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植物基因组编辑专辑 评 述
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基因编辑技术在作物抗病中的应用

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摘要 作物病害常造成粮食的大幅度减产, 严重威胁着全球的粮食安全. 因此, 增强作物抗性, 特别是广谱持久抗病性, 是控制作物病害和保障粮食生产安全的重要策略. 近年来, 基因编辑技术迅速发展, 极大地促进了作物抗病性状的创制和定向优化, 为作物广谱抗病育种提供了全新的途径. 基因编辑技术的应用, 使得科研工作者能够充分挖掘并利用编码膜上模式识别受体蛋白(pattern recognition receptors, PRRs)和胞内核苷酸结合的富含亮氨酸的重复受体蛋白(nucleotide-binding leucine-rich repeat receptors, NLRs)等抗病基因(disease-resistance gene, *R* gene), 这为作物抗病提供了新的策略和遗传资源. 此外, 非典型*R*基因往往赋予作物持久抗病性的同时, 不影响作物产量, 尤其是通过编辑感病基因(disease-susceptibility gene, *S* gene)的方式获得的非典型*R*基因, 为解决作物产量与抗性之间相互制约这一瓶颈提供了新的方案. 本文探讨了多类型基因编辑工具在作物抗病中的最新进展, 重点讨论了多种策略通过基因编辑技术在各种作物抗病中的应用, 例如插入和替换、饱和突变、多重编辑*S*基因、在转录水平和翻译水平调控以及基于人工智能预测介导的基因编辑. 这些策略推进了作物新型抗病基因的挖掘及其分子抗病育种的发展, 同时为打破作物产量与抗性间的平衡提供了新的研究思路. 最后, 我们对基因编辑研究在作物农艺性状改良中的未来发展方向进行了展望, 以便更好地促进基因编辑技术在农作物品种改良中的应用, 助力我国种业振兴和未来农业的可持续发展.

关键词 基因编辑, 植物免疫, 广谱抗病, 水稻, 小麦

1950年全球人口约为25亿, 到2022年底达到80亿, 预计到2050年, 全球人口将从80亿增加到97亿, 并可能在2080年代中期达到近104亿的峰值[1]. 随着全球人口的持续增长, 预计2010~2050年间全球粮食总需求将增长35%~56%[2]. 在满足日益增长的人口粮食需求的同时, 农作物还面临着各种病原微生物的威胁, 这使得全球粮食安全面临着严峻的挑战. 据统计, 目前已知的病原微生物(包括真菌和细菌)超过298000种, 病原菌群体的不断演化、新的病原菌小种的不断出现、人类活动区域的扩大和速度加快、全球气候变化等因素的影响, 作物病害的发生率和严重程度持续增强[3]. 多数作物品种种植多代后抗性逐渐减弱, 导致抗病基因在几年后逐渐丧失抗性, 造成作物产量的长期损失. 因此, 培育优质抗病品种, 提高作物的抗病性, 特别是广谱持久抗病性, 对于保障全球粮食安全至关重要[4].

作物抗病改良的基础是遗传变异, 通过丰富多变的遗传变异类型, 选育出“理想型”株系, 赋予作物对不同病原菌或者是同一病原菌的不同生理小种的广谱抗

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病性的同时, 不影响作物本身的生长与产量. 植物与病原菌长期互作过程中, 进化出两种先天免疫系统, 分别是病原相关分子模式激发的免疫反应(PAMP-triggered immunity, PTI)和效应蛋白激发的免疫反应(effector-triggered immunity, ETI)[5]. PTI主要由细胞表面的模式识别受体(pattern recognition receptors, PRRs)触发, 包括受体激酶(receptor-kinases, RKs)或类受体激酶(receptor-like kinases, RLKs)和类受体蛋白(receptor-like proteins, RLPs), 它们通过识别病原菌或微生物相关分子模式(pathogen-associated molecular patterns, PAMPs or microbe-associated molecular patterns, MAMPs)引起植物产生基础免疫反应, 赋予植物对病原菌的抗性[6,7]. ETI则由细胞内核苷酸结合域富含亮氨酸重复序列受体(nucleotide-binding leucine-rich repeat receptors, NLRs)感知效应因子所触发, 引发更强的免疫反应和局部细胞死亡的超敏反应[8]. 根据N端结构域的不同, 植物NLR分为三类, 分别是CNLs (coiled-coil (CC) NLRs, CNLs)、TNLs (Toll/interleukin-1 receptor (TIR) NLRs, TNLs)和RNLs (powdery mildew 8 (RPW8)-NLRs, RNLs)[9]. 不同的CNL类抗病小体, 如来源于双子叶模式植物拟南芥ZAR1和单子叶植物小麦Sr35, 都在质膜上形成钙离子通道来启动植物的免疫反应[10~12]. 与CNL类抗病蛋白相比, TNL类抗病蛋白的效应结构域TIR具有NAD^+水解酶活性, 产生两类重要信号小分子(pRib-ADP/AMP和ADPr-ATP/ADPR), 激活ADR1/NRG1类RNL抗病蛋白形成的EDS1-PAD4-ADR1和EDS1-SAG101-NRG1通路, 在生物膜上形成钙离子通道来引起细胞死亡[13,14]. 此外, 非典型抗病基因[15]往往赋予植物对病原菌的广谱抗病性, 如一类免疫正调控基因*Fhb7*[16]、*Lr67*[17]和*Lr34*[18]等, 另一类免疫负调控基因或感病基因(disease-susceptibility gene, *S* gene) *MLO*[19]、*RBL1*[20]、*SWEETs*[21]等. 这些基因的高度保守性使其广泛应用于作物抗病中. 因此, 了解作物不同抗病机制, 挖掘新型抗病基因是保障粮食安全的最本质需求.

