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植物生物技术研究进展

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摘要: 生物技术在植物科学领域和作物育种中的应用非常广泛, 能有效促进植物重要性状相关基因和染色体片段的挖掘及应用, 实现植物抗逆、抗病、产量、品质等性状的遗传改良。目前, 利用细胞工程、染色体工程、分子标记等生物技术已成功鉴定大量作物关键性状的候选基因和优异等位变异, 并结合基因工程、全基因组选择、基因编辑和合成生物学等技术实现了作物的性状改良和遗传育种。本文基于植物生物技术在作物遗传育种中的研究现状, 分析其在育种应用中的前景和待解决的问题, 以期为作物生物育种的产业化发展提供参考。

关键词: 生物技术; 作物育种; 基因编辑; 分子标记; 遗传转化

Advances and perspectives in plant biotechnology

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Abstract: Application of biotechnology in plant researches and crop breeding greatly facilitates mining and use of genes and chromosome fragments related to important traits of plants, and would be valuable to genetic improvement of stress and disease resistance, yield, quality, etc. Hitherto, large number of candi-

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date genes and elite allelic variations of valuable traits have been identified through biotechnologies (i.e. cell and chromosome engineering, molecular marker), which were used for crop improvement and genetic breeding via genome editing, genomic selection, and synthetic biology, etc. Here, we summarize the research progress of plant biotechnology in genetic breeding of crops, analyze the perspectives and chanlleging of applying biotechnology in breeding, and wish to promote the industrialization of crop bio-breeding.

Key words: biotechnology; crop breeding; genome editing; molecular marker; genetic transformation

1 植物细胞工程研究进展

植物细胞工程是在细胞水平上对植物进行遗传操作的现代生物技术。目前, 该技术的主要研究内容为通过小孢子培养、体细胞杂交、多倍体高效诱导染色体重组、单倍体育种、人工染色体和基因组编辑等, 突破种间生殖隔离, 实现外源种质优良基因或染色体区段的规模化转移, 克服作物驯化过程引起的驯化瓶颈、选择性清除和遗传背景狭窄等问题。该技术有助于导入野生种或近缘种的遗传物质, 增加染色体重组和基因组遗传变异, 在作物性状改良中具有重要的价值。

1.1 小孢子培养技术在作物育种中的应用现状

小孢子培养技术主要应用于双单倍体(DH)群体构建和作物育种(Dwivedi等2015; Zhang等2023), 近年也被成功用于建立小麦(*Triticum aestivum*)和大麦(*Hordeum vulgare*)的高效基因编辑技术体系(Bhowmik等2018; Han等2020)。如中国农业科学院建立了高效的小孢子培养体系, 缩短了大麦优异种质的纯合时间(Lu等2016; Xu等2021a); 利用甘蓝(*Brassica oleracea*)育性恢复结合小孢子培养技术获得含有*CRa*基因的抗根肿病甘蓝DH系(Zhu等2022)。

1.2 体细胞杂交在作物育种中的研究进展

在体细胞杂交育种方面, 山东大学植物细胞工程与种质创新教育部重点实验室通过体细胞不对称融合将长穗偃麦草(*Thinopyrum ponticum*)的染色体片段引入小麦栽培种, 选育出具高产、抗逆性状的‘山融3号’等新品种, 并结合QTL定位和候选基因*TaSRO1*等功能解析, 为小麦耐盐育种奠定了重要基础(Liu等2014; Wang等2022a)。该实验室曾通过小麦–长穗冰草(*Agropyron elongatum*)的体细胞杂交创建高分子量麦谷蛋白亚基变异的渐渗系(Gao等2010)。东北师范大学通过水稻–菰属(*Zizania*)的不对称体细胞杂交, 创制了可用于水稻新品种培育的材料(Chen等2006; Yang等2012)。杭州师范大学通过原生质体融合获得陆地棉(*Gossypium hirsutum*)和野生种克劳茨基棉(*G. klotzschianum*)的杂种, 并从六倍体融合后代筛选获得高光合效率的种质(Sun等2004; Yu等2012)。扬州大学通过体细胞融合获得甘蓝型油菜(*Brassica napus*)–白芥(*Sinapis alba*)属间杂种, 并从回交后代中选育抗菌核病、黄籽油菜新品系(Li等2012; Jiang等2013)。华中农业大学国家油菜工程技术中心通过油菜与菘蓝(*Isatis indigotica*)的远缘杂交, 创建了具抗炎功效的板蓝根油菜‘菘油1号’; 通过甘蓝型油菜与二倍体芜菁(*B. rapa* subsp. *rapifera*)、近缘种诸葛菜(*Orychophragmus violaceus*)等杂交, 从渐渗系中筛选获得抗根肿病、菌核病的油菜新种质(Shao等2022; Yang等2022a)。该中心和华中科技大学基于萝卜和甘蓝远缘杂交, 创建了抗根肿病和菌核病的异源四倍体萝卜甘蓝(Zhang等2021)。此外, 体细胞杂交可实现对作物细胞质基因组的操作。油菜的Ogura细胞质雄性不育系即源于油菜和萝卜(*Raphanus sativus*)体细胞杂交(Primard-Brisset等2005); 柑橘属(*Citrus*)不同种质的体细胞杂交也可实现优异性状的重组, 如抗黄龙病的胞质杂种(Dambier等2011; Dutt等2021)。

1.3 作物多倍化和驯化育种

在多倍体高效诱导染色体重组方面, 华中农业大学基于大量埃塞俄比亚芥(*B. carinata*)和白菜型油菜(*B. rapa*)品种的杂交, 创建了甘蓝型油菜A^rC^c基因资源库, 并选育出数百个不同谱系的新型甘蓝型油菜自交系和双单倍体, 具有丰富的基因组变异和较强的杂种优势(Zou等2019; Hu等2021)。德国波恩大学通过芸薹属种间杂交创建了六倍体种质

(AABBCC), 发现染色体重组事件会影响该种质的基因组稳定性(Quezada-Martinez等2022)。棉属(*Gossypium*)物种多倍化过程中也发生了大量基因组重组, 且通过不同倍性物种的杂交和多倍化有望实现棉花的抗病、耐旱等性状改良(Paterson等2012; Chen等2015; Yang等2019b)。中国科学院种子创新研究院/遗传与发育生物学研究所基于异源四倍体野生稻(*Oryza alta*)底盘种质资源, 建立野生稻快速从头驯化技术体系, 并通过基因组编辑成功创制了低落粒性、短芒、矮杆、粒长变长、茎秆变粗、抽穗时间短的异源四倍体野生稻(Yu等2021)。马铃薯(*Solanum tuberosum*)在驯化过程中也存在大量高度分化和驯化基因(如调控苦味和块茎形成的基因), 且野生马铃薯(*S. candolleanum*)中含有丰富的抗性基因(Li等2018a)。中国农业科学院深圳农业基因组研究所通过构建番茄(*S. lycopersicum*)、马铃薯泛基因组数据库, 分析了茄属物种进化过程中的基因组结构变异和驯化过程中丢失的遗传力, 为作物复杂性状的遗传机制解析和马铃薯育种改良提供了丰富的遗传变异信息(Hardigan等2017; Zhou等2022a; Tang等2022)。六倍体普通小麦的遗传多样性来源于野生小麦和远缘野草频繁的基因渗入, 且部分渗入片段在群体中的出现频率随驯化和改良而增加(Cheng等2019)。东北师范大学研究发现异源多倍体小麦的部分同源重组(HE)具有与经典同源重组(HR)相似的机制和基因组特征, 但HE多发生在基因编码区, 可促进异源多倍体中新基因/蛋白质的亚/新功能化。该现象在拟南芥(*Arabidopsis thaliana*)、芸薹属、水稻、香蕉(*Musa nana*)和花生(*Arachis hypogaea*)中也普遍存在(Zhang等2020)。Lavia等(2011)利用荧光原位杂交(FISH)技术证明野花生为同源多倍体, 且该属三倍体核型的出现可能是由于染色体的单向有性多倍化。异源四倍体花生栽培种的基因组组装和泛基因组数据也成功注释了与花生重要农艺性状相关的基因及其驯化过程(Bertioli等2019; Zhuang等2019)。利用细胞工程技术有望实现上述野生种质的外源染色体片段或优良基因的渗入, 诱导作物基因组产生丰富的遗传变异, 对作物关键性状的育种改良具有重要意义。但如何克服细胞工程育种中的杂交不亲和、杂种不育及后代分离等问题, 高效聚合野生优异种质资源, 仍是目前作物细胞工程育种关注的热点。此外, 利用人工合成染色体实现定向染色体工程育种(如定向操控着丝粒), 有望为作物精准合成育种提供助力(Birchler等2016; Zhou等2022b)。

