换和2处插入缺失, 其中有1处氨基酸置换和1处插入缺失发生在保守区间。前人报道 $gene11$ 在 $tb1$ 和 $gt1$ 突变体的分蘖芽中表达下调, ChIP-seq结果显示 $gene11$ 是 $TB1$ 的靶基因, 说明 $gene11$ 可能参与了 $TB1$ 和 $GT1$ 调控叶腋分生组织活性维持产生分蘖的过程。综上, 我们认为 $gene11$ 是控制雌穗穗柄节间叶腋分生组织活性维持的一个候选基因。【结论】本研究利用仅香蕉穗表型有差异的掖478<sup>S</sup>和掖478<sup>R</sup>自交系及CSSL群体鉴定到一个控制雌穗穗柄节间叶腋分生组织活性维持的主效位点 $qBE1$ , 并使用高代次级分离群体将定位区间缩小到约750 Kb, 结合时空表达信息和测序结果预测了一个候选基因。

**关键词:** 香蕉穗; 雌穗穗柄节间叶腋分生组织; QTL; 精细定位; 候选基因

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## 玉米新杂优模式“外杂选×内杂选”的创制和新品种选育

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**摘要:** 【目的】黄淮海夏玉米区是我国重要玉米产区, 郑单958及其衍生品种种植面积常年占据该区半数以上。其“改良瑞德×塘四平头”的杂优模式也成为该区玉米品种的主导模式, 导致形成“杂优模式”单一, 种质资源狭窄的现状, 多数品种存在耐密、抗倒性一般, 后期籽粒脱水慢等缺陷, 已不能满足该区玉米生产对品种耐密、抗倒、籽粒后期脱水速率快的新需求。针对上述问题, 本研究的研究目的是解决目前黄淮海夏玉米区主推玉米品种遗传基础狭窄和脆弱的问题, 进一步丰富玉米种质遗传基础。【方法】分别选用黄淮海夏玉米区主推的属于“SS×NSS”杂优模式的先玉335、先玉32T24、先玉1872等国外杂交种和属于“改良瑞德×塘四平头”杂优模式的郑单958、伟科702、浚单20等国内杂交种为基础材料, 利用系谱法或单倍体育种技术分别创制“外杂选”自交系和“内杂选”自交系。在选育过程中采用高密度种植、南北穿梭、定向选择等方法, 使“外杂选”自交系具有抗倒、抗病性、结实性好, 一般配合力高, 籽粒脱水快等优点, “内杂选”自交系具有抗倒、抗病性好, 广适, 一般配合力高等优点。同时, 将自交系基因数据和育种软件运用到自交系创制和品种选育过程中, 大幅提高了工作效率。【结果】创制出WL134、冀42、32T33等耐密、多抗、自身产量高的“外杂选”自交系14份和冀H521、冀H522、冀1877等抗逆、耐密、一般配合力好的“内杂选”自交系9份。利用“外杂选×内杂选”的杂优模式育成冀玉3421、冀玉228、金苑玉389、冀玉911、冀玉912等玉米新品种5个, 其中中国审品种3个, 省审品种2个, 另有多个组合正在参加各级试验。该杂优模式类型玉米新品种均具有耐密、抗倒、高产、稳产等优点。【结论】本单位基于多年育种经验, 创新性的提出并实践了“外杂选×内杂选”的新杂优模式。此杂优模式很好的将国外种质坚韧、抗倒、籽粒脱水快和国内种质抗病、抗逆、广适的特性有机结合, 打破了黄淮海现有主推品种的杂优模式, 有效的解决了黄淮海地区玉米遗传基础狭窄和脆弱的问题, 并育成系列玉米新品种。

**关键词:** 黄淮海夏玉米区; 杂优模式; 外杂选; 内杂选; 玉米新品种; 种质资源

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## Abstract 84

### Analysis of QTLs and candidate genes for tassel symptoms in maize infected with *Sporisorium reilianum*

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**Abstract: 【Objective】** Heat smut is a fungal soil-borne disease caused by *Sporisorium reilianum*, which is a widespread global disease. When the plant infection with this disease, it will affect the development of male and female tassels. Our previous research found that tassel symptoms of maize infected with *Sporisorium reilianum* of some inbred lines with Sipingtuo blood had significantly different and stable heredity at many years of multi-locations. **【Method】** In present study, the QTLs that control the different symptoms of maize tassel infected with *Sporisorium reilianum* were located by two F2 populations which were constructed using three typical inbred lines (Huangzao4, Jing 7 and Chang7-2). The BSA (Bulked Segregation Analysis) method was used to construct extremely mixed gene pools based on the typical symptoms of the tassels.

**【Result】** The QTLs of different symptoms of maize tassel infected with *Sporisorium reilianum* were detected with 869 SSR markers which covering the whole genome of maize. The extremely mixed gene pools were screened with polymorphic markers between the parents. Further other SSR markers were added near the above marker, to detect genotypes in partially single plants in F2 populations that are phenotypic identification. **【Conclusion】** The QTL controlling the tassel symptoms of Huangzao4 and Jing 7 was located in the bin1.06 region, between markers of umc1590 and bnlgl1598, which explained 21.12% of the phenotypic variation with an additive effect of 0.6524. The QTL controlling the tassel symptoms of Jing 7 and chang7-2 were located in the bin2.07 region, between markers of umc1042 and bnlgl1335, which explained 11.26% phenotypic variation with an additive effect of 0.4355. Two candidate genes (*ZmABP2* and *Zm00001D006403*) were identified by a conjoint analysis of label-free quantification proteome sequencings.

**Key words:** QTL; *Sporisorium reilianum*; bulked segregation analysis; maize; tassel symptoms

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## 玉米根腐病抗性相关代谢物挖掘和微生物群落特征解析

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**摘要:** 【目的】禾谷镰孢菌 (*Fusarium graminearum*, Schw., F.g) 引起的玉米根腐病 (Gibberella root rot), 由于表型鉴定困难等导致玉米根腐病抗性机制研究相对滞后, 尤其是, 玉米根腐病抗性相关代谢途径及根际微生物群落的研究尚不多见。【方法】【结果】本研究通过鉴定真菌相对生物量、病级评定及病理切片等方法筛选到两对抗、感材料 (A08 (感)、K09 (抗) 和Y03 (感)、H05 (抗))。代谢组学分析表明, 玉米根腐病抗性与黄酮类 (Flavonoids) 生物合成途径显著相关, 黄酮 (flavone)、类黄酮 (flavonoid)、异黄酮 (isoflavonoid) 主要代谢产物及衍生物含量均在抗病品种中显著增加, 相关的催化合成酶基因在抗病材料中的表达水平显著高于其在感病材料中的表达。其中, 携带甲基化官能团的代谢物或衍生物在抗病品种中的含量显著升高, 比如, 对禾谷镰孢菌菌丝及孢子生长均具有一定抑制性的樱花素 (Naringenin 7-methyl ether) 及异黄酮途径主要代谢物。对抗、感材料的根际土壤和根内微生物多样性分析表明, 黄杆菌 (*flavobacterium*)、寡养单胞菌 (*Stenotrophomonas*)、异样根瘤菌 (*Allorhizobium-Neorhizobium-Pararhizobium -Rhizobium*) 属等主要富集在抗病品种根内及根际土中, 微生物次生代谢途径的功能基因, 特别是抗生素合成相关基因在抗病材料根际土中显著上调。安徽黄杆菌 (*Flavobacterium anhuiense*) 是黄杆菌属中丰度最高的小种, 对禾谷镰孢菌有微弱抑制作用, 并显著增加玉米地上部植株高度。【结论】本研究初步揭示了玉米根腐病抗性相关氧甲基化代谢物及合成酶在抗病品种中的显著富集和表达, 尝试利用抗病材料中显著富集的根际微生物群落, 挖掘有助于根腐病抗性提高的生防菌。本研究以代谢组学和植物-微生物互作为出发点, 为玉米根腐病抗病性研究提供新的微生物资源和研究思路。

**关键词:** 玉米根腐病; 黄酮类代谢途径; 氧甲基化代谢物; 黄杆菌

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## Abstract 86

# Genetic variation in *ZmHLN6* controls husk leaf number and grain dehydration rate in maize

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**Abstract: 【Objective】** The trait of husk leaf number (HLN) is the key factor determining grain dehydration rate at the maturity stage of maize. However, the husk leaf number is a complex quantitative trait, the intensive genetic study of which is relatively poor. **【Method】** Here, we identified a major quantitative trait loci controlling husk leaf number on chromosome 6, explaining 10% phenotype variance. Through multi-year map-based cloning, *qHLN6* is mapped to 2.9-Kb region in the promoter of *ZmHLN6*, which encodes a transcription factor. **【Result】** Comparisons of the NIL-*qHLN6*<sup>PHG</sup> and NIL-*qHLN6*<sup>LJ</sup> alleles indicated that *qHLN6*<sup>PHG</sup> decreased 2~3 husk leaves relative to *qHLN6*<sup>LJ</sup>, which significantly increased grain dehydration rate. Real-time quantitative polymerase chain reaction show that *ZmHLN6* expression was lower in NIL-*qHLN6*<sup>LJ</sup> relative to NIL-*qHLN6*<sup>PHG</sup> in the early stage of maize in the florescence meristem development. Relative to wide-type plants, two independent mutant lines consistently increase ~10 more husk leaves. Thus, these findings indicate that husk leaf number changes are mediated through changes in *ZmHLN6* expression, most likely caused by polymorphisms within the 2.9-Kb region in the promoter of *ZmHLN6*. **【Conclusion】** Furthermore, the analyses of the upstream regulation factors and downstream target genes will be explored thoroughly to construct the comprehensive genetic network controlling the husk leaf number. These results will product important theoretical and practical bass for breeding less-HLN new varieties suitable for mechanical harvesting.

**Key words:** husk leaf number; grain dehydration rate; *ZmHLN6*

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## Abstract 87

# Combining QTL mapping with multiomics profiling reveals genetic control of corn leaf aphid (*Rhopalosiphum maidis*) resistance in maize

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**Abstract: 【Objective】** Corn leaf aphid (*Rhopalosiphum maidis*) is a major maize pest, frequently causing substantial yield losses. Exploring the genetic basis of aphid resistance is important for improving maize yield and quality. **【Method】** Here, we used a maize recombinant inbred line population derived from two parents with different susceptibility to aphids, B73 (susceptible) and Abe2 (resistant), and performed quantitative trait locus (QTL) mapping using aphid resistance scores as an indicator. To further investigate the mechanism of aphid resistance in Abe2, we constructed transcriptome and metabolome libraries from Abe2 and B73 leaves with or without aphid infestation at different time points (0, 6 and 24h). **【Result】** We mapped a stable QTL, *qRTA6*, to chromosome 6 using data from two years' field trials, which explained 40.12%–55.17% of the phenotypic variation. Integrating QTL mapping and transcriptome data revealed three aphid resistance candidate genes (*Zm00001d035736*, *Zm00001d035751*, and *Zm00001d035767*) associated with the hypersensitive response, the jasmonic acid pathway and protein ubiquitination. Integrated transcriptomic and metabolomic analysis revealed that the differentially expressed genes and metabolites were enriched in flavonoid biosynthesis. **【Conclusion】** These findings extend our understanding of the molecular mechanisms controlling aphid resistance, and the QTL and candidate genes are valuable resources for increasing aphid resistance in maize.

**Key words:** Aphid resistance; maize; metabolome; QTL mapping; transcriptome; flavonoids

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## ***Abstract 88***

# **Spatiotemporal Gene Expression Dynamics and Cell Atlas during maize kernel Development**

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**Abstract: 【Objective】** Maize is one of the most widely planted and high-yielding crops in the world. Maize kernels are the storage organs of starch, protein and oil. The accumulation of these substances cannot be achieved without the cooperation of various compartments derived from diploid seed coats from maternal tissue, triploid endosperm and diploid embryo from progeny during kernel developing stage. Little is known in maize, however, about how many cell populations exist, and how the cell populations function and orchestrate with each other, becoming the bottleneck to explore more genes to improve agronomic traits. **【Method】** Laser-capture microdissection (LCM) enables focusing on the interested cells under direct microscopic visualization, but it is difficult to isolate especially cell populations from the interface due to few cell layers and the cell similarity, such as the regions of aleurone, basal endosperm transfer layer (BETL), the endosperm adjacent to scutellum (EAS) and the embryo-surrounding region (ESR), leading to surrounding tissue contamination and insufficient sample collection. The single-cell transcriptome atlas relies heavily on known molecular markers for cell identification because of the loss of spatial information, which inhibits to study certain regions in terms of absence of molecular markers. The spatial transcriptome technique is used to quantify gene expression over the whole tissue section and to preserve spatial information using the unique oligonucleotide barcodes. Here, we pioneered to profile gene expression patterns using Spatial Transcriptomics for the first time and identified eleven functional cell populations distributed in the kernel. **【Result】** We defined the molecular markers of each region based on differential gene expression, which can guide scientists to study the function of interested genes in the target region. We provided a publicly available web resource that could be requested to visualize the electronic RNA in situ hybridization map over the whole tissue section using gene id. **【Conclusion】** Compared with traditional RNA in situ hybridization only doing one gene at a time, the spatial transcriptomics is to quantify all gene expression in a single experiment, which is more accurate, sensitive efficient and higher throughput. The generated landscape of organ-wide gene expression and the comprehensive model in terms of starch, protein and lipid accumulation would facilitate gene annotation and superior gene mining.

**Key words:** maize; spatial transcriptomics; cell populations

## QTG-Miner aids rapid cloning of quantitative trait loci and dissection of co-directional selection of tassel branch number in maize

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**Abstract: 【Objective】** The genetic dissection of agronomic traits is important for crop improvement and global food security. Phenotypic variation of tassel branch number (TBN), a major breeding target, is quantitative and controlled by many quantitative trait loci (QTLs) with major or minor effects. The lack of large-scale QTL cloning methodology constrains the systematic dissection of TBN, which hinders modern maize breeding. **【Method】** We developed QTG-Miner, a multi-omics data-based technique for large-scale and rapid cloning of quantitative trait genes (QTGs) in maize (*Zea mays*). **【Result】** Using QTG-Miner, we cloned and verified seven genes underlying seven TBN QTLs. QTG-Miner performed robustly for both major- and minor-effect TBN QTLs. Selection analysis indicated that a substantial number of genes, such as *lrs1* (*liguleless related sequence1*), and network modules were subject to selection during maize improvement. Co-directional selection signatures were significantly enriched in multiple biological pathways between female heterotic groups and male heterotic groups during modern hybrid maize breeding. **【Conclusion】** In summary, QTG-Miner provides a large-scale approach for rapid cloning of QTGs in crops and sheds light on the genetic basis of TBN for further maize breeding.

**Key words:** QTG-Miner; tassel branch number; network; co-directional selection

## Characterization of regulatory modules controlling leaf angle in maize

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**Abstract:** **【Objective】** Leaf angle is an important agronomic trait determining maize (*Zea mays*) planting density and light penetration into the canopy and contributes to the yield gain in modern maize hybrids. However, little is known about the molecular mechanisms underlying leaf angle beyond the *ZmLG1* (*liguleless1*) and *ZmLG2* (*Liguleless2*) genes. **【Method】** We integrate the methods from genetics, molecular biology, cell biology, functional biology and bioinformatics will help to understand the regulation mechanisms of leaf angle. **【Result】** In this study, we found that the transcription factor (TF) ZmBEH1 (BZR1/BES1 homolog gene 1) is targeted by *ZmLG2* and regulates leaf angle formation by influencing sclerenchyma cell layers on the adaxial side. ZmBEH1 interacted with the TF ZmBZR1 (Brassinazole Resistant 1), whose gene expression was also directly activated by *ZmLG2*. Both ZmBEH1 and ZmBZR1 are bound to the promoter of *ZmSCL28* (SCARECROW-LIKE 28), a third TF that influences leaf angle. **【Conclusion】** LG2-BEH1/BZR1-SCL28 regulatory modules were identified to control the leaf angle in maize. Our study provides gene editing targets for creating optimal maize architecture suitable for dense planting.

**Key words:** maize; leaf angle; regulatory modules

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## **Integrating single-cell and spatial transcriptomics of developing maize ears elucidates key cell-type-specific co-expression networks**

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**Abstract: 【Objective】** The objective of this study was to gain a comprehensive understanding of inflorescence development for crop genetic improvement. **【Method】** In this study, we employed the spatial enhanced resolution omics-sequencing (Stereo-seq) and single cell RNA-seq method to construct a precise spatial transcriptome map of developing maize ear (6mm) primordia. **【Results】** Stereo-seq results identify twelve distinct cell types and their physical distribution. By integrating single-cell RNA transcriptomes, we uncovered a series of spatially-specific networks and hub genes. Furthermore, through detailed clustering of the meristem components, we discovered two MADS-box genes specifically expressed at the apex of determinate meristems. and used CRISPR editing to confirmed their role in stem cell determinacy **【Conclusion】** In conclusion, this study serves as a valuable resource for cereal inflorescence development research. The utilization of the Stereo-seq method facilitated the construction of a precise spatial transcriptome map and enabled the identification of key genes associated with maize ear development and yield improvement. These findings highlight the significance of integrating spatial and single-cell RNA sequencing transcriptomes for advancing our understanding of inflorescence development and genetic enhancement in crops.

**Key words:** maize ear; development; spatiotemporal transcriptome; single cell

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## ***Abstract 92***

### **Mapping of maize Northern leaf blight resistance QTL by BSA-Seq**

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**Abstract:** Northern leaf blight (NLB) is one of the most important foliar diseases in maize, which threatens maize production severely. So far, only a couple of genes conferring NLB resistance have been identified. In this study, an F<sub>2</sub> segregation population consisting of 750 individuals was generated from a cross between a highly resistant maize inbred BJ23 and susceptible inbred BJ76. Bulk-segregant analysis sequencing (BSA-Seq) was performed using a pair of phenotypically contrasting DNA bulks by pooling 60 resistant and 60 susceptible individuals separately. A total of 15.2 million filtered single nucleotide polymorphisms were used for analysis. Seven candidate NLB resistance QTL regions were obtained, which were distributed on chromosomes 2, 3, 4, 6 and 9. The sites with higher peaks are located on chromosomes 3, 4 and 9, with confidence intervals ranging from 0.55 Mb -12.49 Mb in the B73 reference genome. Furthermore, we designed four molecular markers to genotype 442 F<sub>2</sub> individuals and validated QTL effects on *qNLB2-1* in bin 2.06, *qNLB2-2* in bin 2.09, and *qNLB9-1* in bin 9.08. Our results showed that BSA-seq is a promising strategy to reveal reliable QTL in a smaller interval by increasing the size of pool and sequencing depth. In the future, we will conduct fine mapping and gene cloning of target QTL using advanced backcrossing populations.