目前, 基因编辑技术已成为作物抗病育种的强大工具, 并已广泛应用于水稻、小麦、玉米和马铃薯等主要粮食作物的育种中[22]. 基因编辑通过对目标基因进行精确的定点插入、敲除或修改, 实现对靶标基因的定点修饰, 从而优化作物农艺性状, 如增强抗病性、改善品质和提高产量[23]. 随着一系列人工核酸内切酶的出现, 基因编辑工具, 如人工锌指核酸酶(zinc finger endonuclease, ZFN)[24]、类转录激活因子效应物核酸酶(transcription activator-like effector nuclease, TALEN)[25]、规律成簇间隔短回文重复序列及其相关蛋白(clustered regularly interspaced short palindromic repeats/CRISPR-associated, CRISPR/Cas)[26]等快速发展. 在作物中, 各种基因编辑工具, 如不同类型Cas核酸酶介导的CRISPR/Cas基因编辑系统、碱基编辑器(base editors, BEs)、引导编辑器(prime editors, PEs)及其衍生工具PrimeRoot (prime editing-mediated recombination of opportune targets), 使得小的序列插入或缺失(InDels)、单碱基替换和大片段的定向插入等成为可能[27](图1). 目前, 基于CRISPR/Cas9的基因编辑工具应用最为广泛, 它由crRNA、反式激活crRNA(tracrRNA)和Cas9组成, crRNA和tracrRNA结构引导Cas9在靶标位点切割. 2012年, Jinek等人[28]将crRNA和tracrRNA融合为一个小RNA分子(single guide RNA, sgRNA), 在sgRNA的引导下Cas9成功切割目标位点DNA双链. 利用此系统靶向切割DNA双链需满足2个条件: sgRNA 5′端20 nt (nucleotides)的引导序列与靶标DNA互补配对; 靶位点下游必须存在PAM(protospacer adjacent motif). Cas9所识别的PAM序列为5′-NGG-3′. CRISPR/Cas9识别靶标DNA序列后在PAM上游第3、4个碱基之间切割DNA双链, 从而引入DNA双链断裂(double-strand break, DSB), 再利用细胞自身的修复系统对基因组序列进行编辑[29](图1(a)). 除Cas9核酸酶之外, 其他类型核酸酶如Cas12、Cas13及TnpB等核酸酶介导的基因编辑系统也在多种植物中得到应用. 相较于Cas9, Cas12a (Cpf1)核酸酶更小, 识别富含A/T的PAM序列, 且产生的DSB为黏性末端. CRISPR/Cas12a系统中crRNA自身即可加工成熟, 不需要tracrRNA的参与, 因此其sgRNA序列更短(图1(b)), 对于多重基因编辑载体构建更具优势[30]. Cas13是靶向切割单链RNA的核酸酶, 可在RNA层面对靶标进行编辑[31](图1(c)). 因此, Cas13在创制抗RNA病毒作物方面表现出很大潜力, 并已成功应用于多种抗RNA病毒作物的创制[32~34]. 此外, Cas13还具有独特之处, 当Cas13与crRNA及靶向的底物结合形成三元复合体后, 随构象变化, Cas13不仅能切割靶标RNA, 还能够非特异地切割周围任意单链RNA, 这一特性被称为旁切活性(collateral cleavage activity)[35]. Cas12a也具有与Cas13类似的旁切活性[36], 基于这一特点, Cas13和Cas12被改造为快速核酸检测工具, 并成功应用于田间水稻白叶枯、条纹病、黑条矮缩病等多种病原微生物

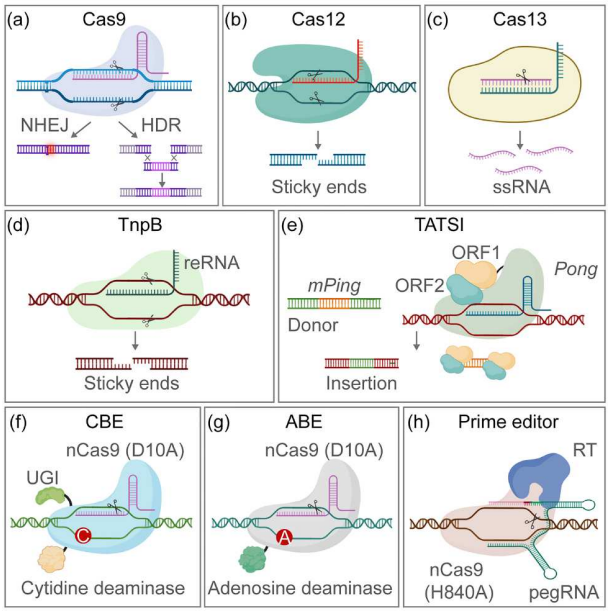

图 1 基于CRISPR/Cas的基因组编辑工具示意图. (a) CRISPR/Cas9; (b) CRISPR/Cas12; (c) CRISPR/Cas13; (d) TnpB; (e) TATSI; (f) 胞嘧啶碱基编辑器; (g) 腺嘌呤碱基编辑器; (h) 引导编辑器. NHEJ, 非同源末端连接; HDR, 同源重组介导的修复; ssRNA, 单链RNA; TnpB, 转座酶B; TATSI, 转座酶辅助靶点整合; *Pong*, 来源水稻的转座酶基因; ORF1, ORF2, 转座酶*Pong*蛋白; *mPing*, 来源水稻的非自主转座元件; nCas9(D10A), RuvC结构域失活的Cas9核酸切口酶; UGI, 尿嘧啶DNA糖基酶抑制剂; Cytidine deaminase, 胞嘧啶脱氨酶; Adenosine deaminase, 腺苷脱氨酶; nCas9(H840A), HNH结构域失活的Cas9核酸切口酶; RT, 逆转录酶; pegRNA, 引导编辑向导RNA. Biorender绘图

Figure 1 Schematic diagram of CRISPR/Cas-based genome editing tools. (a) CRISPR/Cas9. (b) CRISPR/Cas12. (c) CRISPR/Cas13. (d) TnpB, transposase B. (e) TATSI, transposase-assisted target-site integration. (f) Cytosine base editor. (g) Adenine base editor. (h) Prime editor. NHEJ, non-homologous end-joining; HDR, homology-direct repairing; ssRNA, single strand RNA; *Pong*, rice *Pong* transposase; ORF1 and ORF2, rice *Pong* proteins named ORF1 and ORF2; *mPing*, non-autonomous rice transposable elements *mPing*; nCas9 (D10A), nickase Cas9 with the D10A substitution in the RuvC domain; UGI, uracil DNA glycosylase inhibitor; nCas9 (H840A), nickase Cas9 with the H840A substitution in the HNH domain; RT, reverse transcriptase; pegRNA, prime editing guide RNA. Figure created with biorender.com

检测[37,38]. 在对CRISPR系统起源进化研究的过程中发现, 原核生物转座子编码的TnpB是Cas12核酸酶的祖先, 可以在reRNA(right end element RNA)的引导下切割双链DNA, 作用机制与CRISPR/Cas相似, 并且此类蛋白分子量更小, 其有效表达和递送相对更容易(图1(d)). 碱基编辑器分为胞嘧啶碱基编辑器(cytosine base editors, CBEs)和腺嘌呤碱基编辑器(adenine base editors, ABEs). CBEs由nCas9(D10A)和单链DNA胞嘧啶脱氨酶(cytidine deaminase enzyme)及尿嘧啶DNA糖基酶抑制剂(uracil DNA glycosylase inhibitor, UGI)组成(图1(f)). CBEs可以将编辑窗口内的胞嘧啶转换为胸腺嘧啶, 即C-G碱基对转换为T-A碱基对[39]. ABEs由nCas9(D10A)和定向进化的tRNA腺苷脱氨酶(tRNA adenosine deaminase, TadA)组成, 实现A-T碱基到G-C碱基对的转换(图1(g)). 引导编辑器由工程化逆转录酶, Cas9 (nickase Cas9 H840A)和目标位点的引导编辑向导RNA (prime editingguide RNA, pegRNA)组成, 其中nCas9 (H840A)突变使HNH核酸酶结构域失活, 只在目标位点非靶标链上产生单链断裂, pegRNA编码期望编辑序列的逆转录模板和3′端的引物结合位点[40](图1(h)). 将这些CRISPR相关工具与抗病育种相结合, 大幅度缩短了育种周期, 为作物抗病发展提供了全新的途径[41]. 本文重点讨论了基因编辑技术在作物抗病遗传育种改良上取得的重要进展 (表1). 最后, 为了推动基因编辑在作物遗传育种领域的发展, 我们对未来的研究方向进行了展望.