1.4 作物单倍体育种的研究现状

单倍体-双单倍体技术可在固定的遗传背景下加速重组单倍型的纯合, 提高育种效率。传统的单倍体育种始于曼陀罗花药离体培养, 目前已成功应用于玉米(*Zea mays*)、小麦、大麦、黑麦草(*Lolium perenne*)、水稻、高粱(*Sorghum bicolor*)、油菜等物种(Jacquier等2020)。北京市农林科学院利用该技术培育出首个大面积推广的小麦花培新品种‘京花1号’ (胡道芬等1986)。现代单倍体诱导主要通过杂交诱导单性生殖、杂交后引发染色体清除等机制产生单倍体, 包括以诱导系为母本或父本分别诱导产生仅保留父本或母本一套染色体的单倍体。中国农业大学和吉林农业科学院利用该技术培育了玉米单倍体诱导系农大高诱1号和吉高诱系3号, 具有诱导频率高、结实性好等优点(李国良等2008; 才卓等2007)。利用组蛋白着丝粒*CENH3*基因构建的诱导系已成功用于拟南芥、大麦、小麦和玉米的单倍体诱导(Ravi和Chan 2010; Karimi-Ashtiyani等2015; Lv等2020; Wang等2021a)。继先正达公司、法国里昂大学发现花粉特异性磷脂酶基因*ZmMTL*、磷脂酶A基因*ZmNLD*突变分别是玉米Stock6、PK6诱导系可诱导单倍体的原因(Gilles等2017; Kelliher等2017; Liu等2017), 我国在单倍体诱导机制方面也取得了较好的研究进展。华中农业大学和中国农业大学通过玉米诱导系CAU5与常规材料Zheng58杂种胚和胚乳的单细胞测序, 发现精细胞染色体片段化可能是其具诱导功能的原因(Li等2017); 且*ZmPLA1*第四个外显子上4 bp的碱基插入是诱导系CAU5的形成原因(Liu等2017)。水稻和小麦中ZmMTL/ZmPLA1/ZmNLD同源蛋白的功能缺失也可诱发单倍体诱导系(Yao等2018; Liu等2020a, b), 但该蛋白在双子叶植物中不存在同源序列。中国农业大学基于高、低诱导系(CAU5和CAUHOI) F_3群体的图位克隆, 发现编码

DUF679结构域膜蛋白的*ZmDMP*, 并通过基因编辑等证明*ZmDMP*是单倍体诱导的关键基因(Zhong等2019)。*ZmDMP*和*AtLIG4*突变对提高玉米、拟南芥诱导系的诱导效率具有重要作用(Zhong等2019; Tan等2015)。中国农业大学研究发现拟南芥中类*ZmDMP*的*AtDMP8/9*功能缺失也可诱导产生母本单倍体, 为双子叶植物单倍体诱导系的研究奠定了基础(Zhong等2020)。该团队基于玉米诱导系CAU2和高油群体BHO的F_2/F_3代分子标记辅助选择, 筛选获得高油、高诱导效率的新诱导系CHOI4 (Liu等2021a)。Fu等(2018)以芸薹属异源八倍体为花粉供体, 通过杂交获得双单倍体诱导系, 利用其为转送介质可实现对油菜和甘蓝的基因编辑(Li等2021a)。Li等(2022a)利用基因编辑技术创建了甘蓝型油菜*BnaDMP*的纯合突变体, 该突变体的单倍体诱导效率达2.53%。目前单倍体诱导系已成功应用于玉米和番茄育种, 但尚未在其他作物育种中广泛应用(Jacquier等2020)。因此, 利用细胞工程技术和基因编辑技术构建丰富的单倍体诱导系, 对于作物细胞工程育种及提高育种效率尤为重要(Jacquier等2020)。

2 植物染色体工程研究进展

全球气候变化加剧、资源环境过度消耗、极端自然灾害频发以及无法预估的战争破坏, 严重威胁到全球粮食的安全生产(Bentley 2022)。20世纪80年代小麦遗传学家提出“未来谷物改良寄希望于野生基因资源的利用” (Feldman和Sears 1981)。通过远缘杂交和染色体工程的方法对野生近缘物种的染色体(片段)进行附加、代换和消除, 可有效实现外源优异基因的高效转移、鉴定和利用, 在拓展作物遗传多样性、创制高产优质的新种质方面发挥了关键作用(Xiao等2022)。结合现代细胞遗传学、分子生物学和基因组学工具, 高分辨率的FISH、多色荧光原位杂交(M-FISH)、基因组原位杂交(GISH)等技术为鉴定外源染色体片段提供了重要的手段(Albert等2019; Zou等2021)。

2.1 染色体工程在小麦遗传改良中的应用

异源六倍体普通小麦的形成经历了两次远缘杂交和异源多倍化过程, 其染色体组具有较高的兼容性和可塑性。山羊草属(*Aegilops*)、偃麦草属(*Elytrigia*)、黑麦、冰草属(*Agropyron*)、滨麦(*Leymus mollis*)、簇毛麦(*Haynaldia villosa*)和大麦等的祖先种和近缘野生种为小麦遗传改良提供了重要的基因资源(贾子苗等2021)。染色体工程技术在改良小麦抗病、抗逆和品质等方面表现出巨大的应用价值, 应用最成功的范例是1BL.1RS易位系。育种家将黑麦1RS染色体易位到普通小麦, 将黑麦*Lr26/Sr31/Yr9/Sr50/Pm8*等抗病、丰产基因导入小麦, 创制了‘周麦16’、‘百农矮抗58’等优质材料, 已成为我国黄淮麦区大面积推广的主栽品种(Sukumaran等2015; 刘成等2020)。

李振声先生开创了小麦远缘杂交种在生产上大面积推广的先例, 利用染色体工程的方法将长穗偃麦草的优良基因导入小麦, 育成的抗条锈病、抗干热风、优质强筋的‘小偃’系列品种成为我国小麦育种的骨干亲本(李宏伟等2022a)。‘中科166’是我国首个利用长穗偃麦草的外源染色体片段、通过染色体工程及回交选育获得的中抗赤霉病小麦新品种(Guo等2023), 该外源抗病基因可改良亲本‘济麦22’的赤霉病抗性, 协调小麦高产和抗病间的关系。目前, 长穗偃麦草仍是建设滨海草带的重要耐盐碱牧草(李宏伟等2022b; 胥伟华等2022)。西北农林科技大学综合利用十倍体长穗偃麦草、中间偃麦草(*E. intermedia*)、华山新麦草(*Psathyrostachys huashanica*)和滨麦等创新了一批骨干小麦亲本资源, 进一步培育出优质强筋、高产、绿色抗病的国审小麦新品种‘西农511’和‘西农529’, 并在黄淮麦区大面积推广(Zhu等2019)。簇毛麦含有广谱的抗白粉病、黄花叶病毒病和锈病等基因资源, 南京农业大学利用染色体工程的方法创制普通小麦–簇毛麦6VS/6AL易位系, 选育出一批高抗白粉病的新品种(Xing等2018, 2021)。最近构建的染色体水平簇毛麦参考基因组也为小麦遗传改良和广谱抗病性研究提供了重要基因组资源(Zhang等2023)。

粗山羊草(*A. tauschii*)是小麦DD亚基因组的祖先供体种, 其野生群体蕴含丰富的抗性、耐逆、高产和优质基因资源, 是小麦遗传改良的重要二级种质资源库。四川农业大学从国际玉米小麦改良中心(CIMMYT)引进了一批含粗山羊草血缘的人工

合成六倍体小麦资源, 将其与四川本土小麦进行回交或顶交, 培育出在西南麦区广泛使用的‘蜀麦’系列品种(Li等2018b; 郝明等2022)。河南大学对粗山羊草种群的遗传多样性进行系统挖掘, 整合远缘杂交、基因组学、表型组学和快速育种技术, 建立了粗山羊草–普通小麦渐渗系, 实现外源基因的快速转移, 为小麦品种改良提供了重要材料(Zhou等2021b)。中国科学院遗传与发育生物学研究所利用染色体工程创制了一套小麦–黑麦单端体附加系, 并筛选出3RL附加系高抗秆锈Ug99, 为小麦抗病育种提供了新的资源(Liu等2022)。中国农业科学院作物科学研究所创制了小麦–冰草属间杂种, 将冰草的多花多实、高千粒重、广谱抗白粉和锈病基因转入小麦(Li等2016)。

