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植物基因组编辑检测方法

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摘要：以CRISPR/Cas9技术为代表的基因组编辑在生物领域的革命性应用使得生命科学研究迈入新篇章。该技术以其灵活性、易用性且扩展性强等优势，大大加快了基因工程研究，也加速了植物分子育种的步伐。但是，遗传转化过程中产生大量潜在的基因编辑植株，使得早期高通量快速筛选和检测目标编辑植株面临很大挑战。本文综述了近年来植物基因组编辑检测的各种方法，比较了其优缺点和适用范围；同时，还对近几年植物基因组编辑检测方法的发展趋势进行了深入分析和展望，以期对基因组编辑技术在植物中的应用提供参考。

关键词：非同源末端连接；同源重组；PCR/RE；错配切割；Sanger 测序法

Detection methods of genome editing in plants

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Abstract: The life science has entered a new chapter with the revolutionary implementation of the CRISPR/Cas9 genome editing technology in various living organisms. With the unique flexibility, feasibility and extendibility, the CRISPR/Cas9 technology greatly accelerates genetic engineering research, as well as plant molecular breeding. However, it has become a challenge to screen for and identify genome-edited plants at early stages in a rapid and high-through fashion, due to the massive number of plants produced from transformation process. In this review, we summarize the molecular methods developed in the recent years to identify genome-edited plants. We compare their advantages and disadvantages, and the scope of application. In addition, we provide insights of the development trend of detection methods for plant genome editing. The review article will serve as a reference for future genome editing research in plants.

Keywords: non-homologous end joining (NHEJ); homologous recombination (HR); PCR/RE; mismatch cleavage assay; Sanger sequencing

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随着分子生物学技术的发展以及大量物种基因组测序的完成，以序列特异性核酸酶(sequence-specific nucleases, SSNs)为基础的基因组编辑 (genome editing) 在植物中得到迅猛发展。序列特异性核酸酶可以在基因组的特定位置产生DNA双链断裂 (double-strand breaks, DSBs) ，DSBs可以激发生物体的修复机制，从而在基因组的特定位置造成DNA序列的变化，进而改变基因的功能。近年来，成簇的规律间隔的短回文重复序列及其相关系统(clustered regularly interspaced short palindromic repeats/CRISPR-associated 9, CRISPR/Cas9 system)作为最新一代的基因组编辑工具，为植物基因功能研究和作物遗传育种带来了一场全新的技术革命。目前，CRISPR/Cas9技术已经被广泛地应用于各种植物的研究，包括模式植物拟南芥、各种农作物和树木[1~9]。与转基因改良作物相比，基因组编辑技术在最终产品中并不会引入任何外源基因，更类似于传统诱变育种产生的品种，因此成为一种非常受欢迎的作物遗传改良手段。

植物细胞中有两种途径可以修复DSBs：非同源末端连接(non-homologous end joining, NHEJ)和同源重组(homologous recombination, HR)。NHEJ在植物中是一条主要的修复途径，也是一种易错的修复途径，在DNA修复过程中，连接处容易产生少数碱基的插入或缺失，导致基因失活。HR是一条次要的修复途径，需要以同源片段作为模板进行修复，是一条精确的修复途径 [10]。由于HR在植物中的修复效率非常低，因此目前对基因功能的研究和作物改良主要借助NHEJ途径进行基因敲除。对于二倍体植物来说，NHEJ修复之后会产生5种可能的结果：（1）没有产生任何突变；（2）单等位基因突变，只有一个等位基因发生突变，也称杂合突变；（3）双等位基因突变，两个等位基因都发生突变，但是突变的类型不一样；（4）纯合突变，两个等位基因发生相同的突变；（5）嵌合体（chimeric），同一个样品上有3种或3种以上的突变类型。这种修复的多样性和不确定性对基因编辑检测提出更高的要求。对于多倍体植物，由于存在两个以上的等位基因，基因组的情况更为复杂，这对基因编辑的检测带来更高的挑战。本文对目前植物基因组编辑在目标位点的检测方法进行了综述，对各种检测方法进行了详细的介绍和对比，并进一步对各种检测方法的应用趋势进行了分析，以期为植物基因组编辑的应用提供参考。

1 不同类型植物基因组编辑的检测方法

1.1 PCR+RE（restriction endonuclease）方法

1.1.1 PCR/RE 方法

PCR/RE 是应用特异引物 PCR 和限制性酶切相结合而产生的一种检测方法，主要是通过对 PCR 扩增的 DNA 片段进行限制性酶切分析来区分目标位点是否被编辑。PCR/RE 也被称为限制性片段长度多态性 (restriction fragment length polymorphism，RFLP) 分析 [2, 11, 12] 、酶切扩增多态性序列（cleaved amplified polymorphic sequence，CAPS）方法 [13] 。具体操作步骤总结如下：首先设计 300~1700 bp 的扩增子，扩增子包含所要突变的目标片段；之后用限制性内切酶消化该扩增子；最终通过琼脂糖凝胶电泳检测，基于电泳条带的带型进行区分。条带完全被切开，表示该植株没有产生突变；条带部分被切开，表示该植株为杂合突变或嵌合体；条带完全没有被切开，表示该植株为纯合突变或双等位基因突变（图 1）。PCR/RE 方法是目前植物基因组编辑检测中应用最为广泛的方法之一[2, 5~7, 11~71]，尤其是应用在多倍体植物的检测中。目前，小麦基因组编辑的研究大都是应用这种方法检测[5, 27, 43, 53, 67]。与其他检

测方法相比，PCR/RE 方法优点如下：可以检测所有类型的突变，包括 SNP 和各种大小的插入缺失；具有很高的灵敏性；整个过程需要的时间也比较短，只需要几个小时；检测成本也较低；切割产物可以简单地通过琼脂糖凝胶来分辨，非常方便。PCR/RE 方法最大的局限性是在基因组编辑核酸酶切割的位点处需要有限制性酶切位点。

1.1.2 CAPS 衍生方法

CAPS 衍生方法（derived cleaved amplified polymorphic sequence，dCAPS），顾名思义，这种方法是在 CAPS 基础上发展起来的[72]。不同的是，需要在靠近突变位点的地方通过碱基错配引入一个酶切位点。酶切位点的引入可以通过 dCAPS Finder 2.0 软件 (http://helix.wustl.edu/dcaps/dcaps.html)[73] 来实现。设计 dCAPS 标记引物需要考虑以下几方面：首先引物中碱基的错配数目和位置都会影响扩增效率，错配越少，同时离引物 3′端越远，扩增效率越高；其次，错配的空间特性也会影响扩增效率，因为嘌呤与嘌呤的错配没有嘧啶与嘧啶的错配稳定，值得注意的是，所产生的限制性内切酶的切割性能和价格也是一个需要考虑的因素。由于需要错配碱基的引入，所以 dCAPS 的扩增子一般不会太大，通常是 200~300 bp。dCAPS 方法的优点与 PCR/RE 方法类似，但是突破了 PCR/RE 的限制，对 PCR/RE 方法有一定的补充作用。不过在设计上比 PCR/RE 要复杂，所以应用并不是很多[55, 74]。

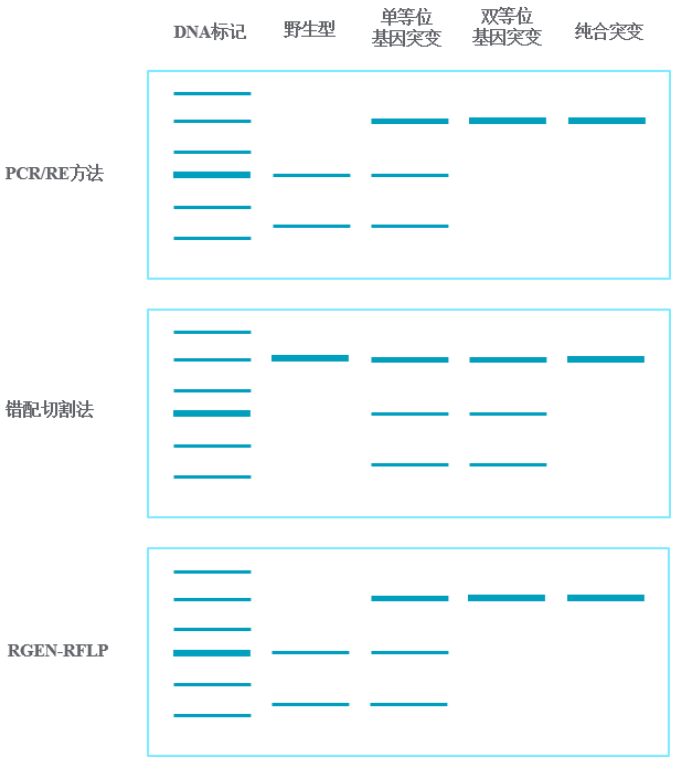

图 1 不同植物基因组编辑检测方法的比较示意图

Fig.1 Comparison of different assays to detect mutations induced by genome editing

1.1.3 限制性酶切 PCR 法

限制性酶切 PCR 法（restriction enzyme-PCR, RE-PCR）是与 PCR/RE 非常相似的一种方法，也是应用特异引物扩增和限制性酶切相结合产生的一种方法。不同之处在于，RE-PCR 是对基因组先进行酶切，然后再对酶切产物进行 PCR 扩增。如果基因组发生突变，相应的片段就不会被切开，因此可以扩增出条带，而野生型因为被切断，所以无法扩增出条带。这种方法的灵敏度与 PCR/RE 一样，目前在植物中应用较多[4, 33, 75~80]。但也有研究将两种方法结合[22, 35]，先对植物基因组进行酶切，然后进行 PCR 扩增，扩增后的产物再次进行酶切（RE-PCR-RE），这样也增加了结果的准确性。RE-PCR 方法的优点和 PCR/RE 类似，同样也受限制性酶切位点的限制。

1. 2 错配切割法

错配切割法（mismatch cleavage assay）是通过错配切割酶识别并切割异源双链核酸分子进行检测的一种方法。具体操作步骤如下：首先是用 PCR 方法扩增基因组的目标区域，然后将扩增子变性、退火产生异源双链核酸分子。这种异源双链核酸分子可以被错配切割酶切开，但是同源双链核酸分子不能被切开，可以通过切开条带的亮度来估算插入缺失的频率（图 1）。错配切割酶的优势在于不受限制性酶切位点的限制。但是该方法经常会低估突变频率，因为它只能检测出单等位基因突变和双等位基因突变类型，不能检测出纯合的基因突变类型。目前，可以应用的错配切割酶有很多种，包括 T7EI、Surveyor（ CelI）和 Cruiser。T7EI 最为常见[23, 24, 27, 36, 57, 67, 75, 81~91]；其次是 Surveyor[37, 42, 59, 92~98]；报道最少的是 Cruiser[36]。这 3 种酶也各有优缺点：T7EI 存在假阳性，因为它能够切割 Holiday 结构和十字（Cruciform）结构；Surveyor 和 Cruiser 特异性稍高一些，但价格相对昂贵。每种方法的灵敏度也不同，T7EI 是 0.5%~5%[99]的突变率可以被检出，Surveyor 大约是 10%[100] 的突变率才可以被检出。