1 基因编辑技术在作物抗病改良中的应用

传统抗病基因的克隆和应用仍面临着一些挑战, 比如克隆周期较长, 抗性可能因病原菌新的生理小种的出现而迅速丧失, 这可能导致抗病基因资源的相对匮乏. 然而, 通过基因编辑技术在作物基因组中原位编辑, 产生各种可遗传的等位基因, 为筛选优异的抗病品种提供了丰富的基因资源库[98]. 基因编辑技术的应用不仅加速了作物抗病品种的创制, 而且不影响作物产量, 从而加快了作物抗病育种的进程.

1.1 插入和替换在作物抗病中的应用

在基因编辑过程中, 目的DNA插入或替换借助于DSB的修复系统, 包括非同源末端连接(nonhomologous end joining, NHEJ)和同源定向修复(homology-directed repair, HDR). 其中NHEJ是DSB最主要的修复途径. 利用NHEJ的定向插入和替换, 已成功在植物中实现了基因的插入、碱基替换和多种调控元件的靶向敲入[99,100]. 然而, NHEJ介导的插入可能会在目标位点的末端引入InDels及插入方向的随机性[101]. 在水稻中, Kumar等人[102]发现了1个核苷酸5′端、化学保护的双链寡脱氧核苷酸显著提高了所有测试的CRISPR靶向位点中靶标插入的精确度和方向控制. 作者通过这种策略将两个不同的转录激活因子样效应子PthXo3和

表 1　基因编辑在作物抗病中的应用

Table 1　Genome editing for disease resistance in crops

植物	编辑工具	靶标基因	抗性	参考文献
水稻	ABE、CBE	*SWEET14*	白叶枯病	[42]
水稻	ABE (rBE14)	*OsTubA2*	除草剂	[43]
水稻	CBE (rBE5)	*Pi-d2*	稻瘟病	[44]
水稻	CRISPR/Cas9	*Bsr-d1*/*Pi21*/*ERF922*	稻瘟病、细菌性枯萎病	[45]
水稻	CRISPR/Cas9	*OsCPK18*/*OsCPK4*	稻瘟病	[46]
水稻	CRISPR/Cas9	*OsERF922*	稻瘟病	[47]
水稻	CRISPR/Cas9	*OsFd1*	稻瘟病、白叶枯病	[48]
水稻	CRISPR/Cas9	*OsSWEET11*/*13*/*14*	白叶枯病	[49]
水稻	CRISPR/Cas9	*Pi21*	稻瘟病	[45]
水稻	CRISPR/Cas9	*Pi21*/*Bsrd1*/*Xa5*	稻瘟病、白叶枯病	[50]
水稻	CRISPR/Cas9	*RBL1*	稻瘟病、稻曲病、白叶枯病	[20]
水稻	CRISPR/Cas9	*ROD1*	稻瘟病、纹枯病、白叶枯病	[51]
水稻	CRISPR/Cas9	*TMS5*/*Pi21*/*Xa13*	稻瘟病、白叶枯病	[52]
水稻	MoBE	*OsACC*	除草剂	[53]
水稻	Prime Editor	*TFIIAγ5*/*xa23*	白叶枯病	[54]
水稻	Prime Editor	*xa23*	白叶枯病	[45]
水稻	PrimeRoot	*PigmR*	稻瘟病	[27]
水稻	STEME	*OsACC*	除草剂	[55]
小麦	CRISPR/Cas9	*TaCIPK14*	条锈病	[56]
小麦	CRISPR/Cas9	*TaeIF4E*	小麦黄花叶病	[57]
小麦	CRISPR/Cas9	*TaMKP1*	条锈病、白粉病	[58]
小麦	CRISPR/Cas9	*TaPsIPK1*	条锈病	[59]
小麦	CRISPR/Cas9	*TaWRKY19*	条锈病	[60]
小麦	TALEN/CRISPR/Cas9	*MLO*	白粉病	[61]
小麦	TALEN	*TaEDR1*	白粉病	[62]
大麦	CRISPR/Cas9	*HvMORC1*/*HvMORC6a*	白粉病、赤霉病	[63]
大麦	CRISPR/Cas9	*PDIL5-1*	大麦黄花叶病、温和花叶病	[64]
玉米	CRISPR/Cas9	*ZmChSK1*	玉米小斑病	[65]
玉米	CRISPR/Cas9	*zmfbl41*	玉米纹枯病	[66]
玉米	CRISPR/Cas9	*Zmfer1*	玉米穗腐病	[67]
玉米	CRISPR/Cas9	*ZmGDIα*	玉米粗缩病	[68]
玉米	CRISPR/Cas9	*ZmNANMT*	小斑病、大斑病、茎腐病	[69]
马铃薯	CRISPR/Cas9	*StDND1*	马铃薯晚疫病	[70]
马铃薯	CRISPR/Cas9	*StNPR3*	马铃薯斑纹片病	[71]
马铃薯	CRISPR/Cas9	*StPM1*	马铃薯晚疫病	[72]
马铃薯	CRISPR/Cas9	*StSR4*	马铃薯晚疫病	[73]
番茄	CRISPR/Cas9	*miR482b*/*miR482c*	番茄晚疫病	[74]
番茄	CRISPR/Cas9	*PMR4*	番茄白粉病	[75]
番茄	CRISPR/Cas9	*SlbBs5*/*SlBs5L*	细菌性斑点病	[76]
番茄	CRISPR/Cas9	*SlBBX20*	番茄灰霉病	[77]
番茄	CRISPR/Cas9	*SlJAZ2*	细菌性叶斑病、灰霉病	[78]
番茄	CRISPR/Cas9	*SlPelo*	番茄黄化曲叶病	[79]
番茄	CRISPR/Cas9	*SlPUB17*	番茄灰霉病、早疫病	[80]
棉花	CRISPR/Cas9	*Gh14-3-3d*	棉花黄萎病	[81]
棉花	CRISPR/Cas9	*GhMYB33*	棉花黄萎病	[82]

(续表1)