**Key words:** maize; Northern leaf blight; BSA-Seq; QTL; disease resistance

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Funding acknowledgement: This work was supported by the Shaanxi Key Research Project (2021ZDLNY01-06) and Yangling Seed Industry Innovation Center Program (YLzy-ym-02).

## **A complete telomere-to-telomere assembly of the maize genome**

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**Abstract:【Objective】**A complete telomere-to-telomere (T2T) finished genome has been the long pursuit of genomic research. **【Method】**Through generating deep coverage ultralong Oxford Nanopore Technology (ONT) and PacBio HiFi reads, we report here a complete genome assembly of maize with each chromosome entirely traversed in a single contig. **【Result】**The 2,178.6 Mb T2T Mo17 genome with a base accuracy of over 99.99% unveiled the structural features of all repetitive regions of the genome. There were several super-long simple-sequence-repeat arrays having consecutive TAG tri-nucleotide repeats up to 235 kb. The assembly of the entire nucleolar organizer region of the 26.8 Mb array with 2,974 45S rDNA copies revealed the enormously complex patterns of rDNA duplications and transposon insertions. Additionally, complete assemblies of all ten centromeres enabled us to precisely dissect the repeat compositions of both CentC-rich and CentC-poor centromeres. **【Conclusion】**The complete Mo17 genome represents a major step forward in understanding the complexity of the highly recalcitrant repetitive regions of higher plant genomes.

**Key words:** Maize; genome; Telomere-to-telomere assembly; centromere; rDNA; satellite DNA

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## Abstract 94

### Genetic variation of *ZmIDH1.5* is associated with drought tolerance in maize

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**Abstract:** Drought stress is a significant limiting factor for maize yield. Identifying the genetic components responsible for drought tolerance in maize is of great importance. Here, we report a genome-wide association study (GWAS) on the maturity stage drought tolerance index of 126 maize materials, identifying an isocitrate dehydrogenase gene, *ZmIDH1.5*, as a key rate-limiting enzyme in the tricarboxylic acid cycle. And, phylogenetic analysis revealed that IDH gene family in plants can be classified into two types, NAD-IDH and NADP-IDH, with distinct gene structures, promoter cis-elements, interacting proteins, tissue expression patterns, and response patterns to abiotic stress. Hence, we propose that different types of *ZmIDH* genes may play distinct roles in maize. Among them, *ZmIDH1.5* belongs to the NAD-IDH subfamily and possesses catalytic activity, participating in the tricarboxylic acid cycle and L-glutamine biosynthesis, with the synthesis of proline depending on glutamine. Tissue-specific quantitative analysis showed specific expression of *ZmIDH1.5* in the anthers, while under 15% PEG stress, its expression in root significantly increased. Further, association analysis revealed that the variation in *ZmIDH1.5* had a significant impact on the anthesis-silking interval, which is the important index for drought resistance. Over-expression of *ZmIDH1.5* in maize resulted in smaller ear, and the performance of overexpressing lines during drought stress was inferior to that of mutant and wild-type materials. These findings suggest that *ZmIDH1.5* responds to drought stress during maize growth and development, although the underlying mechanisms require further investigation.

**Key words:** *ZmIDH*; Drought; phylogenetic analysis; association mapping; function validation

## *Abstract 95*

### **Construction of a Mutant Library for Tropical maize Germplasm**

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**Abstract:** **【Objective】** Mutants are useful tools in genetic researches. Although there have been several maize mutant libraries released, they are temperate germplasm-based and tropical germplasm-based library is not yet available. This study is aimed to develop a tropical maize mutant library. **【Method】** Natural mutants were isolated and collected from selfing lines. Double haploid (DH) mutants isolated from tropical maize DH lines developed from tropical selfing lines by inducing of haploids using haploid inducers and doubled by colchicine subsequently. *Mu* transposon insertion mutants were isolated from active Mutator harboring tropical maize lines. These lines were developed by crossing of tropical maize selfing lines with active Mutator harboring line B73 and backcrossed at least 6 times and self-pollinated. **【Result】** Two hundred independent natural, DH or *Mu* insertion mutations were collected. The observed mutational traits including plant-type, ears, tassels, kernels, fertility and stress tolerant. The identified mutations including endosperm defect, embryo abortion, dwarf, leaf angle, leaf/kernel color, nutritional component. More mutants isolation and identification is ongoing. **【Conclusion】** Tropical germplasm-based maize mutant library is constructing for full utilization of tropical maize germplasm.

**Key words:** maize; tropical germplasm; mutant library; Mutator

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## Abstract 96

### ZEAMAP2: A *Zea* genus multi-omics and multi-modals database for crop design and breeding

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**Abstract: 【Objective】** Maize (*Zea mays* L.) is one of the most important grain crops in the world.

With the maturity of high-throughput sequencing technology, maize genetics and genomics research has produced massive multi-omics data in population scale. Comprehensive using of these data would be great helpful to understand the relationship between genetic variation and complex traits and accelerate further improve maize yield. However, most of the existing databases focus on one or several specific omics data, which makes it difficult to effectively integrate and utilize different databases. **【Method】** In order to implement the principles of autonomous control of localized data, data sharing, continuous updating and data standardization, in this study, we organized and integrated ten omics modules of which most are from a uniform population, including genetics, variations, transcriptomics, genomics, epigenetics, metabolomics, germplasms, phenomics, populations and evolutions to construct a comprehensive information platform of genetic variation mining, function validation and population genetics. **【Result】** We have provided a number of high-quality reference genomes of *Zea* genus, with more than 800 population germplasms, containing 95 millions genetic variations, 22,000 phenotypes, 93,000 QTL, multitudes of GWAS results, selective sweep, spatial transcriptome etc. In addition, ZEAMAP2.0 will also provide user friendly data retrieval, efficient online analysis tools and practical visualization tools. **【Conclusion】** This study could promote the utilization of maize omics data, thus assisting the genetic breeding and improvement of maize.

**Key words:** database; multi-omics; crop design; breeding

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## 玉米胚乳早期发育与细胞命运决定的分子调控网络

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**摘要:** 胚乳是玉米籽粒最主要的营养储藏器官, 胚乳早期发育对玉米粒重和品质形成至关重要。合胞体和细胞化是双受精后胚乳发育的起始阶段, 对胚乳细胞数目及形态建成等有着重要作用, 进而影响籽粒大小、灌浆强度和产量。胚乳细胞分化及各功能区域建成是灌浆及储存物质合成的结构基础, 这一阶段发育产生缺陷将直接导致胚乳发育异常和粒重下降。本课题组瞄准“玉米胚乳早期发育与细胞命运决定的分子调控网络”这一核心科学问题, 克隆影响胚乳早期发育的关键基因, 全面阐释胚乳早期发育及特化细胞性状形成的遗传和分子调控机制, 构建它们的分子调控网络, 为绿色、优质、高产和可持续的玉米遗传改良提供理论基础和模型。本课题组通过反向遗传筛选, 获得多个玉米早期胚乳发育特异基因, 现因课题发展需要, 计划招聘博士后 2-3 名, 课题完成及发表文章预计需要 2-3 年时间。**要求如下:**

(1) 具有植物分子生物学博士学位, 在博士期间从事过基因特别是 QTL 克隆工作, 发表过较高水平研究论文, 具有申请上海超级博士后计划潜力者优先; (2) 对科学探索有浓厚兴趣, 富有创新思维, 动手能力强, 有较强的独立科研工作能力和英文写作能力; 能适应在夏天湿热环境下的长时间授粉工作, 对玉米花粉没有过敏反应, 且立志将来成为教授; (3) 工作勤奋, 主动性强, 富有团队合作精神, 能较长期稳定工作。请应聘者邮件联系 yrwu@cemps.ac.cn。应聘材料将予以保密。符合要求者, 将尽快安排面试。工资及福利等相关待遇按照分子植物卓越中心规定范围的上限执行。

**关键词:** 玉米; 胚乳; 早期发育; 品质; 粒重; 遗传改良

**实验室近两年 5 篇代表性论文:**

1. Huang Y<sup>#</sup>, Wang H<sup>#</sup>, Zhu Y<sup>#</sup>, ..., Wang W<sup>\*</sup>, **Wu Y<sup>\*</sup>** (2022). *Nature*, 612(7939):292-300.
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## *Abstract 98*

### **The maize ontogenetics: complexity, diversity and dynamics**

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**Abstract: 【Objective】** The formation of mature phenotype is related closely to the whole growth and development of organism, so investigating traits from the level of whole growth and development could improve the understanding of dynamic genetic basis of phenotype and expand more genetic resources from the time dimension. **【Method】** We monitored the whole growth status of the maize CUBIC panel using the high-throughput phenotyping platform. **【Result】** Over 1,002,240 RGB images were obtained, and 67 image-based traits (i-traits) from 18 time points were collected. We found the i-traits exhibited four diverse temporal patterns responsive to plant growth. The majority of the QTLs affecting i-traits are time-specific (84%), which largely explained the considerable portions of missing heritability of i-traits in the late growth phase. We simulated the accurate ontogenetic trajectories of maize genotypes, the diversity and genetic architecture of which were systematically understood. We found two genes that functioned differentially upon the plant growth, which greatly influenced the fine tuning of vegetative-reproductive transition in maize. **【Conclusion】** Our results provided the insights into the important traits from the ontogenetic perspectives, which proposed a new route for maize genome breeding via growth complementation.

**Key words:** phenome; GWAS; QTL; growth trajectories; heritability



## Abstract 99

# The coordinated regulation networks of maize *ZmHSFs* in response to heat stress

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**Abstract: 【Objective】** As one of the three most important grain crops in the world, the grain yield and quality of maize are seriously affected by heat stress. The heat shock transcription factors of maize (*ZmHSFs*) are key regulatory factors in response to heat stress. However, it is still lacking around the systematic analysis of regulatory networks of *ZmHSFs* in response to heat stress, and how different *ZmHSFs* subfamily genes cooperatively interacted in response to heat stress need to be further analyzed. **【Method】** To systematically identify the down-regulated genes of *ZmHSFs* family genes in response to heat stress, we mainly applied *in vivo* tsCUT&TAG technology combining with the transcriptome (RNA-seq) and open chromatin region analysis (ATAC-seq) under heat stresses in maize. To dissect the coordinated regulation networks of *ZmHSFs* in response to heat stress, the interacted proteins of the core *ZmHSFs* will be analyzed through high-throughput yeast two-hybrid and bimolecular fluorescence complementation technologies. Finally, the biological functions of the core *ZmHSFs* regulating heat tolerance will be validated by constructing the overexpression and gene-editing materials. **【Result】** By constructing the regulatory networks of *ZmHSFs* under heat stress, we analyzed the differences of *ZmHSFs* subfamily genes participating in the regulation of heat stress, and the interaction networks between different *ZmHSFs* responding to heat stress, and identified several core *ZmHSFs* genes regulating the heat tolerance. Furthermore, we found *ZmHSFs* could collaboratively regulate heat and other abiotic stresses. **【Conclusion】** This project will systematically sort out the regulatory networks of *ZmHSFs* in response to heat stress by a multidisciplinary approach. In addition, this study will identify several new core *ZmHSFs* genes and analyze their biological regulation mechanisms of heat tolerance, thereby providing new gene resources for cultivating new heat tolerance varieties of maize.

**Key words:** Maize; Heat stress; Heat shock transcription factor; Regulation network; tsCUT&TAG

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## ***Abstract 100***

# **ZmHLH-bHLH-type microProteins (miPs) mediate shade avoidance response syndrome (SAR) in Arabidopsis and maize**

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**Abstract:** **【Objective】** Optimization of shade avoidance response (SAR) is an important agronomic measure to increase maize yield per unit area in high-density planting conditions. MicroProteins (miPs) are small (5-25 kDa) and single - domain proteins that directly interact with target protein complexes and accurately disrupt target protein activity. Growing evidence shows that microProteins are efficient and essential bioactive regulators in SAR. However, the molecular mechanisms regulating of SAR in maize are still unclear. **【Method】** Here, we conducted an initial functional characterization of the maize HLH kinds microProteins gene family in regulating light signaling. **【Result】** The maize genome contains 22 distinct HLH miPs, which can be classified into PRE and PAR two subgroups. Quantitative real-time PCR showed that *ZmHLH miPs* gene were differentially expressed in maize tissues, with relatively higher expression in leaves, the expression pattern is similar to that of *AtPIFs*. Two of the *ZmHLH miPs* (*ZmHLH2/9*) could interact with *ZmPIFs* and *AtPIFs* in vivo and vitro. Furthermore, we found that *ZmHLH2* and *ZmHLH9 miPs* directly inhibit the self-associations of *AtPIF4/ZmPIF4s* to repress its oligomerization. Heterologous expression of the *ZmHLH2* and *ZmHLH9 miPs* conferred reduced shade avoidance syndrome in Arabidopsis and could predominantly suppress the PIF4ox shade avoidance syndrome phenotype. Moreover, the *Zmhlh2/9* double mutant generated using the CRISPR/Cas9 technique all showed enhanced mesocotyl elongation in dark-grown seedlings and responded to shade treatment. **【Conclusion】** These data provide new insights into our understanding of the regulatory mechanisms of SAS in maize. Indeed, we believe that harnessing the genetic power of the *ZmHLH miPs* gene family has great potential and a bright future in genetic improvement to increase maize yield.

**Key words:** Maize; Dense planting; Light; Shade Avoidance Response (SAR); microProteins

## **The role of transposon insertion in balancing resistance and yield-related traits in maize**

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**Abstract: 【Objective】** There are many beneficial alleles hidden in teosinte genome, the ancestor of maize. **【Method】** To uncover the hidden alleles in teosinte, a BC<sub>1</sub>F<sub>6</sub> recombinant inbred line (RIL) populations generated from crosses between maize inbred lines (Mo17) and the maize wild relative *Zea mays* ssp. *parviglumis* was developed for quantitative trait locus (QTL) mapping.

**【Result】** Totally, 650 BC<sub>1</sub>F<sub>6</sub> RILs were genotyped by re-sequencing and an over 1 million SNPs were generated for high density genetic map construction. QTL mapping was performed for 19 domestication phenotypic traits, 46,723 gene expression traits and 4,276 metabolism abundance traits in two tissues, respectively. We found that the flower time was shorten and the yield was increased during the domestication of maize. Cis-regulation occurs more frequently and is stronger than trans-regulation. Maize alleles often had higher gene expression level than teosinte, which indicated that domestication tends to increase the gene expression on transcriptome level. The phenotypic variants often controlled by many small effect variants, whereas the gene expression variants often controlled by larger effect variants. Genes that were up-regulated by trans-eQTLs hotspots were enriched in many resistance-related pathways which indicated that the resistance of maize was weaken during the progress of domestication. The nutritional quality of maize was not only reduced in some metabolites, but also increased in some metabolites. The increase in the content of sugars, organic acids and amino acids in maize kernels is associated with an increase in yield. A gene which up-regulated the expression of 468 genes and down-regulated expression of 504 genes was nominated as candidate genes for resistance and yield. Knock out this gene and homologous genes could enhance resistant and reduce yield. A 600 bp transposon element insertion in the promotor of parental *parviglumis* reduced the activity of promotor. The promotor of this gene was significantly selected in teosinte and maize which contained this TE. This TE was derived from *parviglumis* and the frequency was lower during the domestication of maize. We could control the expression of this gene to balance the resistance and yield.

**【Conclusion】** The novel QTLs we identified are good targets for further verification. The developed populations and identified QTLs provide valuable resource for future maize improvement.

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**Key words:** domestication; multi-omics; QTL mapping; resistance; yield; balance

## ***Abstract 102***

# **Fine mapping of a quantitative trait locus for flowering time in maize**

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**Abstract:** Using a large BC<sub>2</sub>S<sub>3</sub> recombinant inbred line (RIL) population derived from a cross between maize and its wild progenitor, teosinte, we performed high-resolution quantitative trait locus (QTL) mapping for flowering time, and fine mapped a flowering time QTL, *qDTP1* using HIF-derived near isogenic lines (NILs). An F<sub>2</sub> population containing 2,269 plants was created by self-fertilizing the HIF family. NIL(maize) and NIL(teosinte) identified using molecular markers were used to validate the effect of *qDTP1*. The re-sults showed that NIL(maize) flowered 4 days earlier than NIL(teosinte) under long-day conditions. Using molecular markers that flank the 3-LOD support interval of *qDTP1*, a total of 71 recombinants were identified from the F<sub>2</sub> population. To more precisely determine the recombination breakpoints, 6 additional markers were developed and used to genotype all recombinants. We further narrowed down *qDTP1* to a 206 kb physical region using 19 recombinant families. Based on the gene annotation of B73 reference genome, there are 6 genes located in the 206kb target region. Our results set important basis of finally cloning and functional characterization of *qDTP1.4*.

**Key words:** maize; flowering time; QTL

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## ***ZmMOS1* 调控玉米籽粒脱水速率的功能研究**

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**摘要:** 玉米作为我国的第一大农作物, 其充足稳定供应对保证我国粮食安全至关重要。近年来, 随着玉米密植栽培、集约化生产等生产模式的改变, 及劳动力成本的上升, 玉米机械化生产趋势势在必行。脱水快、宜机收玉米品种的培育是适应玉米机械化生产的关键。然而长期以来, 玉米籽粒脱水相关的研究基础薄弱、关键基因匮乏, 很大程度上限制了宜机收、脱水快玉米品种的培育。本课题组前期通过CRISPR分析发现, 赤霉素信号相关基因*ZmMOS1*在调控玉米籽粒含水量方面具有重要作用。*Zmmos1*突变后收获时籽粒含水量降低、穗行数减小, 但不影响株型与开花期; 将该基因超量表达后, 收获时籽粒含水量升高、穗行数增多。利用137份自交系转录组数据结合群体遗传学分析发现, *ZmMOS1*基因附近存在显著的cis-eQTL信号, 预示着玉米中存在自然变异或优异单倍型可以调控*ZmMOS1*基因的表达变化。该研究将为玉米籽粒脱水遗传网络建立和脱水快宜机收玉米品种的培育提供重要的理论指导和基因资源。

**关键词:** 玉米; 籽粒含水量; 宜机收

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## Abstract 104

### Genetic variation in *ZmAAA* reduces arsenic in maize kernel

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**Abstract:** **【Objective】** To better understand the genetic components underlying the natural variation and the metabolism of arsenic (As) in the maize kernel, genome-wide association study (GWAS) and linkage mapping were combined to dissect the genetic architecture of As in the maize maturely dry kernel. **【Method】** 63 genetic variants resolved to 12 candidate genes were identified. The peak GWAS signal showed that the natural variation in As Accumulation Associated (*ZmAAA*), encoding a Flavin adenine dinucleotide synthetase, contributes most significantly to the trait. **【Result】** Further analysis showed that a 200 bp insertion in the promoter, confers AsV inducible expression of *ZmAAA* in low-As kernel genotypes. Expression was up-regulated in response to high levels of As. Maize plants transformed with *ZmAAA* gene expressed from a light-induced soybean rubisco promoter (RbcsP) strongly express *ZmAAA* protein in leaves, but not roots, exhibits low-As accumulation in seeds that is most likely due to enhanced AsV reduction in leaves. Taken together, this information provides important genetic insights into the natural variation of maize kernel As content. **【Conclusion】** Our results indicate that *ZmAAA* limits As transport to the grains by sequestering As in the vacuoles of the leaves cells in maize. These results provide new insights for understanding As reduction and thus enhancing the breeding of low-As maize.

