

作物基因组育种

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作物生产是关系到国计民生及粮食安全的关键, 因此培育符合现代农业需求的品种成为当前农业发展的重点方向。传统育种无法对各种有利性状进行广泛筛选, 导致当前育种工作出现瓶颈。近些年, 随着作物分子遗传研究取得迅猛发展, 大量重要农艺性状的调控机制已被解析。这些理论研究成果使广泛开展作物分子鉴定辅助选择育种成为可能。本报告将以水稻这一重要农作物为基础, 结合玉米及小麦的发展前景概述关键分子遗传研究进展和最新的基因组操作技术在作物育种实践中的应用潜力, 分析当前育种的问题及不足, 提出未来基因组育种的分析方法及有效策略。

1 基因组育种理念及发展现状

1.1 自然变异位点挖掘及其育种应用

农作物在自然生长过程中会积累大量的自然变异, 这些变异使植株获得了各种各样的性状, 其中对人类有利的性状经过人工筛选逐渐固定下来, 广泛分布于世界各地的品种中, 而育种的本质就是获得并组合这些有利位点, 经过筛选得到综合性状优良的新品种。然而传统的育种策略主要依赖于育种家的经验观察, 具有主观性强、性状评估效率低等缺点。随着分子生物学的发展, 分子标记鉴定技术逐渐成熟, 可在早期对作物品种系进行筛选, 从而提高育种效率。分子鉴定的前提是获得基因组中调控各种农艺性状的自然变异位点信息, 也称为数量性状调控位点(quantitative trait locus, QTL)。

随着各种作物基因组测序工作的完成, QTL定位乃至克隆逐渐变得可行。其中水稻基因组最先完成, 具有详细的基因组注释数据及分子多态性信息, 2000年水稻中首次成功克隆了抽穗期QTL *Hd1* (Yano等2000), 开启了水稻QTL研究的先河, 随后不断有重要性状的QTL得到克隆, 其中至少有64个重要位点的分子机制得到解析(表1)。该领域的早期研究突破主要由日本科学家完成, 随着中

国对植物领域科研经费投入的增加, 2005年开始, 中国科学家开始崭露头角。近5年, 中国科学家克隆了水稻中多个重要农艺性状位点, 其中包括抗热位点(Li等2015)和抗冷位点(Ma等2015)的首次克隆, 氮肥利用效率位点的挖掘(Hu等2015a; Sun等2014), 杂交制种不育及广亲和性基因的克隆及分子机制解析(Ding等2012; Luo等2013a; Yang等2012; Zhou等2012, 2014), 这些成果具有重大的理论和应用价值, 表明在水稻分子遗传研究领域, 中国已经逐步超越其他国家, 引领国际前沿。而玉米和小麦中克隆的QTL相对较少, 但随着玉米基因组及部分麦类基因组信息的释放及比较基因组学的发展, 这一进程将会加快, 目前, 玉米中的*ZmCCT* (Yang等2013)、*ZmWAK* (Zuo等2015)、*ZmNAC111* (Mao等2015)和*ZmVPP1* (Wang等2016b), 小麦中的*TaGW2* (Su等2011)和*TaGS5* (Wang等2015a)等多个重要QTL都得到克隆和验证。重要农艺性状QTL的克隆为分子标记辅助选择育种提供了筛选靶标, 利用这些位点的序列多态性信息, 育种家可以有效鉴定现有品种的基因信息, 从而根据品种需求进行分子设计改良, 达到精细育种的目的。

1.2 高通量技术在性状及品种筛选中的应用

分子标记是品种鉴定及筛选的主要工具, 用于区分不同的自然变异位点。常规的分子标记技术主要是PCR扩增后直接或酶切后电泳分离, 将序列差异位点转化为可区分的基因型, 包括简单序列重复标记(simple sequence repeat, SSR)、插入/缺失标记(insertion-deletion, InDel)、酶切扩增多态性序列(cleaved amplified polymorphic sequences, CAPS)及衍生酶切扩增多态性序列(derived cleaved amplified polymorphic sequences, dCAPS)等。PCR标记具有准确度高的优点, 但费时费力, 只能对有