植物	编辑工具	靶标基因	抗性	参考文献
油菜	CBE	*BnALS1*/*BnALS3*	除草剂	[83]
油菜	CRISPR/Cas9	*BnaIDA*	油菜菌核病	[84]
油菜	CRISPR/Cas9	*BnaSTOP2*	油菜菌核病	[85]
油菜	CRISPR/Cas9	*BnCRT1a*	黄萎枯死病	[86]
油菜	CRISPR/Cas9	*BnF5H*	油菜菌核病	[87]
油菜	CRISPR/Cas9	*BnHva22c*	油菜黄萎枯死病	[88]
油菜	CRISPR/Cas9	*BnQCR8*	菌核病、灰霉病	[89]
油菜	CRISPR/Cas9	*BnRLK902*	菌核病、灰霉病	[90]
大豆	Cas12-SF01	*GmALS1*/*GmALS3*	除草剂	[91]
大豆	CRISPR/Cas9	*GmTAP1*	大豆根腐病	[92]
大豆	CRISPR/Cas9	*GmTCP19L*	大豆赤霉病	[93]
大豆	CRISPR/CasRx	*GmHC-Pro*	大豆花叶病毒	[94]
辣椒	CRISPR/Cas9	*CaERF28*	炭疽病	[95]
辣椒	CRISPR/Cas9	*CaMLO2*	白粉病	[96]
柑橘	Cas12a/crRNA-RNP	*CsLOB1*	柑橘溃疡病	[97]

TalC的结合元件定向插入到水稻白叶枯抗病基因*Xa23*的隐形等位基因*xa23*上的启动子区域，产生的水稻株系表现出对白叶枯菌不同生理小种的广谱抗病性. 在烟草中, 抗病基因*N'*只对烟草花叶病毒TMV-Cg具有抗性, 而不介导对TMV-U1的抗性. Li等人[103]将CRISPR/Cas系统和原生质体技术优势相结合, *N'*基因的两个抗性相关区域被*N'alata*基因的同源片段替换, 在烟草中实现无缝大片段插入, 赋予烟草对TMV-U1的抗性. HDR介导的基因组编辑可以用来精确地整合或替换作物中目标位点上的所需序列. 但是HDR在DSB修复中的频率远低于NHEJ, 因此在作物中通过HDR途径进行精准修复仍然具有挑战性. 目前, 已有研究表明, 通过串联重复结构介导的同源重组、融合来源于疱疹病毒和T7噬菌体家族的外切酶到Cas9/Cas12a等手段可以提高HDR修复频率, 从而提高基因插入或替换的效率[100,104], 这也为利用HDR途径实现抗病基因精准插入提供了技术手段. 最新研究发现, Liu等人[105]开发出了植物转座酶辅助靶点整合(transposase-assisted target-site integration, TATSI)系统, 将水稻的*Pong*转座酶蛋白与核酸酶Cas9和Cas12a融合表达, 在拟南芥和大豆基因组中实现了高效的靶向插入(图1(e)), 表明这种基于Cas蛋白的融合系统具有广泛的发展空间, 未来通过在各种作物基因组中插入抗病基因, 从而达到赋予作物广谱抗病的目的.

除了基于DSB的基因插入和替换之外, 碱基编辑器及其衍生工具已被用于引入单碱基的多重变异. 2020年, Li等人[55]将胞嘧啶脱氨酶和腺苷脱氨酶融合在nCas9的N端, 构建了5种新型的饱和靶向内源基因突变碱基编辑器(saturated targeted endogenous mutagenesis editors, STEMEs), 同时实现C-G到T-A及A-T到G-C的转换. 此外, 利用STEMEs工具, 研究人员实现了对水稻*OsACC*基因的定向进化, 获得了抗除草剂的材料. 水稻中, *Pi-d2*在第441位氨基酸替换(I441M)导致对稻瘟病菌(*Magnaporthe oryzae*)抗性的丧失[106]. 一个优化的碱基编辑器rBE5系统, 引入人源AID胞嘧啶脱氨酶, 将G>A替换(M441I)引入内源*Pi-d2*基因, 编辑效率为30.8%, 成功创制了Pi-d2$_{Kit}$(M44I)材料, 表现出对稻瘟病的明显抗性[44]. 在碱基编辑器基础上发展起来的引导编辑器, 克服了碱基编辑器只能实现4种碱基转换的问题, 可以完成其余8种形式的碱基颠换以及小片段碱基的精准插入和删除, 是一种精确和高度通用的编辑技术. 迄今为止, PEs已在植物中得到了广泛应用, 为作物遗传改良提供了更为强大的工具. Zong等人[107]通过优化逆转录酶以及在引导编辑器中加入具有核酸伴侣活性的病毒衣壳蛋白, 提高了其在水稻和小麦多个靶位点的编辑效率. 最新研究表明, 在引导编辑器的基础上, 将重组酶系统与双ePPE系统相结合开发了新型编辑工具PrimeRoot, 其插入效率比NHEJ高2~4倍, 该系统在水稻基因组中实现了11.1 kb的DNA片段定点插入. 利用PrimeRoot系统, 成功在水稻肌动蛋白(rice actin1, *OsAct1*)启动子驱动下, 将水稻广谱抗病基因*PigmR*精确整合到水稻基因组中, 赋予水稻对稻瘟病的

广谱抗性(图2(a))[27]. 从自然界中筛选得到的许多天然抗病基因是由单核苷酸多态性(single-nucleotide polymorphism, SNP)差异引起的, 如*Bsr-d1*[108]、*ROD1*[109]. 这些抗病基因在不同品种中往往存在多态性差异, 利用碱基编辑工具对不含抗病基因位点的高产或高品质品系进行定向替换对于培育抗性高、品质好的优质品种是可行的路径. 对于某些不含抗性基因的品系, 可以通过引导编辑器向基因组中定向整合抗病基因, 在不损害产量和品质的前提下进一步提高作物广谱抗病性.

