MEDIRES
Symbol of Quality Research

International Journal of Genetics and Genomic Science
info@mediresonline.org

ISSN:2836-2306 RESEARCH ARTICLE

Overview of CRISPR-Cas9 technologies and its application in crop improvement

Bissah. M.N[1*], Guiderdoni. E[2], Asante. M.D[3], Quain. M.D[3], Ribeiro. P[3], Ochar. K[1], Egbadzor. K[4], Kotey. D.A[1]

[1]*CSIR – Plant Genetic Resources Research Institute, Post Office Box 7, Bunso, Ghana*

[2]*CIRAD, AGAP institute, Avenue Agropolis, BP 5035, 34398 Montpellier, France.*

[3]*CSIR – Crops Resources Research Institute, P. O Box 3785 Fumesua, Kumasi - Ghana*

[4]*Ho Technical University, Ho, Ghana*

***Corresponding Author**: Bissah. M.N., CSIR – Plant Genetic Resources Research Institute, Post Office Box 7, Bunso, Ghana.

Received Date: 02 December 2022; **Accepted Date:** 16 December 2022; **Published date:** 05 January 2023.

Citation: Bissah. M.N., Guiderdoni. E., Asante. M.D, Quain. M.D., Ribeiro. P., Ochar. K., Egbadzor. K, Kotey. D.A1 (2023). Overview of CRISPR-Cas9 technologies and its application in crop improvement. International Journal of Genetics and Genomic Science .1(1). 10.58489/IJGGS.006.

Abstract

Genome editing offers a range of solutions for more efficient development of crops that are productive, adapted to stresses, climate-resilient, and less dependent on agro-inputs. Clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein (Cas9) technology is the current dominant tool used for genome editing. Originally, a Cas9 nuclease was employed to induce a double-strand break in its target site, causing the deletion of a few base pairs, inversion and gene integration to deliver desired changes in organisms. Aside from the primary nuclease activity (knock-in/out), a Base editor system, Gene priming and Cargo chauffeuring activities have been reported to deliver functionalities to specific regions in the DNA such as regulating transcription and fluorescence DNA for visualizing and understanding biological systems. Limitations of the scope of Cas9 activity were also eliminated by the recent development of more Cas9 orthologues (Cpf1-RR and Cpf1-RVR). Cas9 together with the advent of novel base editing tools that enable precise genome modifications and DNA-free genome editing via ribonucleoproteins demonstrate significant promise in the development of future crop improvement strategies. However, large-scale adoption of CRISPR/CAS will require optimizing strategies while accounting for costs, ease of implementation, and potential impacts on production gains. This review focuses on CRISPR application in plants, advances in CRISPR technology, regulations that may disadvantage scientists, resources for the smooth application of CRISPR and the preparedness of Africa to benefit from CRISPR technology.

Keywords: *Genome editing, CRISPR Cas9, Applications, crop improvement*

Introduction

Mutagenesis conferring genome changes in organisms may either turn off (Gene knockouts) or on (Gene knock-in) the function of genes in target regions. This results from a disruption in the synthesis of essential amino acids relevant in diverse metabolic pathways. Genetic mutants are essential for elucidating the genetic and molecular controls of many important biological mechanisms (Fang et al., 2018; Li et al., 2018; Ding et al., 2016; Matsumoto, 2005). The use of mutant-induced genetic variations in plant breeding commenced in the 1960s when radiation and chemical mutagenesis were developed to select favorable genetic combinations. Alterations in the sequence of the Deoxyribonucleic acid (DNA) (single or double-stranded) of organisms are critical for gene disruption (Durland & Ahmadian-Moghadam 2021; Chaudhary et al., 2019). At present, gene transfer and elimination can be achieved through conventional crosses, mutagenesis or through biotechnological approaches like genome editing (Sedlar, 2020; Cardi, 2016). Mutant libraries of several model plants have been generated by physical, chemical, or insertion (T-DNA/transposon insertion) mutagenesis and, more recently, by

genome editing techniques (Lu et al., 2017; Meng et al. 2017).

Traditional mutagenesis approaches often generate random mutations in the host genome, which could often cause deleterious effects that may not be intended and have many drawbacks including large screening populations to identify desired variants (Mohanta et al., 2017; Sikora et al., 2012; El-Gewely et al., 2005). Since the late 2000s four families of engineered and programmable nucleases including Mega-Nucleases, Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector nucleases (TALEN) and the Clustered Regularly Inter-Spaced Short Palindromic Repeats (CRISPR) Cas9 changed mutagenesis from random events to targeted modification of genomes (Mohanta et al., 2017; Gaj et al., 2016; Liang et al., 2014). These developments have limited mutations to specified regions of the genome, which, have helped decipher the function of genes more accurately.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9-associated protein technology is the current dominant genome editing tool used to manipulate and change the DNA of organisms (Ahmad et al., 2020;Wada et al., 2020;Tripathi et al., 2020;Li et al., 2017; Haeussler and Concordet, 2016). The CRISPR technology mimics the surveillance system performed by immune memory in bacteria that enables it to recognize and degrade foreign nucleic acids (Koonin et al., 2017; Doudna and Charpentier, 2014;Fineran and Charpentier, 2012).