**Key words:** arsenic; maize kernel; genome-wide association study

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## 基于玉米多维网络图谱初步解析玉米 APX1 基因功能

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**摘要:** 植物在受到生物或非生物胁迫时, ROS含量会升高从而对细胞结构造成破坏, 甚至导致细胞死亡。因此, 维持ROS稳态对植物生长发育和适应不利环境具有重要意义。抗坏血酸过氧化物酶(APX)是一种重要的抗氧化酶, 其与过氧化氢有较强的结合力, 在维持植物细胞氧化还原稳态方面发挥关键作用。【目的】本研究基于玉米多组学数据构建的网络图谱, 以玉米中APX基因为例, 在整合网络指导下快速挖掘影响重要农艺性状的基因并初步解析其调控网络。【方法】对APX基因家族进行生物信息学分析、基于整合网络图谱中模块的划分选取APX1基因, 通过对其EMS突变体表型调查、全基因组关联分析(GWAS)初定位, 最后进行基因功能验证。【结果】鉴定了玉米中拥有八个完整APX结构域的APX基因, 分析了玉米APX基因家族的功能分化。通过整合网络图谱, 将这些APX基因划分在5个模块中, 处在同一进化分枝的基因在模块的划分中更保守。结合系统发育树发现APX1基因在水稻、玉米中的直系同源基因在调节生长发育和免疫方面均发挥重要作用。GWAS有一致的结果, 进一步通过APX1的EMS突变体表型调查发现突变体与野生型在株高等株型上有显著差异, 差异表达基因主要富集在MAPK信号通路等, 最终我们选择几个主要参与激素信号通路的基因作为潜在的靶基因。为了进一步解析APX1的功能, 在其共表达网络中发现与其共表达最强烈的基因与免疫相关, 对CRISPR材料进行抗病评估, 验证了这一结果。通过玉米不同生长发育时期多组学数据构建的基因调控网络(GRN)和叶片中的ChIP-seq数据筛选了两个与生长发育和免疫相关的上游转录因子下一步进行双荧光素酶报告基因等实验验证。【结论】总之, 基于多组学数据构建的网络图谱, 我们初步解析了玉米APX1基因调控网络, 这为培育理想株型和高抗玉米品种提供了理论依据。

**关键词:** 玉米多组学网络图谱; 抗坏血酸过氧化物酶; 基因功能; 基因调控网络

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## 一种负调控玉米广谱抗病基因 *ZmBRY1* 的功能研究

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**摘要:** 【目的】玉米南方锈病等叶部病害是黄淮海区域影响玉米产量的重要病害, 现有的抗病基因产生的抗性常因病原菌变异易丧失, 而负调控抗性的感病基因的修饰编辑常产生持久广谱抗性。因此, 发掘优良负调控抗性基因是作物病理学和抗性遗传改良的迫切需求。项目组前期在玉米 B73 突变体中筛选定位得到一个 MYB 转录因子 *ZmBRY1*, 初步研究表明该基因具有负调控玉米南方锈病、小斑病和弯孢病等叶部病害广谱抗性功能。因此本研究拟在前期工作的基础上进一步印证不同遗传背景下 *ZmBRY1* 负调控叶部病害广谱抗性的功能。【方法】研究前期主要通过诱导表达模式热图分析, 启动子区域 2000bp 结合元件预测等基础生物学初步判断 *ZmBRY1* 是否受病原菌诱导; 然后对不同背景下的野生型和敲除突变体进行三种不同的病原菌接种, 通过病情等级鉴定及 RT-PCR 分析, 进一步明晰 *ZmBRY1* 负调控玉米广谱抗病功能。最后, 通过活性氧相关检测及激素测定等进行病原菌侵染后 *ZmBRY1* 响应的免疫特性分析。【结果】研究结果表明 *ZmBRY1* 基因主要在成熟的根中表达, 该基因表达蛋白定位于细胞核中。在其启动子区域 2000bp 区域存在多个响应病原菌的顺式作用元件 PRE2 和 GT-1。接种玉米南方锈病、小斑病和弯孢病三种病原菌侵染前后 RT-PCR 分析显示 *ZmBRY1* 受病原菌诱导均显著上调表达, 而敲除突变体 *ZmBRY1* 表达量均很低。同时, 敲除 *ZmBRY1* 基因, 南方锈病从对照高感 (9 级), 提高到中抗 (5 级) 和抗 (3 级) 水平; 小斑病从感 (7 级) 提升到中抗 (5 级) 和抗 (3 级) 的水平; 弯孢也从感 (7 级) 提升到中抗 (5 级) 和抗 (3 级) 的水平。此外, 病原菌侵染前后野生型和敲除突变体 SA、JA 无显著差异, 而病原菌侵染后敲除突变体较野生型 WT 的 ROS 积累明显增加,  $H_2O_2$  含量显著上升, 而 POD 酶活性显著降低, 且三种病原菌表现出类似趋势, 这为 *ZmBRY1* 敲除广谱抗病机制的研究提供了重要依据。【结论】*ZmBRY1* 基因接种玉米南方锈病、小斑病和弯孢病三种病原菌侵染后 *ZmBRY1* 受病原菌诱导均显著上调表达, 且两种不同背景下的敲除突变体均显著增强了玉米对南方锈病、小斑病和弯孢病叶部病害的抗性。因此, *ZmBRY1* 具有负调控叶部病害广谱抗性的功能。

**关键词:** 玉米; MYB 转录因子; 病原菌; 负调控; 广谱抗病

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## Identification of *Fusarium verticillioides* Resistance Alleles in Three Maize Populations With Teosinte Gene Introgression

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**Abstract:** **【Objective】** *Fusarium* ear rot (FER) is a common fungal disease in maize (*Zea mays* L.) caused by *Fusarium verticillioides*. Resistant germplasm resources for FER are rare in cultivated maize; however, teosintes (*Z. mays* ssp. *parviglumis* and *Z. mays* ssp. *diploperennis*), which are wild-type species of maize, have the potential to offer a novel source of resistance alleles to enhance pathogen resistance in modern maize. Therefore, the aim of this study was to identify favorable alleles that confer significant levels of resistance toward FER. **【Method】** Three populations of BC2F8 recombinant inbred lines (RILs) were developed by crossing two different teosintes, *Z. diploperennis* and *Z. parviglumis*, with maize inbred lines B73 and Zheng58, and were screened for FER resistance. **【Result】** We found that *Z. diploperennis* and *Z. parviglumis* had higher resistance toward *F. verticillioides* in the leaves than B73 and Zheng58. However, the resistance toward *F. verticillioides* in the leaf and ear was unrelated among RILs. FER resistance was positively correlated with grain yield in the B73 × *diploperennis* (BD) and Zheng58 × *parviglumis* (ZP) populations, partly because the quantitative trait loci (QTLs) of FER resistance and yield traits were located close together. **【Conclusion】** Four coincident QTLs (qFERbd5.177, qFERbd10.140, qFERzp4.066, and qFERzp5.116) and two highly reliable resistance-yield synergistic QTLs (qFERbd10.140 and qFERzp4.066) were identified in the BD and ZP populations, opening up the possibility of breeding for FER resistance without reducing yield.

**Key words:** *Fusarium verticillioides*; maize; teosinte; QTL; germplasm resources

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## ***Abstract 108***

### **Alleviate light induced ROS accumulation, improve Photosynthetic efficiency of leaves and grain yield of maize**

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**Abstract: 【Objective】** ROS is a by-product of plant photosynthesis. Crop yield reduction caused by various stresses (such as high light, high temperature, cold, drought, salinization) is related to the sharp increase of ROS. The photosynthetic efficiency and yield of crops under adverse conditions can be significantly improved by improving the ability of crops to remove ROS. However, there is little research on this field at present. **【Method】** This project plans to clone a light induced leaf necrosis mutant *nec1* by map and verify its function through the creation of the mutant, and verify the biological function of the gene in improving leaf photosynthetic efficiency through the creation of gene enhanced expression families, ROS accumulation analysis, photosynthetic traits, carbohydrate accumulation analysis; The gene regulatory network was analyzed by molecular biology technology. **【Result】** The results showed that the scavenging mechanism of ROS in chloroplasts participated by corn lipid hydroperoxide lyase could alleviate the accumulation of active oxygen, the "byproduct" of photosynthesis, thus optimizing the physiological environment in chloroplasts, reducing the inhibition of active oxygen on photosynthetic machinery, and significantly improving the Photosynthetic efficiency of maize leaves and field seed weight under low light. **【Conclusion】** The research results will reveal a previously unknown molecular mechanism of ROS as a target to improve photosynthetic efficiency, which not only provides rich gene resources for maize breeding practice, but also explores a new path to improve plant photosynthetic efficiency.

**Key words:** *ZmHPL1*; ROS; JA; photosynthetic efficiency; greenish

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## **The creation of a humidity-sensitive genic male sterile for enhancing hybrid production in maize**

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**Abstract:** **【Objective】** Corn is a widely cultivated crop with heterosis, and hybrid seed production has traditionally relied on detasseling instead of male sterility, which is costly. Here, we created a humidity-sensitive genic male sterile (HGMS) for promoting hybrid production in maize by two-line system. **【Method】** *ZmPTS1* is orthologue of *OsPTS1* which regulated HGMS in rice. In this study, Two approaches were designed to generate HGMS (*zmpts1* mutants), including EMS mutagenesis and CRISPR/Cas9. The fertility of *zmpts1* was assessed for a span of two years in both Beijing and Lingshui. **【Result】** The pollen of *zmpts1* dehydrated completely and devitalised gradually within 90 s after pollen release in the air, ultimately resulting in male sterility. However, *zmpts1* can maintain viability and restore normal fertility when the relative humidity is maintained above 90% after pollen release. In hybrid seed production, The seed heterozygosity rate of *zmpts1* up to 97%, greatly promoting the application of two-line system in maize. **【Conclusion】** Two years of planting experiments in Beijing and Lingshui have shown that the male sterility of *zmpts1* is stable, and its fertility can be effectively restored through artificial pollination and spray humidification. Therefore, *zmpts1* is an ideal humidity-sensitive male sterile material that can broaden the application of the two-line system for hybrid seed production in maize.

**Key words:** Maize; Humidity-sensitive genic male sterility; CRISPR/Cas9; EMS mutagenesis; Hybrid seed production

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## **Genetic Dissection of Maize (*Zea mays* L.) Chlorophyll Content Using Multi-Locus Genome-Wide Association Studies**

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**Abstract:** **【Objective】** The chlorophyll content (CC) is a key factor affecting plant photosynthetic efficiency and the final yield. However, its genetic basis remains unclear. **【Method】** The development of statistical methods has enabled researchers to design and apply various GWAS models, including MLM, MLMM, SUPER, FarmCPU, BLINK and 3VmrMLM. Comparative analysis of their results can lead to more effective mining of key genes. **【Result】** The heritability of CC was 0.86. Six statistical models (MLM, BLINK, MLMM, FarmCPU, SUPER, and 3VmrMLM) and 1.25 million SNPs were used for the GWAS. A total of 140 quantitative trait nucleotides (QTNs) were detected, with 3VmrMLM and MLM detecting the most (118) and fewest (3) QTNs, respectively. The QTNs were associated with 481 genes and explained 0.29%-10.28% of the phenotypic variation. Additionally, 10 co-located QTNs were detected by at least two different models or methods, three co-located QTNs were identified in at least two different environments, and six co-located QTNs were detected by different models or methods in different environments. Moreover, 69 candidate genes within or near these stable QTNs were screened based on the B73 (RefGen\_v2) genome. *GRMZM2G110408 (ZmCCS3)* was identified by multiple models and in multiple environments. The functional characterization of this gene indicated the encoded protein likely contributes to chlorophyll biosynthesis. In addition, the CC differed significantly between the haplotypes of the significant QTN in this gene, and CC was higher for haplotype 1. **【Conclusion】** This study results broaden our understanding of the genetic basis of CC, mining key genes related to CC and may be relevant for the ideotype-based breeding of new maize varieties with high photosynthetic efficiency.

**Key words:** maize (*Zea mays* L.); chlorophyll content; single-locus GWAS; multi-locus GWAS; high photosynthetic efficiency

## ***ZmSPLn* 调控玉米抗旱性的功能分析**

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**摘要：【目的】**玉米是我国第一大作物，玉米高产稳产对保障我国粮食安全至关重要。我国玉米单产仅占欧美国家的 60%，在耕地面积刚性约束的前提下，提高种植密度是提高玉米单产的有效措施。但密植会造成玉米根系对浅耕作层水分和养分的竞争，进而影响植株生长。同时，玉米生育期需水量大且对干旱胁迫敏感，而我国玉米主产区与干旱区域高度重叠，2021 年我国玉米受灾情况 26%来源于干旱，相比其它自然灾害的影响占比最大。因此，挖掘能促进植物根系向纵向生长，以获取土壤深层水分和养分的关键基因，通过遗传改良培育耐密、抗逆新品种是提高玉米产能的有效途径。前期的研究发现玉米转录因子基因 *ZmSPLn* 过表达能抑制根系生长并提高玉米耐旱性，表明 *ZmSPLn* 可能是协同调控玉米根系发育和耐旱性的关键基因。本研究通过栽培学、生理学、细胞学等多层次研究发现 1) *ZmSPLn* 能够应答因干旱导致的土壤板结变硬，上调表达。2) 在土壤变硬的条件下，对照植株根系发育受到明显抑制，*ZmSPLn* 过表达株系则对土壤硬度不敏感，仍能正常生长。而且，初生根、冠根和气生根均能穿透较硬的土壤，继续向纵深生长；在田间，利用回交转育改良的其他自交系和转基因组配杂交种也验证了该表型。3) 原位杂交实验表明 *ZmSPLn* 基因在内皮层和表皮表达；不同硬度下的根尖切片实验表明，土壤硬度增加会明显抑制根尖细胞生长，但在 *ZmSPLn* 过表达株系根尖中，土壤硬度增加反而促进了根尖细胞的生长。4) RNA-seq 分析表明，在干旱土壤硬度增大条件下，*ZmSPLn* 过表达株系根中 GA、ABA 和 Auxin 信号途径基因显著富集。本研究表明，*ZmSPLn* 可能作为一个链接外源信号和内源发育通路的一个关键节点调控因子，应答高硬度土壤，启动不同激素信号通路之间的串扰，进而促进根尖细胞生长发育，增强玉米根系对高硬度土壤的穿透能力，提高玉米抗旱性。

**关键词：**玉米；耐旱；土壤硬度；根系；ABA；生长素

## The map-based cloning of *qrscr1*, a major Southern Rust disease resistance QTL in maize

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**Abstract:** **【Objective】** Southern corn rust, caused by *Puccinia polysora* Underw, is a major disease that significantly impacts corn production. Breeding and planting corn varieties resistant to Southern rust is an effective way to control this disease. The identification and isolation of resistance genes constitutes a crucial step towards achieving this objective. **【Method】** F<sub>2</sub> genetic mapping populations were constructed from highly resistant southern rust inbred line Q319 and highly susceptible inbred line 9801. Inclusive composite interval mapping combined with Bulked Segregant Analysis (BSA-seq) was used to conduct preliminary mapping analysis, and one major QTL for resistance to southern rust was detected which explains about 15% of phenotypic variation. Further, Near Isogenic Lines (NILs) of QTL-*qrscr1* were constructed by continuous backcross and marker-assisted selection, and fine mapping was carried out by using a recombinant-derived progeny testing strategy. **【Result】** The confidence interval of *qrscr1*, based on B73 reference genome data, was found to be 550kb and contained two functional genes. Gene1 encodes a protein involved in the endocytic pathway. Through the sequence alignment of candidate genes, it was discovered that gene1 has several SNPs site differences between the parents. Gene2 encodes a PR-related protein with three tandem repeats in resistant parents and only one in susceptible parents. Currently, we have successfully obtained overexpression and CRISPR/Cas9 knockout strains of Gene1, as well as EMS mutant materials for Gene2. The final target gene can be determined by phenotypic identification in the later stage. Further investigation into the resistance mechanism of the target gene was conducted using RNA-seq, IP-MS, screening a yeast two-hybrid (Y2H) cDNA library and other experimental techniques. **【Conclusion】** In this study, two candidate genes for resistance to southern rust were identified. The identification and cloning of target gene could provide crucial theoretical basis for breeding resistance to southern rust.

**Key words:** maize; southern corn rust; resistance; QTL mapping

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## **Genetic analysis of major effect QTL-*qHNL1* of husk number in maize**

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**Abstract: 【Objective】** The number of husk is one of the important agronomic characters in maize breeding for high yield. It is also an important factor affecting the dehydration rate of maize, and reducing the number of husk reasonably is one of the key objectives of breeding maize varieties suitable for mechanical harvest. However, the genetic mechanism of the character of husk number is poorly understood at home and abroad. **【Method】** In 560 backbone inbred lines, BS351 and US043, which showed stable and extreme husk number traits, were selected to generate multigenerational backcross populations. The number of husk was located by using 10K SNP chip and inclusive composite interval mapping combined with  $F_2$  population, and the major effect QTL-*qHNL1* was mapped. Near Isogenic Lines (NILs) of QTL-*qHNL1* were constructed through continuous backcrossing and molecular marker-assisted selection to verify the genetic effects of the population. Candidate genes were targeted through fine mapping, and target genes were further confirmed by combining with mutant material phenotype. The molecular mechanism of regulating the number of husk will be analyzed by ChIP-seq, RNA-seq and IP-MS methods.

**【Result】** The QTL-*qHNL1* was mapped in the 58Kb region by the method of recombinant-derived progeny testing strategy, and there was only one gene in the region encoding the AP2 family transcription factor, which was named *ZmHNL1*. Through qRT-PCR analysis of NIL<sup>BS351</sup> and NIL<sup>US043</sup>, it was found that there were significant differences in the expression level of *ZmHNL1*. At the same time, combined with the phenotype of EMS mutant premature termination by *ZmHNL1*, it was identified as the target gene, also overexpression and transcriptional inhibition strains have been obtained. A series of experiments will be conducted to find the interacting genes and construct the regulatory network of husk number. **【Conclusion】** In summary, after years of fine mapping, the localization interval was narrowed to 58Kb, and a gene *ZmHNL1*, which codes the transcription factors of AP2 family, was mapped and cloned.