1.2 饱和编辑在作物抗病中的应用

天然抗病基因虽然为作物抗病领域提供了丰富的资源, 但数量有限[110]. 相比之下, 饱和诱变产生了多样化的变异, 建立了新的抗病基因的宝贵资源库. 在饱和诱变中, 对目的基因的随机或靶向突变产生一系列突变等位基因, 通过高通量筛选以获得具有所需性状的等位基因. 饱和诱变是研究基因功能和获得优异等位基因的一种直接策略. Zhang等人[53]开发了新型的多重正交碱基编辑器(multiplexed orthogonal base editor, MoBE)和随机化多sgRNA组装技术. MoBE可以实现在多位点上高效的ABE和CBE多重编辑, 不同sgRNA表达模块的串联使不同靶点和不同编辑器的组合类型便会显著增加. 该研究进一步拓展了基于CRISPR的作物原位定向进化技术体系, 为作物基因组编辑新种质创制和挖掘优良等位变异提供了新的工具支撑. Lee等人[111]利用CRISPR/Cas9系统构建了一系列具有不同分子剂量靶基因的多种突变组合, 多个突变体对黑穗病具有广谱抗病性. 同时, 作者将该策略应用于孤儿品种Boranong, 成功培育出了果实产量显著提高的改良植株. 这表明编辑同源基因能够有效促进多倍体作物的农艺性状改良, 为其工程育种提供了广阔的发展前景. Zhang等人[112]开发了基于TnpB家族成员IsDge10的单基因与多基因编辑工具. 在水稻原生质体中, IsDge10核酸酶系统编辑活性高达15%, 同时在水稻稳定转化中编辑效率最高达到25%, 这表明该系统能够在水稻中介导可遗传的编辑. 除此之外, 该系统在水稻中也可以实现多个位点的同时编辑. 这些基于TnpB衍生的新系统未来有望应用于植物抗病领域中, 推动作物抗病分子育种的发展[112~114]. Sha等人[20]基于CRISPR/Cas9的饱和诱变策略获得了一系列等位基因, 通过表型筛选到一个缺少12 bp的优异等位基因*RBL1*$^{\Delta 12}$, 其赋予水稻对稻瘟病、稻曲病和白叶枯病等多种病害的广谱抗病性, 且不影响水稻产量. *RBL1*基因在其他作物中高度保守, 普适性强, 具有广泛的应用潜力. 该研究表明, 通过基因编辑饱和突变的方法, 获得优异的新型等位基因, 可以用来打破抗性与产量间的平衡. Chen等人[115]建立了解旋酶辅助连续编辑(helicase-assisted continuous editing, HACE)的平台, 通过将解旋酶和脱氨酶融合, 对碱基序列不断脱氨, 可以实现大于1000 bp基因组区域的连续突变. 这项技术为哺乳动物的基因功能学研究提供了平台, 同时也对人类癌症治疗具有重要意义, 为开发新的药物靶点和治疗策略提供了新的可能. 尽管HACE技术尚未在作物改良中得到应用, 但其在基础研究中应用的潜力表明, 未来它可能成为作物抗病改良领域中有价值的编辑工具. 这些饱和编辑方式产生的丰富候选基因资源库为作物分子育种提供了保障, 是实现作物广谱抗病的重要手段(图2(b)).

1.3 编辑感病基因在作物抗病中的应用

在植物与病原菌互作中, *S*基因通过协助病原菌在植物寄主细胞内定殖和繁殖, 促进病原菌的侵染[116]. *S*基因一般是植物生长发育的重要调控因子, 因此简单地敲除*S*基因在提高抗病性的同时往往会影响作物产量. *MLO*基因最早发现在大麦, 功能丧失突变体对白粉病表现出明显的抗病性[117]. 1997年, Büschges等人[118]克隆了*MLO*基因, 发现了该基因编码具有7个跨膜螺旋的跨膜蛋白, 并提出了大麦MLO蛋白在植物免疫中具有负调控作用. *MLO*基因功能在单子叶和双子叶植物中均是高度保守的. 在拟南芥、苹果、葡萄、黄瓜、豌豆、辣椒、烟草和番茄等物种中的同源基因*MLO*, 其突变体同样具有白粉病抗性[119]. 但是, 在大麦和拟南芥中, *MLO*突变会引发叶片细胞壁胼胝质沉积和细胞的自发性死亡. 同时, *mlo*突变体表现出早衰表型. 2014年, Wang等人[61]利用TALEN和CRISPR/Cas9系统靶向敲除六倍体小麦中高度同源的3个感病基因*MLO*, 获得了对白粉病具有广谱持久抗性的功能缺失突变体, 但是植物生长受到严重的影响, 这一负面表型严重限制了其在农业生产中的广泛应用. 为了克服*mlo*完全敲除造成植株矮小的不利性状, 该团队后期筛选到一突变体*Tamlo-R32*, 该材料携带的大片段缺失改变了染色质构象, 在提高白粉病抗性同时不会带来产量损失. 后续研究人员利用CRISPR编辑技术, 在小麦主栽品种中创制相应的基因突变, 仅2~3个月就成功在多个小麦主栽品种中获得了具有广谱白粉病抗性, 且生长和产量

均不受影响的小麦种质. 相比于传统育种方法, 基因组编辑育种极大缩短了育种进程[120]. *TaPsIPK1*是小麦免疫负调控基因, 是条锈菌在侵染寄主过程中劫持的感病因子. Wang等人[59]利用CRISPR/Cas9系统靶向编辑*TaPsIPK1*, 赋予小麦对条锈菌的广谱抗病性且不影响其主要农艺性状. 同样的, *NANMT*基因编码烟酸甲基转移酶, 能够将烟酸转化成葫芦巴碱. 在玉米中, Li等人[69]通过CRISPR/Cas9系统对*ZmNANMT*进行基因编辑, 获得的突变体表现出对玉米小斑病、玉米大斑病和玉米茎腐病的广谱抗病性, 并且玉米产量无显著的负面效应. 此外, *NPR1*是植物激素水杨酸(salicylic acid, SA)的主要调节因子. *NPR1*的同源基因*NPR3*负向调节