The engineered CRISPR-Cas9 system relies on single guide RNA (sgRNA), a small non-coding RNA composed of a crRNA homologous to a genome target region and a tracrRNA that binds to CAS9.

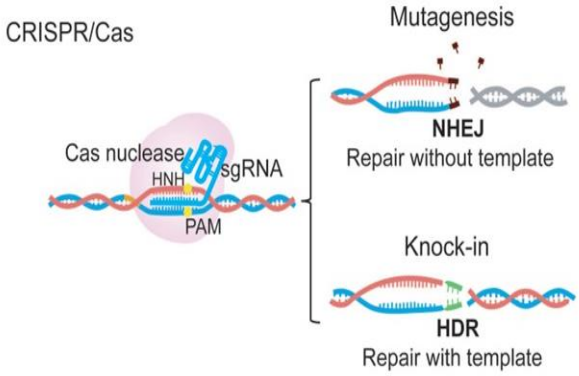
Target sites are generally located in the exon regions of an open reading frame in order to induce effective frameshift mutations (Jinek et al., 2012; Biswas et al., 2019).The target region of CRISPR sgRNA is usually 3 bp upstream of the NGG Proto-spacer Adjacent Motif (PAM) (Meng et al., 2017).

The Cas9 has a bi-lobed architecture, with a large globular recognition lobe (REC) and a small nuclease lobe (NUC) with two nuclease domains, Ruv C andHNH used to edit the DNA at the right position (Li et al., 2018; Endo et al., 2018;Lei et al., 2014;Voytas, 2013)

The CRISPR/Cas9 system can be delivered to targeted cells either indirectly by biological transfer (*Agrobacterium tumefaciens*) or directly by microprojectile bombardment of cultured tissues, electroporation or chemical treatment (Polyethylene glycol -PEG) of protoplast suspensions. Alternatively, it can be delivered as ribonucleoprotein complexes in bombarded tissues or protoplasts (Sharma et al., 2020;Liang et al., 2019;Murovec et al., 2018; Liang et al, 2017; Ding et al., 2016; Doench et al., 2016; Zhang et al., 2016;Krenek et al., 2015;Woo et al., 2015; Mao et al., 2013). However, CRISPR applications in plants have largely depended on *Agrobacterium*-mediated T-DNA transformation, which, is limited to a narrow range of genotypes within a species. The traditional CRISPR genome editing process relies on the double-strand break (DSB) repair capacity of recipient cell types, the promotor under which the Cas9/gRNA is expressed and the regeneration efficiency of the crop (Ding et al., 2016).

The DNA breaks caused by the nuclease are generally repaired by non-homologous end joining (NHEJ) eventually causing deletion and/or insertion of nucleotide(s) or, when a DNA repair template with homology regions with the target site is provided, homology-directed repair (HDR) (Lieber, 2010) (Figure 1). In the NHEJ approach, the cell repair mechanism attempts to rejoin the cut ends but loses or induces a few bases in the process: a situation that can result in gene alterations due to frameshift mutations. In the HDR situation, donor repair templates are supplied and the DSB repair may results in the integration of the donor sequence to allow targeted modification of genes by small to large nucleotide replacement or insertions but its efficiency remains low in flowering plants (Endo et al., 2018; Li et al., 2018; Shibata, 2017; Jiang et al., 2017;Zhang et al., 2017;Gaj et al., 2016; San Filippo et al., 2008).

Fig 1: Repair of double strand break in DNA (Adapted from Wu et al., 2020)



Advances in CRISPR-CAS 9 technology development and applications

The original CRISPR-Cas9 protein uses a combination of an enzyme that cuts DNA (Cas9, a

nuclease) and a guiding piece of genetic material (guide RNA) that specifies the location in the genome to be targeted. The application scope of CRISPR – Cas9 was expanded with the use of new CAS9 variants or CAS proteins with different specificities, notably the PAM, base editing, prime editing and simultaneously targeted mutations (multiplexing) (Wu et al., 2020; Wang et al., 2018; Ding et al., 2016). Recently, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas12a nuclease (Cpf1), dCas9 transcriptional regulators, base editors, PRIME editors and RNA editing tools are widely used in basic research (Wu et al., 2020; Ai, et al., 2022).

Recognition of guide RNA

The original CRISPR-Cas9 technology is based on the commonly used *Streptococcus pyogenes* Cas9 system which recognizes only Proto-spacer adjacent motif (PAM) = NGG. Alternative genome editing technologies including CRISPR *Prevotella and Francisella* 1 (Cpf1) or Cas12a have opened up CRISPR editing to additional areas of the genome have been developed (Shmakov et al.,2017., Gao et al., 2017). The Cas9 and Cpf1 differ in the structural organization as the Cpf1 protein is smaller, use of a shorter guide as the maturation of crRNA by Cpf1 does not require the assistance of trans-activating crRNA (tracrRNA). Whereas Cas9 cleaves the DNA close to the PAM site with blunt ends, Cpf1 cleaves distal to the PAM site and produces staggered ends, which offer the opportunity for further modification at the same would also facilitate HDR. The Cpf1 recognizes TTTV as well as TTTT PAM sites increasing the target range for genome editing for the Cas9 tool (Jiang et al., 2017; Ding et al., 2016; Kleinstiver et al., 2015). The Cas9 and Cas12a both cut DNA, while the Cas13 family has been shown to target RNA (Ai et al. 2022; Tang et al. 2021; Yangs et al. 2019)