收稿 2017-04-20 修定 2017-05-15
资助 国家自然科学基金(31600990)和中国博士后科学基金(2015M581668)。

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表1 2000~2017年间水稻中已克隆的QTL列表

Table 1 Summary of the rice QTLs cloned during 2000–2017

年份	水稻中已克隆的QTL
2000	<i>Hd1</i> (Yano等2000)
2001	<i>Hd6</i> (Takahashi等2001)
2002	<i>Hd3a</i> (Kojima等2002)
2004	<i>Ehd1</i> (Doi等2004)
2005	<i>NiR</i> (Nishimura等2005)、 <i>SKC1</i> (Ren等2005)
2006	<i>Gn1a</i> (Ashikari等2005)、 <i>qSH1</i> (Konishi等2006)、 <i>SH4</i> (Li等2006)、 <i>GS3</i> (Fan等2006)、 <i>Sub1A</i> (Xu等2006)
2007	<i>GW2</i> (Song等2007)、 <i>TAC1</i> (Yu等2007)
2008	<i>qSW5</i> (Shomura等2008; Weng等2008)、 <i>Ghd7</i> (Xue等2008)、 <i>gfl1</i> (Wang等2008)、 <i>PROG1</i> (Jin等2008; Tan等2008)、 <i>qLTG3-1</i> (Fujino等2008)
2009	<i>Dep1</i> (Huang等2009b)、 <i>Bph14</i> (Du等2009)、 <i>SNORKEL1</i> 和 <i>SNORKEL2</i> (Hattori等2009)、 <i>Pi21</i> (Fukuoka等2009)
2010	<i>IPAI</i> (Jiao等2010; Miura等2010)、 <i>DTH8</i> (Wei等2010; Yan等2011)、 <i>SCM2</i> (Ookawa等2010)、 <i>Sdr4</i> (Sugimoto等2010)、 <i>Pb1</i> (Hayashi等2010)
2011	<i>Hd17</i> (Matsubara等2012)、 <i>GS5</i> (Li等2011b)、 <i>pms3</i> (Ding等2012; Zhou等2012)
2012	<i>GW8</i> (Wang等2012)、 <i>GL3.1</i> (Qi等2012; Zhang等2012)、 <i>W4352</i> (Luo等2013a)、 <i>S5</i> (Yang等2012)
2013	<i>DTH2</i> (Wu等2013)、 <i>SPIKE</i> (Fujita等2013)、 <i>Ghd7.1</i> (Gao等2014; Yan等2013)、 <i>spr3</i> (Ishii等2013; Zhu等2013)、 <i>DRO1</i> (Uga等2013)、 <i>PTBI</i> (Li等2013)、 <i>Bph3</i> (Liu等2015)、 <i>An-1</i> (Luo等2013b)
2014	<i>chalk 5</i> (Li等2014)、 <i>tms5</i> (Zhou等2014)、 <i>STV11</i> (Wang等2014a)
2015	<i>GL7</i> (Wang等2015b; Zhou等2015)、 <i>GW6a</i> (Song等2015)、 <i>NRT1.1B</i> (Hu等2015a)、 <i>TT1</i> (Li等2015)、 <i>An-2</i> (Gu等2015)、 <i>LABA1</i> (Hua等2015)、 <i>Cold1</i> (Ma等2015)
2016	<i>GLW7</i> (Si等2016)、 <i>GNPI</i> (Wu等2016)、 <i>TAC3</i> (Dong等2016)、 <i>GS2</i> (Che等2015; Duan等2015; Hu等2015b)、 <i>Hd18</i> (Shibaya等2016)、 <i>GAD1</i> (Jin等2016)、 <i>Bph9</i> (Zhao等2016)、 <i>Bph32</i> (Ren等2016)
2017	<i>Pigm</i> (Deng等2017)、 <i>ipa1-2D</i> (Zhang等2017)、 <i>CTB4a</i> (Zhang等2017)