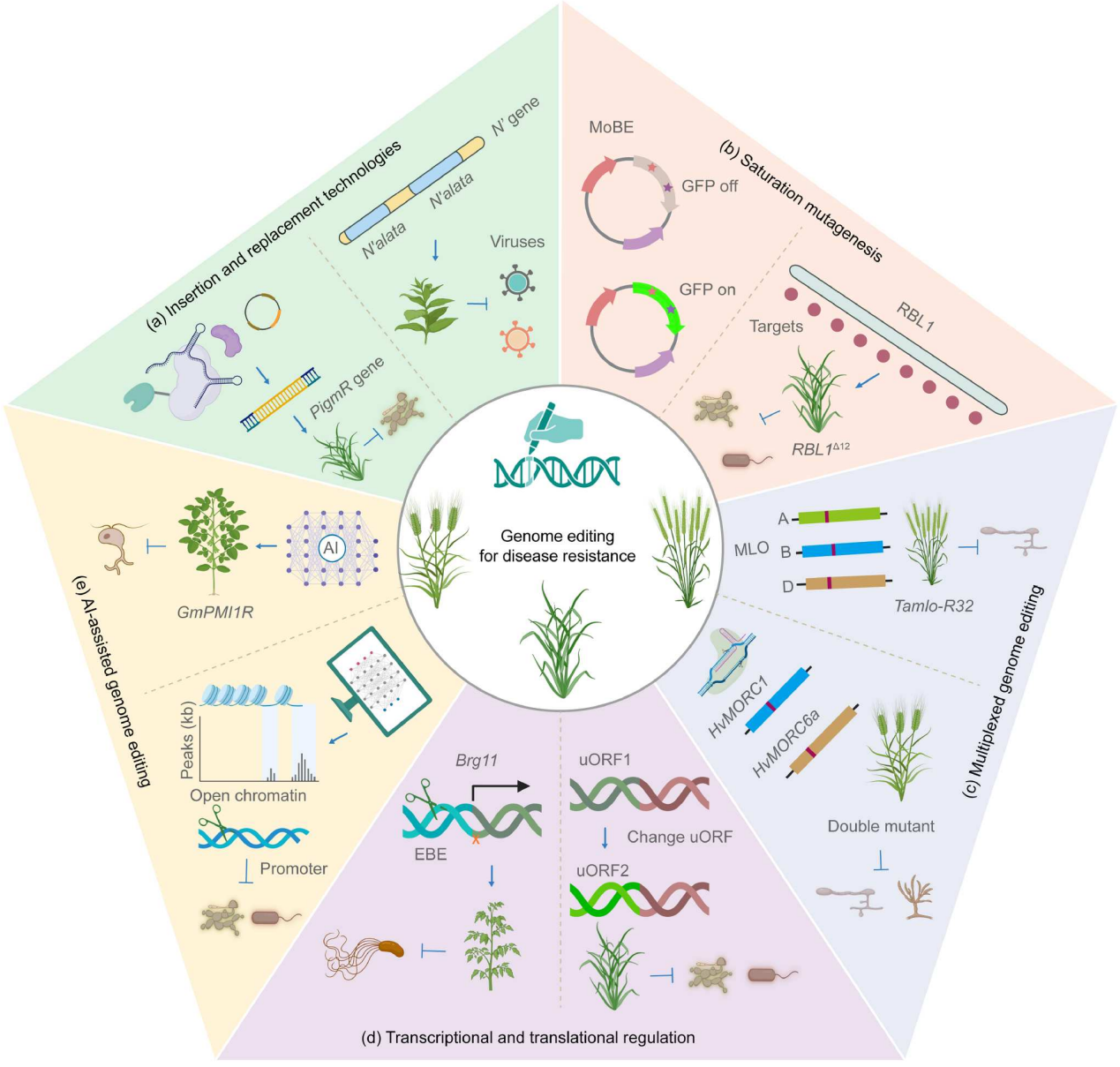

图 2 基因编辑技术在作物抗病改良中的应用. (a) 基于插入和替换编辑在抗病中的应用; (b) 饱和突变产生新型抗病基因; (c) 多重编辑策略实现广谱抗病; (d) 通过基因编辑技术在转录和翻译水平调控基因表达增强作物抗病; (e) 人工智能介导的基因编辑在作物抗病中的应用. AI, 人工智能; uORF, 上游开放阅读框; EBE, 效应蛋白结合元件. Biorender绘图

Figure 2 Genome editing technologies for enhancing disease resistance in crops. (a) Insertion/replacement-based genome-editing techniques for disease resistance. (b) Generation of novel disease-resistance genes via saturation mutagenesis. (c) Multiplexed genome editing strategy to achieve broad-spectrum disease resistance. (d) Enhance disease resistance in crops through genome editing technologies at the transcriptional and translational levels. (e) AI-assisted genome editing for enhancing crop disease resistance. AI, artificial intelligence; uORF, upstream open reading frame; EBE, effector-binding element. Figure created with biorender.com

SA介导的免疫反应. 在马铃薯中, 靶向编辑*NPR3*赋予马铃薯对斑纹片病的抗病性, 且表现出正常的生长[71]. 在柑橘中, *CsLOB1*是柑橘溃疡病的感病基因, Su等人[97]利用Cas12a/crRNA核糖核蛋白转化胚性原生质体来靶向编辑溃疡病感病基因*CsLOB1*, 并且在10个月内从T_0代中培育出无转基因的抗溃疡病柑橘品系. 该柑橘品系已成功获得美国农业部动植物检疫局的监管批准. 这些研究结果表明, 利用基因编辑技术对*S*基因编辑是快速创制平衡抗性与生长突变体材料的手段之一. 在病原菌与植物互作的研究中, 病原菌挟持*S*基因的作用模式或关键位点已经报道, 例如, 水稻黄单胞菌(*Xanthomonas oryzae* pv. *oryzae*, *Xoo*)通过III型分泌系统(type III secretion system, T3SS)在植物细胞内分泌转录激活因子样效应子(transcription activator-like effectors, TALEs), 结合在水稻蔗糖转运蛋白基因*SWEETs*启动子的效应蛋白结合元件(effector binding elements, EBEs)上, 从而激活这些*S*基因的表达, 致使水稻感病. 在植物与病原体的共同进化过程中, *SWEET*蔗糖转运体不仅是植物易感因子家族的代表, 也是各种病原细菌的靶标. *SWEET*基因功能的缺失或抑制会赋予植物抗性. Zaka等人[121]基于TALEN技术对*OsSWEET14*启动子中EBEs进行定向突变, 使病原菌TALEs无法结合靶标, 从而赋予水稻对白叶枯病的明显抗性. 因此, 对病原菌的靶标或作用位点进行定向编辑也是提高植物抗病性的重要策略.

在单基因编辑的基础上, 多重基因编辑同时靶向多个基因, 特别是*S*基因的定点突变, 可以同时增强作物对不同病原菌的抗性, 加速作物广谱抗病育种的研究[122](图2(c)). 多重基因编辑在不同物种中广泛应用, 赋予作物广谱抗病性. 在水稻中, Li等人[52]通过CRISPR/Cas9系统编辑品占中*TMS5*、*Pi21*和*Xa13*基因, 获得三突突变体, 表现出对稻瘟病和白叶枯病的抗性显著增强. 同样地, Tao等人[50]同时敲除水稻的*S*基因*Pi21*、*Bsrd1*和*Xa5*, 提高水稻对稻瘟病和白叶枯病的广谱抗病性. Zhou等人[45]靶向编辑杂交水稻骨干不育系隆科638S三个*S*基因*Bsr-d1*、*Pi21*和*ERF922*, 增强了水稻对稻瘟病和细菌性枯萎病的抗性. Oliva等人[49]对水稻*SWEET11*、*SWEET13*和*SWEET14*三个基因启动子上的EBE区域进行多重编辑, 获得了对多个*Xoo*生理小种具有广谱抗性的水稻材料. 在小麦中, 通过CRISPR/Cas9技术同时编辑小麦*TaCIPK14*的3个同源基因, 获得了*TaCIPK14*突变体, 表现出对小麦条锈病的广谱抗病性[56]. 同样地, 同时突变小麦*TaMKP1*的3个同源基因, *Tamkp1*突变体对小麦条锈病和小麦白粉病的抗性明显增强[58]. 在大麦中, Galli等人[63]通过使用CRISPR/SpCas9系统同时靶向编辑*HvMORC1*和*HvMORC6a*基因, 获得了*hvmorc1*/*6a*双突变体, 该突变体表现出对白粉病真菌(*Blumeria graminis*)和禾谷镰刀菌(*Fusarium graminearum*)的广谱抗性. 在番茄中, 同时敲除*SlBs5*和*SlBs5L*基因实现了对细菌性斑点病的广谱抗性[76]. 这些研究表明, 多重基因编辑是赋予作物广谱抗病性的重要策略.