Nuclease activity

Recently, new and more powerful techniques to edit genes have been developed based on the disabled activity of the Cas9 nuclease. When the nuclease activity of the Cas9 protein is disabled, it is referred to as dead (dCas9). The dead Cas9 can be fused to transcription activators, epigenetic modifiers and Fluorescence-labeled Epitope-tagged to modulate transcription reporter gene function (Veillet et al., 2020; Li et al., 2016; Kleinstiver et al., 2015; Fu et al.,

Multiplex genome editing

Another important development in the CRISPR-Cas9 technology is the ability to effectively express multiple sgRNAs for simultaneous editing of two or more genes (Wang et al., 2018;Ma et al., 2015; Lowder, et al., 2015; Cong et al., 2013; Kurata, et al., 2018; Li et al., 2018; Wang et al., 2017). Multiplexing thus addresses the extremely time-consuming and

2014; Mercer et al., 2012). New technologies have been developed for gene activation, replacement and insertion to satisfy various demands of genome editing for functional genomic studies which eliminate the necessary injury due to the double-strand break (DSB) associated with traditional Cas9 technology.

Base editing

Is the conversion of one base or base pair into another (e.g., A:T to G:C, C:G to T:A) or the introduction of point mutations at specific sites which permits re-code the DNA sequences (Li et al., 2021; Veillet et al., 2020; Li et al., 2018; Kim et al., 2017; Zong et al., 2017). The Adenine (Adenine - Guanine) and cytidine (cytosine-uracil-thiamine) base editors introduce mutations that largely do not disrupt the function of genes (Kim et al., 2017). The cytidine deaminase enzyme removes an amino group from cytosine converting it to uracil, resulting in a U-G mismatch which gets resolved via DNA repair pathways to form U-A base pairs. Subsequently, a T gets incorporated in the newly synthesized strand forming T-A base pairs. This results in C-G to T-A conversion in a programmable manner. Unlike cytidine deaminases, adenine DNA deaminases do not occur in nature. In 2017, David Liu and the group developed ABEs by using Escherichia coli TadA (E. coli TadA) through extensive protein engineering and directed evolution. Escherichia coli TadA is a tRNA adenine deaminase that converts adenine to inosine in the single-stranded anticodon loop of tRNA Arg. It shares homology with the APOBEC enzyme. The procedure involves the conversion of Cas9 nuclease to nickase followed by the fusion of the nickase with the deaminase enzyme (either cytidine deaminase or engineered adenine deaminase for C-G to A-T and A-T to C-G conversions respectively) (Veillet et al., 2020). The base editing technique permits the conversion of alleles by base change, the creation of premature stop codon for knock-out and the creation of new alleles of genes that do not exist in nature and has the advantage of overcoming the long procedure of introgressing new alleles. For instance, the acetolactate synthase (ALS) gene in tomatoes has been edited using Agrobacterium-mediated transformation with a Cytidine base editor changing amino acids essential for the protein to be targeted by the chlorsulfuron herbicide (Veillet et al., 2019). The authors reported the production of chlorsulfuron-tolerant plants with up to 71

laborious bottleneck of conventional cross-breeding methods for pyramiding multiple QTLs (Abdelraman et al., 2021). In China, a CRISPR/Cas9-mediated multiplex genome editing system was used to simultaneously mutate three major genes in rice which negatively regulated grain weight including Grain Width 2 (GW2), Grain Width 5 (GW5) and Thousand-Grain Weight 6 (TGW6)(Dai et al., 2016). All three genes generated mutants (gw5tgw6 and gw2gw5tgw6) which showed notably larger grain sizes than that of the wild-type (Dai et al., 2016). In hexaploid bread wheat, CRISPR-Cas9 has been used to modify multiple alleles to confer heritable resistance to powdery mildew (Wang et al. 2014b, Wang et al. 2018).

Transgene free edited products

An important feature of the agricultural application of CRISPR-Cas9 technology is the delivery of non-transgenic traits in crops. In seed-propagated crops, the CRISPR/Cas9 transgene can be easily eliminated through Mendelian segregation in progenies. This process alone distinguishes the technique from the class of GMOs and hence absorbs CRISPR edits from rigorous testing regimes. The transformation of protoplasts followed by plant regeneration allows either the delivery of a ribonucleoprotein complex or the transient expression of delivered plasmid DNA without DNA integration into the host genome. Such methods are particularly useful for vegetatively propagated crops. Woo et al. (2015)have optimized CRISPR/Cas9 ribonucleoproteins (RNPs) to completely avoid transgene integration. Veillet et al. (2019) reported 12.9% and 10

Applications of CRISPR technologies in crop improvement

3.1 CRISPR-Cas9 genome editing methods have been exploited in several spheres of research including breeding and development of agricultural crops, animals and human health genetic applications (Ahmad et al., 2020; Tripathi et al., 2020; Wada et al., 2020; Bao et al., 2019; Ding et al., 2016). In agriculture, CRISPR-Cas9 has been applied to modify and understand the functions of genes controlling major agronomic, nutritional and economic traits in a vast array of crops to achieve improved yield performance, enhanced nutritional quality (bio-fortification) as well as biotic and abiotic stress tolerance (Johnsson et al., 2019; Romero and Gatica-Arias, 2019; Mishra and Zhao, 2018; Mohanta et al., 2017; Zhang et al., 2017). There are several efforts of gene function analysis in important crops including Banana, cassava, maize, millet rice, sorghum, soybean, tobacco and tomatoes. Table 1 shows some of the current applications of CRISPR/Cas9 systems in crops are for studying gene function, improving agricultural traits, creating male-sterility mutants, and inducing haploids.