**Key words:** Maize; Husk number; QTL; Map-based cloning; Transcription factor

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## Global Landscape of Alternative Splicing in Maize Response to Low Temperature

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**Abstract: 【Objective】** Maize (*Zea mays* L.) is an important food crop planted across the world, and low-temperature stress can affect maize germination. Alternative splicing (AS) is widely present in plants under abiotic stress; however, the response of AS to low temperature stress in maize remains unclear. **【Method】** In this study, RNA-seq data obtained from maize embryo tissues with low temperature and normal-temperature treatments were used to identify alternative splicing genes in maize under low temperatures and the mining of alternative splicing genes in response to low temperature stress. Maize mutant materials were used to validate alternatively spliced genes.

**【Result】** AS events were distributed on each chromosome, approximately 2.05-2.09 AS events per gene. Seven genes only had AS in low-temperature-resistant inbred lines. A total of 278 KEGGs and 46 GOs were enriched based on overlapping AS genes, which were associated with hormone and oxidoreductase activity. The mutant was used to verify the function of AS gene *ZmWRKY48*, and the relative germination rate, relative seedling length, relative radicle length, and relative radicle surface area of the mutant decreased by 15.16%-19.87% compared with the normal line. **【Conclusion】** This study demonstrated the transcriptional regulation mediated by AS genes between different resistant inbred lines. AS genes were distributed on each chromosome. Overlapping AS genes in the four inbred lines were primarily enriched in pathways such as the redox state and cell wall. The AS of the WRKY transcription factor could play an important role in regulating low temperatures in maize. The function of the *ZmWRKY48* gene was verified with the mutant line, and the results indicated that the AS of *ZmWRKY48* was associated with low-temperature resistance in maize. These results are important for studying the regulatory mechanism of maize.

**Key words:** maize; low temperature; alternative splicing; transcriptome profiling; pathway analysis

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## 玉米大刍草杂交衰退分子机理初步解析

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**摘要:** 【目的】杂交衰退 (Hybrid decay) 是在玉米与特定地域大刍草回交过程中发现的遗传学现象, 表现出生长势和繁殖力的显著下降, 遗传方式不遵循孟德尔法则, 伴随跨代传递的表型遗传特征, 本研究尝试利用遗传学和生物信息学的方法对杂交衰退的分子机理进行初步探索。【方法】将杂交衰退植株及其轮回亲本与遗传基础广泛的关联自交群体杂交, 以  $F_1$  代的结实率为调查对象, 筛选抑制杂交衰退的自交系; 在全基因组范围内对抑制杂交衰退的位点进行关联分析; 选取抑制效果显著的自交系与杂交衰退植株杂交构建分离群体, 以雌穗结实率为调查表型, 对抑制杂交衰退的位点进行连锁分析; 利用  $20\times$  杂交衰退的 3 代基因组数据, 对杂交衰退特异的高拷贝序列进行组装, 分析结构特征和基因组中的定位; 通过与已知表观遗传突变体杂交的方法分析杂交衰退依赖的表观遗传路径。【结果】关联分析结果显示抑制位点与杂种优势种群的选择位点高度相关。连锁分析结果显示调控结实率的位点由单基因控制, 杂交衰退特异的高拷贝序列具有串联重复结构特征, 其在基因组的位置与调控结实率的位点吻合, 基于此确定候选基因, 其可能通过与转录酶互作的方式调控下游基因。【结论】杂交衰退具有类似副突变的表观遗传调控方式, 除了顺式抑制候选基因, 还可以通过串联结构的增强子序列, 反式抑制候选基因, 造成非孟德尔分离的现象。候选基因可能与转录聚合酶互作, 导致多个重要农艺性状的显著变化。该研究为玉米近缘野生资源利用、表观遗传调控机理解析和杂种优势利用提供参考思路。

**关键词:** 杂交衰退; 表观遗传; 大刍草

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## 玉米 *ZmEL1* 在雌穗发育中的功能研究

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**摘要:** 玉米是我国第一大作物, 在保障我国粮食安全以及国民经济中占据重要地位。穗长和每行粒数是玉米重要的产量性状, 因此研究玉米雌穗的穗长和行粒数的调控机制有助于进一步提高玉米产量。本课题组前期通过对 EMS 突变体材料表型观察发现一个与玉米雌穗发育负相关的基因 *ZmEL1*。*ZmEL1* 编码一个细胞膜定位的离子通道蛋白, 在玉米中的表达具有广普性, 在雌穗和籽粒中的表达较高。该基因突变后雌穗的穗长、每行粒数、穗重以及每穗粒重显著高于对照, 而穗粗及穗行数与对照相比降低, 此外突变体籽粒的粒长、粒宽以及百粒重也显著高于对照; 将该基因过表达后发现过表达植株与对照相比, 穗长、每行粒数以及穗重减小。利用 137 份自交系雌穗转录组数据结合群体遗传学分析发现, *ZmEL1* 基因附近存在显著的 cis-eQTL 信号, 利用 14 份已发表的玉米自交系基因组分析 *ZmEL1* 基因区域和上下游 10kb 的序列, 发现该基因启动子区存在转座子插入, 预示着玉米中存在自然变异或优异单倍型可以调控 *ZmEL1* 基因的表达变化。本研究将丰富玉米雌穗发育的调控网络, 为提高玉米雌穗大小进而提高产量提供理论指导和基因资源。

**关键词:** 玉米; 雌穗大小; 籽粒大小

## **ZmSPL13 and ZmSPL29 Act Together to Promote Vegetative and Reproductive Transition in Maize**

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**Abstract:** Flowering time is a key agronomic trait determining environmental adaptation and yield potential of crops. The regulatory mechanisms of flowering in maize still remain rudimentary. In this study, we identified two homologous *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) transcription factors *ZmSPL13* and *ZmSPL29*, most closely related with Arabidopsis *SPL3/4/5*, act as potential regulators of the vegetative phase transition and floral transition by using a combination of biochemical approaches, cell biology, molecular biology and genetic methods. Here, we show that both *ZmSPL13* and *ZmSPL29* are preferentially expressed in leaf phloem, vegetative and reproductive meristem. We show that vegetative phase change and flowering time are moderately delayed in the *Zmspl13* and *Zmspl29* single knockout mutants, and more significantly delayed in the *Zmspl13/29* double mutants. Consistently, the *ZmSPL29* overexpression plants display precocious vegetative phase transition and floral transition, thus early flowering. We demonstrate that *ZmSPL13* and *ZmSPL29* directly upregulate the expression of *ZmMIR172C* and *ZCN8* in the leaf, and of *ZMM3* and *ZMM4* in the shoot apical meristem, to induce juvenile-to-adult vegetative transition and floral transition. These findings establish a consecutive signaling cascade of the maize aging pathway by linking the miR156-SPL and the miR172-G115 regulatory modules and provide new targets for genetic improvement of flowering time in maize cultivars.

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## **Two teosintes made modern maize**

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**Abstract: 【Objective】** Despite its global importance, the origins of maize and its closest wild relatives remained the topic of vigorous debate for nearly a century. Molecular analyses ultimately concluded that maize was domesticated once from a common ancestor with its closest extant relative, the lowland wild grass *Zea mays* ssp. *parviglumis*. But neither the current genetic model nor earlier models based on archaeological data account for the totality of available data, and recent work has highlighted the potential contribution of a second wild relative, the highland *Zea mays* ssp. *mexicana*. **【Method】** Here we present a detailed population genetic analysis of the contributions of both wild taxa to modern maize diversity using the largest sample of traditional maize varieties sequenced to date. **【Result】** We show that all modern maize can trace its origin to an ancient admixture event between domesticated ancient maize and *Zea mays* ssp. *mexicana* in the highlands of Mexico some 4,000 years after domestication began. We show that variation in admixture is a key component of modern maize genetic and phenotypic diversity, both at the level of individual loci and as a factor driving a substantial component of additive genetic variation across a number of agronomic traits. **【Conclusion】** Our results clarify the long-debated origin of modern maize, highlight the potential contributions of crop wild relatives to agronomic improvement, and raise new questions about the anthropogenic mechanisms underlying multiple waves of dispersal throughout the Americas.

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## **QTL mapping for northern leaf blight and southern leaf blight resistance in maize**

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**Abstract:** Northern leaf blight (NLB) caused by the hemibiotrophic pathogen *Exserohilum turcicum*, and southern leaf blight (SLB) caused by the necrotrophic fungus *Cochliobolus heterostrophus* are important foliar diseases reducing maize production worldwide. The genetic basis of resistance to NLB and SLB are complex and can be greatly affected by the environment and genetic background. Identifying quantitative trait loci (QTL) and genes for resistance to these diseases will lay a foundation for maize resistance breeding. Here, a maize recombinant inbred line population derived from a cross between PH4CV (susceptible parent) and KB020 (resistant parent) was used to identify QTLs associated with NLB and SLB resistance across six environments. Plants in all environments were artificially inoculated. The correlation coefficient between NLB and SLB resistance was 0.58. A total of 46 and 44 QTLs were identified for NLB and SLB resistance respectively. Two major QTLs for NLB resistance were located in bins 3.04 (*qNLB3-1*,  $R^2 = 14.36\%$ ) and 10.04 (*qNLB10-1*,  $R^2 = 14.26\%$ ), which were stable in multiple environments. In the same way, stable QTLs with major effect for SLB resistance were located in bins 3.04 (*qSLB3-2*,  $R^2 = 16.73\%$ ) and 4.05 (*qSLB4-1*,  $R^2 = 13.32\%$ ). QTLs for NLB and SLB resistance were identified to be co-localized in bins 2.06, 3.04, 4.05 and 6.07. Using segregation populations derived from residual heterozygous lines, effects of *qNLB3-1*, *qSLB3-2*, and *qSLB4-1* were validated. Alleles from the resistant parental line KB020 at each locus could significantly improve disease resistance. Fine mapping and characterization of these genes will allow a better understanding of multiple disease resistance and facilitate disease resistance breeding in maize.

**Key words:** maize; northern leaf blight; southern leaf blight; QTL

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## **Multi-view genomic best linear unbiased prediction: a promising solution for post-omics data integration**

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**Abstract: 【Objective】** The concept that different perspectives can be used to describe the same thing constitutes multiple views of an object. Multi-view data of an object is widely existent in the real world, such as in breeding, where a sample can be represented by its genes, expression levels, metabolism, phenotype and so on. Utilizing the complementarity between multi-view data can improve algorithm performance to some extent. Therefore, we propose a multi-view GBLUP prediction method that integrates multiple omics or data types for phenotype prediction, and can extract useful information from high-dimensional multi-view data. **【Method】** In this work, the multi-view GBLUP prediction method assigns different weights to different types of data and uses the weighted multi-type data to obtain a multi-view kinship matrix, which is then incorporated into the GBLUP model for phenotype prediction. To obtain the optimal combination of weights, we employ a differential evolution algorithm for iterative learning and introduce an early stopping mechanism. Specifically, our research includes: 1) proposing a method for calculating the kinship matrix; 2) integrating the multiple types of data with weights to obtain a kinship matrix; 3) using the differential evolution algorithm to find the optimal combination of weights for the multiple types of data. The specific process of an iteration of the algorithm is as follows: first randomize an initial population consisting of NP individuals, each representing a combination of weights for multiple types of data, then perform mutation and crossover operations on the initial population to simulate recombination and drift in nature, and finally select individuals with high phenotype prediction accuracy by comparing the initial population with the individuals after mutation and crossover. (4) setting a stopping criterion to stop learning when the maximum prediction accuracy difference between adjacent iterations is less than 0.0001. **【Result】** In numerical experiments, the multi-view GBLUP method was tested using four datasets: Rice210, Maize368, Maize282, and Tomato332. The results indicated that this method can improve prediction accuracy in different species and datasets, with the highest prediction accuracy reaching 0.87, 0.66, 0.91, and 0.40, respectively. Compared with single-type data, the multi-view GBLUP method can improve the prediction accuracy by up to approximately 5 percentage points at most. **【Conclusion】** We propose a method for integrating multiple types of data for phenotype prediction and use differential evolution algorithm to quickly and easily obtain the optimal weights for the multiple types of data. This method provides a new approach to the comprehensive use of multiple types of big data.

**Key words:** multiview; differential evolution algorithm; genomic BLUP; predictive ability

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## Abstract 121

### Natural variations of *ZmZEP1* alter carotenoids in maize kernels

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**Abstract: 【Objective】** Carotenoids are a group of fat-soluble pigments that are widely found in fresh vegetables and fruits, which play a crucial role in human health. Zeaxanthin as a kind of carotenoid plays an important role in antioxidation and eye health. Vitamin A deficiency (VAD) is a human health issues in poor and underdeveloped regions. As maize is the only staple crop that contains provitamin A carotenoids among three cereal crops, elevating the level of carotenoids in maize kernels through biofortification is a promising and effective strategy to combat global VAD. *ZEP1*, an enzyme encoding a zeaxanthin epoxidase, is mainly responsible for the conversion of zeaxanthin to antherxanthin and violaxanthin through continuous oxidation reactions. **【Method】** In this study, we performed phylogenetic analysis, subcellular localization experiments, candidate gene association analysis, expression pattern analysis and constructed CRISPR/Cas9 and overexpressing materials of *ZmZEP1* to identify the natural variations of *ZmZEP1* that effect carotenoid content in maize kernels. **【Result】** Phylogenetic analysis indicated *ZmZEP1* is relatively conserved in *Gramineae*. The subcellular localization results indicated that T2 transcript of *ZmZEP1* may not be functional. Expression pattern analysis found that *ZmZEP1* was constitutively expressed. We resequenced *ZmZEP1* in 508 maize inbred lines and identified 221 polymorphic sites in the first exon and 3'UTR of *ZmZEP1* that were significantly associated with zeaxanthin content via candidate gene association analysis. Knockout of *ZmZEP1* increased zeaxanthin, while overexpressing *ZmZEP1* decreased zeaxanthin. As a result, *ZmZEP1* plays a crucial role in regulating zeaxanthin content. **【Conclusion】** These results indicated that natural variations of *ZmZEP1* influence zeaxanthin content. Further investigations will be conducted to confirm the causal variations of *ZmZEP1* such as enzyme activity and other experiments among different haplotypes. Additionally, we will develop corresponding molecular markers to provide a molecular basis for improving the carotenoid content and selecting new maize breeding materials with high carotenoid content.

**Key words:** carotenoids; *ZmZEP1*; natural variations; association analysis



## **Genetic basis underlying carotenoid variation in maize kernels revealed by linkage and association mapping**

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**Abstract: 【Objective】** Carotenoids play a vital role in various physiological and biochemical processes in plants and are essential for animals and human. Maize, a widely cultivated crop, is rich in carotenoids and serves as a staple food in many countries. Increasing the provitamin A carotenoid content in maize can help alleviate vitamin A deficiency. Elucidating the genetic basis of carotenoid content in maize kernels is crucial for developing biofortification strategies to address undernutrition issues. **【Method】** In this study, we utilized six recombinant inbred line populations (RILs) and employed three different methods including single linkage mapping (SLM), joint linkage mapping (JLM) and RIL-based genome-wide association study (RIL-based GWAS) to reveal the genetic basis of carotenoid content in maize kernels. **【Result】** Our analysis identified 80, 22 and 43 loci associated with carotenoid content by SLM, JLM and RIL-based GWAS, respectively. Most of the QTLs only explained small part of phenotypic variation which indicated that carotenoid content was regulated by minority of major loci and a majority of minor loci. Additionally, the contribution of additive effects was found to play a predominant role in the genetic basis of carotenoid content, as the total phenotypic variation explained by epistatic effects was considerably smaller. Pathway enrichment analysis highlighted the crucial roles of genes involved in carotenoid-related pathways. Certain loci exhibited superior variations derived from lower-level carotenoid founders rather than higher-level founders, indicating the incomplete aggregation of superior alleles in high-level carotenoid inbred lines. *ZmPTOX*, falling within the *qTC2-1* locus, identified consistently by all three methods, was found to be associated with the content of various carotenoids, including lutein,  $\beta$ -cryptoxanthin,  $\alpha$ -carotene,  $\beta$ -carotene, total carotenoid and total provitamin A carotenoid. This result was confirmed by fine mapping and gene-editing. *ZmPTOX* encodes a putative plastid terminal oxidase and produces PQ-9 for phytoene desaturase (PDS). Variants in the promoter of *ZmPTOX* may positively regulate carotenoid content by modulating expression levels of *ZmPTOX*. **【Conclusion】** Our findings shed light on the genetic basis of carotenoid content in maize kernels, and provide potential avenues for enhancing carotenoid content in maize kernels through biofortification strategies.

**Key words:** Carotenoid; QTL mapping; *ZmPTOX*; Natural variation; Maize

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# The ZmPP2C26-ZmMPK3/7 module regulates drought tolerance in maize

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**Abstract:** Serine/threonine protein phosphatase 2C (PP2C) dephosphorylates proteins and plays crucial roles in plant growth, development, and stress response. In this study, we characterized a clade B member of maize PP2C family, i.e., ZmPP2C26, that negatively regulated drought tolerance by dephosphorylating ZmMAPK3 and ZmMAPK7 in maize. The ZmPP2C26 gene generated ZmPP2C26L and ZmPP2C26S isoforms through untypical alternative splicing. ZmPP2C26S lost 71 amino acids including a MAPK interaction motif and showed higher phosphatase activity than ZmPP2C26L. ZmPP2C26L directly interacted with, dephosphorylated ZmMAPK3 and ZmMAPK7, and localized in chloroplast and nucleus, but ZmPP2C26S only dephosphorylated ZmMAPK3 and localized in cytosol and nucleus. The expression of ZmPP2C26L and ZmPP2C26 was significantly inhibited by drought stress. Meanwhile, the maize *zmp2c26* mutant exhibited enhancement of drought tolerance with higher root length, root weight, chlorophyll content, and photosynthetic rate compared to wild type. However, overexpression of ZmPP2C26L and ZmPP2C26S significantly decreased drought tolerance in *Arabidopsis* and *rice*, respectively. Likewise, the expression of ZmMPK3 and ZmMPK7 was significantly induced by drought stress. Overexpression of ZmMPK3 and ZmMPK7 significantly enhanced drought tolerance in *Arabidopsis* and *rice*, respectively. Phosphoproteomic analysis revealed that the ZmPP2C26 protein also altered the phosphorylation level of proteins involved in photosynthesis. This study suggests that the ZmPP2C26-ZmMPK3/7 module regulates drought tolerance and provides insights into understanding the mechanism of PP2C in response to abiotic stress.