1.3 Sanger 测序法

Sanger 测序（Sanger sequencing）是基于一代测序技术来检测基因组编辑情况的方法。目前关于植物基因组编辑的研究中，该方法所占的比例很高[5, 52, 55, 57, 60, 61, 68, 74, 90, 101~162]（图 2）。用来检测基因组编辑的方法分为两种：一种是 PCR 产物直接测序，通过测序图谱的峰值来判断是否发生编辑，该方法被称为 Sanger sequencing chromatograms。多数情况下，这种方法只能知道是否发生了突变，不能判断突变的确切类型。Brinkman 等[163]和 Liu 等[164]分别开发了相关的软件可以将测序的峰图进行解码，进而分辨出具体的突变类型。但是，这种方法只能分辨同时含有两种基因型的突变，超过两种以上的突变无法用该软件解码。由于这些软件的推广，目前越来越多的研究采取 Sanger 测序的方法来检测基因组编辑[129, 137, 138, 144]。另外一种方法是将 PCR 产物进行克隆测序[9, 162]，这种方法得到的结果更为精确，可以确切判断具体的突变类型，同时也可以测定两种以上的突变类型。实际上，大部分的研究通过其他的方法进行初筛之后，最终还是选择 Sanger 测序来确定最终的突变类型[11~27, 81~91]。

1.4 下一代测序法

随着测序技术的发展，费用更低、通量更高的下一代测序技术（next-generation sequencing, NGS）应运而生。下一代测序的核心思想是边合成边测序，通过捕捉新合成的末端的标记或者合成过程释放的特殊标记来确定 DNA 序列。目前仍在使用的平台有 4 个：

Illumina 平台、Roche 454 平台（ 已经退市）、Ion torrent - Proton / PGM 平台和 PacBio 平台。植物基因组编辑主要使用 Roche 454 和 Illumina 两种精度更高的测序平台，大部分研究使用了 Illumina 平台[46, 53~54, 57, 85~86, 96, 142, 165~176]，少数研究使用了 Roche 454 平台[118, 177~180]，极少数研究使用了 PacBio 平台[181]。这与测序技术的更新换代有着密切的联系，随着 454 平台的淡出，更多的基因编辑检测会使用 Illumina 平台。与 Sanger 测序相比，下一代测序可以同时产生成千上万的数据，通量大大提高。由于涉及到大量并行数据读取，下一代测序需要的时间会更长（Ion torrent 除外），后续的数据分析也更复杂。并且，下一代测序技术读长都比较短，并不适合大片段缺失的检测。

1.5 TaqMan 方法

TaqMan 方法是在 PCR 体系中加入一个与目标区域互补的荧光探针，探针的 5′端标记一个发光基团，3′端标记一个淬灭基团。当进行 PCR 扩增时，探针与目标区域特异地结合在一起。随着 PCR 的延伸，聚合酶的 5′外切活性会将探针切下，释放出发光基团，继而发出的荧光信号可以被检测到，从而通过荧光信号的强弱推测植物体内目标基因的拷贝数[182]。如果目标基因发生突变，拷贝数就会相应改变，从而可以推算出基因编辑的情况[183~187]。TaqMan 的通量非常高，通常以 96/384 孔板的形式操作，灵敏度也很高，是一种可以高通量检测植物目标区域突变的方法。当然，这种方法也有其局限性，不能够区分双等位基因突变和纯合突变。同时，TaqMan 需要昂贵的仪器和试剂，例如合成不同荧光染料标记的探针，并不适合少量样品操作。所以目前报道的研究中，只有几家大规模的农业生物公司如杜邦先锋[182~185]和先正达[186, 187]应用了 TaqMan 方法。

1.6 扩增片段长度多态性

扩增片段长度多态性（amplified fragment length polymorphism，AFLP）的基本原理：通过对基因组DNA酶切片段的选择性扩增来检测DNA酶切片段长度的多态性。现在用于基因组编辑检测的AFLP标记已经不完全等同于之前的AFLP，而是指特异的引物扩增出来的不同长度的DNA片段，通过片段的长度来揭示基因组目标位点是否发生突变。这种标记只适用于大片段的缺失，不适用于小片段的插入或缺失，也不能检测出碱基的替换，适用范围比较有限。目前只有少数几篇植物基因组编辑的文章用到了这种方法[94,188,189]。

1.7 PCR单链构象多态分析法

单链构象多态分析法(single-strand conformational polymorphism, SSCP)是利用DNA单链构象具有多态性的特点，结合PCR技术进行多态分析的方法。在低温下，DNA单链呈现一种由内部分子相互作用形成的空间折叠，当其中一个碱基发生变化时，整条单链的空间构象也会发生变化，从而影响DNA分子在非变性胶中的迁移率，这样就可以区分不同的核酸分子。该方法可以有效地检测小片段的插入缺失和错配突变。Zheng 等[190]于2016年将该方法成功地应用于水稻基因组编辑的检测，灵敏度可以达到10%。Zhou 等[191]将该方法与RFLP相比，发现SSCP灵敏度更高。与其他DNA检测方法不同，SSCP检测的是单链DNA，几乎可以检测所有类型的突变，与PCR/RE相比，该方法不受酶切位点的限制，但是并不能确定最终的突变类型，只适用于初筛。

1.8 RGEN 介导的 RFLP

RGEN（RNA-guided endonucleases）介导的 RFLP 是通过 CRISPR/Cas9 或者 CRISPR/Cpf1 对目标片段的切割来判断基因组编辑情况的方法[192, 193]。RGEN 是包含了 Cas9 蛋白或者 Cpf1 蛋白和 gRNA 的复合物，可以特异性切割基因组编辑的目的位点。具体操作步骤：扩增基因组编辑的目标区域，然后再加入 RGEN 进行切割，如果之前已经被编辑就不能切开，没有编辑的可以被切割，通过琼脂糖凝胶可以清楚地区分基因组被编辑的情况（图 1）。与错配切割的方法相比较，该方法可以检测出纯合的突变类型，也能够区分单等位基因突变和双等位基因突变类型，但是不能够区分双等位基因突变和纯合突变的个体。当然该方法多用于检测 CRISPR 系统所产生的基因突变，少数情况下适用于 TALEN 产生的突变[193]。而且相比于限制性内切酶，CRISPR/Cas9 酶或者 CRISPR/cpf1 酶价格更高，所以该方法在植物基因组编辑的应用也很有限[85, 193]。

1.9 高分辨率片段分析方法

高分辨率片段分析（high-resolution fragment analysis, HRFA）是结合 PCR 和毛细管电泳的一种方法。具体操作步骤是采用 96 孔板进行 DNA 提取、PCR 扩增，然后毛细管电泳，就可以分辨出不同类型的突变。或者在扩增引物上添加荧光标记，引物覆盖目标位点，加上荧光检测可以区分不同大小的产物。该方法的灵敏度比较高，可以区分小到 1 bp 的插入或缺失。另外，也可以区分多种不同大小的突变。这种方法的局限是不能检测出含有单碱基替换的突变，也不能区分具有相同大小插入缺失的突变。通过设计不同长度的扩增片段，同时结合不同的荧光标记，HRFA 方法可以很容易地分析多个基因位点发生突变的情况。该方法更适用于多倍体植物的检测，Andersson 等[194]利用该方法一次检测了 4 个基因位点的突变。

1.10 高分辨率溶解曲线分析方法

高分辨率溶解曲线分析（high-resolution melting analysis, HRMA）方法是一种基于单核苷酸溶解温度不同而形成不同溶解曲线的检测方法。具体步骤：设计 PCR 引物进行扩增，扩增子大小一般是 90~200 bp，应用实时定量 PCR 加上相应的荧光染料，然后通过分析溶解曲线的方法来分析突变类型。该方法具有极高的灵敏度，并且在 PCR 之后不需要酶切和电泳分析，可以在几分钟之内得到结果，特别适用于高通量操作。Thomas 等[195]最早将这种方法应用在斑马鱼（*Barchydanio rerio* var）基因组编辑的检测，灵敏度可以达到 5%。Hilioti 等[196]将该方法应用于植物基因组编辑的检测。

1.11异源双链泳动分析法

异源双链泳动分析法（heteroduplex mobility assay, HMA）是根据野生型和突变型DNA分子经过变性和退火后会产生异源双链DNA分子，按照同源双链分子和异源双链分子在非变性聚丙烯酰胺凝胶中电泳的泳动速度不同来区分基因组有没有发生突变。另外，也可以利用微芯片电泳系统来进行区分。与错配切割方法相比，省去了酶切步骤，避免了切割不完全导致的假阳性，但是容易错过大片段的缺失突变。Ota等[197]最早将该方法应用在斑马鱼基因组编辑的检测，之后该方法在植物基因组编辑检测中得到推广[41,42, 49]。

1.12 单等位基因识别 PCR

单等位基因识别 PCR（simple allele-discriminating PCR，SAP）的原理是基于 ARMS (amplification refractory mutation system)，即 PCR 的延伸是基于 3′末端与模板的完全匹配，

当 3′端不完全匹配时候，会有不稳定的效应。在有些情况下，3′末位的单碱基错配并不能够很好地区分野生型和突变型，需要在倒数第二位引入错配来增加 PCR 的特异性。Bui 等[198]最初用 SAP 方法区分拟南芥的突变体和野生型，之后 Morineau 等[120]将该方法用于基因组编辑检测。这种方法不受限制性内切酶的限制，但对 PCR 引物的设计有较高的要求。而且 CRISPR/Cpf1 系统的突变多为多碱基的缺失，所以不适合用这种方法进行检测。

1.13 ACT-PCR

Hua 等 [199] 通过控制 PCR 成功的两个关键因素，即特异的引物以及适宜的退火温度实现了基因组编辑的检测，这种方法被称为 ACT-PCR（at critical temperature PCR）。在临界退火温度下，特异引物不能和突变体严格匹配，也就不能有效地进行扩增，因此可以用来区分野生型和基因编辑的突变体。该方法只需要进行一次常规的 PCR 反应，便可以很快地检测出成功编辑的个体，是一种简单准确、快速经济的方法，不过这也对 PCR 引物的设计和扩增条件提出较高要求。利用 ACT-PCR，研究人员不仅在水稻中鉴定出基因编辑的个体，同时也在斑马鱼中成功鉴定出基因组编辑的个体，表明 ACT-PCR 的应用不受物种的限制[199]。

1.14 微滴数字 PCR

微滴数字PCR（droplet digital PCR, ddPCR）被称为第三代PCR，可以利用有限稀释、终点PCR和泊松分布来实现核酸浓度的绝对定量。该方法最早应用在人类细胞系基因组编辑检测方面[200, 201]，随后Gao等[202]将该方法应用在植物基因组编辑检测。与错配切割法相比，该方法需要很少的模板量，具有很高的灵敏度，适合高通量操作。