The role of genomics in plant research and sustainable crop production is imminent and it will become even more crucial in the future. Two key applications of CRISPR that will revolutionize agriculture more than the green revolution are the fixing of hybrid and enhancing diversity to improve adaptation.

Promoting apomixes for hybrid vigor preservation through the CRISPR/Cas9 editing

Hybrid vigor is a phenomenon in which progenies display greater performance than parents for a specific trait. This phenomenon has been established in many crops; however, the phenotype is usually lost in the following generations (Wang, 2020). F2 progeny seeds from hybrid often experience trait segregation, which lowers global plant performance. Hence farmers are compelled to renew hybrid seeds which sour the cost of production. Presently, hybrid seed production in rice relies on the implementation of male sterility systems whereas hybrid maize production relies on manual emasculation. Thus, year in and year out, huge resources are committed to a labor-intensive practice in maize and the development of male sterile lines in order to produce hybrid seeds

Which translates into a high seed cost for hybrids (Yuan et al., 2020). To eternalize the benefits of useful hybrids, hybrids must be propagated by seeds so that the benefit of hybrids can reach a large number of farmers. Alternative male sterility systems can be generated by CRISPR/Cas9. In rice, through the use of CRISPR/Cas9, sterile male lines have been obtained by mutating the OsGELP34 gene which encodes a putative GDSL lipase that plays a vital role in rice male reproduction (Yuan et al. 2020). An elite rice restorer line using the nptII gene as a plant selection marker. Agrobacterium-mediated genetic transformation (Chakraborty et al., 2016).

Apomixis is an asexual reproductive process that bypasses female meiosis and fertilization to produce embryos identical to the maternal parent (Hojsgaard, et al., 2019; Barcaccia and Albertini, 2013). Apomitic reproduction generates clonal seeds and offers tremendous potential in permanently preserving hybrid vigor. (Wang, 2020;Sailer et al., 2016; Sajid et al., 2021; Koltunow and Grossniklaus, 2003). This kind of reproductive process has been documented in

Table1: CRISPR/Cas9 AND ITS APPLICATION IN CROP IMPROVEMENT

Crop	Trait	Target Gene	Reference
Banana	Bacteria wilt resistance	Banana Xanthomonas wilt (BXW)	Tripathi et al. 2020),
	Endogenous banana streak virus resistance	eBSV	Tripathi, et al. (2022)
	Plantain	PDS gene	Ntui et al.,(2020)
Cassava	Cyanogen content	CYP79D1, YP79D2,	Lyons et al., (2021)
	Brown Streak	EIF4E, nCBP-1, nCBP-2	Gomez et al., (2019)
	Phytoene desaturase (carotenoid biosynthesis)	MePDS	Gomez et al. (2019)
	Cassava mosaic disease resistance	GBSS, PTST1, SBE2, CYP79D1, CYP79D2, and MePDS	Odipio et al., (2017)
Maize	Grain yield under drought stress conditions	ARGOS8	Shi et al. (2016)
	Male sterility		Jiang, et al. (2022)
	Haploid induction	ZmPLD3	Li et al. (2021)
		ZmPLA1	Liu et al. (2019)
		ZmDMP	Zhong et al. (2019
	Adaptation to high altitudes	ZmCCT9	Huang et al. (2018)
	Male sterility33	ZmMs33	Xie et al. (2018)
	Stomatal conductance and photosynthesis	ZmCST1	Wang et al. (2019b)
	Small kernel	RNAPIII subunit	Zhao et al. (2020)
	EMS mutants	ZmNRPC2	Thiruppathi (2020)
	Yield - inflorescence branching.	TPPs RAMOSA3 and TPP4	Claeys et al. (2019)
Millet	Chlorsulfuron-resistant plants. Herbicide tolerance	ALS2 gene SiFMBP , SiDof4 , SiBADH2 , SiGBSS1 , and SiIPK1	Svitashev et al. (2015) Liang et al. (2022)
Potato	Potato Virus Y Resistance Spectrum	IF4E1	Lucioli et al. (2022)
	Potyvirus Potato virus Y (PVY).	eIF4Es: eIF4E1-S, eIF4E1-T eIF4E2-S eIF4E2-T	Le et al. (2022)
Rice	Haploid induction	OsMATL	Yao et al. (2018)
	Lodging resistance	HTD1	Lacchini et al. (2020)
	Grain yield	GS3, GW2 & GN1 A	Lacchini et al. (2020)
	Photosynthetic efficiency, yield	Hexokinase OsHXK1	Zheng et al. (2021)
	Enhanced Rice Blast Resistance	Gene OsERF922. Ethylene-responsive Transcription Factor (ERF)	Wang et al. (2016)
	Nutrient Use Efficiency	NtUE	Sathee et al. (2022)
	Drought and salt tolerance	OsADR3	Li et al. (2021)
	Proline (Pro) synthesis	OsP5CS (Δ 1 -pyrroline-5-carboxylate synthetase)	Zhang et al. (2019)
	Stress response	(PYL1–PYL6 and PYL12) and group II (PYL7–PYL11 and PYL13) PYL	Miao et al (2018)
		NRT1.1B	Li, X. (2018)
	Bacteria blight	SWEET, Bsrk-1,	Oliva et al. 2019; Zhou et al. (2018)
	Glutinous Rice	Wx Gene	Fie et al. (2019)
	High amylose rice	SBEIIb, SBEI	Sun et al. (2017)
Sorghum	Carotenoid biosynthesis	Cinnamyl alcohol dehydrogenase (CAD) and phytoene desaturase (PDS)	Liu et al. (2019)
	Fragrance	SbBADH2	Zhang et al. (2022)
Soybean	Drought tolerance (polyethylene glycol (PEG)-simulated)	GmHdz4	Zhong et al. (2022)
	Flowering time	GmFT2a	Chen et al. (2017)
Tobacco	Seed Lipid Content	NtAn1	Tian et al. (2021)
	High oleic tobacco	NtFAD2–2	Tian et al. (2020)
	Biomass accumulation (Carboxylation rate -Rubisco)	rbcS_S1a, rbcS_S1b and rbcS_T1 a	Donovan et al. (2020)
	PVY- resistant tobacco	Ntab0942120	Ruyi et al. (2021)
Tomato	Stress-tolerance	SP and SP5G	Li et al. (2018d)
	Longer shelf life, fruit size	ALC gene	Yu et al. (2017)
	Anthocyanin biosynthetic Flavonoid biosynthesis	DcMYB7/DcbHLH3; MYB12, PSY1, MYB12, and SGR1	Yang et al. (2019) Yang et al. (2022)
	Chlorosulfuron-tolerance	ALS gene	Veillet et al. (2019)