**Key words:** Maize; drought stress; protein phosphatase 2C; MAPK; alternative splicing

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## **Validation and Characterization of Maize Stalk Rot Resistance QTL**

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**Abstract:** Stalk rot is one of the most serious soil-borne diseases in maize worldwide, causing significant yield losses each year. Disease resistance to maize stalk rot is a complex quantitative trait, controlled by many genes and greatly affected by genetic background and environment. Here, we performed QTL mapping for stalk rot resistance against *Fusarium graminearum* using 222 F<sub>7:8</sub> recombinant inbred lines generated from maize inbred lines KA105 (resistant) and HZ4 (susceptible). All the lines were artificially inoculated and evaluated using the root-inoculation method at two locations over two years. Five QTL in chromosomes 2, 5, 7, and 10 were identified from at least three environments, each of which explained 7.8-15.6% of the phenotypic variations. To confirm the existence and allele effects of target QTL, five F<sub>2</sub> populations derived from five residual heterozygous lines (RHLs), in which selected QTL were heterozygous with a uniform genetic background, were assessed for stalk rot resistance in replicated trials and genotyped with markers in the QTL intervals. Resistance alleles from KA105 at each of the five QTL could significantly enhance stalk rot resistance. Fine mapping of the QTL in chromosome 7 using RHLs narrowed down the region to a 2.1 Mb interval. The results facilitate further QTL cloning and breeding for stalk rot resistance maize lines.

**Key words:** maize; stalk rot; *Fusarium graminearum*; QTL

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## **SNP-based bulk segregant analysis revealed disease resistance QTLs associated with northern corn leaf blight in maize**

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**Abstract: 【Objective】** The present study focused on the identification and characterization of major QTLs associated with NCLB in maize by utilizing a segregating population (F<sub>2</sub>) developed from GML71 (resistant parent) and Gui A10341 (susceptible parent). The genomic DNA from 30 plants of each category resistant and susceptible (identified after inoculation) was pooled for downstream analysis. **【Method】** QTLs with candidate genes related to Corn spot resistance were identified and annotated by combining the phenotype with genotype to provide a genetic basis for NCLB disease in two contrasting parental genotypes using Nextgeneration sequencing (NGS)-based bulked-segregant analysis (BSA). **【Result】** We further characterized each QTL and identified genes residing in the QTL regions. A total of 48, 131, 74, 405, 716, 1844, 19, 36, 82, and 69 genes were identified in Q1-1, Q2-1, Q2-2, Q2-3, Q2-4, Q2-5, Q3-1, Q3-2, Q5-1, and Q5-2, respectively. The identified genes were further subjected to GO term ontology and KEGG pathways analysis. **【Conclusion】** We identified ten QTLs on Chr 1, Chr 2, Chr 3, and Chr 5 with a 99% confidence interval associated with NCLB resistance. Moreover, we screened 265 non-synonymous SNP-containing genes and narrowed them down to 27 candidate genes with differential expression patterns in NCLB contrasting genotypes (susceptible and resistant). Our study provides a genetic basis for quantitative disease resistance against NCLB in maize. Further functional characterization of candidate genes based on the provided information can yield significant insights into the NCLB resistance mechanisms in maize.

**Key words:** maize; QTL; northern corn leaf blight

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### **Genetic basis analysis of plant hormones regulating the development of maize tassels**

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**Abstract: 【Objective】** Maize is the largest crop in China, which has various uses such as food, feed, and industrial production. However, the current corn production in China is far from meeting domestic demand. There is a negative correlation between traits such as the number of male ear branches and grain yield in maize, and reducing male ear branches is also an important selection goal and direction in the maize breeding process. In the process of plant branching development, it is jointly regulated by various factors such as plant hormones, environment, and genetic factors, among which plant hormones play a key role in regulating plant branching development. **【Method】** In this project, 388 temperate maize related populations were used to determine the hormone content in tassel about 5mm in size by HPLC-MS/MS method. Using genome-wide association study strategy, key genes controlling the number of tassel branches and endogenous hormone content in maize were located and cloned, their biological functions and molecular mechanisms were analyzed, and excellent alleles were excavated and created. **【Result】** The phenotypic investigation in three places in two years showed that the number of male spike branches in this population varied from 1 to 20, with a broad heritability of 0.93. In Xiangyang, Nantong and Ezhou, the heritability explained by SNP was 0.59, 0.68 and 0.74 respectively. A total of 179 loci were detected that were significantly associated with the number of branches. Based on the LD distance, they were divided into 38 loci, and the proportion of alleles that reduced the number of branches in inbred lines with fewer branches was significantly higher than that in inbred lines with more branches. At the same time, the content of 11 auxin substances in inbred lines with fewer branches was significantly higher than that in inbred lines with more branches; On the contrary, its zeatin and GA15 content were significantly lower than those of inbred lines with more branches; The content of five abscisic acids in inbred lines with fewer branches was significantly higher than that in inbred lines with more branches. Significant differences in the number of male spike branches were detected between the two alleles in 7 out of 22 loci significantly associated with ABA content, with high abscisic acid content and low number of male spike branches. Based on this, we have screened a large number of candidate genes, mainly involving hormone response, hormone synthesis, transportation and other pathways. **【Conclusion】** Different plant hormones regulate branch development in different ways, and the inhibition of branch development by auxin may be related to its apical dominance; The hormone ABA closely related to stress response may inhibit branching development.

**Key words:** maize; hormones; tassel morphology; development

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## **Title Genomic prediction of the performance of hybrids and the combining abilities for line by tester trials in maize**

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**Abstract:****【Objective】**The two most important activities in maize breeding are the development of inbred lines with high values of general combining ability (GCA) and specific combining ability (SCA), and the identification of hybrids with high yield potentials. Genomic selection (GS) is a promising genomic tool to perform selection on the untested breeding material based on the genomic estimated breeding values estimated from the genomic prediction (GP).**【Method】**In this study, GP analyses were carried out to estimate the performance of hybrids, GCA, and SCA for grain yield (GY) in three maize line-by-tester trials, where all the material was phenotyped in 10 to 11 multiple-location trials and genotyped with a mid-density molecular marker platform.

**【Result】**Results showed that the prediction abilities for the performance of hybrids ranged from 0.59 to 0.81 across all trials in the model including the additive effect of lines and testers. In the model including both additive and non-additive effects, the prediction abilities for the performance of hybrids were improved and ranged from 0.64 to 0.86 across all trials. The prediction abilities of the GCA for GY were low, ranging between  $-0.14$  and  $0.13$  across all trials in the model including only inbred lines; the prediction abilities of the GCA for GY were improved and ranged from  $0.49$  to  $0.55$  across all trials in the model including both inbred lines and testers, while the prediction abilities of the SCA for GY were negative across all trials. The prediction abilities for GY between testers varied from  $-0.66$  to  $0.82$ ; the performance of hybrids between testers is difficult to predict.

**【Conclusion】**GS offers the opportunity to predict the performance of new hybrids and the GCA of new inbred lines based on the molecular marker information, the total breeding cost could be reduced dramatically by phenotyping fewer multiple-location trials.

**Key words:** Maize; Genomic selection; Line-By-Tester; General combining ability; Specific combining ability

## **Expression profiles of *ZmDGK5* gene in maize under cold stress**

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**Abstract:** **【Objective】** Diacylglycerol kinase (DGK) is a unique phospholipid kinase that catalyzes the phosphorylation of DAG to produce PA. However, The potential functions of *ZmDGK5* gene in the plant growth stage and tissues and the profiles of *ZmDGK5* response in maize remains obscure. **【Method】** In this study, the expression profiles of *ZmDGK* gene in response to cold stress was analyzed, and the *ZmDGK5* gene in maize DGK subfamily I, which is significantly responsive to cold stress, was used to confirm *ZmDGK5* expression and its promoter in the induced expression of cold stress. The localization of maize *ZmDGK5* in maize cells was analyzed by laser confocal microscopy of GFP fluorescence signals using GFP fluorescent protein fusion expression and transient expression of protoplasts. **【Result】** RT-qPCR test showed that *ZmDGK5* was up-regulated 4.5 times after 4°C cold treatment; *ZmDGK5* promoter-driven GUS activity was observed in different growth stages and tissue sites of *Arabidopsis*. GUS activity was higher in the whole plant at day 1 and day 3 after germination. After seedling germination, GUS activity was observed in leaf veins and growth spots on days 5,7, and 14. At flowering time, rosette leaves, stems, stems, leaves, buds, petals, filaments, anthers, styles, horns and roots all showed strong GUS activity. GUS staining was shallow in transgenic *Arabidopsis* when treated without cold temperature (22°C). After 4°C treatment for 3d, the GUS signal was significantly enhanced. The GFP protein has a strong fluorescent signal throughout the cell and does not contain vacuoles and chloroplasts. However, the *ZmDGK5*-GFP fusion protein only had a GFP fluorescence signal in the vesicles. **【Conclusion】** The expression of *ZmDGK5* in *Arabidopsis* has some tissue specificity, and *ZmDGK5* gene expression activity was enhanced after cold treatment, indicating that cold stress induced the expression of *ZmDGK5* gene and that *ZmDGK5* gene may be involved in maize response to cold stress.

**Key words:** Maize (*Zea mays* L.); Cold stress; Diacylglycerol kinase (DGK)

## **Natural variation of *ZmCCT11* determines maize mesocotyl domestication and altitude adaptation**

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**Abstract:** **【Objective】** China, 20% areas planted with maize are in mountain. When planted maize in these areas, deep sowing is a good way to provide seeds sufficient water supply and stable temperature, which will be favorable for germination. Elongation of mesocotyl play a vital role in maize emergence from soil, but the mechanism controlling this process is not clear so far.

**【Method】** Here, we investigated the mesocotyl lengths of a maize association panel containing 380 inbred lines. **【Result】** Based on genome wide association analysis, we mapped and cloned *ZmCCT11* which encodes a transcription factor of CCT family. *ZmCCT11* was localized to the nucleus, and has transcriptional transactivation activities. Over expression of *ZmCCT11* leads to a longer mesocotyl. Two “UGUA” motifs were found in *ZmCCT11* 3’UTR, which could be the binding sites of ZPUM5 (RNA binding protein). Our study proved that ZPUM5 could regulate the protein accumulation of *ZmCCT11* by binding to the “UGUA” motifs. We found that *ZmCCT11* accumulated more in EMS mutant of *ZPUM5* (*zpum5*). *zpum5* showed a longer mesocotyl, which was similar to *ZmCCT11*-OE lines. Results of RNA-seq showed that genes involved in auxin signaling were up-regulated in *ZmCCT11*-OE lines. The maize inbred lines were divided into TypeA and TypeB based on four SNPs which are significantly ( $P < 1 \times 10^{-6}$ ) associated with maize mesocotyl length. Mesocotyls from TypeA were longer than those from TypeB. Experiments based on F<sub>2</sub> and BC<sub>3</sub>F<sub>2</sub> populations further proved that TypeA is favorable in promoting mesocotyl elongation. The haplotypes of maize landraces from Americas and their associations with the environments were determined. The results revealed that frequency of TypeA was higher in high altitude. Moreover, the frequency of TypeA in teosinte differed from that in maize, indicating that *ZmCCT11* was selected in domestication and improvement and TypeA could be a favorable haplotype for maize planting in higher altitude. **【Conclusion】** Our results provided new genetic resources and molecular markers for breeding maize cultivars suitable for planting in areas with higher altitudes.

**Key words:** Maize; Mesocotyl; GWAS; altitude adaptation



## Abstract 130

### Molecular Mechanism Analysis of Maize *miR169*-*ZmNuC* Module of Controlling Resistance to Rough Dwarf Disease

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**Abstract:** **【Objective】**Maize rough dwarf disease (MRDD) is a worldwide viral disease caused by Rice black streaked dwarf virus (RBSDV) in the main production areas of China. With global warming and the outbreak of grey planthoppers, the disease is becoming increasingly severe and is developing in northern China, including in provinces such as Heilongjiang. **【Method】** This study takes the maize *miR169*-*ZmNuC* module discovered by the research team in the early stage as the research object. Construction of *miR169* overexpression and suppression expression vector, target gene overexpression and crispr editing vector, transformation of maize inbred line B104, phenotypic identification after artificial inoculation, analysis of molecular mechanism of maize *miR169* and its target gene *ZmNuC* regulating rough dwarf disease resistance by combining transcriptome sequencing, metabolome, interaction between *ZmNuC* and genes in maize, and changes in histiocyte morphology and hormone content, verifies the function of the module on maize rough dwarf disease resistance, and preliminarily analyzes its regulatory molecular mechanism. **【Result】** The 5' RACE validation confirmed that *ZmNuC* gene was the target gene of *miR169*. Overexpression of *miR169* and editing of *ZmNuC* gene could significantly improve maize rough dwarf disease resistance, and the disease resistance level was increased from fourth level to second level. The analysis of micromorphological changed found that the *miR169*-*ZmNuc* module improved resistance to rough dwarf disease by regulating photosynthesis through maintaining chloroplast morphology, and the analysis of hormone levels found that the *miR169*-*ZmNuc* module improved resistance to rough dwarf disease by regulating gibberellin and jasmonic acid. **【Conclusion】** *miR169* could improve maize rough dwarf disease resistance, while *ZmNuC* played a negative role in regulation.

**Key words:** maize rough dwarf disease; *miR169*; *ZmNuC* gene; Function verification; Mechanism analysis

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## ***Abstract 131***

### **Transcription factor NCR1-activated root-to-shoot nitrate transport conferring high nitrogen use efficiency in maize**

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**Abstract:** Nitrogen (N) is one of essential nutrient elements for plant growth and development, and it also determines crop yield. Maize has maximum planting area and total yield in China, thus it demands a huge number of nitrogen fertilizer. Therefore, genetic improvement of maize nitrogen utilization efficiency (NUE) is urgently required. However, the molecular mechanisms of maize NUE are little known. Using GWAS method, we have identified a transcription factor gene *ZmNCR1* that is involved in the regulation of root-to-shoot nitrate transport in maize. Preliminary data indicated that *ZmNCR1* directly regulates the expression of nitrate transporter gene *ZmNRT* that may modulate root-to-shoot nitrate transport. Besides, *ZmNCR1* may also regulate the expression of the genes involved in nitrate absorption, reduction and ammonium assimilation. These results suggest that *ZmNCR1* may act as a core factor that regulates maize NUE. In this project, we will characterize the physiological functions and regulatory mechanisms of *ZmNCR1* in maize nitrate transport and metabolism, then identify the excellent allelic variation of *ZmNCR1*. The prospective results of this project would provide the theory and approach for the improvement of maize NUE.

**Key words:** nitrogen; nitrate transport; maize; transcription factor; gene expression

## 玉米种质资源苗期耐盐性鉴定及关联分析

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**摘要:** 玉米是我国第一大作物, 提高我国玉米生产能力, 尽快摆脱进口玉米的依赖性, 是保障我国粮食安全的首要任务。盐碱地是我国极为重要的后备耕地资源, 培育耐盐碱玉米新品种, 合理利用盐碱地是增加我国玉米产量的有效措施。但由于玉米是盐敏感作物, 同时种质资源间对盐敏感程度变异很大, 筛选鉴定耐盐种质资源, 为耐盐玉米新品种提供优良育种材料和理论依据具有重要意义。【材料与方法】本研究选取 350 份来自中美两国不同育种年代的玉米自交系进行苗期耐盐性鉴定, 200mM NaCl 处理 20 天后统计株高、叶长、叶宽、叶片含氮量和叶绿素含量等指标, 继续处理 5 天, 统计存活率和地上部鲜重。然后, 计算各指标的盐胁迫抑制百分比, 根据该数值对各个玉米材料进行综合评价。再结合覆盖玉米全基因组的 SNP 标记对相关性状开展全基因组关联分析 (Genome wide analysis system, GWAS), 筛选显著的 SNP 位点和候选基因, 并通过等位变异效应分析鉴定主效 SNP 位点及其优异等位变异。【结果】研究发现, 在高盐环境下, 玉米株高、叶长、叶宽等形态学指标发生明显变化, 且不同材料间变异很大, 通过该研究建立了玉米苗期耐盐性筛选和评价体系。利用该体系, 对 350 份玉米自交系耐盐性进行了分级, 鉴定到了多个极度耐盐和盐敏感的材料。利用各种形态学指标和叶片氮素含量及叶绿素含量进行 GWAS 分析, 获得了多个显著的 SNP 位点, 同时总结了玉米耐盐性相关的 QTL 热点区间, 目前正在对结果进行进一步的分析, 挖掘调控玉米耐盐性的关键候选基因。该研究将为培育耐盐玉米新品种提供材料信息和优良基因源。

**关键词:** 玉米; 耐盐性; 种质资源鉴定; 关联分析

## ***Abstract 133***

### **A *DUF1230* gene confers quantitative resistance to southern leaf blight and gray leaf spot in maize**

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**Abstract:** Southern leaf blight (SLB) and gray leaf spot (GLS) are two important maize foliar diseases causing significant yield losses. Genes conferring multiple disease resistance (MDR) are valuable in crop improvement. Previous study using the maize Nested Association Mapping (NAM) population identified a disease resistance QTL against SLB in bin 8.03. Candidate region-based association analysis was performed using the 282 association panel. The most significant marker was located on the flanking region of the gene *ZmDUF1230*, which were in the same linkage disequilibrium block. *ZmDUF1230* encodes a domain of unknown function 1230 (DUF1230) protein, which is conserved in the green lineage and diatoms. EMS mutants and knock-out lines using CRISPR-Cas9 editing showed enhanced resistance to both SLB and GLS. *ZmDUF1230* is localized in the nucleus, cytoplasm and chloroplast. Through large-scale transcriptome co-expression analysis and yeast two-hybrid screening, we identified a *ZmDUF1230*-interacting protein named FTSH5, which is a chloroplast-localized metal peptidase. We hypothesize that *ZmDUF1230* may provide MDR to SLB and GLS by improving chloroplast stability through interacting with FTSH5.