2.2 基因编辑助力作物染色体工程育种

基因编辑技术的快速发展使传统染色体工程育种焕发了新的面貌, 利用CRISPR/Cas系统在拟南芥和玉米中实现了精准的染色体大片段缺失、倒位、易位和重复(Ronspies等2022)。德国科学家首先实现了拟南芥基因组不同位点的可遗传倒位(Schmidt等2019), 随后利用卵细胞特异启动子表达Cas9蛋白靶向诱导可传递的18 kb染色体易位(Beying等2020), 并在异染色质区靶向逆转了1.1 Mb hk4S倒位, 重塑了该区域的减数分裂重组模式(Schmidt等2020)。美国杜邦公司利用CRISPR/Cas9技术实现了优质玉米自交系PH1V5T染色体75.5 Mb的臂间倒位, 实现大量遗传变异的染色体区域重组, 为玉米育种提供了新的基因资源(Schwartz等2020)。通过CRISPR/Cas9技术固定或打破染色体上的基因遗传连锁、重构染色体基因组, 在作物遗传改良和精准染色体工程育种领域表现出巨大的应用潜力。如何进一步突破现有瓶颈, 在作物尤其是多倍体小麦中实现高效、定向、稳定的染色体重排是未来的重要探索方向(Ronspies等2021; Zhou等2022b)。

3 农作物分子标记开发和利用研究进展

DNA分子标记是实现作物精准育种、提高育种效率的关键技术, 先后经历了以分子杂交、PCR扩增及可视化检测、高通量测序及序列解码比对为核心技术的代际发展历程(胡亚亚等2018; 陈星和高子厚2019; 徐云碧等2020)。在作物遗传改良中, 该技术主要应用于针对特定性状目标基因型的跟踪转育、靶向全基因组范围的遗传背景评估、及根据性状和标记的数学相关性建立全基因组选择模型等。近年来, 我国在作物优异种源鉴定和关键基因克隆方面取得了较好的进展, 已定位或克隆了大批具有重要育种应用价值的功能基因, 并建立了相应的分子标记育种体系。

3.1 作物重要性状相关基因的定位、克隆及连锁分子标记的开发

通过正向遗传学手段鉴定和挖掘优异等位基因是开发选择标记的前提。在水稻产量相关性状方面, 已克隆了调控种子大小的*GS3* (Fan等2009)、*GW6* (Shi等2020), 穗粒数的*DEP1* (Huang等2009)、*FZP* (Bai等2017), 株型的*TAC1* (Yu等2007)、*Nal1* (Qi等2008), 以及影响产量和生育期或抗逆性的多效基因*Ghd7* (Xue等2008)、*IPA1* (Jiao等2010)。在小麦中克隆了调控株高的*Rht24* (Tian等2022)、粒型相关的*Tasg-D1* (Cheng等2020), 雄性不育基因*Ms1* (Wang等2017)、*Ms2* (Xia等2017)。在玉米中克隆了调控叶夹角的*UPA1* (Tian等2019)、*UPA2* (Tian等2019)、*ZmIBH1-1* (Cao等2020), 籽粒相关的*Urb2* (Wang等2018a)、*SCC4* (He等2019a)、*KNR6* (Jia等2020)等, 以及两个关键产量杂种优势基因*ZmACO2*和*ARGOS1* (Wang等2023a)。在大豆(*Glycine max*)中克隆了调控开花的*GmPRR37* (Wang等2020a)、*GmPRR3b* (Li等2020a), 雄性不育基因*GmMs1* (Nadeem等2021)、*GmMs3* (Hou等2022), 及调控籽粒大小和品质的*GmST05* (Duan等2022)。这些基因的挖掘及标记开发为选育高产品种提供了重要的支持。在品质方面, 水稻中已克隆了控制香味的*Badh2* (Chen等2008), 控制直链淀粉合成的Wx^{la} (Zhou等2021a)、Wx^{lv} (Zhang等2019a), 控制垩白的*chalk5* (Li等2014)、*WCR1* (Wu等2022c), 以及控制蛋白含量的*qGPC-10* (Yang等2019a)。小麦中定位到*Qsdss-1A*、*Qsdss-1B.1*、*Qsdss-1B.2*和*Qsdss-5D* (Chang等2022)等控制加工品质的位点。玉米中克隆了*THP9* (Huang等2022)等调控蛋白品质和含量的基因。大豆中克隆了*POWR1* (Goettel等

2022)等调控蛋白含量和产量的基因。在抗性方面, 已克隆了水稻抗稻瘟病的*Pi2* (Zhou等2006)、*Pi9* (Qu等2006)等, 抗白叶枯病的*Xa23* (Wang等2014)等, 抗褐飞虱的*Bph6* (Guo等2018)、*Bph14* (Du等2009)、*Bph30* (Shi等2021)等生物抗性基因, 以及耐冷的*bZIP73* (Liu等2018)、*COLD1* (Ma等2015b), 耐热的*TT1* (Li等2015)、*TT2* (Kan等2022)、*TT3* (Zhang等2022), 耐旱的*DROT1* (Sun等2022)、*LG3* (Xiong等2018), 耐盐碱的*SKC1* (Ren等2005)、*qSE3* (He等2019b)、*STH1* (Xiang等2022)等非生物抗性基因。小麦中已克隆了抗叶枯病*Stb6* (Saintenac等2018)、*Stb16q* (Saintenac等2021), 抗白粉病*Pm2* (靳玉丽等2022)、*Pm24* (Lu等2020a)、*Pm41* (Li等2020b)等生物抗性基因, 以及抗热胁迫的*TaSINA* (Thomelin等2021)等。玉米中已获得*qRgls1.06* (Sun等2021)、*qGLS8* (Qiu等2021)等抗灰斑病、*qMrdd8* (Liu等2016)等抗粗缩病的主效QTL, 以及抗南方锈病的*RppK* (Chen等2022a)等。大豆中已克隆了抗花叶病毒的*Rsc4* (Yin等2021), 耐盐的*GmSALT3* (Guan等2014)等基因。上述基因位点及其分子标记将有助于作物抗逆品种选育。

3.2 分子标记在作物遗传改良中的应用成效

3.2.1 分子标记在作物产量改良中的应用

利用分子标记辅助选择可加速作物的产量性状改良。李家洋团队分离了水稻理想株型基因*IPA1*, 提出理想株型与杂种优势相结合的育种思路, 并利用分子标记辅助选择等技术将*IPA1*优异等位变异导入籼/粳稻骨干亲本, 育成株型理想、抗逆性强、增产显著、且适宜长江中下游稻区种植的‘嘉优中科’系列水稻品种(Zhang等2017a; 余泓等2018)。Tian等(2019)借助分子标记辅助选择将控制大刍草玉米(*Z. mays* ssp. *parviglumi*)叶夹角的*UPA2*等位基因回交导入玉米杂交种‘农大108’双亲中, 获得耐密植、高产的改良品种。Jia等(2020)利用该技术将*KNR6*优良等位基因型导入玉米品种‘郑单958’的父母本中, 改良后的父本、母本及所配制的杂交种产量均得到显著提升。Song等(2022)利用分子标记辅助选择结合快速育种技术将常规品种‘ZS11’的长角果、大粒等位基因*BnaA9.CYP78A9a*导入甘蓝型油菜骨干恢复系621R中, 获得了角果长和千粒重显著改良的新恢复系材料, 为高产油菜育种提供了优异资源。

3.2.2 分子标记在作物品质改良中的应用

罗世友等(2020)通过分子标记辅助选择结合系谱选育, 将香味基因*Badh2*导入水稻品种‘赣晚籼30号’中, 选育出优质香稻品种‘九香粘’。李荣田等(2020)利用功能标记对香味基因*fgr*进行前景选择, 结合双亲间具多态性的SSR标记进行背景选择, 培育出具香味且其他性状与‘空育131’相似的早粳稻空育131 (*fgr*)导入系。杨瑞芳等(2020)结合分子标记辅助选择和表型选择, 将控制高抗性淀粉合成的主效基因*sbe3-rs*导入普通水稻品种, 选育出高产、高抗性淀粉的水稻新品种‘优糖稻2号’、‘优糖稻3号’等。郭聚领等(2021)通过分子标记前景选择和背景选择的策略, 将高油酸等位基因*fad2*导入甘蓝型油菜波里马细胞质温敏不育系616A及其恢复系L-35R中, 获得油酸含量高于75%的改良株系, 为高油酸油菜育种奠定了基础。