many flowering plant species, although no major seed crops have been shown to be capable of apomixis. The ability to generate maternal clones and therefore rapidly fix desirable genotypes is an important breeding strategy to preserve superior allele combinations from heterosis. Apomixis breeding has followed three approaches that include (i) introgression of apomixis or its components from related species (ii) mutagenesis and (iii) genetic engineering approaches (Fiaz et al., 2021; Kaushal et al., 2004). Sexual reproduction is controlled by a cluster of genes, therefore Apomixis requires coordinated deregulation of several genes involved in reproduction (Hand and Koltunow 2013; Hojsgaard and Hörandl (2015). A number of genes play a critical role in apomixis, for instance, BBM and APOSTAT are candidate genes for the induction and maintenance of apomictic events. AtREC8 and AtSCC3 genes have been found to be essential to the monopolar orientation of the kinetochores during meiosis (Chelysheva et al., 2005). The MTL gene leads to haploid induction whereas the DYAD gene is a regulator of meiotic chromosome organization and is required for progression through female meiosis (Yang et al. 2019). Recently, CRISPR/Cas9-mediated genome editing has been used to induce mutations to divert the natural sexual pathway toward apomixis. The dyad mutant of Arabidopsis is defective in female meiosis and produces diploid embryo sacs following disrupted meiosis (Ravi et al. 2008). Centromeres are specified by a centromere-specific histone 3 (CENH3) protein in plants. An apomictic effect is produced in Arabidopsis lines that contain a manipulated centromere-specific histone (CENH3) (Ravi and Chan 2010). In rice partial apomixis was achieved almost two decades ago by triggering parthenogenesis in MiMe-generated unreduced female gametes by ectopic expression of a male-specific OsBBM (Kaushal et al., 2004; Marimuthu et al., 2011). Wang (2019) reported multiplex editing of three (key meiotic genes Omission of Second Division (OSD1), Absence of pairing and recombination (PAIR1), and Meiotic Recombination Protein (REC8) in hybrid rice to produce clonal diploid gametes. In three separate development, CRISPR/Cas9-mediated genome editing was used to synchronously knockout three genes in rice, PAIR1, OsREC8, and OsOSD1 (Khanday et al., 2019), OsSPO11-1, OsREC8, and OsOSD1 (Xie et al., 2019) and OSD1, SPO11/PAIR1 and REC8 (Muliet et al., 2016). A common limitation of these reports are the efficiency of seed production and paucity of pathogenesis thus additional parthenogenesis or an inducer without fertilization is needed for MiMe phenotypes to achieve apomixis and resolve the issue of low fertility (Yin et al., 2022). Specific expression of BBM and MATL genes promotes the elimination of the paternal genome after fertilization whereas editing of BBM1 expression or disruption of MTL leads to clonal seed production and heritability for multiple generations. However, a mutation in the MTL gene leads to haploid induction at the expense of seed production. To resolve the issue of low fertility it has been suggested that Mutation in BBM and MATL can be used in combination with MiMe to obtain apomictic plants (Khanday et al 2018, Wang et al 2019)