2 植物基因组编辑检测方法的现状及发展趋势

本文总结了目前植物基因组编辑领域的所有检测方法，并对近几年国内外具有较高影响力期刊中近 200 篇文章所涉及的检测方法进行了分类归纳。图 2 显示不同方法在植物基因组编辑研究中的应用次数，可以看出不同实验室使用的方法比较集中。PCR/RE 和 Sanger 测序占据非常大的比例，下一代测序技术和错配切割法应用也比较广泛。其他方法的应用相对较少，有的方法仅被报道过一次[120, 194, 196, 199]，说明这些方法需要进一步优化。当然，大部分研究应用了不止一种方法，而是结合多种方法来分析不同的目标基因，说明每种方法各有优缺点，有的研究甚至使用了 3 种及以上的方法来检测基因组编辑的情况[42, 55, 57]。

选择何种方法进行植物基因组编辑的检测，与诸多因素有关，现总结如下：

（1）基因组编辑效率。如果突变频率很低，花费较高的 Sanger 测序方法不适用；如果突变频率高，高通量检测方法不具有优势，科研人员可以直接利用 Sanger 测序，从少量的样品中就可以得到需要的突变类型。

（2）植物的倍性。有些检测方法不适用多倍体植物检测，例如 TaqMan 方法在多倍体小麦的检测中很难设计出合适的引物和探针，而 PCR/RE 和 NGS 却是不错的选择。目前，关于小麦基因组编辑的研究也大多利用这两种方法 [5, 27, 43, 53, 67] 。

（3）基因组编辑工具。大部分方法都适用于 CRISPR/Cas9 系统，因为这些方法也大都是针对该酶而开发的，尤其是 RGEN 介导的 RFLP。但是，如 Base editing 这样的工具，由于其造成的变化限定在特定的范围，科研人员更倾向于利用下一代测序的方法来检测。由于

CRISPR/Cpf1 系统一般会造成多碱基的缺失，很难用 SAP 或者 ACT-PCR 方法设计出合适的引物进行检测。

本文统计了 2013~2017 年间植物基因组编辑检测方法的发展趋势（图 3）。为了便于统计，本文将 PCR/RE、dCAPS 和 RE-PCR 统一归为 PCR+RE，而把数量很少的检测方法归为其他。从表 3 中可以看出，Sanger 测序所占的比例由 2013 年的 9.5%上升到 2017 年的 45.2%。PCR+RE 方法所占的比例在逐渐下降，由 2013 年的 66.7%下降到 2017 年的 27.4%。推测原因如下：（1）测序技术的快速发展，成本有所降低；（2）Brinkman 和 Liu 等[163,164]开发的解码软件大大简化了 Sanger 测序的流程，加快了 Sanger 测序直接用作基因编辑检测手段的推广；（3）测序的结果能更直接地反映突变的类型，所有的筛选方法初筛之后最终都需要测序来确定突变类型，如果成本和通量差不多，那人们将会首选 Sanger 测序。

下一代测序技术由于高准确性、高通量、高灵敏度以及低运行成本等突出优势，在基因组编辑检测中的应用也越来越广泛，呈现出一种上升的趋势。未来如果能将时间缩短，并且简化分析流程，该方法将是一个非常不错的选择。从图 3 也可以看出，2015 年和 2016 年报道了很多新的方法用来检测植物基因组编辑，但是这些方法却没有得到推广，这也对科研人员开发新的检测方法提出了更高的要求。

随着越来越多植物基因组测序的完成，解读与改造基因的功能更为紧迫，从低等的苔藓到高等的树木，从二倍体的拟南芥到多倍体的小麦，以 CRISPR/Cas9 为代表的基因组编辑技术无不显露出其强大的优势，这也对植物基因组编辑的检测方法提出更高的挑战。目前，还有一些检测方法在其他物种的基因组编辑中也有报道[203, 204]，但在植物中尚未尝试。将来可以根据植物基因组编辑的具体情况，考虑是否可以开发为植物基因组编辑的检测方法。

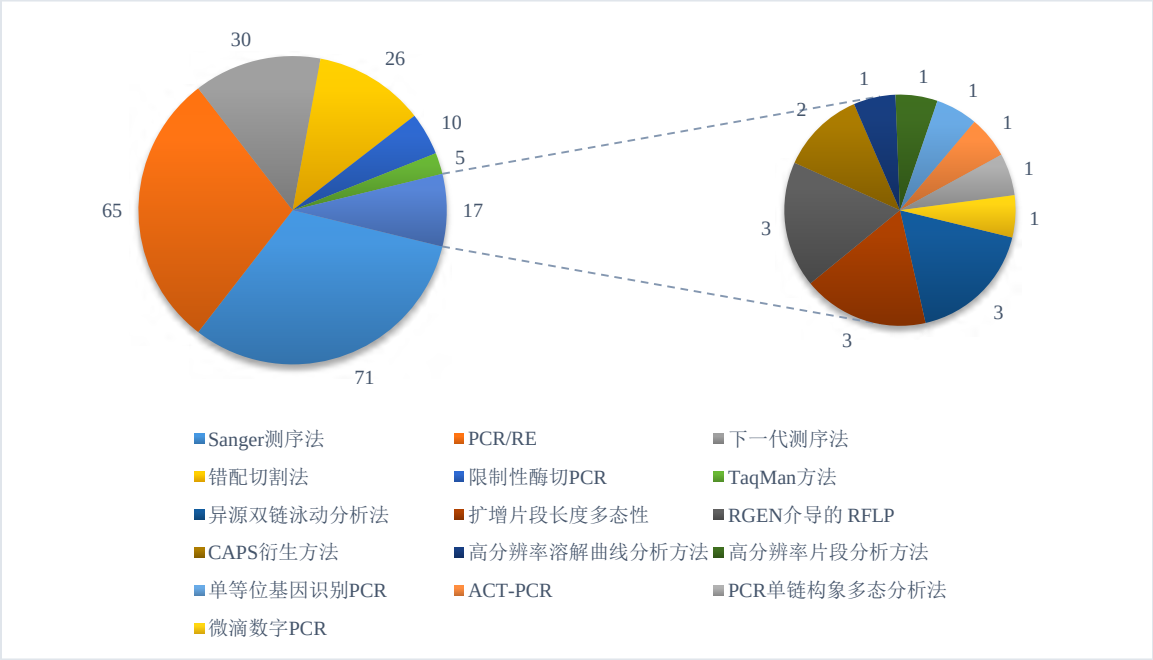

图 2 植物基因组编辑检测方法应用次数分布双饼分布图

Fig.2 Double pie distribution graphs showing application frequencies of genome editing detection methods

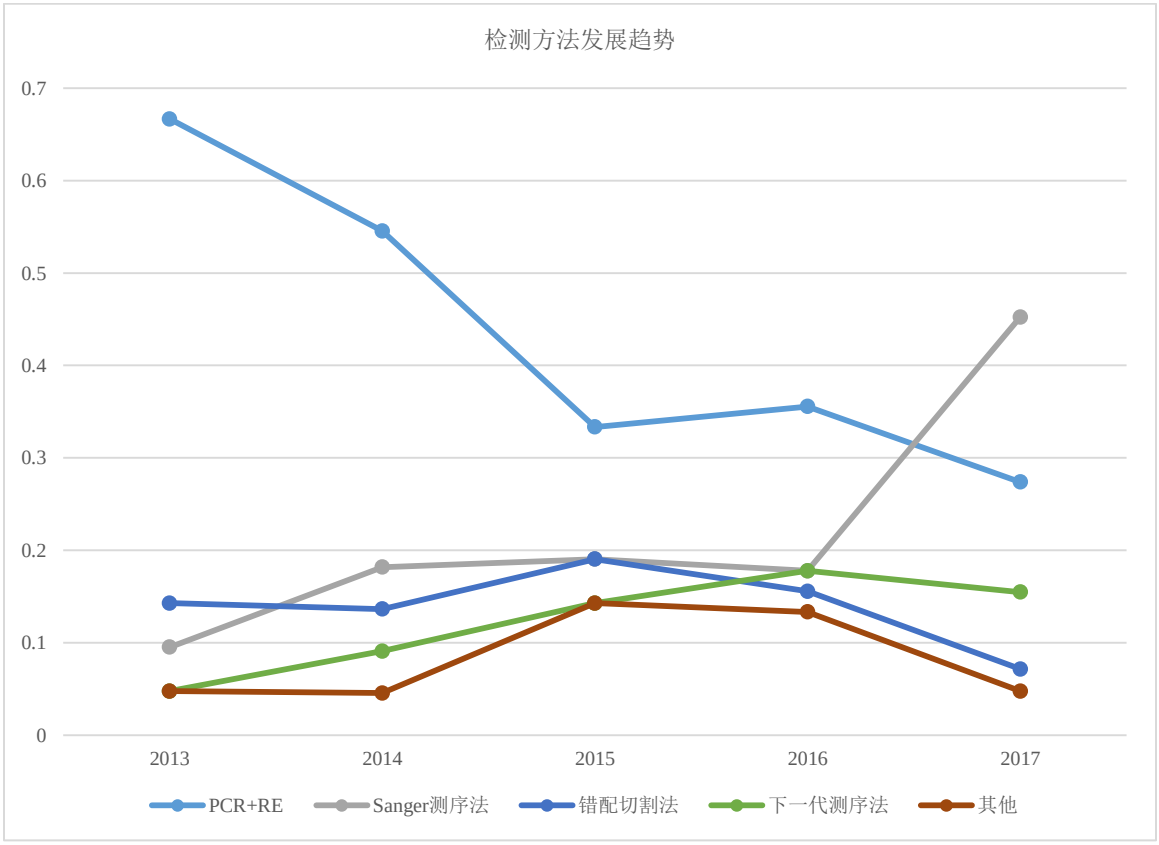

图 3 植物基因编辑检测方法发展趋势

Fig.3 The trend for detection methods in plant genome editing

每个点代表应用此种检测方法的研究占该年所有研究总数的比例。

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参考文献(References)

[1] Li JF, Norville JE, Aach J, McCormack M, Zhang D, Bush J, Church GM, Sheen J. Multiplex and homologous recombination-mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9. *Nat Biotechnol*, 2013, 31(8): 688-691.

[2] Feng Z, Zhang B, Ding W, Liu X, Yang DL, Wei P, Cao F, Zhu S, Zhang F, Mao Y, Zhu JK. Efficient genome editing in plants using a CRISPR/Cas system. *Cell Res*, 2013, 23(10): 1229-1232.

[3] Lozano-Juste J, Cutler SR. Plant genome engineering in full bloom. *Trends Plant Sci*, 2014, 19(5): 284-287.