1.4 基于转录和翻译水平调控基因表达在作物抗病中的应用

基因表达受到转录水平和翻译水平上的调控, 根据基因表达水平的不同产生不同等级的表型变化, 这对于作物产生新型的理想性状至关重要[123](图2(d)). 基因表达受多个区域元件的调控, 包括启动子、5′上游开放阅读框(5′-uORF)以及5′和3′非翻译区(5′和3′UTRs). 基于CRISPR/Cas9和CRISPR/Cas12a基因编辑系统, Wu等人[124]靶向水稻HPPD抑制剂抗性基因*OsHPPD*的3′-UTR区域设计了10个靶位点进行编辑, 获得了水稻HPPD抑制性除草剂抗性材料. *RNG1*和*RNG3*的表达水平与水稻稻瘟病抗性呈负相关. Xu等人[125]利用CRISPR/Cas9技术分别对两个基因的3′-UTR区域进行编辑, 发现了*RNG1*和*RNG3*的表达量水平影响着水稻对稻瘟病和白叶枯病的抗性. 启动子顺式调控元件变异引起基因表达水平的改变, 因此也被用以提高抗病性. 青枯雷尔氏菌(*Ralstonia solanacearum*)菌株转录激活物样蛋白RipTAL结合在植物*Brg11*基因启动子的EBE上, 转录激活下游易感基因*Bs4C*以增加宿主易感性. 通过基因编辑技术靶向*Brg11*基因启动子EBE区, 赋予番茄对青枯菌的抗性, 且对番茄的农艺性状无影响[126]. 基于CRISPR/Cas9技术, Liu等人[127]靶向玉米*CLE*基因启动子区并设计9个靶位点, 获得多个提高玉米产量的株系. 除此之外, 改变目的蛋白的翻译调控水平是实现精准调控作物抗病能力的重要策略之一. Tian等人[128]开发了一种基于CRISPR技术的“uORF类型转换系统(CRISPR-aTrE-uORF)”, 其中包括uORF生成、uORF去除和uORF类型转变, 利用该系统可以产生不同等级翻译水平的突变体. 例如将水稻谷氨酰胺合成酶基因*OsGS2*的uORF系统改变, 从Type1 uORF变为Type2 uORF增加了对*GS2*的翻译抑制作用, 提高了水

稻的广谱抗病性, 且不影响水稻正常生长. Xu等人[129]发现uORF-TBF1介导的翻译调控可以用来精确调节NPR1的蛋白质表达, 从而提高水稻对稻瘟病、白叶枯病和细菌性条斑病的抗性, 而不影响其他农艺性状. 该研究为未来作物抗病改良提供了新的思路.

1.5 人工智能介导的基因编辑在作物抗病中的应用

通过整合各种基因的表型、功能以及其他组学数据, 形成一个丰富的信息调控网络模型, 人工智能(artificial intelligence, AI)预测介导的基因编辑为实现精准编辑提供了强有力的支撑. 近年来, 前沿的生物技术与信息技术共同推动着这种革新逐渐进入作物育种领域. 研究者通过采用这种策略快速挖掘作物性状关联调控基因和预测性状变异, 从而实现智能、高效地培育新品种, 改善作物的农艺性状. AI在优化基因编辑系统和预测潜在遗传变异对表型的影响上具有很好的应用潜力[130](图2(e)). 脱氨酶样蛋白质能够催化核酸中核苷酸和碱基的脱氨作用, 是碱基编辑器应用中重要的一环. 目前, 只有少数针对单链DNA的载脂蛋白B mRNA编辑酶催化多肽(apolipoprotein B mRNA editing catalytic polypeptide-like, APOBEC)、激活诱导的胞嘧啶脱氨酶(activation-induced cytidine deaminase, AID)类脱氨酶以及一个针对双链DNA的脱氨酶(DddA)被用于生成CBEs, 它们蛋白体积大, 使得通过腺相关病毒(adeno-associated virus, AAV)病毒载体进行递送变得困难. Huang等人[131]通过AlphaFold2辅助的蛋白质结构预测和聚类的方法分析脱氨酶的功能及内在的联系, 挖掘更高效的脱氨酶应用于基因编辑, 创制新型编辑体系, 减少脱靶效应的影响, 实现精准编辑, 促进作物广谱抗病的研究. 新开发的脱氨酶mini-Sdd7在大豆中表现出高效的编辑效率, 与其他脱氨酶的编辑系统相比, mini-Sdd7有10~30倍的巨大提升. 并且采用新研发的Sdd7对大豆进行编辑, 成功获得了稳定遗传的杂合子大豆植株. 这项研究工作展示了基于人工智能优化脱氨酶提高了基因编辑的效率, 这为未来的农业育种工作作出了重要贡献. 除此之外, AI介导的基因编辑已经逐渐成为通过微调目标基因的表达水平来产生所需性状的有效策略. 研究者们通过ATAC-seq(assay for transposase-accessible chromatin using sequencing)、MNase-seq(micrococcal nuclease digestion with deep sequencing)、ChIP-seq(chromatin immunoprecipitation sequencing)和启动子顺式元件编辑等可用数据信息库的预测, 利用基因编辑技术操纵抗病基因的表达水平, 在不影响作物生长的前提下提高作物广谱抗病性. 在蛋白水平, AlphaFold[5,132]、Rosetta-Fold[133]和ESMfold[134]是3个高度精确的蛋白质结构预测工具. 最近的研究表明, AlphaFold3在结构预测的准确性上取得了新突破, 对药物设计具有重要的指导意义. 与AlphaFold2相比, AlphaFold3在模型架构、数据处理和应用范围上都实现了显著的提升和扩展[135]. 这些工具的预测能力对于指导蛋白突变设计具有重要意义, 有望在作物基因组编辑中实现作物广谱抗病方面发挥关键作用.

2 展望

提高作物抗病性, 特别是广谱抗病性, 对于保障全国粮食安全至关重要. 解析作物抗病遗传基础, 挖掘新型抗病基因, 阐明潜在抗病机制是抗病分子育种成功的关键. 培育广谱持久抗性且稳产的作物品种是作物抗病育种的重要导向, 利用基因编辑技术培育广谱持久抗病性品种是作物抗病性改良的重要策略, 基因编辑技术已然成为种质创新的有效途径. 本文重点聚焦于基因编辑在作物抗病中的应用, 特别是如何利用对*R*基因和*S*基因的精细调控打破作物抗病和产量间的平衡. 例如, 编辑水稻*RBL1*[20]以及小麦*MLO*等*S*基因, 可以在实现广谱抗病性的同时不影响产量[136]. 越来越多的研究表明, *S*基因编码的蛋白一般是病原菌致病因子的重要靶点, 是打破作物抗病和产量间的平衡的重要途径, 是创制广谱持久抗性且稳产材料的宝贵资源.