Enhancing fitness of crops through Genetic recombination

Genetic recombination is an important process for the maintenance of genetic diversity in organisms and provides the gene combinations needed to survive in changing fitness optimum in changing environments (Blary and Jenczewski 2019). Genetic recombination facilitates natural selection in addressing bottlenecks for evolution in an ecological context. Meiotic recombination is needed for homologous pairing during meiosis. It also ensures that offsprings differ in their fitness and decrease the risk factor for the population. Several genes including RECQ4, FANCM and FIGL1 are anti-cross-over genes, the mutation of which allows an increase in Cross over number (Zhang et al., 2017; Mieulet et al., 2016). Mieulet et al. (2016) explored the effects of mutating the orthologues of FANCM3, RECQ44 or FIGL15 on recombination in three distant crop species, rice (*Oryza sativa*), pea (*Pisum sativum*) and tomato (*Solanum lycopersicum*). The authors reported that the single recq4 mutation increased crossovers by about three-fold in these crops, suggesting that manipulating RECQ4 may be a universal tool for increasing recombination in plants.

Regulation of genome-edited products

The development of genome editing technologies within a safe and ethical framework requires global governance. Presently the Americas and Australians do not regulate genome-edited products without foreign gene integration but the European Union considers genome-edited products as GMOs, hence they are highly regulated (Halford, 2019; Callaway, 2018). Africa and most parts of Asia are yet to come up with regulatory mechanisms. In the European Union (EU), organisms obtained through “recent methods” of mutagenesis must be classified as Genetically Modified Organisms (GMOs) and must

follow stringent release procedures including prohibitive costs, lengthy assessments and approval timelines and labeling requirements (Jorasch, 2020; Murovec et al., 2018; Wolt et al., 2016; Voytas and Gao, 2014). On the contrary, the United States Department of Agriculture (USDA) lifted regulations on genetically edited crops, including CRISPR materials that do not contain CRISPR/Cas9 anymore are considered non-GMO. For instance, genome-edited products like high oleic-modified soybeans and cold storable potatoes without transgenes have been field evaluated and commercialized as non-GMO products (Wu et al., 2020; Haun et al., 2014).

Genome editing application in crop agriculture includes the enhancement of nutritional value, improving tolerance to stresses and domestication of crops. Some genome-edited products do not fall under the classification of GMOs. This necessitates that we develop guidelines to enable us to effectively regulate such without putting undue rigor on the products that do not fall under the category of GMO. In many countries, there is a lack of clarity as to the GMO or non-GMO status of genome-edited crop germplasm (Schulman et al., 2020). In Africa, no regulations are in place, probably because not much is being done in this field except for South Africa, Kenya, and Uganda (Ogaugwu et al., 2019; Tandoh, 2017). The safety application of genome-editing technology is a matter of discussion in the scientific community and many scientists have called for its regulation (Huang et al., 2016; Gantz and Bier 2015). However, the dominant view is that an international framework under which countries retain the right to make their own decisions is put in place.

Importance of genome editing for Africa's agriculture

In Sub-Saharan Africa, the majority of people depend on agriculture for food, nutrition and income security. Agriculture is constrained by the changing pattern of climatic variables and the increasing reduction of arable lands. Climate change is predicted to impact crop productivity through additional stress on crops. In addition, pests and disease outbreaks that are devastating to farmers are becoming more common. Consequently, food crop production in many parts of Africa where agricultural activities are largely rain-fed will be affected. Many of the current crop varieties are less resilient to such conditions and may cause total crop failure, reduced income returns and consequently rising poverty, which, will hamper the attainment of Sustainable Development Goals (SDGs) number 1 and 2. Hence the need to become both climate-proof and climate-smart through improved agricultural practices and resource-efficient varieties has become even more important. CRISPR-Cas 9 technology presents different strategies for crop improvement for greater resilience and better adaptation and its most important applications for food crops' improvement. Many of the production factors impeding agricultural productivity in Africa are affected by multi-gene-controlled traits, which are difficult to improve and gene introgression is currently difficult to achieve. Multiplexed gene editing and transcriptional activation or repression of plant endogenous genes could be exploited to address complex traits while avoiding linkage drag during breeding and gene pyramiding. Major genes that negatively regulate important agronomic traits can be addressed through gene knockouts. The potential advantages of combining heterosis and apomixis in agricultural crops will have immediate benefits that is likely to dwarf the gains of the green revolution. In principle, CRISPR technology should facilitate the enhancement of traits and provide much-needed climate resilience for Africa.