**Key words:** maize; multiple disease resistance; domain of unknown function 1230; southern leaf blight; gray leaf spot

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## **The comprehensive analysis of ceRNA expression profile revealed the response mechanism of maize germination to low temperature**

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**Abstract: 【Objective】** Non-coding RNAs include circRNAs (circRNAs), long-chain non-coding RNAs (lncRNAs), and microRNAs (miRNAs), which are involved in abiotic stress processes in plants. Similarly, non-coding RNAs can also participate in plant abiotic stress as competitive endogenous RNAs (ceRNAs), but the mechanism is still unclear. **【Method】** In this study, the response of Zao8-3 and Ji853 varieties to low temperature was studied by three high-throughput sequencing methods: RNA-Seq, small RNA and degradation group. The novel lncRNAs and circRNAs obtained from transcriptome were studied. **【Result】** Under low temperature stress, 146 lncRNAs and 48 circRNAs were differentially expressed in maize germination stage, respectively. The analysis of microRNA group and degradation group showed that zma-miR156h-3p, zma-miR169o-3p\_L-1R+1, PC-3p-15424\_419 and other three important differentially expressed miRNAs had a total of 7 target genes that could meet the gene expression trend in both high-tolerance and high-sensitive materials. In addition, the lncRNA/CircRNA-miRNA-mRNA regulatory network of maize low-tolerant inbred (zao8-3) and low-temperature-sensitive inbred (ji853) under low-temperature stress was constructed through the joint analysis of several histologies to understand the mechanism of maize germination response to low temperature. It is worth noting that lncRNA (MSTRG.18187.1) interacting with miR169 and miR156, and circRNA384 interacting with PC-3p-249755\_28. In addition, GO enrichment and KEGG pathway analysis revealed that the lncRNA/circRNA-miRNA network is mainly involved in Rho guanyl-nucleotide exchange factor activity, cell wall, integral component of membrane response to cold and other pathways. **【Conclusion】** It is worth noting that lncRNA (MSTRG.18187.1) interacting with miR169 and miR156, and circRNA384 interacting with PC-3p-249755\_28. These results provide a new perspective for studying the response of potential target-recognized non-coding RNA networks to low temperature regulation.

**Key words:** maize; low temperature; Germination; ceRNA

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## *ZmMAPK16* 基因平衡生长发育与镰刀菌抗性的机制

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**摘要:** 【目的】镰刀菌引发的玉米穗腐病 (ear rot) 和茎腐病 (stalk rot), 是世界玉米产区普遍发生的一类真菌病害, 严重影响玉米产量和籽粒品质, 对食用、饲用和贮藏安全带来极大威胁。目前, 对穗腐和茎腐病抗性的有效基因研究还很少, 生产上缺乏抗镰刀菌且高产优质的玉米品种。所以挖掘抗镰刀菌的基因是十分有必要的。【方法】通过对实验室现有的转基因材料进行镰刀菌抗性鉴定, 筛选出具有抗性的转基因材料。再利用分子生物学方法对该基因开展进一步研究, 解析该基因在抗镰刀菌方面的分子机制。【结果】通过对转基因玉米材料离体叶片接种轮枝镰孢菌 (XY-1), 鉴定出 *ZmMAPK16* 基因与镰刀菌抗性相关, 表现为突变体 *mapk16* 较野生型敏感, 过表达材料抗性与野生型类似, 同时田间穗腐病表型与其保持一致。同时 *ZmMAPK16* 的缺失导致植株变矮但不影响产量, 过表达 *ZmMAPK16* 导致植株变高产量增加。通过 IP-MS、酵母双杂交筛库等方法, 筛选鉴定出 3 个互作蛋白: CNR8、JAZ 和 E3-ligase。同时在 BiFC 实验过程中, 发现 E3-ligase 与 MAPK16 共注射会导致烟草叶片卷曲坏死, 暗示该过程可能与活性氧爆发相关。【结论】以上结果暗示 *ZmMAPK16* 基因可能通过与 E3-ligase、JAZ 的互作从而促使植物体一些生理生化反应发生变化, 从而促进玉米生长发育与镰刀菌抗性。

**关键词:** 玉米; MAPKs; 抗病

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## **Genetic basis of the differentiation of important agronomic traits between maize and teosinte**

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**Abstract: 【Objective】**The introgression regions from *mexicana* to maize may not only contribute to the adaptation of maize from lowland to highland environment, but also help to improve the yield potential and other important agronomic traits. **【Method】** In this study, a Mo17-*mexicana* introgression population (hereafter, TM population) was used to investigate the genetic basis underlying maize domestication and improvement of 20 morphological traits. **【Result】** A total of 123 QTL were detected for the above 20 traits. The alleles of 39 QTL from *mexicana* increase flowering time, branch number etc., and vice versa for those of the remaining 84 QTL. Trait-QTL network revealed that the traits with high correlations, such as ear traits, seem to be selected simultaneously during maize domestication or improvement, and could be controlled by pleiotropic or linked loci/genes. Colocalization analysis of genome-wide selected genomic regions and identified QTL indicated that adaptive and yield-related traits were strongly selected during domestication and improvement. A QTL affecting multiple ear traits was cloned as a ULTRAPETALA (ULT) transcription factor *EL3*. *EL3* may negatively regulate ear length by influencing ear development and hormone regulation related genes, and was strongly selected during domestication. **【Conclusion】** In this study, we systematically investigated the differentiation between modern maize and its wild relative, and identified a transcription factor *EL3*, that controls ear length.

**Key words:** maize; *mexicana*; QTL; selection; *EL3*

## 玉米花粉壁形成及响应高温的代谢与遗传调控解析

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**摘要:** 【目的】近年来极端高温天气频发, 授粉季高温直接影响玉米花粉的育性保持, 严重时将导致减产甚至绝收。花粉壁结构与花粉的逆境适应关系密切, 但玉米花粉壁结构组成及其和高温耐受性的关系还不清晰。【方法】采用质谱和核磁共振技术对玉米花粉外壁孢粉素的组分进行了解析。【结果】首次在玉米花粉外壁中鉴定到系列酚类代谢物, 包括耐高温标记代谢物, 如木质素。群体测定发现孢粉素中木质素含量在热带玉米材料里整体积累要高于温带玉米材料。木质素合成突变体的花粉对高温敏感。通过mGWAS分析鉴定到与这些高温标记代谢物含量高度相关的SNP及系列候选基因, 并对候选基因*ZmVAMP726*的功能进行了验证。【结论】首次发现玉米孢粉素中含有H、G、S衍生的木质素单体, 并且组成形式非常有特点, 和常规的次生壁明显有很大区别, 这可能是造就孢粉素坚硬质地的原因。这些单体的含量和组成与玉米花粉对高温的耐受性相关。*ZmVAMP726*在小麦、高粱等其他重要经济作物及模式植物拟南芥里可能功能保守, 对农作物花粉高温环境下的育性保持至关重要。

**关键词:** 玉米花粉壁; 高温; 孢粉素; 酚类代谢物; 木质素

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## Abstract 138

### The genetic architecture of prolificacy in maize revealed by association mapping and bulk segregant analysis

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**Abstract: 【Objective】** The development of maize plants with multiple ears, or prolificacy, is crucial for enhancing maize yield and breeding specialty maize varieties, such as baby corn. To achieve this goal, it is important to understand the genetic basis of ear number (EN). **【Method】** Here, we employed a genome-wide association study (GWAS) to analyze the genetic basis of EN in maize. The analysis was conducted using 492 maize inbred lines across five different environments with a wide range of EN variability. **【Result】** Our results demonstrated significant differences in genetic, environmental, and interaction effects. The broad-sense heritability ( $H^2$ ) of EN was 0.60. We performed a GWAS using a Q+K model, which revealed 527 significant single nucleotide polymorphisms (SNPs) ( $P \leq 10^{-4}$ ), involved 290 QTLs, and total 806 genes located in them. Of these SNPs, 18 (3.40%,  $R^2 \geq 10\%$ ) were classified as major effect loci and 509 (96.60%,  $R^2 \leq 10\%$ ) were classified as minor effect loci. This suggests that EN is a quantitative trait that is controlled by both major and minor genes. To further investigate the genetic basis of EN, we performed a bulk segregant analysis (BSA) using the few-ears line Zheng58 and the multi-ears line 647 as parents to construct an  $F_2$  population. Our BSA results identified one significant quantitative trait locus (QTL), *qBEN1*, located between 10.24 Mb-24.04Mb on chromosome 10. Importantly, when combining the GWAS and BSA results, four co-located QTLs, involving six genes, were identified. Three of them were expressed in vegetative meristem, shoot tip, internode and tip of ear primordium, with *ZmEN1* having the highest expression level in these tissues. Our findings suggest that *ZmEN1* may play a crucial role in promoting axillary bud and tillering to encourage the formation of prolificacy. Notably, *ZmEN1* was located in *qGEN261*, which encodes an unknown auxin-like protein. Haplotype analysis of *ZmEN1* revealed significant differences between different haplotypes, with inbred lines carrying hap6 having more EN. **【Conclusion】** Overall, this is the first report about using GWAS and BSA to dissect the genetic architecture of EN in maize and provides new insight about it, which can be valuable for breeding specialty maize varieties and improving maize yield. Additionally, our findings offer useful resources for future research on maize genetics.

**Key words:** prolificacy; specialty maize; genetic architecture; genome-wide association study;

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bulk segregant analysis

## **Identification of *ZmLEA* genes in maize and screening of dehydration related genes**

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**Abstract:****【Objective】** The objective of this study was to identify *ZmLEA* gene members in maize and screen genes related with kernel dehydration rate in maize . To study the relationship between the LEA family and kernel dehydration, this study conducted identification and expression pattern analysis of the LEA family, as well as its expression changes in the later stages of grain development. **【Method】** This study used bioinformatics methods to identify and analyze *LEA* genes in maize, and then screened genes related to grain dehydration through transcriptome data. Finally, the function of the relevant *LEA* gene was verified through phenotype of mutant, wild-type, and transgenic materials. **【Result】** There are 52 *ZmLEA* genes present in the maize genome. Based on phylogeny analysis with Arabidopsis, rice and sorghum, 52 *ZmLEA* gene family members were identified, and there were divided into 8 subgroups. Combining with the published transcription data, fourteen *ZmLEA* genes were predicted to be involved in the regulation of maize kernel moisture content and kernel dehydration rate. **【Conclusion】** This study further interpreted the characteristics of the *ZmLEA* genes, and laid a foundation for further research on the function of *ZmLEA* genes in regulating the genes related to grain moisture content and grain dehydration rate in maize.

**Key words:** Maize; Kernel moisture content; Kernel dehydration rate; LEA family

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## **Natural variation of *ZmCGT1* is responsible for the isoorientin accumulation in maize silk**

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**Abstract:****【Objective】**Maize silk contains high levels of flavonoids and is widely used to promote human health. Isoorientin, a natural C-glycoside flavone rich in maize silk, has attracted considerable attention due to its potential value. Although different classes of flavonoid have been well characterized in plants, the genes involved in the biosynthesis of isoorientin are largely unknown in maize.**【Method】**Here, we used targeted metabolic profiling of isoorientin on the silks in an association panel consisting of 294 maize inbred lines, and identified a gene *ZmCGT1* by genome-wide association analysis (GWAS).**【Result】**The *ZmCGT1* protein was characterized as a 2-hydroxyflavanone C-glycosyltransferase and can C-glycosylate 2-hydroxyflavanone to form flavone-C-glycoside after dehydration. Moreover, overexpressing *ZmCGT1* increased isoorientin level and RNA-mediated interference *ZmCGT1* decreased accumulation of isoorientin in maize silk. Further, two nucleotide polymorphisms A502C and A1022G, which led to amino acid changes, I168L and E341G, respectively, were identified to be functional polymorphisms responsible for natural variation of isoorientin.**【Conclusion】**Taken together, we identified a gene, *ZmCGT1*, which plays an important role in isoorientin biosynthesis and provides the insights of the genetic basis of the natural variations of isoorientin level in maize silk. The identified favorable alleles CG of *ZmCGT1* may be further used for genetic improvement of nutritional quality in maize.

**Key words:** Maize silk; mGWAS; isoorientin; *ZmCGT1*; C-glycosyltransferase; functional polymorphism

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## **Cloning and Functional Analysis of Maize Transcription Factor *ZmbZIP18* Gene**

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**Abstract:****【Objective】**This study focused on gene cloning and candidate gene association analysis in order to gain comprehensive insights into the characterization and molecular mechanisms of *ZmbZIP18* in maize. **【Method】** A combination of bioinformatics analysis and molecular biology techniques was employed to conduct preliminary investigations into the molecular functions of *ZmbZIP18* including phylogenetic analysis, subcellular localization, tobacco live imaging, yeast two-hybrid (Y2H) assays, and fluorescence quantification. Additionally, data from prior studies conducted by our research group were utilized for polymorphism analysis and candidate gene association analysis of *ZmbZIP18* in an association panel with 164 maize inbred lines. **【Result】** Phylogenetic analysis and sequence alignment confirmed that *ZmbZIP18* is a putative maize gene encoding a TGA transcription factor. Subcellular localization in tobacco verified that it was located in nucleus. Transactivation assays revealed its transcriptional activation activity and provided insights into the location of its activation domains. Furthermore, in vivo imaging experiments using the LUC reporter system in tobacco demonstrated the specific binding of *ZmbZIP18* to the TGACG motif. Moreover, candidate gene association analysis identified 16 polymorphic loci within the *ZmbZIP18* gene that are associated with grain moisture content. **【Conclusion】** This study successfully cloned *ZmbZIP18*, and validated *ZmbZIP18* as a typical TGA gene involved in drought and ABA stress responses, showing similar expression patterns. Through candidate gene association analysis, we identified three significant loci, SNP429, InDel1218, and SNP2865, within the *ZmbZIP18* gene that exhibited a highly significant correlation with grain moisture content and dehydration rate. These findings provide valuable genetic resources and a theoretical basis for enhancing stress resistance and grain dehydration characteristics in maize varieties.

**Key words:** Maize; Transcription factor; TGA, *ZmbZIP18*; Candidate gene association analysis

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## ZmMAB10 调控玉米耐热性的机理解析

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**摘要:** 【目的】近些年来全球气候变暖已经成为世界面临的主要环境问题。研究表明, 高温胁迫影响玉米苗期生长和产量。据统计, 全球年平均气温每升高1℃, 会使玉米减产7.4%。因此探究玉米响应高温胁迫的调控机理和创制耐热种质资源, 对于培育耐热玉米新品种具有重要意义。【方法】实验室前期针对玉米株型、光周期、激素合成与信号转导以及育种选择等过程的相关基因进行了敲除或者过表达材料的构建。对实验室已有转基因材料V1期进行高温筛选, 45℃处理3天, 而后进行恢复培养并统计其成活率。【结果】我们发现一个对高温极度敏感的材料, *ZmMAB10-OE*。该材料经高温处理后, 存活率显著低于对应野生型。该基因为MATH-BTB蛋白家族成员, 一般认为该家族参与形成Cullin3-RING泛素连接酶复合物, CRL3。但我们发现其不与玉米CUL3互作, 所以*ZmMAB10*不参与CRL3的形成。*ZmMAB10*可以与自己或水稻中的同源蛋白互作, 形成同源或异源二聚体。有意思的是, 我们对*ZmMAB10*进行pan-gene分析时, 发现*ZmMAB10*的编码区包含一个2-bp Indel, 造成蛋白翻译的提前终止, 导致BTB结构域缺失, 我们将两种单倍型分别命名为, *ZmMAB10-BTB*和*ZmMAB10-btb*。分析两种基因型编码蛋白的亚细胞定位发现, *ZmMAB10-BTB*主要定位在细胞膜, 而*ZmMAB10-btb*定位于细胞核和细胞膜。下一步将研究两种单倍型对玉米耐高温的生物学功能。【结论】筛选到一个高温敏感的过表达材料, 预计可以通过基因组编辑该基因而创制耐热玉米种质, 同时也为探究不参与CRL3形成的MATH-BTB蛋白功能提供遗传材料。研究结果将丰富玉米响应高温胁迫的调控机理, 并为培育耐高温玉米品种提供种质资源。

**关键词:** 玉米; 高温胁迫; MATH-BTB; 种质资源

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## **The mechanism of *AOF5* gene on the regulation of maize attracting Asian corn borer**

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**Abstract: 【Objective】** Asian corn borer is one of the most important pests of maize, occurs throughout the whole growth stages of maize and leads to massive yield and seed quality reduction. For long-term field management, the treatment dealing with corn borer is mainly relying on chemical pesticides, which seriously cause environmental pollution and threat human health. Among all kinds of approaches, developing insect-resistant crops has becoming an effective way, and the identification of new insect-resistance genes and germplasms are critical.

**【Method】** In this work, we found that two maize inbred lines with different backgrounds attracted different degrees of corn borer. We used bioinformatics to analyze the differential genes and metabolites. **【Result】** Through the combined analysis of transcriptome and metabolome, we identified the *AOF5* gene. For this reason, we generated an CRISPR maize mutant *aof5*, and showed that this mutant has a strong ability to resist corn borer. **【Conclusion】** This work has shed a light on the mechanism of maize resistance to corn borer and would pave a novel way for breeding new insect-resistant germplasm of maize.

**Key words:** maize; insect resistance; corn borer; *AOF5*; molecular mechanism

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## Abstract 144

### Structural modification of (3E)-4,8-dimethyl-1,3,7-nontriene enhances its ability to kill *Plutella xylostella* insect pests

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**Abstract: 【Objective】** Plant secondary metabolites and their modified derivatives play an important role in the discovery and development of novel insecticides. The natural plant product (3E)-4,8-dimethyl-1,3,7-nontriene (DMNT) has been proven to be able to effectively repel and kill the lepidopteran insect pest *Plutella xylostella*. **【Method】** In this study, four oxygenated derivatives of DMNT were synthesized by allylic hydroxylation and subsequent etherification or esterification.

**【Result】** Bioassays on *P. xylostella* larvae showed that the compounds DMNT-OCH<sub>3</sub>, DMNT-OCy and DMNT-OAc were more toxic to the larvae than DMNT alone. The most pronounced effect was observed for DMNT-OCH<sub>3</sub>, which showed a 22.23% increase in lethality at a concentration of 0.25 μM. Moreover, the peritrophic matrix (PM) barrier in the insect midgut was more severely damaged by DMNT-OCH<sub>3</sub>, DMNT-OCy and DMNT-OAc than by DMNT. The median lethal concentration (LC<sub>50</sub>, 48 h) of DMNT-OCH<sub>3</sub>, DMNT-OCy and DMNT-OAc on *P. xylostella* was determined to be 0.98, 1.13 and 1.11 mg/mL, respectively, which is much lower than the commercial insecticides eucalyptol (2.89 mg/mL) and thymol (2.45 mg/mL). **【Conclusion】** These results suggested that oxygenated DMNT derivatives offer a significantly improved killing effect over DMNT on *P. xylostella*. This work has provided a basis for further design, structural modification and development of DMNT as botanical insecticides.