3.2.3 分子标记在作物逆境改良中的应用

病虫害和极端环境导致的逆境是制约作物生长的主要限制因子之一。利用分子标记辅助作物抗逆育种已经取得显著成果。Jiang等(2015)通过分子标记辅助选择将抗稻瘟病基因*Pi2*和抗白叶枯病基因*Xa23*导入水稻不育系GZ63-4S, 获得抗性改良的新不育系Hua1015S, 并配制出优良的抗病新品种。Sandhu等(2019)结合分子标记辅助选择和表型选择, 将3个抗旱QTL导入‘Swarna’, 获得抗旱水稻品种。Yang等(2022b)通过分子标记及低密度单核苷酸多态性(SNP)育种芯片的选择, 将抗稻瘟病基因*Pi2*、抗白叶枯病基因*Xa7*/*Xa23*和抗褐飞虱基因*Bph14*/*Bph15*导入水稻光温敏核不育系丰39S, 获得兼抗稻瘟病、白叶枯病和褐飞虱的近等基因系。牛付安等(2023)利用水稻高密度基因芯片对恢复系和不育系中抗稻瘟病和白叶枯病基因进行单倍型分析, 通过测交配组选育出聚合*Pi2*、*Pita*、*Pib*、*Pi9*、*Pi54*、*Pikm*、*Pit*等抗稻瘟病基因和抗白叶枯病基因*Xa21*的杂交粳稻新品种‘申优28’。高月等(2022)分别以携带抗白粉病基因*Pm21*、*Pm35*的小麦品种‘金禾9123’和‘普冰01’为供体, 以携带抗白粉病基因*Pm52*的‘良星99’为受体, 利用标

记辅助选择回交转育, 获得*Pm21*+*Pm52*或*Pm35*+*Pm52*的改良株系, 为小麦抗病育种提供了重要资源。方桃红等(2022)利用分子标记辅助选择将小麦抗条锈病基因*Yr52*转育到三个主栽感病小麦品种中, 获得抗病性显著提高的改良品种。Xu等(2020a)利用分子标记辅助选择将玉米抗粗缩病的主效位点*qMrdd8*导入多个骨干自交系, 获得了抗性改良的新材料。Chen等(2022a)利用抗南方锈病基因*RppK*对大面积推广的玉米杂交种‘京科968’的亲本进行分子标记辅助选择, 组配出抗南方锈病的改良品种。战宗祥等(2015)将抗根肿病基因*PbBa8.1*转育至感病品种‘华双5号’, 培育出我国首个抗根肿病的油菜品种‘华双5R’。李倩等(2021)利用分子标记将大白菜材料‘CR Shinki’中的*CRb*抗病位点导入油菜杂交种‘华油杂62’的恢复系Bing409中, 培育出我国首个抗根肿病的油菜杂交种‘华油杂62R’。

4 植物基因工程研究进展

4.1 形态发生调节因子促进植物转化

从细胞培养中再生转基因植株高度依赖于受体物种、基因型和组织类型。一些植物发育调节因子可通过触发细胞和组织再生, 影响植物转化效率。在油菜中异位表达细胞全能性调节因子*BBM*, 可在无外源生长调节剂的情况下促进细胞增殖、生长和发育(Jha和Kumar 2018)。*WUS*异位表达也可促进体细胞胚胎发生和扩大分生组织。利用*BBM*和*WUS2*的差异表达, 已成功改良了顽固高粱品种的遗传转化(Nelson-Vasilchik等2018)。为缓解*BBM*和*WUS2*组成型表达引起的异常表型, 研究者使用玉米特异性启动子驱动*BBM*和*WUS2*在未成熟玉米胚中表达, 所获胚体可直接发芽成植株(Lowe等2018)。拟南芥发育调节因子*PLT5*、*WIND1*和*WUS*可促进金鱼草(*Antirrhinum majus*)和番茄的转化, 且其中*PLT5*诱导的转化效率最高。此外, *PLT5*可显著改善白菜型油菜的芽再生和转化, 促进甜椒(*Capsicum annum*)转基因愈伤组织和体细胞胚胎的形成(Lian等2022)。在甜菜(*Beta vulgaris* ssp. *vulgaris*)愈伤组织中异位表达拟南芥的生长调节因子*AtGRF5*, 可加速芽的形成、提高转化效率, 促进顽固甜菜品种产生稳定的转化体。在油菜、大豆、向日葵(*Helianthus annuus*)和玉米中, *GRF*也可促进转化细胞增殖、提高再生能力和转化效率(Kong等2020)。为了有效介导CRISPR/Cas蛋白进入植物细胞, 研究者将形态发生调节因子和三元载体系统组合, 在增强玉米转化方面具有加性效应(Zhang等2019b)。利用再生相关基因*TaWOX5*已成功转化了5个常见的小麦品种, 且过表达*TaWOX5*显著提高了小麦、黑麦、大麦和玉米等作物的转化效率和基因编辑效率, 降低对基因型的依赖性(Wang等2022b)。尽管过表达植物形态调节因子能有效提高转化效率, 仍需建立新的方法以提高转化的有效和易用性, 使其适用于更广泛的顽固物种和基因型。通过结合小麦GRF4–GIF1融合蛋白表达的转化策略, 显著提高了小麦、水稻和柑橘等的再生效率, 降低了对小麦基因型的依赖(Debernardi等2020)。

4.2 农杆菌转化技术的简化和创新

农杆菌(*Agrobacterium*)介导的植物转化(AMT)是一种高效、低成本的基因传递系统, 可将大的DNA片段转移到植物染色体中, 是植物转化的首选技术。但无效的农杆菌感染阻碍了该技术在许多单子叶作物中的应用。通过工程根癌农杆菌表达丁香假单胞菌(*Pseudomonas syringae*)的III型分泌系统(T3SS)可增加农杆菌转化, 且单独递送丁香假单胞菌效应子AvrPto、AvrPtoB或HopAO1可抑制宿主防御反应, 成功将小麦、苜蓿(*Medicago truncatula*)和柳枝稷(*Panicum virgatum*)的转化效率提高2~4倍。该系统也可递送组蛋白H2A-1, 对于改良顽固物种的转化尤为重要(Raman等2022)。

棉花中建立了连续再生驯化策略(SRA): 利用转基因后代群体中分离出的阴性单株(无T-DNA插入)的种子为下一次遗传转化的受体, 并将该过程重复3~4次, 即能显著提高农杆菌介导的棉花遗传转化、植株再生的效率(Li等2019)。为突破传统植物基因组修饰对特定基因型的依赖, 棉花中建立了顶端分生组织(SAM)细胞介导的高效转化系统(SAMT), 实现了陆地棉、海岛棉(*G. barbadense*)和亚洲棉(*G. arboreum*)等顽固基因型的稳定遗传转化, 并基于该系统获得了CRISPR/Cas介导的棉花基因编辑植株(Ge等2022)。尽管棉花茎尖转化法

具有不受基因型限制、转化周期短等优点, 但仍存在遗传不稳定、转化效率低等不足, 这可能是由于农杆菌与受体的共生关系导致的假阳性(肖荣等2022)。

为了克服传统组织培养过程的困难和复杂性, 研究者开发了Fast-TrACC和直接递送(DD)两种方法, 可在双子叶植物中递送*WUS2*、*BBM*等发育调节因子(DR) (Cody等2022)。上述方法可共同递送DR和基因编辑载体等, 在诱导的分生组织中实现转基因过程, 其对靶标基因的修饰可稳定遗传。发根农杆菌介导(ARM)转化是基于转基因根与野生型芽形成复合植物的一种技术。大豆中的一步ARM转化法操作简单、转化效率高, 每株复合植株的转基因毛状根数量约为传统ARM转化的3倍(Fan等2020)。此外, 研究者基于发根农杆菌开发了一个极简单的CDB (cut-dip-budding)递送系统, 实现了橡胶草(*Taraxacum koksaghyz*)、小冠花(*Coronilla varia*)、甘薯(*Ipomoea batatas*)等顽固植物的遗传转化。该系统可在非无菌条件下实现有效的转化或基因编辑(Cao等2022)。

4.3 遗传因子递送系统的改良

植物细胞壁限制了外源性生物分子的递送效率。传统的植物DNA递送方法存在宿主物种限制、转化效率低、组织损伤或无法避免非目的DNA整合等不足。开发高效、简单和安全的基因递送方法在植物生物技术领域尤为重要。