自然诱变和人工诱变产生的群体中含有丰富的高密度等位基因突变, 这些突变包括复杂的单碱基替换或缺失以及大规模的染色体缺失. 这些变异为我们从多样化的突变群体中筛选特定类型的突变提供了便利, 成为挖掘广谱抗病基因的重要资源. 例如, 天然隐性抗病突变体*rod1*[109]源自粳稻TP309育种群体, 而广谱抗病突变体如*spl11*[137]、*oscul3a*[138]和*mlo*[139]等则源自人工诱变群体. 这些遗传资源对于快速鉴定和克隆广谱抗病基因具有重要的价值. 然而, 传统抗病基因的克隆周期较长, 且这些突变体可能存在生长缺陷. 基因编辑技术的发展显著加速了植物抗病育种的进程, 在优化植物抗病基因表型方面发挥着重要作用, 尤其是对于具有多效性且与植物生理功能连锁的抗病基因的编辑, 可以减少其对植物生理功能的负面影响. 例如, 通过人工诱变获得的*mlo*突变体虽然具有广谱抗病性, 但表现出早衰的表型, 生长受到严重限制, 这为基因编辑靶向

优化提供了可利用基因，充分发挥抗病基因在农业生产中应用的可能性. 利用基因编辑技术靶向*MLO*基因, 不仅可以赋予小麦广谱抗病性, 还能挽救产量损失, 从而加速了植物抗病育种进程[120]. 同时，基因编辑优化系统使得*MLO*基因具有跨物种实现广谱抗性的巨大潜力, 对作物抗病领域发展具有重大意义[15]. 此外，人工智能和基因编辑的结合，有可能为作物抗病品种培育带来新的突破. 在动物中，BE-DICT、ABEdeepoff、CBEdeepoff等工具已预测靶标基因的编辑效率[140]，但植物中高通量基因编辑技术尚处于起步阶段，已有的基因编辑数据量尚难以满足精准预测工具的要求，亟需开发新的高通量基因编辑工具、产生一系列突变类型并评估其表型效应，使植物基因编辑技术进入AI的时代, 促进基因编辑育种在作物抗病中的应用[141].

基因编辑技术，特别是基于CRISPR系统的系列编辑工具的开发，对基础研究和生物育种具有重大意义. 但是, 基因编辑系统本身也存在一些局限性. 在遗传转化方面，基因编辑工具的有效递送是基因编辑工作中的关键一环. 利用传统的递送方式将基因编辑工具递送至植物细胞中，以产生稳定遗传并消除相关作用元件的无转基因基因编辑植物品系，往往需要依赖复杂繁琐的组织培养及后代筛选过程，并且大部分植物不易进行遗传转化或世代周期较长. 近年来, 越来越多的有效递送系统被开发应用于不同植物中. 切-浸-萌芽(cut-dip-budding，CDB)递送系统是一种无需组织培养的转化方法，克服了传统技术由于植物组织培养过程带来的困难，在草本植物、块根植物、木本植物和叶插繁殖能力的多肉植物中实现了基因编辑工具的递送, 展现了CDB递送系统的广泛应用潜力[142]. 在底层工具酶方面, Tnp或其他微型的可编程核酸酶的应用有望进一步丰富植物基因编辑工具，相比较Cas蛋白，更能实现高效的编辑效率[114]. 在植物基因编辑中，高效率的精准无痕基因替换和敲入仍是一大技术瓶颈. Prime-Root和转座酶的应用为解决这一基因敲入和染色体大片段操控提供新的策略.

基因编辑技术凭借其精准修饰基因组的能力，在多种作物的抗病育种中得到了广泛应用，为实现病害的绿色防控提供了宝贵的资源. 2022和2023年, 农业农村部先后发布了《农业用基因编辑植物安全评价指南(试行)》和《农业用基因编辑植物评审细则(试行)》, 进一步明确基因编辑植物的分类依据和简化评审的标准, 规范基因编辑作物的评价, 为基因编辑技术在作物改良中的实际应用迈出了重要一步，为基因编辑作物的商业化推广和应用奠定了基础. 2024年, 农业农村部发布了农业基因编辑生物安全证书(生产应用)批准清单，其中包括中国科学院遗传与发育生物学研究所联合齐禾生科开发的抗白粉病高产小麦. 这体现了基因编辑技术在提高作物抗病性方面的潜力，也标志着该技术在农业生产中的实际应用和推广的可能性，有望在将来为农业生产带来革命性的变化.

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Summary for “基因编辑技术在作物抗病中的应用”

Genome editing for disease resistance in crops

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Plant diseases significantly reduce crop yield and quality, threatening global food security. Cultivating disease-resistant crops is essential for environmentally friendly disease control and food security. Thus, to enhance crop disease resistance, particularly broad-spectrum disease resistance (BSR), is vital for sustainable agriculture. Disease-resistance (*R*) genes encoded by pattern recognition receptors (PRRs) and nucleotide-binding leucine-rich repeat receptors (NLRs) confer BSR to a range of pathogens in plants. Additionally, atypical *R* genes, which do not encode immune receptors, often exhibit race non-specific and multipathogen resistance without yield penalty in crops. Understanding the underlying mechanisms of plant immunity, including pattern-triggered immunity (PTI), effector-triggered immunity (ETI) and atypical *R* gene-mediated immunity, provide new strategies for breeding disease-resistant crops. Over the past decade, genome editing has played an increasingly important role in crop breeding. Genome editing tools, such as diverse CRISPR/Cas systems, base editors, prime editors, and their derived tools, have proven highly efficient in generating single nucleotide polymorphisms (SNPs), insertions and deletions (InDels), substitutions, and large fragment deletions. These tools have significantly advanced functional genomics studies, particularly in the cloning, characterization, and engineering of BSR genes. They have also provided a wide range of strategies for enhancing disease resistance in crops. In contrast to traditional crop breeding approaches that rely on screening natural genetic variation and combining elite traits through cross-breeding, genome editing has significantly broadened the genetic diversity available for breeding disease-resistant crops and fostered innovation for disease resistance in crops. This advancement enables research on disease resistance in crops to break current barriers and progress to the next generation.

In this review, we briefly introduce the principles and recent advancements of various genome editing tools. In addition, we provide examples of disease resistance achieved through CRISPR/Cas systems in multiple crops, such as rice, wheat, barley, maize, potato, tomato, soybean, pepper, cotton, and citrus. We then highlight diverse strategies employed to achieve disease resistance, for example, targeted insertion or replacement for disease resistance, saturation mutagenesis for elite *R* alleles, multiplexed genome editing of disease-susceptibility (*S*) genes for BSR, transcriptional and translational control of *R* and *S* genes for balancing between crop growth and immunity, and artificial intelligence (AI)-guided genome editing for precise editing, which accelerates the molecular breeding strategies for obtaining novel *R* alleles and generating disease-resistant crops. We also emphasize the potential applications of advanced genome editing tools in precisely introducing diverse *R* genes into crops for disease resistance in the future. Finally, we discuss the attitudes and principles of relevant domestic and foreign policies on the application of genome-edited crops. In summary, using genome editing strategies makes it possible to develop crops with durable and broad-spectrum disease resistance and promote sustainable agriculture in the future, ultimately contributing to global food security.

genome editing, plant immunity, broad-spectrum disease resistance, *Oryza sativa*, *Triticum aestivum* L.

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