**Key words:** *Plutella xylostella*, DMNT, DMNT modifications, insecticidal activity, allylic oxidation

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## ***Abstract 145***

### **ZmBET5L1 inhibits primary root growth and decreases osmotic stress tolerance by mediating vesicle aggregation and tethering in maize**

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**Abstract: 【Objective】** Based on current predictions, in the next 30 years approximately 50% of the cultivable land will suffer from drought and 20 million hectares of cultivated land will be affected by salt and alkaline soil, which can cause osmotic stress and reduce crop yield, thus limiting crop production worldwide. Improving osmotic stress tolerance is critical to help crops to thrive and maintain high yields in adverse environments. **【Method】** We characterized a core subunit of the transport protein particle (TRAPP) complex, ZmBET5L1, in maize using knowledge-driven data mining and genome editing. **【Result】** We found that ZmBET5L1 can interact with TRAPP I complex subunits and act as a tethering factor to mediate vesicle aggregation and targeting from the endoplasmic reticulum to the Golgi apparatus. *ZmBET5L1* knock - out increased the primary root elongation rate under 20% polyethylene glycol - simulated osmotic stress and the survival rate under drought stress compared to wild - type seedlings. In addition, we found that *ZmBET5L1* moderates PIN1 polar localization and auxin flow to maintain normal root growth. *ZmBET5L1* knock - out optimized auxin flow to the lateral side of the root and promoted its growth to generate a robust root, which may be related to improved osmotic stress tolerance. **【Conclusion】** Together, these findings demonstrate that *ZmBET5L1* inhibits primary root growth and decreases osmotic stress tolerance by regulating vesicle transport and auxin distribution. This study has improved our understanding of the role of tethering factors in response to abiotic stresses and identified desirable variants for breeding osmotic stress tolerance in maize.

**Key words:** knowledge - driven breeding; maize (*Zea mays* L.); osmotic stress; tethering factor; vesicle trafficking

## ***Abstract 146***

### ***Defective kernel 66* encodes a GTPase essential for kernel development in maize**

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**Abstract:** Mitochondria is a semi-autonomous organelle that provides energy for cell activities through oxidative phosphorylation. In this study, we identified a *defective kernel 66* (*dek66*)-mutant maize with defective kernels. We characterized the candidate gene *DEK66*, encoding a ribosomal assembly factor, locating in mitochondria, and possessing GTPase activity (the GTPase belonged to ribosome biogenesis GTPase A family). In the *dek66* mutant, abolishment of mitochondrial structure and function led to the accumulation of reactive oxygen species and promoted programmed cell death in endosperm cells. Furthermore, transcript level of most of the key genes associated with nutrient storage, mitochondrial respiratory chain complex, and mitochondrial ribosomes in the *dek66* mutant were significantly altered. **【Conclusion】** Collectively, the results suggested that *DEK66* is essential for the development of maize kernels via affecting the mitochondrial function. This study provided a reference to understand the impact of mitochondrial ribosomal assembly factor in maize kernel development.

**Key words:** oxidative phosphorylation; GTPase activity; programmed cell death

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## **The mechanism of *RTA25* gene on the regulation of maize resistance to aphids**

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**Abstract:** Corn leaf aphid is one of the common limiting factors affecting corn planting and production in China. It can directly affect the growth and development of corn by needling and sucking corn plants, and spread plant viruses, which seriously affected the yield and quality of corn crops. Therefore, it is of great theoretical and practical significance to explore the key genes of maize aphid defense, reveal the molecular mechanism of maize aphid defense, and study the biocontrol technology of maize aphid for maize production and yield improvement. To this end, we screened and identified a maize mutant *Zmrta25* with significant resistance to aphids. The mutant showed a strong ability to inhibit the growth and reproduction of aphids. To further validate the role of *RTA25*, we obtained an Arabidopsis mutant *Atrta25*. Both mutants showed insect resistance phenotypes consistent with *Zmrta25*, confirming that *RTA25* is involved in the regulation of plant resistance to aphids. Our protein BiFC analysis indicated that *RTA25* interacts with the IOR1 protein, which has been reported to be involved in insect resistance in maize. In addition, GC-MS analysis identified a volatile substance that may have an attractive effect on aphids. This study revealed the mechanism of aphid resistance in plants and opened up a new way for the breeding of new insect resistant maize varieties.

**Key words:** maize; insect resistance; aphid; molecular mechanism

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## **Cloning and functional analysis of *ZmDT6* controlling drought tolerance in maize**

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**Abstract: 【Objective】** Drought is the most important abiotic adversity factor limiting maize production, causing yield losses of up to 20%-30% annually. With global warming and increasing shortage of agricultural water resources, drought is becoming a more prominent problem. Cloning drought-tolerant genes and breeding drought-tolerant maize is an effective way to secure grain production worldwide. **【Method】** We cloned a drought tolerance gene *ZmDT6* by genome-wide association analysis, which was highly expressed in roots, silk and female spikelet, and could be induced by drought stress. We characterized the drought tolerance of *ZmDT6* overexpression and CRISPR/Cas9 gene knockout lines, and EMS mutant (B73 background) under the field condition.

**【Result】** *ZmDT6* localized in the plasma-membrane. *ZmDT6* overexpression resulted in shortened ASI, reduced plant and ear height, suppressed female ear development, and reduced ear biomass and yield under both well-water and water-stress field conditions. Both *ZmDT6* knockout plants and EMS mutants showed increased plant height, female ear and grain yield. Further study revealed that difference in plant height might be due to high expression of *ZmDT6* gene in the lower internodes at elongation stage, resulting in reduced nodes number below ear position. In addition, *ZmDT6* gene overexpression reduced yield-related traits such as ear length and width, row number, kernel number, length and width. *ZmDT6* controlling drought tolerance might be through regulating the water loss rate of detached leaves and root development in maize.

**【Conclusion】** Our study provided a promising target for stress-tolerant and high-yielding maize biological breeding.

**Key words:** maize; drought tolerance; plant height; ear biomass; grain yield

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## *ZmSMAX1* 负调控玉米与 AM 真菌共生功能研究

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**摘要:** 【目的】丛枝菌根真菌 (Arbuscular mycorrhizae, AM) 是一种可以与90%的陆地生物形成互利共生关系的根际微生物。在植物与AM真菌共生之前, 两个合作者之间会相互识别, 触发了彼此的基因重塑, 才能形成定殖。玉米作为重要的粮食作物, 也是AM真菌很好的寄主。但目前对于玉米中与AM真菌共生过程相关基因了解尚少。*SMXL*家族对植物生长发育, 激素信号传导等多种方面都具有重要的作用, 近期*OsSMAX1*基因被发现负调控水稻与AM真菌共生, 因此本研究旨在探究*ZmSMAX1*在玉米中调节菌根共生信号途径中的作用。

【方法】对不同物种已报道的*SMXL*家族基因进行进化树分析筛选出玉米中可能参与共生途径的*ZmSMAX1*, 通过生物信息学对其基因结构、蛋白结构组进行分析, 通过转录组数据差异表达分析预测, 进行组织表达模式、时空表达模式分析, 确定在接种AM真菌后*ZmSMAX1*基因具有差异表达。利用百脉根毛状根转化体系进行启动子诱导表达模式分析, 明确*ZmSMAX1*基因是否受AM真菌诱导表达及其定位。利用玉米EMS诱变突变体材料, 进行接种实验对其功能进行研究。【结果】*ZmSMAX1*是一个共生相关的负调控因子, 在进化分析上与*OsSMAX1*聚于一支, *ZmSMAX1*蛋白结构和水稻*OsSMAX1*相似有3个预测结构域, 在N段有一个核定位信号(NLS), 和一个参与伴侣蛋白相互作用的双ClpN结构域和两个P-loop ATP酶。时空表达分析表明接种AM真菌35天和60天后*ZmSMAX1*表达量显著增加。启动子诱导表达模式分析表明*ZmSMAX1*受AM真菌诱导表达, 定位于丛植周围。利用EMS诱变*zmsmax1*突变体材料进行接种实验, 结果表明接种AM真菌后*zmsmax1*突变体侵染率及侵染强度均显著高于野生型, 并且接种后其突变体生物量及茎粗均显著高于野生型。【结论】*ZmSMAX1*是一个共生相关的负调控因子, 负调控玉米与AM真菌共生, 促进植株在接种AM真菌后生长发育。

**关键词:** 玉米; 丛枝菌根真菌; 负调控; 共生

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## ***ZmBRA1* 调控玉米根系夹角与抗倒伏性的功能研究**

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**摘要:**【目的】玉米是我国的第一大农作物，其充足稳定供应对保证我国粮食安全至关重要。我国玉米主产区，如北方春播玉米区和黄淮海夏播玉米区，在每年的6-9月份普遍易受暴风雨的袭击并造成玉米倒伏，严重威胁玉米安全生产。当前，抗倒高产玉米新品种的培育已成为我国玉米生产面临的重大产业需求。已有研究表明，由根系向重力性决定的气生根夹角在玉米倒伏抗性调控方面发挥着关键作用。【方法】本课题通过多种生物学及遗传学手段验证“*ZmBRA1*调控玉米根系向重力性以及根系夹角的遗传机理”，并通过群体遗传学方法鉴定其优异单倍型。【结果】本课题前期通过CRISPR分析发现，*ZmBRA1*基因在调控玉米气生根夹角方面具有重要作用；*Zmbra1*突变后气生根夹角显著高于野生型、地上根系覆盖范围更广；通过根系向重力性敏感度分析发现，*Zmbra1*突变体较野生型材料重力敏感性降低，这可能是其气生根夹角变大的重要原因；利用137份自交系转录组数据结合群体遗传学分析发现，*ZmBRA1*基因附近存在显著的cis-eQTL信号，预示着玉米中存在自然变异或优异单倍型可以调控*ZmBRA1*基因的表达变化。【结论】该研究将为玉米根系构型遗传网络建立和玉米抗倒伏育种提供重要的理论指导和基因资源。

**关键词:** 玉米；抗倒伏；向重力性；气生根夹角

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## 玉米 AGS1 调控籽粒大小的功能研究

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**摘要:** 籽粒的生长发育直接影响玉米的产量和品质。因此挖掘影响玉米籽粒发育的关键基因并解析其作用机理, 能够为玉米产量和品质的遗传改良提供理论依据和优良的基因资源。AAA (ATPases Associated with Diverse Cellular Activities) 家族 ATPase 是一大类具有 ATP 水解活性的蛋白家族, 参与多种生理过程, 包括基因转录、蛋白翻译、细胞内转运和各种代谢调控等。目前有关作物中 AAA-ATPase 基因家族功能研究较少。本研究利用玉米籽粒突变体, 整合基于表型的转录组和籽粒粒型、产量相关 QTL 分析, 筛选获得与籽粒大小相关的 AAA-ATPase 家族成员 AAA-ATPase related to Grain Size1 (*ZmAGS1*)。在此基础上, 创制 *ZmAGS1* 过表达转基因株系并获得纯合 EMS 突变体, 籽粒表型鉴定显示, 过表达 *ZmAGS1* 导致籽粒变大, 而突变体导致籽粒变小。利用石蜡切片对突变体的籽粒进行组织结构分析, 结果显示突变体胚乳细胞变小, 提示 *ZmAGS1* 参与调控胚乳细胞的伸长, 进而影响籽粒的大小。通过进化树分析, 该基因属于 AAA-ATPase 家族的一员, 编码微管切割蛋白 Katanin 的 p60 亚基。亚细胞定位结果显示该基因定位于细胞质和细胞核。为进一步探索 *ZmAGS1* 的作用机理, 通过原核表达及纯化获得了 AGS1 蛋白, 酶活测定结果表明 AGS1 具有 ATPase 活性。后续研究中, 我们将进一步筛选 *ZmAGS1* 的互作蛋白和作用底物, 阐明其调节籽粒大小的分子机理, 同时挖掘其优良等位变异, 验证其在提高籽粒大小和粒重中的应用效果。本研究结果为 ATPase 参与调控籽粒发育的机制提供重要支持, 增进对玉米籽粒生长发育分子机理的理解。

**关键词:** 玉米; 籽粒; AAA-ATPase; Katanin; 细胞大小

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## **The ZmDT9 protein improves maize drought resistance at flowering stage**

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**Abstract: 【Objective】** Maize is the only monoecious crop plant that develops male (tassel) and female (ear) inflorescences at different positions of the same plant. When drought occurs during flowering stage, it typically results in asynchronous development of male and female inflorescences, leading to a larger anthesis to silking interval (ASI) and greatly reducing yields. Therefore, maintaining ASI under drought conditions is essential for maize drought resistance.

**【Method】** To identify new quantitative trait loci (QTLs) associated with maize ASI under drought conditions, we performed a QTL mapping using populations of maize inbred line CIMBL55 crossed with Mo17. **【Result】** There are three QTLs on chromosome 2, 9, 10, namely *qDT2*, *qDT9* and *qDT10*, respectively, were significantly associated with drought-induced ASI trait. Among them, *qDT9* (drought tolerance 9) with the largest effect was selected for further positional cloning. Resultantly, the *qDT9* locus was narrowed down to a 2,245-base pair (bp) located in the promoter region of a gene encoding an auxin-responsive protein. The near-isogenic lines (NILs) for *qDT9* (NIL-*qDT9*<sup>CIMBL55</sup> and NIL-*qDT9*<sup>Mo17</sup>) differed in ASI under flowering-stage drought conditions, indicating a role of *qDT9* in regulating drought-induced ASI. *ZmDT9* was mainly expressed in silks and repressed by drought. However, a 609-bp insertion was found in the *DT9*<sup>CIMBL55</sup> promoter which probably reduced in the gene expression. Knocking out *ZmDT9* resulted in a larger ASI under drought conditions compared with wild-type plants. Further analysis found that *ZmDT9* interacted with two plasma membrane (PM) localized phosphatases and inhibited their activities. It probably facilitated the PM H<sup>+</sup>-ATPase activity and promoted the cell growth of silks. **【Conclusion】** Taken together, this research reported a positive role of *ZmDT9* in maintaining synchronous development of male and female inflorescences, and provided a promising target for genetic improvement for maize drought resistance at flowering stage.

**Key words:** QTL mapping; ASI; *ZmDT9*



## **Cloning and functional analysis of maize transcription factor *ZmARR2* gene**

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**Abstract:** *ZmARR2*, a key response regulator in the cytokinin two-component system, plays an important role in plant growth and development and response to various abiotic stress. A good example is B-type response regulators (Type-B ARR), which is beneficial to improving the stress resistance in Arabidopsis. However, the exact mechanism of *ZmARR2* in maize is still little unknown, which may limit the process of breeding high resistance maize varieties in maize. Therefore, to understand the functional characterization of *ZmARR2* in maize, a combination of bioinformatics analysis and molecular biology techniques was employed to conduct preliminary investigations of *ZmARR2* in maize. Phylogenetic analysis and sequence alignment showed that *ZmARR2* had the highest similarity with rice *OsORR22*, and was closely related to rice, but far related to Arabidopsis. It was localized to the nucleus, and expressed in a variety of tissues with the highest expression level was discovered in the bracts at flowering stage. Meanwhile, transactivation analysis illustrated its transcriptional activation activity and provided insights into the location of its activation domain. The main-root in overexpressing of *ZmARR2* was significantly shorter than that of wild type, and its promoter region contained ABER or DRE core elements. Also, real-time fluorescence quantitative PCR results showed that *ZmARR2* was down-regulated in roots treated by both ABA and PEG. All these results laid a foundation for further analysis of *ZmARR2* function and its regulatory network.

**Key words:** *ZmARR2*; expression pattern analysis; phylogenetic analysis; abiotic stress

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## ***Abstract 154***

### **Genome assembly of a high-oil maize inbred line and genetic dissection of kernel oil content**

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**Abstract:** High-oil maize results from a long-term artificial selection. Maize oil is high in energy and levels of polyunsaturated fatty acids, which makes high-oil maize a popular resource for food, feed, and bioenergy. Understanding the genetic basis of oil biosynthesis and accumulation in maize kernels lays a solid foundation for improving the oil quantity and quality in maize kernels. In this study, we generated a genome of a high-oil inbred line, BY815, with the estimated genome size of 2,221 Mb. More than 95.86% of the assembled genome were anchored onto 125 contigs in ten pseudochromosomes. The high BUSCO (98.6%) and long terminal repeat (LTR) assembly index (37.46), as well as great contig N50 (118.11 Mb) indicated that we had assembled a high-quality high-oil maize genome. In addition, we identified 38,984 high-confidence protein-coding gene models in BY815 on the basis of the transcriptome data from 32 tissues across the whole developing stages. We further compared BY815 genome with two genomes of regular maize inbred lines, and identified 1,490,335 and 1,328,886 presence-absence variants (PAVs), 244 and 236 inversion, 42,229 and 35,890 translocation for B73 and MO17, respectively. Subsequently, we will construct a pan-genome using BY815 and all public maize genomes, mine the SVs in a maize association panel consisting 456 inbred lines, perform the associations of all identified SVs with oil content and compositions, and final elucidate the molecular mechanism of some important SVs regulating oil synthesis and accumulation.

**Key words:** genome assembly; high-oil maize; structure variants; genetic dissection

## Exploring the genetic basis and function of leaf chromatin accessibility in maize

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**Abstract: 【Objective】** The non-coding regions are important components of genome and play critical roles in gene expression regulation. The ATAC-seq technique makes it viable to obtain chromatin accessibility regions on the genome and promotes the understanding in the regulatory function of non-coding regions. **【Method】** In this study, we performed a genome-wide association analysis using ATAC-seq and other omics data of 215 maize inbred lines. **【Result】** We observed extensive variation in open chromatin regions (OCRs) by identifying 82,174 OCRs across population in which over 6,000 OCRs showed variation greater than 4-fold. Genome-wide association analysis with 7,443,172 SNPs reveals that 18,428 OCRs have genetic variants with an average heritability of 0.55. And 2,463 (44%) of the genetic variants located within OCRs were predicted to disrupt TF binding sites (motif), indicating that chromatin accessibility may be associated with TF motif disruption. Using association analysis and co-localization analysis of OCRs and genes, 1,990 OCR-gene regulatory pairs, with 78.91% of OCRs regulating one gene expression were identified and 46.8% of which (933) were located more than 40kb apart, indicating that many long-range regulatory elements may exist in the maize genome. OCRs were significantly enriched in QTLs for agronomic traits including flowering and yield, as well as metabolic traits such as amino acids, sugars, and fatty acids. Through joint analysis with other omics data, we identified two OCRs which regulate the expression of *fad7* (fatty acid desaturase), ultimately affecting the content of C18:3 (linolenic acid). **【Conclusion】** These results not only provide deep insights into the genetic basis and regulation mechanism of leaf chromatin accessibility in maize, but also enrich our understanding of non-coding regions regulating complex traits in maize.