鉴于合成纳米结构可将生物大分子转移至哺乳动物细胞中, 工程嵌合肽和碳纳米颗粒有望在植物基因传递中发挥功能(Kumar等2020)。研究表明, 具有特殊阳离子和疏水结构域的工程肽和纳米圆柱形的精氨酸功能化单壁碳纳米管可在质粒DNA凝结时通过烟草(*Nicotiana tabacum*)根的细胞屏障, 实现目的基因的递送(Golestanipour等2018)。通过对功能化的高纵横比碳纳米管(CNT)表面化学物质的优化, 获得一种单壁CNT (SWNTs), 可保护DNA免受细胞核酸酶降解, 并以植物物种非依赖性的方式递送质粒。该递送平台无需转基因整合, 无毒性或组织损伤, 适用于单、双子叶植物(Demirer等2019)。棉花中研发了基于磁性纳米颗粒基因载体的花粉磁珠转化方法, 也适用于辣椒(*Capsicum annuum*)、南瓜(*Cucurbita moschata*)、西葫芦(*C. pepo*)和百合(*Lilium brownii*) (Zhao等2017)。通过纳米磁转化和花粉介导法相结合, 可提高转化效率、缩短转基因周期、实现多基因协同转化, 加速转基因新品种的培育, 在作物遗传学、合成生物学和生物反应器等领域具有广泛应用前景。但有研究质疑纳米磁珠能否在单子叶植物(如玉米、高粱等)花粉中瞬时转化(Vejlupkova等2020)。Wang等(2022c)借助纳米磁珠将外源基因通过花粉萌发孔导入玉米花粉, 解决了玉米转化中依赖组培体系、严重受基因型限制等问题, 证明了花粉磁转在植物中的可行性。为避免外源基因向杂草亲属扩散, 研究者利用脂质交换包膜渗透机制设计了壳聚糖络合单壁碳纳米管, 选择性地将质粒DNA递送到不同物种的叶绿体上, 实现了芝麻菜(*Eruca sativa*)、豆瓣菜(*Nasturtium officinale*)、烟草、拟南芥叶绿体的靶向转基因(Kwak等2019)。在基因沉默方面, 已研发了一种简单高效的siRNA递送方法, 即利用氧化石墨烯纳米颗粒(GONs)-siRNA复合物将siRNA递送到细胞中, 实现高效、短暂的基因沉默(Li等2022c)。此外, 有研究利用硒纳米颗粒(SeNPs)减少油菜中镉(Cd)积累, 提高植株对Cd胁迫的抗性(Qi等2021)。综上, 纳米颗粒作为生物传感器和基因载体具有重要的应用价值。但其在植物基因转化中仍存在不能稳定转化、不易降解、具有毒性作用等问题。未来需对这些问题加以改善, 拓宽纳米技术在作物育种中的应用前景。

病毒载体系统是外源基因体内递送和瞬时表达的理想工具。浙江大学李正和团队首次利用苦苣菜(*Sonchus oleraceus*)黄网病RNA病毒载体, 在烟草中递送CRISPR/Cas组分, 实现了高效基因编辑(Ma等2020)。但该病毒载体尚未能在作物中得到广泛应用。该团队基于一种广寄主范围的番茄斑萎病毒(TSWV)建立的瞬时递送系统, 开发了广谱RNA病毒载体递送CRISPR/Cas核酸酶的新方法, 成功向烟草、番茄、辣椒、花生和酸浆(*Physalis alkekengi*)的不同品种稳定递送CRISPR/Cas分子。该载体可携带荧光报告基因示踪病毒侵染, 并可通过串联表达多个向导RNA实现高效的多重基因编辑(Liu等2023)。该系统具备广泛的宿主范围和非基

因型依赖性, 可望推动基因编辑在作物育种中的应用。基于大麦条纹花叶病毒(BSMV)介导的gRNA传递系统, 成功创造了编辑*TaHRC*的抗赤霉病小麦(Chen等2022b)。

鉴于大多数植物存在转化困难、生长周期长等问题, 研究者将嫁接与移动CRISPR剪辑工具相结合, 实现了跨物种的远距离基因编辑。TLS (tRNA like squence)可作为RNA信号在植物内长距离移动。将TLS元件与CRISPR/Cas9序列融合形成移动的CRISPR/Cas9 RNA, 再将野生型接穗嫁接到含CRISPR/Cas9 RNA的植物根部, 可实现编辑工具从根部到嫩枝、花朵的移动编辑。利用拟南芥为砧木表达Cas9和gRNA转录本, 再将其与野生白菜型油菜进行嫁接, 也可实现跨物种基因编辑(Yang等2023)。上述可移动基因编辑系统为未来分子精准设计育种提供了快速高效的平台。

5 植物基因编辑的研究进展

基因编辑技术能对植物基因组进行精准修饰, 定向改变目标性状, 为作物遗传改良提供了有力的技术支撑。目前应用较为广泛的编辑系统主要包括锌指核酸酶(ZFNs)、类转录激活因子效应物核酸酶(TALENs)和CRISPR/Cas系统(Voytas和Gao 2014; Shan等2018; Woo等2015; Christian等2010)。这些核酸酶可在靶位点引起DNA双链断裂(DSB), 进而通过DNA修复实现精确的基因组编辑。CRISPR/Cas系统因具有简单、高效、低成本等优势, 已成为应用最广泛的编辑系统。该系统可实现基因敲除、碱基编辑、基因定点替换、插入和引导编辑, 已成为作物基因功能研究和性状改良的重要工具(Li和Xia 2020; Ma等2015a; Manghwar等2019)。

5.1 基因敲除

CRISPR/Cas系统引起基因组DNA双链断裂, 通过非同源末端修复(NHEJ)或同源重组修复(HDR)对基因组进行修复。基因敲除即是通过NHEJ使目标基因产生随机插入、缺失或碱基替换, 导致基因移码或提前终止(Cong等2013; Zetsche等2015; Hiom 2010; Puchta和Fauser 2014)。利用该技术对控制植物重要性状(产量、品质、抗逆性等)的负向调控基因进行定点敲除, 创制性状改良的突变体, 已成为植物遗传改良和育种的重要策略。如对水稻*OsPYLs*基因家族的三个成员编辑突变后, 水稻的生物量和产量都显著增加(Miao等2018)。通过敲除水稻和小麦淀粉分支酶基因*OsSBEIIb*和*TaSBEIIa*, 可创制高抗性淀粉的新种质(Li等2021b; Sun等2017)。同时敲除玉米淀粉合成基因*ZmWX*与*ZmSH2*, 成功创制了甜糯玉米新材料(Dong等2019)。敲除*OsVQ25*基因可显著提高水稻对稻瘟菌和白叶枯菌的广谱抗性(Hao等2022)。利用基因编辑技术敲除棉花腺体形成的关键基因获得了无棉酚的种质资源(Li等2021c); 对棉花的不饱和脂肪酸合成通路的关键基因*GhFAD2*进行敲除, 获得的棉花种子亚油酸含量降低而油酸含量大幅提升的种质资源(Chen等2021)。

此外, 基因编辑技术也有力推动了传统育种技术的发展。通过敲除玉米单倍体诱导基因*PLA1/MTL*、*DMP*和*PLD3*等, 创制高效的单倍体诱导系, 可提高单倍体育种效率(Kelliher等2017; Li等2021d; Zhong等2019)。在小麦、水稻、油菜、谷子(*Setaria italica*)等作物中, 也通过编辑相关同源基因建立单倍体诱导技术体系(Liu等2020a; Yao等2018; Zhong等2022; Cheng等2021)。在水稻无融合生殖研究方面, 通过敲除减数分裂相关基因(*Mike*), 同时在卵细胞中异位表达*BBM1*基因或敲除*MTL*基因, 可使后代产生倍性相同、无融合生殖的种子, 有望实现一系法杂交水稻制种(Khanday等2018; Wang等2019a)。基因编辑技术也使野生近缘植物的快速从头驯化成为可能, 通过对四倍体野生稻的落粒基因*qSH-1*、芒长基因*An-1*、绿色革命基因*SD1*、粒长基因*GS3*以及理想株型基因*IPA1*进行基因编辑, 获得了上述性状快速驯化的系列突变体, 为四倍体野生稻的生产利用奠定了基础(Yu等2021)。

5.2 碱基编辑

SNP是最常见的遗传变异来源, 是植物中多种性状变异的遗传基础。因此, 实现单核苷酸定点替换的碱基编辑技术对植物遗传改良具有重要意义。2016年, 基于胞嘧啶脱氨酶的、可实现C:G到T:A转换的碱基编辑系统(CBE)首先被报道(Komor等2016)。随后, 相继研发了可实现A:T到G:C转换的腺嘌呤碱基编辑器(ABE), 以及C:G到G:C转换的