[4] Jiang W, Zhou H, Bi H, Fromm M, Yang B, Weeks DP. Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in *Arabidopsis*, tobacco, sorghum and rice. *Nucleic Acids Res*, 2013, 41(20): e188.

[5] Upadhyay SK, Kumar J, Alok A, Tuli R. RNA-guided genome editing for target gene mutations in wheat. *G3 (Bethesda)*, 2013, 3(12): 2233-2238.

[6] Liang Z, Zhang K, Chen K, Gao C. Targeted mutagenesis in *Zea mays* using TALENs and the CRISPR/Cas9 system. *J Genet Genom*, 2014, 41(2): 63-68.

[7] Jia H, Wang N. Targeted genome editing of sweet orange using Cas9/sgRNA. *PLoS One*, 2014, 9(4): e93806.

[8] Gao W, Long L, Tian X, Xu F, Liu J, Singh PK, Botella JR, Song C. Genome editing in cotton with the CRISPR/Cas9 system. *Front Plant Sci*, 2017, 8: 1364.

[9] Fan D, Liu T, Li C, Jiao B, Li S, Hou Y, Luo K. Efficient CRISPR/Cas9-mediated targeted mutagenesis in populus in the first generation. *Sci Rep*, 2015, 5: 12217.

[10] Puchta H. The repair of double-strand breaks in plants: mechanisms and consequences for genome evolution. *J Exp Bot*, 2005, 56(409): 1-14.

[11] Sugano SS, Shirakawa M, Takagi J, Matsuda Y, Shimada T, Hara-Nishimura I, Kohchi T. CRISPR/Cas9-mediated targeted mutagenesis in the liverwort *Marchantia polymorpha* L.

***Plant Cell Physiol*, 2014, 55(3): 475-481.**

[12] Yan L, Wei S, Wu Y, Hu R, Li H, Yang W, Xie Q. High-efficiency genome editing in *Arabidopsis* using YAO promoter-driven CRISPR/Cas9 system. *Mol Plant*, 2015, 8(12):1820-1823.

[13] Mikami M, Toki S, Endo M. Comparison of CRISPR/Cas9 expression constructs for efficient targeted mutagenesis in rice. *Plant Mol Biol*, 2015, 88(6): 561-572.

[14] Lloyd A, Plaisier CL, Carroll D, Drews GN. Targeted mutagenesis using zinc-finger nucleases in *Arabidopsis*. *Proc Natl Acad Sci USA*, 2005, 102(6): 2232-2237.

[15] Zhang F, Maeder ML, Unger-Wallace E, Hoshaw JP, Reyon D, Christian M, Li X, Pierick CJ, Dobbs D, Peterson T, Joung JK, Voytas DF. High frequency targeted mutagenesis in *Arabidopsis thaliana* using zinc finger nucleases. *Proc Natl Acad Sci USA*, 2010, 107(26): 12028-12033.

[16] Curtin SJ, Zhang F, Sander JD, Haun WJ, Starker C, Baltes NJ, Reyon D, Dahlborg EJ, Goodwin MJ, Coffman AP, Dobbs D, Joung JK, Voytas DF, Stupar RM. Targeted mutagenesis of duplicated genes in soybean with zinc-finger nucleases. *Plant Physiol*, 2011, 156(2): 466-473.

[17] Zhang Y, Zhang F, Li X, Baller JA, Qi Y, Starker CG, Bogdanove AJ, Voytas DF. Transcription activator-like effector nucleases enable efficient plant genome engineering. *Plant Physiol*, 2013, 161(1): 20-27.

[18] Wendt T, Holm PB, Starker CG, Christian M, Voytas DF, Brinch-Pedersen H, Holme IB. TAL effector nucleases induce mutations at a pre-selected location in the genome of primary barley transformants. *Plant Mol Biol*, 2013, 83(3): 279-285.

[19] Pater S, Pinas JE, Hooykaas PJJ, Zaal BJ.. ZFN-mediated gene targeting of the *Arabidopsis* protoporphyrinogen oxidase gene through Agrobacterium-mediated floral dip transformation. *Plant Biotechnol J*, 2013, 11(4): 510-515.

[20] Shan Q, Wang Y, Chen K, Liang Z, Li J, Zhang Y, Zhang K, Liu J, Voytas DF, Zheng X, Zhang Y, Gao C. Rapid and efficient gene modification in rice and *Brachypodium* using TALENs. *Mol Plant*, 2013, 6(4): 1365-1368.

[21] Shan Q, Wang Y, Li J, Zhang Y, Chen K, Liang Z, Zhang K, Liu J, Xi JJ, Qiu JL, Gao C. Targeted genome modification of crop plants using a CRISPR-Cas system. *Nat Biotechnol*, 2013, 31(8): 686-688.

[22] Nekrasov V, Staskawicz B, Weigel D, Jones JD, Kamoun S. Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease. *Nat Biotechnol*, 2013, 31(8): 691-693.

[23] Qi Y, Li X, Zhang Y, Starker CG, Baltes NJ, Zhang F, Sander JD, Reyon D, Joung JK, Voytas DF. Targeted deletion and inversion of tandemly arrayed genes in *Arabidopsis thaliana* using zinc finger nucleases. *G3 (Bethesda)*, 2013, 3(10): 1707-1715.

[24] Christian M, Qi Y, Zhang Y, Voytas DF. Targeted mutagenesis of *Arabidopsis thaliana* using engineered TAL effector nucleases. *G3 (Bethesda)*, 2013, 3(10): 1697-1705.

[25] Baltes NJ, Gil-Humanes J, Cermak T, Atkins PA, Voytas DF. DNA replicons for plant genome engineering. *Plant Cell*, 2014, 26(1): 151-163.

[26] Jiang W, Yang B, Weeks DP. Efficient CRISPR/Cas9-mediated gene editing in *Arabidopsis thaliana* and inheritance of modified genes in the T2 and T3 generations. *PLoS One*, 2014, 9(6): e99225.

[27] Shan Q, Wang Y, Li J, Gao C. Genome editing in rice and wheat using the CRISPR/Cas system. *Nat Protoc*, 2014, 9(10): 2395-2410.

[28] Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu JL. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nat Biotechnol*, 2014, 32(9): 947-951.

[29] Xing HL, Dong L, Wang ZP, Zhang HY, Han CY, Liu B, Wang XC, Chen QJ. A CRISPR/Cas9 toolkit for multiplex genome editing in plants. *BMC Plant Biol*, 2014, 14: 327.

[30] Jiang WZ, Brueggeman AJ, Horken KM, Plucinak TM, Weeks DP. Successful transient expression of Cas9 and single guide RNA genes in *Chlamydomonas reinhardtii*. *Eukaryot Cell*, 2014, 13(11): 1465-1469.

[31] Lor VS, Starker CG, Voytas DF, Weiss D, Olszewski NE. Targeted mutagenesis of the tomato PROCERA gene using transcription activator-like effector nucleases. *Plant Physiol*, 2014, 166(3): 1288-1291.

[32] Gao J, Wang G, Ma S, Xie X, Wu X, Zhang X, Wu Y, Zhao P, Xia Q. CRISPR/Cas9-mediated targeted mutagenesis in Nicotiana tabacum. *Plant Mol Biol*, 2015, 87(1-2): 99-110.

[33] Sun X, Hu Z, Chen R, Jiang Q, Song G, Zhang H, Xi Y. Targeted mutagenesis in soybean using the CRISPR-Cas9 system. *Sci Rep*, 2015, 5: 10342.

[34] Shan Q, Zhang Y, Chen K, Zhang K, Gao C. Creation of fragrant rice by targeted knockout of the OsBADH2 gene using TALEN technology. *Plant Biotechnol J*, 2015, 13(6): 791-800.

[35] Feng C, Yuan J, Wang R, Liu Y, Birchler JA, Han F. Efficient targeted genome modification in maize using CRISPR/Cas9 system. *J Genet Genomics*, 2016, 43(1): 37-43.

[36] Du H, Zeng X, Zhao M, Cui X, Wang Q, Yang H, Cheng H, Yu D. Efficient targeted mutagenesis in soybean by TALENs and CRISPR/Cas9. *J Biotechnol*, 2016, 217: 90-97.

[37] Endo M, Mikami M, Toki S. Biallelic gene targeting in rice. *Plant Physiol*, 2016, 170(2): 667-677.

[38] Sun Y, Zhang X, Wu C, He Y, Ma Y, Hou H, Guo X, Du W, Zhao Y, Xia L. Engineering herbicide-resistant rice plants through CRISPR/Cas9-mediated homologous recombination of acetolactate synthase. *Mol Plant*, 2016, 9(4): 628-631.

[39] Tang X, Zheng X, Qi Y, Zhang D, Cheng Y, Tang A, Voytas DF, Zhang Y. A single transcript CRISPR-Cas9 system for efficient genome editing in plants. *Mol Plant*, 2016, 9(7): 1088-1091.

[40] Gao X, Chen J, Dai X, Zhang D, Zhao Y. An effective strategy for reliably isolating heritable and Cas9-free *Arabidopsis* mutants generated by CRISPR/Cas9-mediated genome editing. *Plant Physiol*, 2016, 171(3): 1794-1800.

[41] Kaya H, Mikami M, Endo A, Endo M, Toki S. Highly specific targeted mutagenesis in plants using *Staphylococcus aureus* Cas9. *Sci Rep*, 2016, 6: 26871.

[42] Osakabe Y, Watanabe T, Sugano SS, Ueta R, Ishihara R, Shinozaki K, Osakabe K. Optimization of CRISPR/Cas9 genome editing to modify abiotic stress responses in plants. *Sci Rep*, 2016, 6: 26685.

[43] Zhang Y, Liang Z, Zong Y, Wang Y, Liu J, Chen K, Qiu JL, Gao C. Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. *Nat Commun*, 2016, 7: 12617.

[44] Chandrasekaran J, Brumin M, Wolf D, Leibman D, Klap C, Pearlsman M, Sherman A, Arazi T, Gal-On A. Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. *Mol Plant Pathology*, 2016, 17(7): 1140-1153.

[45] Li J, Meng X, Zong Y, Chen K, Zhang H, Liu J, Li J, Gao C. Gene replacements and insertions in rice by intron targeting using CRISPR-Cas9. *Nat Plants*, 2016, 2: 16139.

[46] Cigan AM, Singh M, Benn G, Feigenbutz L, Kumar M, Cho MJ, Svitashev S, Young J. Targeted mutagenesis of a conserved anther-expressed P450 gene confers male sterility in monocots. *Plant Biotechnol J*, 2017, 15(3): 379-389.

[47] Jiang WZ, Henry IM, Lynagh PG, Comai L, Cahoon EB, Weeks DP. Significant enhancement of fatty acid composition in seeds of the allohexaploid, *Camelina sativa*, using CRISPR/Cas9 gene editing. *Plant Biotechnol J*, 2017, 15(5): 648-657.