限的遗传位点进行分析。随着二代测序技术的发展,基因组重测序成本已经降到极低水平,利用相关技术对品种进行基因型鉴定已经成为当前育种的一个潮流。2009年, Huang等(2009a)在水稻中首次建立了低覆盖度重测序检测基因型的技术体系,大大降低了鉴定成本。随后他们利用该技术对世界各地品种进行基因型鉴定,并进行表型关联分析,最终找到一系列性状关联变异位点(Huang等2010, 2011)。通过对野生稻的测序鉴定,他们还首次解析了不同水稻亚种的起源问题(Huang等2012)。随后该团队成功解析了中国一系列杂交水稻品种的遗传构成,结合大量F₂群体分析,最终找到了决定杂种优势的关键遗传位点(Huang等2015, 2016b)。

除了重测序技术,基因芯片技术也逐渐开始应用。2014年,张启发团队成功设计出一个全基因组芯片(RICE6K)用于水稻基因组育种,该芯片还同时覆盖28个重要性状位点的功能型变异位点(Yu等2014)。利用该芯片,该团队在2015年和2016年分别培育出了抗白叶枯病的雄性不育系(Jiang等

2015)及抗飞虱的恢复系(Wang等2016a),并用于杂交稻制种,证明芯片技术利用在基因组育种中的可行性。除了RICE6K,他们同时发展了一套覆盖度更高的芯片(RiceSNP50),可用于品种分类、鉴定及性状关联分析,具备更好的分辨精度(Chen等2014)。

1.3 诱变及基因编辑技术快速改良品种

在品种筛选过程中,不仅可利用已有的自然变异位点进行性状改良,还可人为诱导突变产生有利性状。近年来在水稻中发现了多个通过诱变方法产生的有利位点,其中包括调控粒形的*GS6* (化学诱变)(Sun等2013),调控穗型的*LGI* (物理诱变)(Li等2011a)以及调控株型的*PAY1* (化学诱变)(Zhao等2015)。这些突变位点可直接用于品种培育,从而加速了育种进程,且可发现自然群体中不存在的新突变,然而上述诱变方法只能随机对基因组进行改造,需要进行大量筛选才能获得符合预期的突变类型。

2012年,杨兵课题组利用转录激活因子样效应子(transcription activator-like effector, TALE)成

功实现了对水稻白叶枯敏感基因*OsHIN3*的定点突变,快速获得了水稻抗性植株(Li等2012)。这种对特定基因定点改造的技术通称为基因编辑技术。高彩霞研究组利用该方法改造了水稻中的香米基因(Shan等2015),并首次实现同时对小麦中3个同源感病基因的敲除,获得了符合生产需求的作物品系(Wang等2014b)。随后出现了新一代CRISPR/Cas9突变技术,该方法借助RNA指导靶向序列,设计简单且效率高,在多个物种中都可实现有效敲除,成为当前基因编辑领域广泛使用的技术。目前,水稻、玉米及小麦等作物都成功实现了对不同基因位点的编辑,且多个研究组开始尝试利用基因编辑技术改造重要性状QTL位点。水稻中已完成了对*Gn1a*、*DEP1*、*GS3*、*IPA1*和*TMS5*等位点的敲除(Li等2016b; Shen等2016; Zhou等2016),其中*TMS5*是中国杂交水稻制种广泛应用的温敏雄性不育基因,利用基因编辑技术,研究人员快速获得了11个籼稻或粳稻背景的不育系,加速了育种进程。

人们通过回交方法可以将用于基因编辑的转基因片段分离,同时保留突变位点,被认为可有效避免转基因食品安全问题,但回交分离依然是一个相对耗时的过程。最近,高彩霞课题组尝试利用CRISPR/Cas9 RNA转化植株,可直接获得不含转基因片段的小麦敲除品系(Zhang等2016),而美国先锋种业则利用基因枪技术将CRISPR/Cas9 RNA-蛋白复合体导入玉米中,也获得了不含转基因片段的基因编辑植株,从而规避了转基因问题(Svitashev等2016)。由于自然变异还存在功能获得型变异,基因敲除无法获得目标性状,2016年,高彩霞课题组利用CRISPR/Cas9介导的同源修复原理成功解决了这一难题,他们将功能片段置换到除草剂敏感基因区段,获得了抗除草剂水稻植株(Li等2016a),该技术被称为knock-in,大大拓展了可用于基因编辑改造的性状范围。因此,基因编辑技术将成为未来育种领域的重要手段,而“基因组编辑作物”管理框架目前也逐渐提上日程(Huang等2016a)。