Africa is endowed with a rich diversity of plant species that are used for food, feed and fuel. Most of these species are harvested from the wild, while others are underutilized and have not received enough scientific research recognition. These underutilized, semi-domesticated species together with their wild relatives are deficient in economically important domestication traits like germination inhibition, ripening, shattering and long processing time (Lemmon et al., 2018; Li et al., 2018d). CRISPR genome editing techniques could be applied to confer domestication traits on this germplasm to broaden crop genetic diversity, widen the food options and better adapt to climate variations (Fernie and Yan, 2019; Hua et al., 2019). Selection for desirable agronomic traits could bring more diversity to the table to address food and nutritional security. The CRISPR technique can also help to address the setback in yield when current varieties are crossed with ancestral ones to introgress genes for resistance to stresses. On the African continent, CRISPR is being applied in the research of Banana *X*anthomonas Wilt Disease (BXW), striga resistance, Maize Lethal Necrosis (MLN), Cassava Mosaic Disease (CMD) and salt tolerance. Banana *Xanthomonas* wilt indiscriminately affects banana cultivars with up to 100% yield loss (Blomme et al., 2014). The technique can be extended to other crops which have consequences on the food security and incomes of farmers. CRISPR-Cas9 is currently being used in the diagnosis and management of viral

diseases in bananas (Tripathi et al., 2021; Tripathi et al., 2020). The International Institute of Tropical Agriculture (IITA) in Kenya has demonstrated the local capacity to apply CRISPR-Cas9 genome editing to the development of disease tolerance to BXW disease. In Uganda, CRISPR-Cas9 is being used in research focused on overcoming the deadly Cassava Brown Streak Virus (CBSD) which is a damaging disease of cassava plants grown mainly in East Africa. These breeding efforts notwithstanding, African scientists currently have an unequal capacity, limited opportunities and incentives to realize the contributions that gene editing can make to address the intrinsic challenges plaguing African agriculture. The biggest drawback to fully exploiting the benefits of genome editing is the lack of sufficient resource allocation in the agricultural sector by various governments in Africa. Failing to take advantage of gene editing research could result in creating further inequalities in science, agricultural productivity and health in Africa. The number of people affected by hunger across Africa rose by 10 million people from 2018 to 2019 (FAO, 2020) with eight years remaining to the SDG 2 goal of 2030.

Conclusion and Perspectives

CRISPR-Cas9 represents a significant change in how mutations are made and presents a lot of possibilities hitherto not possible with conventional breeding for correcting abnormalities and improving the performance of current varieties. The future and the role of genome editing in plant research and sustainable crop production are eminent. Africa is at the inception -stages of using genome editing technology to improve crop production and utilization challenges. The predominant view in the literature is that CRISPR technology is simple and easy to apply mainly because the engineering advances have laid the groundwork for the creation, refinement and implementation of genome-modifying tools in advanced countries. However, a broad application of genome editing will rely on enhanced knowledge of genome organization and function, germplasm characterization, gene function characterization and identification of favorable variation in crops and their wild relatives. This should be complemented with phenotyping, omics and systems biology studies pursued across large-scale germplasm collections of important staples. There is also a need to commence the development of enabling technologies such as plant transformation protocols and tissue culture regeneration for important crops in order to take advantage of the opportunities in genome editing technologies. Comprehensive integration of these technologies is lacking in most African countries. However, there are regional centers, various CGIAR Centers, and universities that have equipped laboratories and institutions that support capacity-building in modern technological applications in Africa. For instance, the Biosciences eastern and central Africa - International Livestock Research Institute (BecA - ILRI) Hub in Nairobi, Kenya, is a shared agricultural research platform that exists to increase access to affordable, world-class research facilities. The Facility houses Genomics, Bioinformatics and Breeding platforms and offers research-related and capacity-building opportunities to African researchers. The African Orphan Crop Consortium (AOCC), Divseek Project, MoBreed and CultiVar are important initiatives that are helping to build the capacity of African scientists. The focus of the AOCC is to genetically sequence de novo and assemble and annotate 101 African Orphan Crops. The sequence information Generated from this project is openly accessible to all interested parties. DivSeek International Network aims to facilitate the generation, integration and sharing of data and information related to plant germplasm enhancement of useful exotic germplasm. The cultivar project is funded through ANR (the French National Research Agency) under the "Investissements d'avenir" programme and coordinated by Agropolis Fondation under the frame of I-SITE MUSE (ANR-16-IDEX-0006). Cultivar's main goals include the implementation of crop improvement as an impact pathway through leveraging new technologies and concepts. Educational institutions such as the West African Center for Crop Improvement (WACCI), African Center for Crop Improvement (ACCI), Makerere University Regional Centre of Excellence for Crop Improvement (MarCCI) and The UC Davis-African Plant breeding Academy are providing training to breeders in Africa to accelerate the use of modern plant breeding techniques by scientists in Africa. However, after acquiring skills in these modern techniques, scientists are unable to apply the skills to their local research because of a lack of supporting facilities. Different investments and policy actions from both national governments and development partners are needed to boost the current efforts at using CRISPR technology to address the myriad of challenges facing agriculture in Africa.

Acknowledgment

The Management of the CSIR-Plant Genetic Resources Research Institute (PGRRI) are acknowledged for their support.

Compliance with ethical standards

N/A

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the publication of this article. All authors read and gave their consent for the manuscript to be published.

Authors' contribution

Bissah drafted and prepared the manuscript. Asante, Ochar, Quain, Egbadzor, Ribeiro and Kotey wrote parts of the manuscript and provided a critical review.