[48] Tian S, Jiang L, Gao Q, Zhang J, Zong M, Zhang H, Ren Y, Guo S, Gong G, Liu F, Xu Y. Efficient CRISPR/Cas9-based gene knockout in watermelon. *Plant Cell Rep*, 2016, 36(3):399-406.

[49] Endo A, Masafumi M, Kaya H, Toki S. Efficient targeted mutagenesis of rice and tobacco genomes using Cpf1 from *Francisella novicida*. *Sci Rep*, 2016, 6: 38169.

[50] Li J, Sun Y, Du J, Zhao Y, Xia L. Generation of targeted point mutations in rice by a modified CRISPR/Cas9 system. *Mol Plant*, 2017, 10(3): 526-529.

[51] Gil-Humanes J, Wang Y, Liang Z, Shan Q, Ozuna CV, Sánchez-León S, Baltes NJ, Starker C, Barro F, Gao C, Voytas DF. High-efficiency gene targeting in hexaploid wheat using DNA replicons and CRISPR/Cas9. *Plant J*, 2017, 89(6): 1251-1262.

[52] Lu Y, Zhu JK. Precise editing of a target base in the rice genome using a modified CRISPR/Cas9 system. *Mol Plant*, 2017, 10(3): 523-525.

[53] Liang Z, Chen K, Li T, Zhang Y, Wang Y, Zhao Q, Liu J, Zhang H, Liu C, Ran Y, Gao C. Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. *Nat Commun*, 2017, 8: 14261.

[54] Tang X, Lowder LG, Zhang T, Malzahn AA, Zheng X, Voytas DF, Zhong Z, Chen Y, Ren Q, Li Q, Kirkland ER, Zhang Y, Qi Y. A CRISPR-Cpf1 system for efficient genome editing and transcriptional repression in plants. *Nat Plants*, 2017, 3: 17018.

[55] Soyk S, Müller NA, Park SJ, Schmalenbach I, Jiang K, Hayama R, Zhang L, Van Eck J, Jiménez-Gómez JM, Lippman ZB. Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. *Nat Genet*, 2016, 49(1): 162-168.

[56] Minkenberg B, Xie K, Yang Y. Discovery of rice essential genes by characterizing a CRISPR-edited mutation of closely related rice MAP kinase genes. *Plant J*, 2017, 89(3): 636-648.

[57] Zong Y, Wang Y, Li C, Zhang R, Chen K, Ran Y, Qiu JL, Wang D, Gao C. Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. *Nat Biotechnol*, 2017, 35(5): 438-440.

[58] Kaya H, Numa H, Nishizawa-Yokoi A, Toki S, Habu Y. DNA methylation affects the efficiency of transcription activator-like effector nucleases-mediated genome editing in rice. *Front Plant Sci*, 2017, 8: 302.

[59] Ueta R, Abe C, Watanabe T, Sugano SS, Ishihara R, Ezura H, Osakabe Y, Osakabe K. Rapid breeding of parthenocarpic tomato plants using CRISPR/Cas9. *Sci Rep*, 2017, 7(1): 507.

[60] Shen L, Hua Y, Fu Y, Li J, Liu Q, Jiao X, Xin G, Wang J, Wang X, Yan C, Wang K. Rapid generation of genetic diversity by multiplex CRISPR/Cas9 genome editing in rice. *Sci China Life Sci*, 2017, 60(5): 506-515.

[61] Shimatani Z, Kashojiya S, Takayama M, Terada R, Arazoe T, Ishii H, Teramura H, Yamamoto T, Komatsu H, Miura K, Ezura H, Nishida K, Ariizumi T, Kondo A. Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. *Nat Biotechnol*, 2017, 35(5): 441-443.

[62] Kaya H, Ishibashi K, Toki S. A split *Staphylococcus aureus* Cas9 as a compact genome-editing tool in plants. *Plant Cell Physiol*, 2017, 58(4): 643-649.

[63] Nakajima I, Ban Y, Azuma A, Onoue N, Moriguchi T, Yamamoto T, Toki S, Endo M. CRISPR/Cas9-mediated targeted mutagenesis in grape. *PLoS One*, 2017, 12(5): e0177966.

[64] Li CX, Liu CL, Qi XT, Wu YC, Fei XH, Mao L, Cheng BJ, Li XH, Xie CX. RNA-guided Cas9 as an *in vivo* desired-target mutator in maize. *Plant Biotechnol J*, 2017, 15(12): 1566-1576.

[65] Holme IB, Wendt T, Gil-Humanes J, Deleuran LC, Starker CG, Voytas DF, Brinch-Pedersen H. Evaluation of the mature grain phytase candidate HvPAPhy_a gene in barley (*Hordeum vulgare* L.) using CRISPR/Cas9 and TALENs. *Plant Mol Biol*, 2017, 95(1-2): 111-121.

[66] Yu Z, Chen Q, Chen W, Zhang X, Mei F, Zhang P, Zhao M, Wang X, Shi N, Jackson S, Hong Y. Multigene editing via CRISPR/Cas9 guided by a single-sgRNA seed in *Arabidopsis*. *J Integr Plant Biol*, 2018, 60(5): 376-381.

[67] Kim D, Alptekin B, Budak H. CRISPR/Cas9 genome editing in wheat. *Funct Integr Genomics*, 2018, 18(1): 31-41.

[68] Meng X, Hu X, Liu Q, Song X, Gao C, Li J, Wang K. Robust genome editing of CRISPR-Cas9 at NAG PAMs in rice. *Sci China Life Sci*, 2017, 61(1): 122-125.

[69] Klimek-Chodacka M, Oleszkiewicz T, Lowder LG, Qi Y, Baranski R. Efficient CRISPR/Cas9-based genome editing in carrot cells. *Plant Cell Rep*, 2018, 37(4): 575-586.

[70] Kanazashi Y, Hirose A, Takahashi I, Mikami M, Endo M, Hirose S, Toki S, Kaga A, Naito K, Ishimoto M, Abe J, Yamada T. Simultaneous site-directed mutagenesis of duplicated loci in soybean using a single guide RNA. *Plant Cell Rep*, 2018, 37(3): 553-563.

[71] Ji X, Li F, Yan Y, Sun HZ, Zhang J, Li JZ, Peng T, Du YX, Zhao QZ. CRISPR/Cas9 system-based editing of phytochrome-interacting factor OsPIL15. *Sci Agric Sin*, 2017, 50(15): 2861-2871.

季新，李飞，晏云，孙红正，张静，李俊周，彭廷，杜彦修，赵全志. 基于 CRISPR/Cas9 系统的水稻光敏色素互作因子 *OsPIL15* 基因编辑. 中国农业科学, 2017, 50(15): 2861-2871.

[72] Hodgens C, Nimchuk ZL, Kieber JJ. indCAPS: A tool for designing screening primers for CRISPR/Cas9 mutagenesis events. *PLoS One*, 2017, 12(11): e0188406.

[73] Neff MM, Turk E, Kalishman M. Web-based primer design for single nucleotide polymorphism analysis. *Trends Genet*, 2002, 18(12): 613-615.

[74] Lee J, Nonaka S, Takayama M, Ezura H. Utilization of a genome-edited tomato (*Solanum lycopersicum*) with High gamma aminobutyric acid content in hybrid breeding. *J Agric Food Chem*, 2018, 66(4): 963-971.

[75] Xie K, Yang Y. RNA-guided genome editing in plants using a CRISPR-Cas system. *Mol Plant*, 2013, 6(6): 1975-1983.

[76] Jia H, Wang N. Xcc-facilitated agroinfiltration of citrus leaves: a tool for rapid functional analysis of transgenes in citrus leaves. *Plant Cell Rep*, 2014, 33(12): 1993-2001.

[77] Wang S, Zhang S, Wang W, Xiong X, Meng F, Cui X. Efficient targeted mutagenesis in potato by the CRISPR/Cas9 system. *Plant Cell Rep*, 2015, 34(9):1473-1476.

[78] Chen X, Lu X, Shu N, Wang S, Wang J, Wang D, Guo L, Ye W. Targeted mutagenesis in cotton (*Gossypium hirsutum* L.) using the CRISPR/Cas9 system. *Sci Rep*, 2017, 7: 44304.

[79] Jia H, Xu J, Orbović V, Zhang Y, Wang N. Editing citrus genome via SaCas9/sgRNA system. *Front Plant Sci*, 2017, 8: 2135

[80] Pu Yan, Liu C, Li JY, Aerzu Guli TS, Hu Y, Liu XD. Different SlU6 promoters cloning and establishment of CRISPR/Cas9 mediated gene editing system in tomato. *Sci Agric Sin*, 2018, 51(2):315-326

蒲艳，刘超，李继洋，阿尔祖古丽·塔什，胡燕，刘晓东. 番茄 U6 启动子的克隆及 CRISPR/Cas9 基因编辑体系的建立. 中国农业科学, 2018, 51(2):315-326.

[81] Kim JM, Kim D, Kim S, Kim JS. Genotyping with CRISPR-Cas-derived RNA-guided endonucleases. *Nat Commun*, 2014, 5: 3157.

[82] Zhou H, Liu B, Weeks DP, Spalding MH, Yang B. Large chromosomal deletions and heritable small genetic changes induced by CRISPR/Cas9 in rice. *Nucleic Acids Res*, 2014, 42(17): 10903-10914.

[83] Hyun Y, Kim J, Cho SW, Choi Y, Kim JS, Coupland G. Site-directed mutagenesis in *Arabidopsis thaliana* using dividing tissue-targeted RGEN of the CRISPR/Cas system to generate heritable null alleles. *Planta*, 2015, 241: 271-284.

[84] Ji X, Zhang H, Zhang Y, Wang Y, Gao C. Establishing a CRISPR-Cas-like immune system conferring DNA virus resistance in plants. *Nat Plants*, 2015, 1: 15144.

[85] Woo JW, Kim J, Kwon SI, Corvalán C, Cho SW, Kim H, Kim SG, Kim ST, Choe S, Kim JS. DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. *Nat Biotechnol*, 2015, 33(11): 1162-1164.

[86] Subburaj S, Chung SJ, Lee C, Ryu SM, Kim DH, Kim JS, Bae S, Lee GJ. Site-directed mutagenesis in Petunia × hybrida protoplast system using direct delivery of purified recombinant Cas9 ribonucleoproteins. *Plant Cell Rep*, 2016, 35(7): 1535-1544.

[87] Eid A, Ali Z, Mahfouz MM. High efficiency of targeted mutagenesis in arabidopsis via meiotic promoter-driven expression of Cas9 endonuclease. *Plant Cell Rep*, 2016, 35(7): 1555-1558.

[88] Char SN, Neelakandan AK, Nahampun H, Frame B, Main M, Spalding MH, Becraft PW, Meyers BC, Walbot V, Wang K, Yang B. An *Agrobacterium*-delivered CRISPR/Cas9 system for high-frequency targeted mutagenesis in maize. *Plant Biotechnol J*, 2017, 15(2): 257-268.