碱基编辑器(CGBE) (Gaudelli等2017; Kurt等2021)。这些碱基编辑系统不引起DNA双链断裂、不依赖于HDR或供体DNA, 具有简单、高效的优点。此外, 研究人员通过改造胞嘧啶/腺嘌呤脱氨酶、优化碱基编辑器的表达、筛选新的Cas蛋白等方式, 进一步提高单碱基编辑效率、缩小编辑窗口、扩展编辑范围, 并降低脱靶频率等(Zong等2017; Kim等2017; Nishida等2016; Hua等2019; Ren等2018, 2021; Walton等2020; Koblan等2018; Qin等2019; Richter等2020; Tan等2022; Yan等2021)。

碱基编辑系统已被广泛应用于多种植物的遗传改良研究(Bharat等2020)。如通过对水稻、小麦、番茄、马铃薯、西瓜(*Citrullus lanatus*)、油菜和拟南芥等植物的*ALS*基因进行单碱基替换, 获得了一系列抗除草剂的植株(Li等2017; Zong等2017; Ren等2017; Shimatani等2017; Tian等2018; Veillet等2019; Wu等2020)。在棉花中利用ABE碱基编辑器精准编辑株型调控基因——*GhPEBP*, 获得了果枝短、株型紧凑的理想株型棉花材料(Wang等2022)。在油菜中对*BnaIDA*进行编辑, 获得“花不落”油菜新种质, 可有效阻断菌核病的传播途径(Wu等2022b)。

传统的基因诱变存在靶向性低、突变随机性高等缺点, 碱基编辑系统可对关键基因位点进行饱和氨基酸突变, 实现重要目标基因的定向进化。研究者通过融合胞嘧啶脱氨酶和腺嘌呤脱氨酶, 建立了饱和靶向内源基因突变的双碱基编辑器STEME, 并利用该系统对水稻*OsACC*基因进行饱和突变, 筛选到抗除草剂水稻新材料(Li等2020c)。基于此策略, 研究者相继开发了pDuBE1、AGBE等更高效、灵活的双碱基编辑器, 为植物基因的定向进化研究提供技术支撑(Xu等2021b; Liang等2022)。

5.3 HDR介导的基因精准编辑

植物遗传改良需要将优异等位基因精准引入基因组中, HDR介导的基因组精准编辑技术可实现优异等位基因的定点替换或插入。目前, 利用CRISPR/Cas9和CRISPR/LbCpf1系统均已实现了植物基因的精准替换, 如对水稻OsALS第548和627位两个氨基酸进行等位替换, 赋予了水稻对磺酰脲类除草剂的抗性(Sun等2016; Li等2020d)。利用RNA转录本为修复模板, 实现了HDR介导的*OsALS*等位基因替换, 首次在植物细胞中建立了以转录本为修复模板介导的同源重组修复体系(Li等2019)。虽然在多种植物中已有HDR介导的基因组精准编辑的报道, 然而该技术的应用仍受制于HDR的低效率和植物细胞中供体模板的低递送力(Luo等2016)。为此, 已开展多项研究尝试提高HDR效率。如通过突变LbCas12a的氨基酸(D156R), 获得编辑效率更高的ttLbCas12a, 在烟草中实现了更高效的基因敲入(Huang等2021)。采用卵细胞和早期胚胎特异性启动子驱动Cas9表达, 并结合二代转化策略, 在拟南芥中提高了HDR介导的基因敲入和替换效率(Miki等2018)。通过对供体DNA片段进行硫代修饰和磷酸化修饰, 开发了重复片段介导的同源重组(TR-HDR)方法, 将修饰片段靶向敲入至目标位点, 制造串联重复结构, 进而诱导TR-HDR实现基因替换, 效率最高可达11.4%。这一技术将促进HDR介导的基因精准编辑在植物育种中的应用(Lu等2020b)。

5.4 引导编辑

2019年, 一种新型的基因精准编辑技术——引导编辑(prime editing)技术问世, 它将nCas9 (H840A)和经过工程化改造的M-MLV RT逆转录酶融合, 在编辑链的单链切口处通过PBS (primer binding site)序列引导逆转录酶工作, 利用包含目标序列的逆转录模板(RT template)将突变精确引入基因组中(Anzalone等2019)。引导编辑系统可在不产生DSB和不引入DNA供体的情况下, 实现小片段靶向插入、删除及12种类型的碱基替换。已有多项研究构建了适用于植物表达的引导编辑工具, 并成功在水稻、小麦、玉米等作物中实现了基因精准编辑, 但编辑效率普遍较低(Lin等2020; Li等2020e; Tang等2020; Xu等2020b, c; Hua等2020; Jiang等2020)。为了突破编辑效率的限制, 研究人员进行了多种尝试。如通过开发pegFinder和PrimeDesign等软件, 优化pegRNA的设计流程(Chow等2020; Kim等2020)。PBS的$T_{\rm m}$值对引导编辑效率影响较大, 当$T_{\rm m}$值为30ºC左右时, 引导编辑效率在多数水稻内源位点中达到最高。采用2个pegRNA分别靶向靶序列两条链的策略, 可显著提高编辑效率(Lin等2021)。在Cas9的N端融合逆转录酶M-MLV, 并在RT模板中引入同义错配碱基, 可使编辑效率提高10倍以上(Xu等2022a)。

通过在pegRNA的3'端添加RNA motifs增强引导编辑系统的稳定性, 可建立更高效的PPE3-evopreQ1引导编辑系统(Zou等2022)。此外, 通过构建基于潮霉素抗性基因的代理引导基因编辑器PE3-HS和基于双草醚抗性基因的编辑器PE3-AS、以及基于这两基因的双代理引导基因编辑器PE3-DS, 可将编辑效率提高50倍, 同时实现水稻多个内源基因的精准编辑(Li等2022b)。

5.5 植物基因编辑研究展望

综上, 以CRISPR/Cas系统为代表的基因编辑技术发展迅速, 已成为植物基因功能研究和遗传改良的重要工具。其中CRISPR/Cas介导的基因敲除和单碱基编辑技术应用最为广泛; 而HDR介导的基因精准编辑的效率仍较低, 限制了其应用。研究人员已采取多种策略来提高植物中的HDR效率(Miki等2018; Aird等2018; Carlson-Stevermer等2017; Wolter等2018)。CRISPR相关转座酶可高效的将DNA整合到细菌基因组中, 也为提高植物中HDR效率提供了参考(Klompe等2019; Strecker等2019)。此外, 植物细胞递送也是影响基因编辑效率的重要因素之一。目前主要的递送方法为基因枪和农杆菌介导的遗传转化, 虽然已有报道利用*WUS*、*BBM*、*GRF-GIF*等发育调控基因可在一定程度上提高植物遗传转化和再生效率, 但仍需要开发更为高效、简便的递送系统(Lowe等2016; Debernardi等2020)。最近, 利用烟草脆裂病毒(TRV)、大麦条纹花叶病毒(BMSV)、菜豆矮缩病毒(BeYDV)等病毒载体的递送系统已在玉米、小麦、烟草、棉花等作物中成功实现了可遗传的靶向基因编辑(Hu等2019; Ellison等2020; Ma等2020; Li等2021e, 2022c)。另外, 多种纳米材料能够高效递送遗传物质或蛋白质, 具有高效率、无毒性和无转基因整合的优势。但该方法在植物中的研究和应用仍有待深入(Staahl等2017; Lee等2017)。

尽管我国在植物基因编辑技术研发和应用中取得了一系列重要进展, 但ZFNs、TALEN和CRISPR/Cas9系统等主流基因编辑工具的底盘技术专利均掌握在美国等发达国家手中, 我国基因编辑育种产业将面临“卡脖子”的风险。因此, 需要进一步加强基因编辑领域基础研发和技术创新的投入, 加快开发原创性的基因编辑工具, 建立具有自主知识产权的基因编辑技术体系。