**Key words:** ATAC-seq; Chromatin accessibility; Genome-wide association analysis; Transcription factor; Complex traits

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## 第六届全国玉米生物学学术研讨会通讯录

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24	省部共建作物逆境适应与改良国家重点实验室
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## 「企业简介」

北大荒垦丰种业股份有限公司是一家集研发、生产、加工、销售、服务和进出口业务于一体，具有完整产业链、多作物经营的现代化大型国有控股种业公司。注册资本4.732亿元。是中国种子行业首批AAA级信用企业、农业农村部首批“育繁推一体化”企业，国家级高新技术企业，国家农业产业化重点龙头企业、黑龙江省农业产业化重点龙头企业，农业农村部重点实验室、国际种子检验协会（ISTA）会员、海关AEO高级认证企业，2021年被授予“十年AAA信用企业”称号。2022年以105.65亿元的品牌价值成功入选世界品牌实验室《中国500最具价值品牌》。在农业农村部评选的69家“国家农作物种业阵型企业”中，垦丰种业同时承担水稻强优势和玉米、大豆补短板三大作物任务。

垦丰种业研发投入强度始终保持在年总营收的5%左右，已建成分子育种七大平台：高通量种子切片分选平台、高通量DNA提取平台、高通量SNP基因型鉴定平台、高通量SSR基因型鉴定平台、高通量基因芯片平台、高通量二代测序平台、高通量作物表型鉴定平台，达到国际先进水平，为作物种质资源鉴定、新品种选育提供理论与技术支撑。

垦丰种业以成为国内领先、世界一流的大型种业集团为目标，始终践行于“科技与服务，创造美好生活”的企业使命，通过提供优良品种及种植解决方案，为农民创造价值，改善农村环境，推动现代农业发展！



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## 科学研究试剂

### 核酸提取纯化



- 组织保存
- RNA提取、基因组DNA提取
- DNA产物纯化与凝胶回收
- 质粒提取、外泌体提取、磁珠法提取
- 自动化核酸提取仪

### PCR系列



- 高保真PCR、普通PCR
- 高产量、快速、长片段、热启动PCR
- 直接扩增、等温扩增、多重扩增

### 克隆载体构建



- 快速克隆
- 拓扑克隆
- 快速定点突变
- 传统TA克隆
- 化学感受态细胞

### 逆转录系列



- 通用型逆转录酶/试剂盒
- RT-qPCR专用预混液
- 一步法RT-PCR专用预混液
- miRNA逆转录专用试剂
- 5' RACE & 3' RACE扩增

### qPCR系列



- 染料法荧光定量专用预混液
- 探针法荧光定量专用预混液
- 一步法RT-qPCR预混液
- miRNA定量专用预试剂

### 细胞系列



- 细胞转染
- 细胞凋亡检测
- 细胞增殖检测
- 双荧光素酶报告基因检测
- 支原体检测与清除

### 蛋白系列

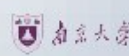


- Western Blot
- Bradford 蛋白定量
- BCA蛋白定量
- 蛋白胶

### 高通量试剂



- DNA建库、RNA建库
- 单细胞系列
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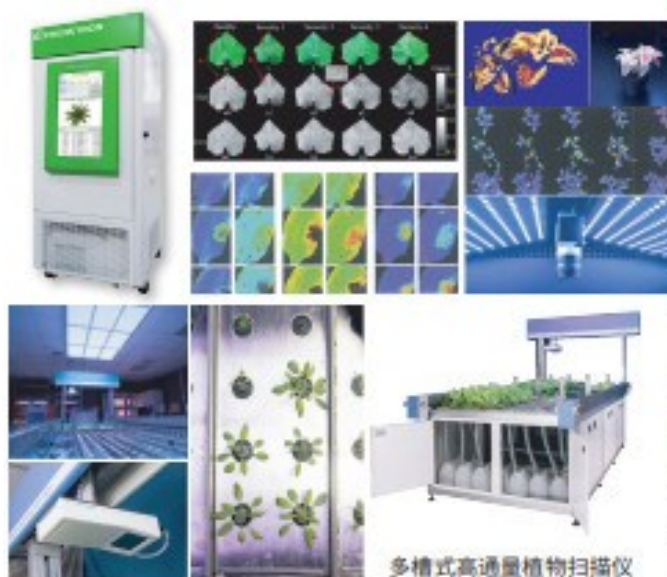


植物生长箱系列



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Phenotron系列



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赛默飞旗下Invitrogen及Applied Biosystems品牌自成立以来，致力于生物学各个分支学科的深入研究和创新，尤其在分子生物学领域，推出了多种创新型研究工具。从样本制备到核酸检测，全流程覆盖，加速您的科学研究。

### 样品制备

- 土壤DNA提取
- PureLink快速核酸纯化试剂盒
- MagMAX磁珠法核酸纯化试剂盒
- Kingfisher核酸纯化系统
- DNeasy磁珠法
- RNA提取试剂产品

### 反转录

- Superscript III反转录酶
- PrimeOUT逆转录RNA酶抑制剂

### PCR

- Applied Biosystems PCR仪
- Supermix II 高保真DNA聚合酶
- Platinum II Taq聚合酶/PCR反应混合物
- Platinum 高保真PCR反应混合物
- Applied Biosystems PCR耗材
- Invitrogen引物合成

### 高通量测序

- Illumina Power Prep 高通量测序系统
- Illumina 高通量测序试剂
- Illumina 高通量测序耗材
- Invitrogen DNA文库
- Illumina DNA文库

### 分子克隆

- TOPO克隆试剂盒
- Invitrogen感受态细胞
- Gateway克隆系统
- Gibson Assembly
- HifiX克隆试剂盒

### 基因芯片

- 多基因、多芯片、高通量检测
- 基因分型、12K-24K SNP位点
- 高通量检测、高通量检测系统

### 二代测序

- 多基因、多芯片、高通量检测
- 高通量检测、高通量检测系统
- 高通量检测、高通量检测系统

### 一代测序

- 多基因、多芯片、高通量检测
- 高通量检测、高通量检测系统
- 高通量检测、高通量检测系统

### 定量PCR

- 多基因、多芯片、高通量检测
- 高通量检测、高通量检测系统
- 高通量检测、高通量检测系统

### 数字PCR

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农业液相芯片开创者

博瑞迪是一家专注于动植物分子检测 and 育种相关技术开发与应用的创新型企  
业，致力于为我国种业发展提供高通量、低成本的精准基因型分析和分子检测技术，  
推动我国动植物育种技术从传统向分子跨越发展。

700,000+  
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500+  
育种单位

300+  
液相芯片

100+  
物种



作物



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## 软件系统

智能化信息管理系统MIS、生产分析平台MBAP、博瑞云大数据育种平台等，满足百万级样本数据分析大数据应用，实现从样本采集到报告的全程信息化管理。

## iMBP 智慧育种工厂

采用分布式流水线设计，覆盖核酸提取、建库、测序等全套分子育种检测流程，可实现7×24h无人值守的不同制作业，单日通量高达6000份，单样本检测通量超百万级。平台核心技术均为自主研发。



## 液相芯片

博瑞迪拥有液相芯片产品300余款，覆盖100多个物种，累计检测样本超70万份，具有高通量、强通用性、位点配置灵活、高敏感性、高特异性等优势。

## 试剂耗材

自主研发试剂涵盖核酸提取、文库构建、杂交捕获、定量化检测分子检测全流程，配套国产化标准耗材可适配于自动化设备。

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## 公司简介 About Us

三泰生物（集团）成立于2014年，总部位于上海张江高科技园区，生产部坐落于上海北大门的江苏省南通市北高新产业园区。公司总面积超过20000m<sup>2</sup>，实验室面积超过5000m<sup>2</sup>，工厂15000m<sup>2</sup>，直接控股子公司5家。

三泰生物致力于植物学、农学、医学、动物、食品及环境、微生物代谢物检测以及植物遗传转化技术的开发，帮助生命科学领域的科研人员跨越分析检测的技术障碍，获得可靠的实验数据。公司目前配备有淀粉/多糖解析平台、精准靶向检测平台、代谢组学平台、高通量测序平台、蛋白质组学平台和植物遗传转化平台，已为近千家高校、科研院所及企业提供了可靠的实验数据，累计参与文章近两千篇，累计影响因子已经破万。

公司秉承“专业、专注、专心”的理念，坚持着“不让技术成为研究和研发的障碍”的信念，努力成为广大科研从业者最专业的技术合作伙伴。

### 打造科研一体化服务平台 淀粉性质解析+转录组+蛋白组+代谢组+精准靶向



淀粉性质全指标测定

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代谢物精准靶向测定

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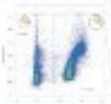
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## 实验室级花粉活力分析仪 **Ampha Z40**

采用微流控阻抗式细胞技术 (IFC) 的花粉活力分析仪,能够在微流体精确参考条件下,实现流动态花粉细胞的高通量、连续无损阻抗检测,获得细胞活性、数量、浓度、大小等统计结果。具有非标记、多参数、低污染和检测速度快等显著优势,可大大减少时间消耗并降低成本。



### 特点

- 通用性** 适用于所有植物花粉,已测试250+种植物花粉
- 高效性** 标准化测试方法 2 min 内提供准确的花粉信息
- 灵活性** 根据研究主题匹配检测协议,适用于多个花粉研究和常规检测的环节

### 科学研究

- 品种筛选** 多品种对比  
环境响应:气候变化  
优化授粉
- 小孢子培养** 鉴定小孢子发育时期  
监测小孢子活性  
估算小孢子数量  
产胚率可预测  
优化培养体系
- 产量预测** 花粉活力与结实率的关系  
标准化授粉

### 新品繁育

- 亲本选择  
遗传性分析  
雄性不育系育种  
筛选雄性不育系  
检测F<sub>1</sub>代育性恢复  
GW育种  
小孢子培养

### 生产研究

- 花粉质量管控  
花期生长 花粉采集  
花粉播种 花粉处理  
花粉两水合  
优化授粉  
非生物因素响应  
气候变化

### 种子生产

- 花粉质量检测  
优化授粉  
花粉活力动态变化  
花粉活力时效  
授粉窗口期 最佳授粉期  
花粉量动态变化  
确保授粉质量 提高种植比例

## 面对未来农业的植物表型平台设计、咨询和解决方案



实验室内对幼苗、愈伤组织等的  
自动化高通量表型测量

实验室高通量植物表型平台  
**HyperAlpert**



温室、气候室内从幼苗到成熟植株的  
高通量表型测量

温室型高通量植物表型平台  
**PhenoAlpert HT**



野外田间、大型温室内  
对植株群体表型测量

田间高通量植物表型平台  
**PhenoWatch**



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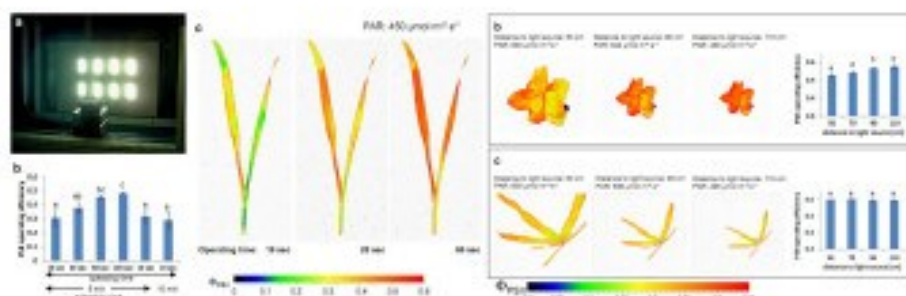
## 易科泰光谱成像技术及其应用

易科泰生态技术公司与 PSI (叶绿素荧光成像及植物表型分析技术)、Specim (高光谱成像技术)、SSI 植物/土壤呼吸测量技术) 等国际先进仪器技术公司合作, 致力于“生态-农业-健康”科研与应用技术解决方案, 特别是光谱成像技术及其应用、光合-呼吸-碳通量测量监测、植物种质资源与表型分析技术:

- 高光谱成像技术
- 叶绿素荧光成像技术
- 多光谱荧光成像技术
- 高光谱荧光成像技术
- 植物表型分析
- 种质资源鉴定
- 植物光生物学研究
- 遗传育种与生态栽培研究



自左至右依次为: FluorCam 叶绿素荧光成像系统、PhenoTron-HSI 多功能高光谱成像系统、PhenoTron-PTS 植物表型分析平台、PhenoTron 复式作物种质资源表型分析平台 (智能 LED 光源与表型成像分析)



FluorCam 叶绿素荧光成像系统: 德国莱布尼茨植物遗传与栽培作物研究所 IPK 用于玉米、烟草等光合能力评估与育种 (Tschiersch, 2017)





## 公司简介 COMPANY PROFILE

武汉艾迪晶生物科技有限公司（艾迪晶生物）成立于2019年4月，位于武汉市东湖新技术开发区光谷生物城武汉生物技术研究院，占地面积1600平方米。

艾迪晶生物汇聚了一批由顶尖的功能基因组学和分子生物学领域的院士专家顾问、教授、博士、硕士组成的人才团队，依托高通量的分子设计平台及植物遗传转化体系，致力于基因编辑技术的开发与应用，为功能基因挖掘和研究提供“一站式”的技术服务。

我们秉承“探索自然，服务人类”的宗旨，不断研发和创新，以促进现代基因工程的科学研究与应用，实现生物研究和生物产业的协同发展。

## 分子生物学平台



## 遗传转化平台

## 育种技术服务平台

我司依托自有分子生物学平台、遗传转化平台和育种基地可实现水稻、玉米和大豆在保持原品种优良特性的基础上，对特定性状进行改良。对已选到突变结果和表型的材料，通过PCR鉴定，突变材料不含外源载体元件，即启动子、抗性基因、Cas9基因。



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基因组测序服务

助力客户发表

**Nature**

**THP9 enhances seed protein content and nitrogen-use efficiency in maize**

[Yongcai Huang](#), [Haihai Wang](#), [Yidong Zhu](#), [Xing Huang](#), [Shuai Li](#), [Xingguo Wu](#), [Yao Zhao](#), [Zhigui Bao](#), [Li Qin](#),  
[Yanaka Jin](#), [Yahui Cui](#), [Guanglin Ma](#), [Qiao Xiao](#), [Qiang Wang](#), [Jianhua Wang](#), [Yuxiang Yang](#), [Hongshu Liu](#)



## 公司简介 Company profile

武汉迈维特生物科技有限公司 (简称迈维代谢), 总部坐落于武汉国家生物产业基地—光谷生物城生物创新园, 另设上海/嘉善华东研发中心、长沙诊断产品研发与生产中心。

近年来, 公司相关技术成果曾多次在 Cell, Nature Genetics, Nature Communications, PNAS, National Science Review, Circulation Research 等国际学术期刊发表。

 20000平 自有实验室面积	 ISO9001 通过认证	 300+ 实验研发团队	 50+ 公司拥有设备数	 450+ CT+BI+IT 团队	 60% 研发技术人员占比
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## 公司书籍



## 公司参与高水平学术成果

Zhu et al, Cell, 2016, 175:269	Gong et al, Nat Commun, 2020, 11:2629	Fang et al, Mol Plant, 2016, 9:136	Wu et al, Plant Biotechnol J, 2020
Zhang et al, Cell, 2020, 183:1	Wang et al, Nat Commun, 2020, 12:5949	Deng et al, Mol Plant, 2020, 8:111	Ning et al, Nat Genet, 2023, 55:348
Chen et al, Nat Genet, 2014, 46:124	Zhan et al, Nat Plant, 2020, 6:5447	Chen et al, Mol Plant, 2021, 6:1769	Chen et al, PLANT PHYSIOL, 2023, 184:251
Chang et al, Nat Genet, 2015, 47:1838	Gong et al, PNAS, 2023, 120:26320	Fang et al, Mol Plant, 2023	Shu et al, New Phytol, 2023
Peng et al, Nat Commun, 2017, 8:1375	Li et al, Mol Plant, 2020, 13:1209	Huan et al, Mol Plant, 2021	Wang et al, PLANT PHYSIOL, 2022, 184:664
Wu et al, Nat Commun, 2014, 5:3638	Zeng et al, Nat Plant, 2020, 13:112	Zhang et al, Plant Biotechnol J, 2020	
Chen et al, Nat Commun, 2016, 7:12277	Wang et al, Mol Plant, 2020, 12:899	Ping et al, Plant Biotechnol J, 2020	

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# 铁岭旭日农业技术开发有限公司

铁岭旭日农业技术开发有限公司(铁岭东升)成立于2011年。是一家以实现作物育种高效化、精准化、信息化、机械化为目标,集农作物分子检测、单倍体生产、遗传转化、育种软件开发销售及育种机械研发生产销售等全方位为一体的科技型育种服务公司。

## 分子检测服务

具体开展业务为定向捕获测序(SNP)、品种真实性检测(SSR)、转基因检测(荧光PCR)、转基因转化体真实性检测、少量位点SNP的KASP功能位点检测等。

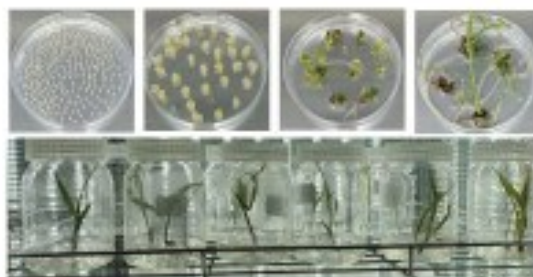


## 遗传转化服务

目前实验室已建立完整的遗传转化体系,转化体年转化量500+。

转化周期为6~8个月。

全年开展转化业务,可转化国内多数骨干自交系。



## 单倍体生产服务

我公司为全国最大的单倍体生产服务商,采用组培方式进行加倍处理,获取DH纯系周期为6~8个月,并可全年开展业务。



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全基因组重测序是对已知基因组序列的物种进行基因组测序,并在此基础上对个体或群体进行差异性分析。

助力快速发文

至今完成10万+的动植物重测序样本，合作提名发表文章100余篇，累计影响因子1,000+

- 文章题目: Whole-genome resequencing of 445 *Lactuca* accessions reveals the domestication history of cultivated lettuce
- 期刊名称: *Nature Genetics*
- 研究单位: 深圳华大生命科学研究院, 荷兰瓦赫宁根大学等
- 研究结果: 依托自主测序平台(DNBSEQ)揭示栽培生菜起源于野苣荬菜(*L. scariola*)及栽培芜菁。仅展示部分结果



郵箱: info@cnmcc.cn



## 公司简介

北京博美兴奥科技有限公司位于北京市昌平区中科院谷园，公司自成立以来一直致力于玉米等作物的遗传转化服务，始终坚持以“助力作物研发，推动中国农业发展”为己任。多年来已服务于中国农业大学、中国农业科学院、中国科学院、浙江大学、山东大学、上海植物生理研究所、河南大学、华中农业大学、北京师范大学等多家高校和研究所，得到了业内老师的高度评价和赞扬。

## 载体构建



Gateway



maize 干扰



定点突变



编辑率可达 80%



## 技术优势

成熟稳定的玉米遗传转化体系。采用农杆菌介导法，抗性筛选基因可选用草铵膦或者草甘膦，自交率可达到 80%，全年可提供 B104 和 B73 为受体的遗传转化服务，提供≥10 份独立的阳性转化事件，可提供加代繁殖服务。

## 服务流程和实验周期



地址：北京市昌平区中科院谷园 网址：www.bmsai.com 电话：185-1312-8781/156-0079-1929

## ACKNOWLEDGEMENTS