[89] Xu R, Qin R, Li H, Li D, Li L, Wei P, Yang J. Generation of targeted mutant rice using a CRISPR-Cpf1 system. *Plant Biotechnol J*, 2017, 15(6): 713-717.

[90] Begemann MB, Gray BN, January E, Gordon GC, He Y, Liu H, Wu X, Brutnell TP, Mockler TC, Oufattole M. Precise insertion and guided editing of higher plant genomes using Cpf1 CRISPR nucleases. *Sci Rep*, 2017, 7(1): 11606.

[91] Li C, Chen C, Chen H, Wang S, Chen X, Cui Y. Verification of DNA motifs in *Arabidopsis* using CRISPR/Cas9-mediated mutagenesis. *Plant Biotechnol J*, 2018, 16(8): 1446-1451.

[92] Osakabe K, Osakabe Y, Toki S. Site-directed mutagenesis in *Arabidopsis* using custom-designed zinc finger nucleases. *Proc Natl Acad Sci USA*. 2010, 107(26): 12034-12039.

[93] Li T, Liu B, Spalding MH, Weeks DP, Yang B. High-efficiency TALEN-based gene editing produces disease-resistant rice. *Nat Biotechnol*, 2012, 30(5): 390-392.

[94] Mao Y, Zhang H, Xu N, Zhang B, Gou F, Zhu JK. Application of the CRISPR-Cas system for efficient genome engineering in plants. *Mol Plant*, 2013, 6(6): 2008-2011.

[95] Zhang Z, Mao Y, Ha S, Liu W, Botella JR, Zhu JK. A multiplex CRISPR/Cas9 platform for fast and efficient editing of multiple genes in *Arabidopsis*. *Plant Cell Rep*, 2015, 35(7): 1519-1533.

[96] Zhu J, Song N, Sun S, Yang W, Zhao H, Song W, Lai J. Efficiency and inheritance of targeted mutagenesis in maize using CRISPR-Cas9. *J Genet Genomics*, 2016, 43(1): 25-36.

[97] Ren C, Liu X, Zhang Z, Wang Y, Duan W, Li S, Liang Z. CRISPR/Cas9-mediated efficient targeted mutagenesis in Chardonnay (*Vitis vinifera* L.). *Sci Rep*, 2016, 6: 32289.

[98] Yin X, Biswal AK, Dionora J, Perdigon KM, Balahadia CP, Mazumdar S, Chater C, Lin HC, Coe RA, Kretzschmar T, Gray JE, Quick PW, Bandyopadhyay A. CRISPR-Cas9 and CRISPR-Cpf1 mediated targeting of a stomatal developmental gene EPFL9 in rice. *Plant Cell Rep*, 2017, 36(5): 745-757.

[99] Kim Y, Kweon J, Kim A, Chon JK, Yoo JY, Kim HJ, Kim S, Lee C, Jeong E, Chung E, Kim D, Lee MS, Go EM, Song HJ, Kim H, Cho N, Bang D, Kim S, Kim JS. A library of TAL effector nucleases spanning the human genome. *Nat Biotechnol*, 2013, 31(3): 251-258.

[100] Vouillot L, Th ́elie A, Pollet N. Comparison of T7E1 and surveyor mismatch cleavage assays to detect mutations triggered by engineered nucleases. *G3 (Bethesda)*, 2015, 5(3): 407-415.

[101] Li C, Unver T, Zhang B. A high-efficiency CRISPR/Cas9 system for targeted mutagenesis in Cotton (*Gossypium hirsutum* L.). *Sci Rep*, 2017, 7: 43902.

[102] Miao J, Guo DS, Zhang JZ, Huang QP, Qin GJ, Zhang X, Wan JM, Gu HY, Qu LJ. Targeted mutagenesis in rice using CRISPR-Cas system. *Cell Res*, 2013, 23(10): 1233-1236.

[103] Feng ZY, Mao YF, Xu NF, Zhang BT, Wei PL, Yang DL, Wang Z, Zhang ZJ, Zheng R, Yang L, Zeng L, Liu XD, Zhu JK. Multigeneration analysis reveals the inheritance, specificity, and patterns of CRISPR/Cas-induced gene modifications in *Arabidopsis*. *Proc Natl Acad Sci USA*, 2014, 111(12): 4632-4637.

[104] Zhang H, Zhang J, Wei P, Zhang B, Gou F, Feng Z, Mao Y, Yang L, Zhang H, Xu N, Zhu JK. The CRISPR/Cas9 system produces specific and homozygous targeted gene editing in rice in one generation. *Plant Biotechnol J*, 2014, 12(6): 797-807.

[105] Haun W, Coffman A, Clasen BM, Demorest ZL, Lowy A, Ray E, Retterath A, Stoddard T, Juillerat A, Cedrone F, Mathis L, Voytas DF, Zhang F. Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. *Plant Biotechnol J*, 2014, 12(7): 934-940.

[106] Brooks C, Nekrasov V, Lippman ZB, Van Eck J. Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. *Plant Physiol*, 2014, 166(3): 1292-1297.

[107] Xu RF, Li H, Qin RY, Li J, Qiu CH, Yang YC, Ma H, Li L, Wei PC, Yang JB. Generation of inheritable and "transgene clean" targeted genome-modified rice in later generations using the CRISPR/Cas9 system. *Sci Rep*, 2015, 5: 11491.

[108] Fan D, Liu T, Li C, Jiao B, Li S, Hou Y, Luo K. Efficient CRISPR/Cas9-mediated targeted mutagenesis in populus in the first generation. *Sci Rep*, 2015, 5: 12217.

[109] Ma X, Zhang Q, Zhu Q, Liu W, Chen Y, Qiu R, Wang B, Yang Z, Li H, Lin Y, Xie Y, Shen R, Chen S, Wang Z, Chen Y, Guo J, Chen L, Zhao X, Dong Z, Liu YG. A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. *Mol Plant*, 2015, 8(8): 1274-1284.

[110] Ma X, Chen L, Zhu Q, Chen Y, Liu YG. Rapid decoding of sequence-specific nuclease-induced heterozygous and biallelic mutations by direct sequencing of PCR products. *Mol Plant*, 2015, 8(8): 1285-1287.

[111] Zhang B, Yang X, Yang C, Li M, Guo Y. Exploiting the CRISPR/Cas9 system for targeted genome mutagenesis in petunia. *Sci Rep*, 2016, 6: 20315.

[112] Butler NM, Baltes NJ, Voytas DF, Douches DS. Geminivirus-mediated genome editing in potato (*Solanum tuberosum* L.) using sequence-specific nucleases. *Front Plant Sci*, 2016, 7: 1045.

[113] Nishitani C, Hirai N, Komori S, Wada M, Okada K, Osakabe K, Yamamoto T, Osakabe Y. Efficient genome editing in apple using a CRISPR/Cas9 system. *Sci Rep*, 2016, 6: 31481.

[114] Qi W, Zhu T, Tian Z, Li C, Zhang W, Song R. High-efficiency CRISPR/Cas9 multiplex gene editing using the glycine tRNA-processing system-based strategy in maize. *BMC Biotechnol*, 2016, 16(1): 58.

[115] Xu R, Yang Y, Qin R, Li H, Qiu C, Li L, Wei P, Yang J. Rapid improvement of grain weight via highly efficient CRISPR/Cas9-mediated multiplex genome editing in rice. *J Genet Genomics*, 2016, 43(8): 529-532.

[116] Blanvillain-Baufumé S, Reschke M, Solé M, Auguy F, Doucoure H, Szurek B, Meynard D, Portefaix M, Cunnac S, Guiderdoni E, Boch J, Koebnik R. Targeted promoter editing for rice resistance to *Xanthomonas oryzaepv.oryzaereveals* differential activities forSWEET14-inducing TAL effectors. *Plant Biotechnol J*, 2017, 15(3): 306-317.

[117] Shen L, Wang C, Fu Y, Wang J, Liu Q, Zhang X, Yan C, Qian Q, Wang K. QTL editing confers opposing yield performance in different rice varieties. *J Integr Plant Biol*, 2018, 60(2): 89-93.

[118] Demorest ZL, Coffman A, Baltes NJ, Stoddard TJ, Clasen BM, Luo S, Retterath A, Yabandith A, Gamo ME, Bissen J, Mathis L, Voytas DF, Zhang F. Direct stacking of sequence-specific nuclease-induced mutations to produce high oleic and low linolenic soybean oil. *BMC Plant Biol*, 2016, 16: 225.

[119] Zhou H, He M, Li J, Chen L, Huang ZF, Zheng SY, Zhu LY, Ni ED, Jiang DG, Zhao BR, Zhuang CX. Development of commercial thermo-sensitive genic male sterile rice accelerates hybrid rice breeding using the CRISPR/Cas9-mediated TMS5 editing system. *Sci Rep*, 2016, 6: 37395.

[120] Morineau C, Bellec Y, Tellier F, Gissot L, Kelemen Z, Nogué F, Faure JD. Selective gene dosage by CRISPR-Cas9 genome editing in hexaploid *Camelina sativa*. *Plant Biotechnol J*, 2017, 15(6): 729-739.

[121] Hu X, Wang C, Liu Q, Fu Y, Wang K. Targeted mutagenesis in rice using CRISPR-Cpf1 system. *J Genet Genomics*, 2017, 44(1): 71-73.

[122] Kishi-Kaboshi M, Aida R, Sasaki K. Generation of gene-edited chrysanthemum morifolium using multicopy transgenes as targets and markers. *Plant Cell Physiol*, 2017, 58(2): 216-226.

[123] Liu C, Li X, Meng D, Zhong Y, Chen C, Dong X, Xu X, Chen B, Li W, Li L, Tian X, Zhao H, Song W, Luo H, Zhang Q, Lai J, Jin W, Yan J, Chen S. A 4-bp insertion at ZmPLA1 encoding a putative phospholipase a generates haploid induction in maize. *Mol Plant*, 2017, 10(3): 520-522.

[124] Yuan J, Chen S, Jiao W, Wang L, Wang L, Ye W, Lu J, Hong D, You S, Cheng Z, Yang DL, Chen ZJ. Both maternally and paternally imprinted genes regulate seed development in rice. *New Phytol*, 2017, 216(2): 373-387.

[125] Chen Y, Wang Z, Ni H, Xu Y, Chen Q, Jiang L. CRISPR/Cas9-mediated base-editing system efficiently generates gain-of-function mutations in *Arabidopsis*. *Sci China Life Sci*, 2017, 60(5): 520-523.

[126] Minkenberg B, Xie K, Yang Y. Discovery of rice essential genes by characterizing a CRISPR-edited mutation of closely related rice MAP kinase genes. *Plant J*, 2017, 89(3): 636-648.

[127] Wang M, Lu Y, Botella JR, Mao Y, Hua K, Zhu JK. Gene targeting by homology-directed repair in rice using a geminivirus-based CRISPR/Cas9 system. *Mol Plant*, 2017, 10(7): 1007-1010.