6 植物全基因组选择研究进展

全基因组选择(GS)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统计模型, 进而对表型未知的育种群体做出合理的预测和选择(Meuwissen等2001)。与分子标记辅助选择育种技术相比, GS育种技术无需鉴定与目标性状显著相关的位点, 即使单个位点的效应很小, 导致表型变异的全部遗传效应也都能够被高密度的遗传标记捕获, 并且能够在得到个体基因型时即对其育种值进行评估, 可大大缩短育种周期, 提高育种效率, 实现从经验育种到基因组精准育种的飞跃, 已成为动植物育种的一项革命性技术(Hickey等2017)。GS在动物育种, 尤其是奶牛育种中取得了巨大成功, 自2009年开始奶牛的选育已由基因组选择主导。近年来随着高通量测序技术的发展和测序成本的下降, 在植物育种中利用GS技术已成为可能, 特别是植物的杂种育种中, 杂交种的基因型可以由亲本基因型进行推断, GS的优势更加突出。国内外已开展了多种植物的全基因组选择方法与应用研究。科迪华和拜耳等跨国企业在育种流程中应用全基因组选择技术提高玉米选育效率, 并配套研发了高通量和无损伤的单粒种子基因型鉴定技术设备。CIMMYT已在全球玉米育种计划中实施全基因组选择技术, José Crossa团队设计了一种多亲本群体的快速轮回基因组选择, 既能保持遗传多样性, 又能在短时间内获得高遗传增益(Zhang等2017b)。Fu等(2022)提出了在育种实践中整合全基因组选择育种技术与双单倍体育种技术的方法。Cui等(2020)提出了利用已知表型的杂交种作为训练群体跨群预测未知杂交种表型的水稻育种策略。

6.1 全基因组预测准确性的影响因素

在实际育种中, 全基因组选择的成功与否很大程度依赖于基因组预测的准确性。影响预测准确性的因素有很多, 如群体大小、标记密度、亲缘关系、目标性状遗传力以及标记和QTL间连锁不平衡(LD)程度等(Crossa等2017)。一般而言, 随着训

练群体和标记密度的增加, 基因组预测的准确性随之增加, 但达到一定程度后准确性难以提高。通过统计方法筛选训练群体和分子标记, 能够提高预测的准确性, 并降低成本(Guo等2019)。有研究表明训练群体占整个群体的2%~13%可以建立有效的GS模型。训练群体和测试群体的亲缘关系对基因组预测的准确性也有一定影响, 对于遗传上相似的群体能够获得较高的预测准确性, 反之亦然(Lorenz和Smith 2015)。在玉米双亲杂交群体中, 与随机增加其他材料相比, 在训练群体中增加来自双亲的半同胞家系材料, 预测的准确性更高(Riedelsheimer等2013)。然而, 如果亲缘关系利用过度, 遗传变异将受到限制, 进而影响遗传增益。此外, 标记和QTL之间的LD程度也会影响基因组预测的准确性, 随着世代的增加, 标记和QTL间的LD会逐渐降低, 在基因型测定后的前两个世代基因组预测的准确性下降较快, 其他世代下降速度则相对减慢, 随着世代的增加, 遗传力较高性状的基因组预测准确性降低较慢(Meuwissen等2001)。不同性状的基因组预测准确性大不相同, 这主要是由遗传力不同导致的, 遗传力和预测准确性通常呈正相关(Wang等2018b)。

除上述遗传因素外, 统计模型是影响基因组预测准确性的重要因素。进行基因组预测时面临的是“大*P*, 小*n*”问题, 即标记数目远大于样本量, 这种情况容易导致多重共线性和过度参数化。为了解决这些问题, 已发展出了很多基因组预测模型, 包括基因组最佳线性无偏估计(GBLUP)、贝叶斯方法、支持向量机、再生核希尔伯特空间(RKHS)、随机森林、深度学习等多种方法。其中GBLUP从整体上分析样本间的遗传关系, 将所有位点赋予相同的遗传方差, 因此在实际数据的分析中有较强的稳健性, 对微效多基因控制的数量性状分析更具优势(VanRaden 2008)。BayesA、BayesB和Bayesian LASSO等选择压缩算法则对大部分位点的效应进行压缩, 因此擅长捕获基因组上的显著效应(González-Recio和Forni 2011; Pérez和Campos 2014)。各种贝叶斯方法的主要区别在于它们选择了不同的先验分布, 继而产生不同的压缩程度。模拟研究表明, 选择压缩算法对QTL的数目较为敏感, 当性状由较少数目的QTL控制时, 预测准确性较高; 当影响数量性状的QTL数目很多时, 预测准确性会下降(Wang等2015)。随机森林和RKHS方法更擅于捕获非加性效应, RKHS利用高斯核函数拟合模型, 模型可通过贝叶斯框架下的抽样方法求解, 也可根据混合线性模型求解(De los Campos等2010)。支持向量机是一种典型的非参数方法, 核函数的选择是支持向量机的关键, 需要反映训练样本的分布特性(Maenhout等2007)。深度学习则是包含多个隐藏层的多层感知器, 能够捕捉数据中蕴含的复杂非线性关系, 近年来在作物多环境、多组学预测研究中获得了一定成效(Montesinos-López等2021)。

6.2 提升全基因组预测准确性的主要策略

全基因组选择虽然为作物育种提供了新的前景, 但是对受环境影响较大的产量及其相关的数量性状来说, 基因组预测的准确性仍较低(徐扬等2021)。研究人员已尝试采取以下一些策略来提高对复杂性状的预测力。(1)将功能标记纳入GS模型。在GS模型中加入已知基因或SNPs的先验信息, 有可能揭示复杂性状的遗传结构。在缺乏基因先验信息时, GWAS鉴定的显著位点也可以被视为固定效应协变量。GWAS和GS联合策略很大程度上依赖于特定性状的遗传结构, 该策略更适合少数大效应QTL控制的性状(Bian和Holland 2017)。(2)构建基因型与环境互作GS模型。作物育种中经常进行多环境试验, 整合基因型和环境互作(G×E)可以利用环境之间的相关信息。多项研究表明, 与单一环境下的模型相比, 将G×E效应纳入模型能够有效提高预测能力(Crossa等2016; Cuevas等2017)。徐云碧团队提出了基因组环境组集成预测(iGEP)新策略, 并指出未来育种应当在基因型、表型和环境型信息支撑下, 准确预测特定基因型在特定环境下的表现(Xu等2022b)。(3)开展多性状联合预测。多性状联合分析既可利用性状之间的遗传相关信息, 又可利用环境相关信息, 尤其利于一些低遗传力性状的选择。Cheng等(2018)提出了一种通用的多性状BayesCπ和BayesB方法, 同时开发了一个开源软件JWAS来实现多性状GS的分析。计算复杂度是多性状模型的主要限制, 通过整合HAT方法与BLUP模型, 建立双变量GS (2D GS)模型, 可大幅提

高计算效率(Wang等2021b)。此外, 选择指数可利用性状间的遗传相关性构建一个综合指标进行多性状的联合选择, Wang等(2019b)提出了一种基于选择指数的GS方法, 该方法能够利用与目标性状相关的多个辅助性状及其蕴含的目标性状遗传信息, 构建选择指数以实现对目标性状的辅助预测。(4)整合多组学信息。传统的基因组预测无法捕获基因的复杂互作及其下游调控, 随着多组学技术的快速发展, 利用转录组、代谢组等组学信息开展预测已逐渐得到关注。Meyer等(2007)首次在拟南芥中用代谢物预测生物量, 预测值与真实值的相关系数达到0.58。Riedelsheimer等(2012)利用285份玉米自交系的基因组和代谢组数据及570份测交种的表型数据, 预测了7个农艺性状的一般配合力, 发现130个代谢物的预测效果不亚于使用5万个SNP标记。Westhues等(2017)开展了玉米杂交种表型的多组学预测研究, 发现基因组和转录组数据的联合预测效果最佳。徐辰武团队建立了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, 并首次提出了将亲本表型信息纳入水稻杂交种表型预测的新策略(Xu等2020d)。李慧慧团队提出利用植物多组学数据进行全基因组预测的深度学习方法(Wang等2023b)。

6.3 全基因组选择育种面临的挑战

尽管GS技术拥有巨大应用前景, 我国的GS育种发展仍面临着众多挑战, 主要包括以下几点: (1)基因分型成本是GS技术推广和应用的重大障碍, 许多研究机构和育种企业由于高昂的基因分型成本而无法大规模应用; (2)前沿基因组选择育种模型原创性不足, 育种决策支持软件和平台开发与应用不足; (3)高效表型精准鉴定技术尚不完善, 转录组、代谢组、环境组等多维数据尚未有效应用于基因组选择育种; (4)种业资源较为分散, 缺乏足够的历史数据积累和规范化的管理, 从而使不同研究的数据和结果难以相互利用, 限制了基因组选择效率的提高。因此, 未来可以考虑开发全基因选择育种专用芯片, 降低单个基因分型成本至百元内; 进一步强化人工智能、大数据等信息技术的交叉集成, 创新发展适用于多组学、多环境等多维数据的全基因组选择模型; 制定多维度数据采集、分析、存储与管理的标准与规范, 协同建立通用的育种大数据平台, 实现育种信息的充分共享与利用。全基因组选择育种技术的优化升级将为作物育种的精准化、高效化和规模化发展提供不竭动力。