2 基因组育种探索的若干问题

2.1 有效组装不同位点获得有利性状

尽管当前许多自然变异位点的分子机制已经得到解析,然而如何有效利用这些位点是今后探索的重点。不同品种涉及到复杂的遗传构成及种

植环境,分子理论研究往往只对一个位点进行性状评估,不能真实反映相关位点的育种价值。水稻*depl1*位点被认为是一个增产位点,可通过增加穗粒数提高单株产量;不同研究则发现该位点在某些遗传背景下具有减产效应,此时等位的*Dep1*能更有效地增加产量(Yi等2011; Zhou等2009)。类似地,后续研究也发现*SPIKE*对产量的效应存在环境依赖性,大田种植条件下该位点并不增产(Adriani等2016)。另外,有些基因发挥作用存在上位性互作,形成调控网络才能产生目标性状,仅对一个位点进行评估往往无法反映育种组合中的实际效应。

因此,对已有位点进行全方面的遗传评估是保证基因组育种有效进行的前提,具体可从以下几点进行:(1)获得重要性状位点在不同品种背景下的近等基因系或替换系材料,评估遗传背景对性状的影响;(2)将不同株系材料种植于不同栽培环境及地区,评估不同环境下性状是否稳定;(3)组合不同有利位点及其等位,探索位点间的竞争性及互补性(如源-库-流平衡)。目前,中国科学院先导项目已经着手对相关内容进行探索,期望该项目能够全面探索不同位点的组合效应,为今后基因组育种工作提供理论指导。另外,基于多位点组合互作原理,基因组选择(genomic selection)育种理论可为今后育种实践提供一种变换的方法(Goddard和Hayes 2009),直接筛选育种群体中的基因组片段组合。

2.2 不同资源与技术的合理组合

当前理论研究与技术突破已经使基因组育种进入一个快速发展的时代,我国现在也在积极促进这一进程,多家单位和企业进入这一领域,包括中国科学院(作物分子模块育种先导项目)、中国农科院、华中农业大学、中种集团等,这些研究机构代表了我国在基因组育种领域的前沿力量,使我国具备了与国外种业公司竞争的实力,一些基因编辑技术公司也在兴起,可为基因组育种提供各种基因编辑材料。尽管如此,中国作物育种目前仍主要采用传统方法,大部分育种家尚没有开展基因组育种的设备及技术支持,如何建立一个有效平台整合不同育种资源是今后基因组育种普遍开展的关键。或许我们可以借鉴美国植物育种家协会(National Association of Plant Breeders)的模式,将不同层面的育种资源和机构联系在一起,及

时了解育种领域的新问题及新需求, 并培养出符合基因组育种需求的新型人才。

3 基因组育种展望与对策

近些年, 随着中国植物分子领域的发展, 我们逐渐具备了全面开展基因组育种的理论基础和技术支撑, 在作物育种方面不断取得突破。袁隆平科研团队成功实现了华南双季稻亩产1 500 kg的壮举, 表明在水稻高产育种上我国已经达到世界领先水平。随着人们物质生活水平的提高及劳动力结构的变化, 今后的育种重点不应仅关注高产, 更应该关注多抗、优质以及优化农业资源结构, 如再生稻品种的培育和推广应用既可节省人力投入, 又能增加农民收入。华中农大张启发院士还提出了“资源节约、环境友好”的绿色超级稻理念及其育种策略(Zhang 2007), 为今后育种理论探索提供了新的方向。相信随着更多符合上述理念的QTL位点的挖掘及综合效应评估, 我们可以在植株生长早期对各种性状进行筛选, 实现绿色超级稻的分子精细设计育种。

2013年, 孟山都公司开发了种子芯片技术, 可将包含预期位点组合的种子直接筛选出来, 极大地提高了育种选择效率, 该方法值得我们借鉴学习。目前“种业自主创新”已经成为国家科技重大专项“十三五”发展规划的重要内容, “七大农作物育种”试点专项正在积极开展。可以预见, 我国将在基因组育种领域取得更多突破。

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Received 2017-04-20 Accepted 2017-05-15

This work was supported by the National Natural Science Foundation of China (Grant No. 31600990) and China Postdoctoral Science Foundation (Grant No. 2015M581668).

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