[128] Ryder P, McHale M, Fort A, Spillane C. Generation of stable nulliplex autopolyploid lines of *Arabidopsis thaliana* using CRISPR/Cas9 genome editing. *Plant Cell Rep*, 2017, 36(6): 1005-1008.

[129] Li X, Zhou W, Ren Y, Tian X, Lv T, Wang Z, Fang J, Chu C, Yang J, Bu Q. High-efficiency breeding of early-maturing rice cultivars via CRISPR/Cas9-mediated genome editing. *J Genet Genomics*, 2017, 44(3):175-178.

[130] Wang M, Mao Y, Lu Y, Tao X, Zhu JK. Multiplex gene editing in rice using the CRISPR-Cpf1 system. *Mol Plant*, 2017, 10(7): 1011-1013.

[131] Nekrasov V, Wang C, Win J, Lanz C, Weigel D, Kamoun S. Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. *Sci Rep*, 2017, 7(1): 482.

[132] Kapusi E, Corcuera-Gómez M, Melnik S, Stoger E. Heritable genomic fragment deletions and small indels in the putative ENGase gene induced by CRISPR/Cas9 in barley. *Front Plant Sci*, 2017, 8: 540.

[133] Cai YP, Chen L, Liu XJ, Guo C, Sun S, Wu CX, Jiang BJ, Han TF, Hou WS. CRISPR/Cas9-mediated targeted mutagenesis of GmFT2a delays flowering time in soya bean. *Plant Biotechnol J*, 2018, 16(1): 176-185.

[134] Lu HP, Liu SM, Xu SL, Chen WY, Zhou X, Tan YY, Huang JZ, Shu QY. CRISPR-S: an active interference element for a rapid and inexpensive selection of genome-edited, transgene-free rice plants. *Plant Biotechnol J*, 2017, 15(11): 1371-1373.

[135] Zhang Y, Bai Y, Wu G, Zou S, Chen Y, Gao C, Tang D. Simultaneous modification of three homoeologs of TaEDR1 by genome editing enhances powdery mildew resistance in wheat. *Plant J*, 2017, 91(4): 714-724.

[136] Wang Y, Geng L, Yuan M, Wei J, Jin C, Li M, Yu K, Zhang Y, Jin H, Wang E, Chai Z, Fu X, Li X. Deletion of a target gene in Indica rice via CRISPR/Cas9. *Plant Cell Rep*, 2017, 36(8): 1333-1343.

[137] Hu X, Meng X, Liu Q, Li J, Wang K. Increasing the efficiency of CRISPR-Cas9-VQR precise genome editing in rice. *Plant Biotechnol J*, 2018, 16(1): 292-297.

[138] Xie Y, Niu B, Long Y, Li G, Tang J, Zhang Y, Ren D, Liu YG, Chen L. Suppression or knockout of SaF/SaM overcomes the Sa-mediated hybrid male sterility in rice. *J Integr Plant Biol*, 2017, 59(9): 669-679.

[139] Meng X, Yu H, Zhang Y, Zhuang F, Song X, Gao S, Gao C, Li J. Construction of a genome-wide mutant library in rice using CRISPR/Cas9. *Mol Plant*, 2017, 10(9): 1238-1241.

[140] Yu QH, Wang B, Li N, Tang Y, Yang S, Yang T, Xu J, Guo C, Yan P, Wang Q, Asmutola P. CRISPR/Cas9-induced Targeted Mutagenesis and Gene Replacement to Generate Long-shelf Life Tomato Lines. *Sci Rep*, 2017, 7(1): 11874.

[141] Lu Y, Ye X, Guo R, Huang J, Wang W, Tang J, Tan L, Zhu JK, Chu C, Qian Y. Genome-wide targeted mutagenesis in rice using the CRISPR/Cas9 system. *Mol Plant*, 2017, 10(9): 1242-1245.

[142] Sánchez-León S, Gil-Humanes J, Ozuna CV, Giménez MJ, Sousa C, Voytas DF, Barro F. Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. *Plant Biotechnol J*, 2018, 16(4): 902-910

[143] Zhang F, LeBlanc C, Irish VF, Jacob Y. Rapid and efficient CRISPR/Cas9 gene editing in Citrus using the YAO promoter. *Plant Cell Rep*, 2017, 36(12): 1883-1887.

[144] Wang L, Chen L, Li R, Zhao R, Yang M, Sheng J, Shen L. Reduced drought tolerance by CRISPR/Cas9-mediated SlMAPK3 mutagenesis in tomato plants. *J Agric Food Chem*, 2017, 65(39): 8674-8682.

[145] Odipio J, Alicai T, Ingelbrecht I, Nusinow DA, Bart R, Taylor NJ. Efficient CRISPR/Cas9 genome editing of phytoene desaturase in cassava. *Front Plant Sci*, 2017, 8: 1780.

[146] Rodríguez-Leal D, Lemmon ZH, Man J, Bartlett ME, Lippman ZB. Engineering quantitative trait variation for crop improvement by genome editing. *Cell*, 2017, 171(2): 470-480.e8.

[147] Ali Z, Eid A, Ali S, Mahfouz MM. Pea early-browning virus-mediated genome editing via the CRISPR/Cas9 system in *Nicotiana benthamiana* and *Arabidopsis*. *Virus Res*, 2018, 244: 333-337.

[148] Deng L, Wang H, Sun C, Li Q, Jiang H, Du M, Li CB, Li C. Efficient generation of pink-fruited tomatoes using CRISPR/Cas9 system. *J Genet Genomics*, 2018, 45(1): 51-54.

[149] LeBlanc C, Zhang F, Mendez J, Lozano Y, Chatpar K, Irish VF, Jacob Y. Increased efficiency of targeted mutagenesis by CRISPR/Cas9 in plants using heat stress. *Plant J*, 2018, 93(2): 377-386.

[150] Hummel AW, Chauhan RD, Cermak T, Mutka AM, Vijayaraghavan A, Boyher A, Starker CG, Bart R, Voytas DF, Taylor NJ. Allele exchange at the EPSPS locus confers glyphosate tolerance in cassava. *Plant Biotechnol J*, 2018, 16(7): 1275-1282.

[151] Lin CS, Hsu CT, Yang LH, Lee LY, Fu JY, Cheng QW, Wu FH, Hsiao HC, Zhang Y, Zhang R, Chang WJ, Yu CT, Wang W, Liao LJ, Gelvin SB, Shih MC. Application of protoplast technology to CRISPR/Cas9 mutagenesis: from single-cell mutation detection to mutant plant regeneration. *Plant Biotechnol J*, 2018, 16(7): 1295-1310.

[152] Zhang J, Zhang H, Botella JR, Zhu JK. Generation of new glutinous rice by CRISPR/Cas9-targeted mutagenesis of the Waxy gene in elite rice varieties. *J Integr Plant Biol*, 2017, 60(5): 369-375.

[153] Ren B, Yan F, Kuang Y, Li N, Zhang D, Zhou X, Lin H, Zhou H. Improved base editor for efficiently inducing genetic variations in rice with CRISPR/Cas9-guided hyperactive hAID mutant. *Mol Plant*, 2018, 11(4): 623-626.

[154] Wang ZP, Wang SB, Li DW, Zhang Q, Li L, Zhong CH, Liu YF, Huang HW. Optimized paired-sgRNA/Cas9 cloning and expression cassette triggers high-efficiency multiplex genome editing in kiwifruit. *Plant Biotechnol J*, 2018, 16(8): 1424-1433.

[155] Li C, Chen C, Chen H, Wang S, Chen X, Cui Y. Verification of DNA motifs in *Arabidopsis* using CRISPR/Cas9-mediated mutagenesis. *Plant Biotechnol J*, 2018, 16(8): 1446-1451.

[156] Liang Y, Zeng X, Peng X, Hou X. Arabidopsis glutamate:glyoxylate aminotransferase 1 (Ler) mutants generated by CRISPR/Cas9 and their characteristics. *Transgenic Res*, 2018, 27(1): 61-74.

[157] Hua K, Tao X, Yuan F, Wang D, Zhu JK. Precise A.T to G.C base editing in the rice genome. *Mol Plant*, 2018, 11(4): 627-630.

[158] Yan F, Kuang Y, Ren B, Wang J, Zhang D, Lin H, Yang B, Zhou X, Zhou H. High-efficient A.T to G.C base editing by Cas9n-guided tRNA adenosine deaminase in rice. *Mol Plant*, 2018, 11(4): 631-634.

[159] Zhou WJ, Tian XJ, Ren YK, Wei XJ, Gao Y, Xie LH, Liu HZ, Bu QY, Li XF. Breeding of early-maturatity and fragrant rice via CRISPR/Cas9 mediated genome editing. *Soils Crops*, 2017, 6(2): 146-152.

周文甲，田晓杰，任月坤，魏祥进，高扬，谢黎虹，刘华招，卜庆云，李秀峰. 利用CRISPR/Cas9 创造早熟香味水稻. 土壤与作物, 2017, 6(2): 146-152.

[160] Yuan WX, Wang XM, Li DY, Zhou J, Yan QC, Chen JP. Application of the technology of CRISPR/Cas9 edit rice gene. *Acta Agric Zhejiangensis*, 2017, 29(5): 685-693.

原文霞，王栩鸣，李冬月，周洁，严成其，陈剑平. 利用CRISPR/Cas9 技术靶向编辑水稻基因. 浙江农业学报, 2017, 29(5): 685-693

[161] Wang BK, Zhang H, Hong RK, Zhang JW, Yang R, Luo Q, Zeng QC. Wx gene editing via CRISPR/Cas9 system in rice. *Chin J Rice Sci*, 2018, 32(1): 35-42.

汪秉琨，张慧， 洪汝科， 张锦文，杨睿，罗琼，曾千春. CRISPR/Cas9 系统编辑水稻 Wx 基因. 中国水稻科学，2018, 32(1): 35-42.

[162] Liu X, Pang AY, Liu CL, Ruan Y. Obtaining the Knock-out Mutant of AtLCR67 Gene in Arabidopsis thaliana by CRISPR-Cas9 Gene Editing Technique. *Mol Plant Breed*, 2017, 15(6): 311-316.

刘询，庞奥运，刘春林，阮颖. 利用CRISPR-Cas9 基因编辑技术获得AtLCR67 基因敲除突变体. 分子植物育种，2017, 15(6): 311-316.

[163] Brinkman EK, Chen T, Amendola M, van Steensel B. Easy quantitative assessment of genome editing by sequence trace decomposition. *Nucleic Acids Res*, 2014 42(22): e168.

[164] Liu W, Xie X, Ma X, Li J, Chen J, Liu YG. DSDecode: A web-based tool for decoding of sequencing chromatograms for genotyping of targeted mutations. *Mol Plant*, 2015, 8(9): 1431-1433.