7 植物合成生物学研究进展

近年来, 随着分子生物学、系统生物学等技术的快速发展, 利用植物为底盘的合成生物学研究也取得了长足的进步(邵洁等2020)。植物合成生物学已成为农业、工业和医疗等领域的研究热点。

7.1 光合效率提升

产量和品质是作物生产的两个关键要素。提升作物产量是植物合成生物学研究的第一要务, 而提高光合作用效率则是提升农作物产量的重要途径之一。合成生物技术体系的出现和发展为系统改造光合作用、提高光合效率, 从而改良作物产量提供了一条崭新的途径(Long等2005, 2015; Zhu等2010; 张立新等2017)。

植物光合作用研究的核心是揭示光合系统高效吸能、传能、转能和固碳的分子调控机制, 进而优化光合系统、提高光能利用效率和生物量产率。研究表明优化以下三个主要光合作用过程能有效提升光合效率。(1)光能高效吸收、传递。主要包括优化捕光天线系统; 提高光合膜上的电子传递效率; 增强电能转化为ATP和NAD(P)H的效率(Blankenship等2011; Pesaresi等2009; Ort等2011; Kirst等2014)。(2)光能高效利用。主要包括降低非光化学淬灭等能量损耗; 增强光保护、减轻光抑制等带来的光合效率下降(Zhu等2004; Kromdijk等2016; Liu等2012; Peng等2006; Leister 2012)。(3)提高光合碳同化效率。主要包括提高RuBisCO酶的羧化活性; 引进CO_2浓缩机制; 减少碳损耗、降低光呼吸(Giordano等2005; Occhialini等2016; Price等2013; Prins等2016; Xin等2015; 张智胜和彭新湘 2016)。

在利用合成生物学策略提高作物光合效率方面, Kromdijk等(2016)通过促进叶黄素循环中紫黄质和玉米黄质的转换、增加光系统II亚基数量, 最终提高光合作用效率和生物质产量。South等(2019)使用合成生物学的方法重新设计光呼吸途径, 使转基因烟草的生长量比野生型提高40%。华南农业大学

彭新湘团队在水稻中建立了新的光呼吸旁路, 使光合作用效率、生物质产量和氮含量等显著提高, 为水稻增产提供了新的途径(Shen等2019; Wang等2020b)。中国科学院分子植物科学卓越创新中心郭房庆课题组突破传统光合作用的研究思维, 将叶绿体D1蛋白编码基因加上热诱导开关, 并融合到细胞核基因组中, 发现其可以显著提高拟南芥、水稻、烟草的耐热能力, 促进植物生长和光系统II修复, 提高生物质积累和作物产量, 且相关转基因水稻的大田产量提高20% (Chen等2020)。随着光合作用研究在理论认知和工程化改造水平上取得的根本性突破, 利用合成生物学手段优化提高光合效率正从概念验证转入生产实践阶段。

7.2 作物营养强化

在提升产量的基础上, 进一步实现重要作物的营养强化是植物合成生物学研究的重要目标。作物营养强化的最经典案例是在水稻胚乳中表达类胡萝卜素合成基因, 实现胚乳中类胡萝卜素的大量合成, 产生黄金大米(Ye等2000; Paine等2005), 开创了植物合成生物学的新时代。随着多基因组装技术的进一步发展(Zhu等2020), 研究者可组装更复杂的代谢途径, 如在水稻胚乳中定向合成虾青素和花青素等化合物, 产生赤晶米和紫晶米等营养强化大米(Zhu等2017, 2018)。在其他主粮(如玉米)中也实现了花青素、虾青素等营养物质的大量合成(Zhu等2018; Liu等2021b)。

近年来, 番茄等高产园艺植物也成为营养强化的对象。通过在果实中特异表达金鱼草中调控花青素合成的转录因子基因*AmDelila*和*AmRosea1*, 英国约翰英纳斯中心团队成功创制了在果实成熟阶段大量合成花青素的紫番茄(Butelli等2008)。紫番茄除了有很高的营养价值(Butelli等2008), 还具备耐储存和抗灰霉病的特点(Zhang等2013)。这表明植物合成生物学除实现作物营养强化外, 还可提升农艺性状。此外, 在番茄果实中特异表达拟南芥转录因子*AtMYB12*, 可引导代谢流流向莽草酸途径, 创制出合成重要苯丙烷类化合物的通用底盘(Zhang等2015b)。目前, 基于该系统已实现了在番茄果实中合成白藜芦醇、大豆异黄酮和L-多巴胺等营养物质(Zhang等2015b; Breitel等2021)。基因编辑技术的兴起和发展也将作物营养强化推入新的阶段。以番茄为例, 通过特异性敲除竞争性支路或者去除关键合成酶的活性抑制区域, 获得了维生素D富集(Yang等2022c)和富含γ-氨基丁酸的新品种(Nonaka等2017)。中国科学院遗传与发育研究所李传友团队利用基因编辑技术对花青素、类胡萝卜素和叶绿素途径进行随机编辑和组合, 快速定向创制七种不同果色的番茄材料(Yang等2022d)。2021年, 日本政府批准了富含γ-氨基丁酸的番茄新品种上市, 使其成为全世界首个上市的基因编辑食品(Nonaka等2017; Waltz 2021)。随着世界各国对基因编辑食品立法的完善和技术的不断进步, 未来将有更多的营养强化食品进入市场。

7.3 基于植物底盘的蛋白表达体系

与传统的微生物和动物底盘相比, 植物生长只需要阳光、空气、水和土壤。近年来, 如何利用植物底盘, 通过分子农场生产药用重组蛋白已成为植物合成生物学的一个新研究方向(Fausther-Bovendo和Kobinger 2021)。2012年, 辉瑞公司的戈谢病治疗蛋白药物是全世界上市的第一款以植物为底盘生产的药用蛋白(Pelosi等2012)。虽然植物底盘的生长周期和产量还不能和优化的微生物底盘相比, 但随着近年瞬时转化技术的不断提升, 利用植物底盘生产重组蛋白的周期已缩短至3周内, 为大规模的快速生产提供了必要条件(Sainsbury 2020)。

疫苗是植物生产的重要重组蛋白产品。利用植物底盘瞬时表达系统可快速生产病毒样颗粒(VLP), 其产物可在减少依赖佐剂的情况下有效激发动物体的免疫反应(Fausther-Bovendo和Kobinger 2021)。目前, 在兽用疫苗领域快速发展的基础上, 人用疫苗领域已有多项植物源疫苗进入三期临床实验(Ward等2020)。自2019年底新型冠状病毒(COVID-19, 简称新冠)疫情爆发以来, 如何快速安全的开发和生产新冠疫苗成为世界各国关注的热点(Mouro和Fischer 2022; Fausther-Bovendo和Kobinger 2021)。其中, 葛兰素史克(GSK)和Medicago公司基于烟草瞬时表达系统生产了植物源新冠疫苗Covifenz。该疫苗已于2022年2月获加拿大卫生部批准成为全世界第一款上市的植物源新冠疫苗(Mouro和Fischer 2022)。

7.4 基于叶绿体转基因技术的植物合成生物学研究

近年来, 基于叶绿体转基因技术的合成生物学研究也成为热点之一。通过精确的同源重组在叶绿体基因组中插入大量外源基因, 使叶绿体成为一个非常好的合成生物学研究的载体(Bock 2015; Yang等2022c)。植物的次生代谢产物合成往往需要不同的细胞器。如萜类合成中的甲基赤藓糖-4-磷酸(MEP)途径存在于叶绿体中, 因此在叶绿体中针对萜类物质进行代谢工程可以取得显著成效(Fuentes等2018)。以烟草、生菜等作为底盘, 利用叶绿体转基因技术实现了青蒿酸(Fuentes等2016)、虾青素(Harada等2014)、维生素E (Yabuta等2013)等重要次生代谢物的大量合成。此外, 通过叶绿体转基因技术提升植保素含量或者引入双链RNA, 可以显著改良植株的抗虫性(Zhang等2015a; Gnanasekaran等2016; Wu等2022a)。

除了以上领域, 近年来植物合成生物学在环境检测传感器、减少农作物化肥使用量、生物能源等领域也取得了重要进展(邵洁等2020)。未来, 随着国家相关政策的完善以及与其他生物技术的深度融合, 植物合成生物学技术将迎来进一步的发展。

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