[165] Jacobs TB, LaFayette PR, Schmitz RJ, Parrott WA. Targeted genome modifications in soybean with CRISPR/Cas9. *BMC Biotechnol*, 2015, 15: 16.

[166] Zhou X, Jacobs TB, Xue LJ, Harding SA, Tsai CJ. Exploiting SNPs for biallelic CRISPR mutations in the outcrossing woody perennial Populus reveals 4-coumarate:CoA ligase specificity and redundancy. *New Phytol*, 2015, 208(2): 298-301.

[167] Kim H, Kim ST, Ryu J, Choi MK, Kweon J, Kang BC, Ahn HM, Bae S, Kim J, Kim JS, Kim SG. A simple, flexible and high-throughput cloning system for plant genome editing via CRISPR-Cas system. *J Integr Plant Biol*, 2016, 58(8): 705-712.

[168] Peterson BA, Haak DC, Nishimura MT, Teixeira PJ, James SR, Dangl JL, Nimchuk ZL. Genome-wide assessment of efficiency and specificity in CRISPR/Cas9 mediated multiple site targeting in *Arabidopsis*. *PLoS One*, 2016, 11(9): e0162169.

[169] Svitashev S, Schwartz C, Lenderts B, Young JK, Mark Cigan A. Genome editing in maize directed by CRISPR-Cas9 ribonucleoprotein complexes. *Nat Commun*, 2016, 7: 13274.

[170] Jia H, Zhang Y, Orbović V, Xu J, White FF, Jones JB, Wang N. Genome editing of the disease susceptibility gene CsLOB1 in citrus confers resistance to citrus canker. *Plant Biotechnol J*, 2017, 15(7): 817-823.

[171] Malnoy M, Viola R, Jung MH, Koo OJ, Kim S, Kim JS, Velasco R, Nagamangala Kanchiswamy C. DNA-free genetically edited grapevine and apple protoplast using CRISPR/Cas9 Ribonucleoproteins. *Front Plant Sci*, 2016, 7: 1904.

[172] Kim H, Kim ST, Ryu J, Kang BC, Kim JS, Kim SG. CRISPR/Cpf1-mediated DNA-free plant genome editing. *Nat Commun*, 2017, 8: 14406.

[173] Filler Hayut S, Melamed Bessudo C, Levy AA. Targeted recombination between homologous chromosomes for precise breeding in tomato. *Nat Commun*, 2017, 8: 15605.

[174] Čermák T, Curtin SJ, Gil-Humanes J, Čegan R, Kono TJY, Konečná E, Belanto JJ, Starker CG, Mathre JW, Greenstein RL, Voytas DF. A multipurpose toolkit to enable advanced genome engineering in plants. *Plant Cell*, 2017, 29(6): 1196-1217.

[175] Butt H, Eid A, Ali Z, Atia MAM, Mokhtar MM, Hassan N, Lee CM, Bao G, Mahfouz MM. Efficient CRISPR/Cas9-Mediated Genome Editing Using a Chimeric Single-Guide RNA Molecule. *Front Plant Sci*, 2017, 8: 1441.

[176] Wang PC, Zhang J, Sun L, Ma YZ, Xu J, Liang SJ, Deng JW, Tan JF, Zhang QH, Tu LL, Daniell H, Jin SX, Zhang XL. High efficient multisites genome editing in allotetraploid cotton (*Gossypium hirsutum*) using CRISPR/Cas9 system. *Plant Biotechnol J*, 2018, 16(1): 137-150.

[177] Shukla VK, Doyon Y, Miller JC, DeKelver RC, Moehle EA, Worden SE, Mitchell JC, Arnold NL, Gopalan S, Meng X, Choi VM, Rock JM, Wu YY, Katibah GE, Zhifang G, McCaskill D, Simpson MA, Blakeslee B, Greenwalt SA, Butler HJ, Hinkley SJ, Zhang L, Rebar EJ, Gregory PD, Urnov FD. Precise genome modification in the crop species Zea mays using zinc-finger nucleases. *Nature*, 2009, 459(7245): 437-441.

[178] Fauser F, Schiml S, Puchta H. Both CRISPR/Cas-based nucleases and nickases can be used efficiently for genome engineering in *Arabidopsis thaliana*. *Plant J*, 2014, 79(2): 348-359.

[179] Schiml S, Fauser F, Puchta H. The CRISPR/Cas system can be used as nuclease for in planta gene targeting and as paired nickases for directed mutagenesis in *Arabidopsis* resulting in heritable progeny. *Plant J*, 2014, 80(6): 1139-1150.

[180] Jung JH, Altpeter F. TALEN mediated targeted mutagenesis of the caffeic acid O-methyltransferase in highly polyploid sugarcane improves cell wall composition for production of bioethanol. *Plant Mol Biol*, 2016, 92(1-2): 131-142.

[181] Vu GTH, Cao HX, Fauser F, Reiss B, Puchta H, Schubert I. Endogenous sequence patterns predispose the repair modes of CRISPR/Cas9-induced DNA double-stranded breaks in *Arabidopsis thaliana*. *Plant J*, 2017, 92(1): 57-67.

[182] Ingham DJ, Beer S, Money S, Hansen G. Quantitative real-time PCR assay for determining transgene copy number in transformed plants. *Biotechniques*, 2001, 31(1): 132-134, 136-140.

[183] Li Z, Liu ZB, Xing A, Moon BP, Koellhoffer JP, Huang L, Ward RT, Clifton E, Falco SC, Cigan AM. Cas9-guide RNA directed genome editing in soybean. *Plant Physiol*, 2015, 169(2): 960-970.

[184] Svitashev S, Young JK, Schwartz C, Gao H, Falco SC, Cigan AM. Targeted mutagenesis, precise gene editing, and site-specific gene insertion in maize using Cas9 and guide RNA. *Plant Physiol*, 2015, 169(2): 931-945.

[185] Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M, Hakimi SM, Mo H, Habben JE. ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant Biotechnol J*, 2017, 15(2): 207-216.

[186] Kelliher T, Starr D, Richbourg L, Chintamanani S, Delzer B, Nuccio ML, Green J, Chen Z, McCuiston J, Wang W, Liebler T, Bullock P, Martin B. MATRILINEAL, a sperm-specific phospholipase, triggers maize haploid induction. *Nature*, 2017, 542(7639): 105-109.

[187] Yao L, Zhang Y, Liu C, Liu Y, Wang Y, Liang D, Liu J, Sahoo G, Kelliher T. OsMATL mutation induces haploid seed formation in indica rice. *Nat Plants*, 2018, 4(8): 530-533.

[188] Ordon J, Gantner J, Kemna J, Schwalgun L, Reschke M, Streubel J, Boch J, Stuttmann J. Generation of chromosomal deletions in dicotyledonous plants employing a user-friendly genome editing toolkit. *Plant J*, 2017, 89(1): 155-168.

[189] Deng L, Wang H, Sun C, Li Q, Jiang H, Du M, Li CB, Li C. Efficient generation of pink-fruited tomatoes using CRISPR/Cas9 system. *J Genet Genomics*, 2018, 45(1): 51-54.

[190] Zheng X, Yang S, Zhang D, Zhong Z, Tang X, Deng K, Zhou J, Qi Y, Zhang Y. Effective screen of CRISPR/Cas9-induced mutants in rice by single-strand conformation polymorphism. *Plant Cell Rep*, 2016, 35(7): 1545-1554.

[191] Zhou J, Deng K, Cheng Y, Zhong Z, Tian L, Tang X, Tang A, Zheng X, Zhang T, Qi Y, Zhang Y. CRISPR-Cas9 based genome editing reveals new insights into microRNA function and regulation in rice. *Front Plant Sci*. 2017, 8: 1598.

[192] Kim JM, Kim D, Kim S, Kim JS. Genotyping with CRISPR-Cas-derived RNA-guided endonucleases. *Nat Commun*. 2014, 5: 3157.

[193] Liang Z, Chen KL, Yan Y, Zhang Y, Gao CX. Genotyping genome-edited mutations in plants using CRISPR ribonucleoprotein complexes. *Plant Biotechnol J*, 2018, doi: 10.1111/pbi.12938.

[194] Andersson M, Turesson H, Nicolia A, Fält AS, Samuelsson M, Hofvander P. Efficient targeted multiallelic mutagenesis in tetraploid potato (*Solanum tuberosum*) by transient CRISPR-Cas9 expression in protoplasts. *Plant Cell Rep*, 2017, 36(1): 117-128.

[195] Thomas HR, Percival SM, Yoder BK, Parant JM. High-throughput genome editing and phenotyping facilitated by high resolution melting curve analysis. *PLoS One*, 2014, 9(12): e114632.

[196] Hilioti Z, Ganopoulos I, Ajith S, Bossis I, Tsaftaris A. A novel arrangement of zinc finger nuclease system for *in vivo* targeted genome engineering: the tomato LEC1-LIKE4 gene case. *Plant Cell Rep*, 2016, 35(11): 2241-2255.

[197] Ota S, Hisano Y, Muraki M, Hoshijima K, Dahlem TJ, Grunwald DJ, Okada Y, Kawahara A. Efficient identification of TALEN-mediated genome modifications using heteroduplex mobility assays. *Genes Cells*, 2013, 18(6): 450-458.

[198] Bui M, Liu Z. Simple allele-discriminating PCR for cost-effective and rapid genotyping and mapping. *Plant Methods*, 2009, 5: 1.

[199] Hua Y, Wang C, Huang J, Wang K. A simple and efficient method for CRISPR/Cas9-induced mutant screening. *J Genet Genomics*, 2017, 44(4): 207-213.

[200] Miyaoka Y, Chan AH, Judge LM, Yoo J, Huang M, Nguyen TD, Lizarraga PP, So PL, Conklin BR. Isolation of single-base genome-edited human iPS cells without antibiotic selection. *Nat Methods*, 2014, 11(3): 291-293.

[201] Findlay SD, Vincent KM, Berman JR, Postovit LM. A digital PCR-based method for efficient and highly specific screening of genome edited cells. *PLoS One*, 2016, 11(4): e0153901.

[202] Gao R, Feyissa BA, Croft M, Hannoufa A. Gene editing by CRISPR/Cas9 in the obligatory outcrossing *Medicago sativa*. *Planta*, 2018, 247(4): 1043-1050.

[203] Kc R, Srivastava A, Wilkowski JM, Richter CE, Shavit JA, Burke DT, Bielas SL. Detection of nucleotide-specific CRISPR/Cas9 modified alleles using multiplex ligation detection. *Sci Rep*, 2016, 6: 32048.

[204] Falabella M, Sun L, Barr J, Pena AZ, Kershaw EE, Gingras S, Goncharova EA, Kaufman BA. Single-Step qPCR and dPCR detection of diverse CRISPR-Cas9 gene editing events *in vivo*. *G3 (Bethesda)*, 2017, 7(10): 3533-3542.

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