References

1. Abdelrahman, M., Wei, Z., Rohila, J. S., & Zhao, K. (2021). Multiplex genome-editing technologies for revolutionizing plant biology and crop improvement. *Frontiers in Plant Science*, *12*, 721203.
2. Ahmad, S., Wei, X., Sheng, Z., Hu, P., & Tang, S. (2020). CRISPR/Cas9 for development of disease resistance in plants: recent progress, limitations and future prospects. *Briefings in Functional Genomics*, *19*(1), 26-39.
3. Ai, Y., Liang, D., & Wilusz, J. E. (2022). CRISPR/Cas13 effectors have differing extents of off-target effects that limit their utility in eukaryotic cells. *Nucleic Acids Research*, *50*(11), e65-e65.
4. Anders, C., Bargsten, K., & Jinek, M. (2016). Structural plasticity of PAM recognition by engineered variants of the RNA-guided endonuclease Cas9. *Molecular cell*, *61*(6), 895-902.
5. Bao, A., Burritt, D. J., Chen, H., Zhou, X., Cao, D., & Tran, L. S. P. (2019). The CRISPR/Cas9 system and its applications in crop genome editing. *Critical reviews in biotechnology*, *39*(3), 321-336.
6. Barcaccia, G., & Albertini, E. (2013). Apomixis in plant reproduction: a novel perspective on an old dilemma. *Plant reproduction*, *26*, 159-179.
7. Biswas, S., Li, R., Yuan, Z., Zhang, D., Zhao, X., & Shi, J. (2019). Development of methods for effective identification of CRISPR/Cas9-induced indels in rice. *Plant cell reports*, *38*, 503-510.
8. Blary, A., & Jenczewski, E. (2019). Manipulation of crossover frequency and distribution for plant breeding. *Theoretical and Applied Genetics*, *132*, 575-592.
9. Nakato, V. (2014). Guy Blomme, Kim Jacobsen, Walter Ocimati, Fen Beed, Jules Ntamwira, Charles Sivirihauma, Fred Ssekiwoko. *Eur J Plant Pathol*, *139*, 265-281.
10. Callaway, E. (2018). CRISPR plants now subject to tough GM laws in European Union. *Nature*, *560*(7716), 16-17.
11. Cardi, T. (2016). Cisgenesis and genome editing: Combining concepts and efforts for a smarter use of genetic resources in crop breeding. *Plant Breeding*, *135*(2), 139-147.
12. Chakraborty, M., Sairam Reddy, P., Laxmi Narasu, M., Krishna, G., & Rana, D. (2016). Agrobacterium-mediated genetic transformation of commercially elite rice restorer line using npt II gene as a plant selection marker. *Physiology and molecular biology of plants*, *22*, 51-60.
13. Chaudhary, J., Deshmukh, R., & Sonah, H. (2019). Mutagenesis approaches and their role in crop improvement. *Plants*, *8*(11), 467.
14. Chelysheva, L., Diallo, S., Vezon, D., Gendrot, G., Vrielynck, N., Belcram, K., ... & Grelon, M. (2005). AtREC8 and AtSCC3 are essential to the monopolar orientation of the kinetochores during meiosis. *Journal of cell science*, *118*(20), 4621-4632.
15. Cai, Y., Chen, L., Liu, X., Guo, C., Sun, S., Wu, C., ... & Hou, W. (2018). CRISPR/Cas9-mediated targeted mutagenesis of GmFT2a delays flowering time in soya bean. *Plant biotechnology journal*, *16*(1), 176-185.
16. Claeys, H., Vi, S. L., Xu, X., Satoh-Nagasawa, N., Eveland, A. L., Goldshmidt, A., ... & Jackson, D. (2019). Control of meristem determinacy by trehalose 6-phosphate phosphatases is uncoupled from enzymatic activity. *Nature plants*, *5*(4), 352-357.
17. Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., ... & Zhang, F. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science*, *339*(6121), 819-823.
18. Dai, W. J., Zhu, L. Y., Yan, Z. Y., Xu, Y., Wang, Q. L., & Lu, X. J. (2016). CRISPR-Cas9 for in vivo gene therapy: Promise and hurdles. *Molecular Therapy-Nucleic Acids*, *5*, e349.
19. Ding, Y., Li, H., Chen, L. L., & Xie, K. (2016). Recent advances in genome editing using CRISPR/Cas9. *Frontiers in plant science*, *7*, 703.
20. Doench, J. G., Fusi, N., Sullender, M., Hegde, M., Vaimberg, E. W., Donovan, K. F., ... & Root, D.

E. (2016). Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nature biotechnology*, *34*(2), 184-191.

21. Doman, J. L., Raguram, A., Newby, G. A., & Liu, D. R. (2020). Evaluation and minimization of Cas9-independent off-target DNA editing by cytosine base editors. *Nature biotechnology*, *38*(5), 620-628.
22. Donovan, S., Mao, Y., Orr, D. J., Carmo-Silva, E., & McCormick, A. J. (2020). CRISPR-Cas9-mediated mutagenesis of the Rubisco small subunit family in Nicotiana tabacum. *Frontiers in Genome Editing*, *2*, 28.
23. Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, *346*(6213), 1258096.
24. Durland, J., & Ahmadian-Moghadam, H. (2021). Genetics, mutagenesis. In *StatPearls [Internet]*. StatPearls Publishing.
25. El-Gewely, M. R., Fenton, C., Kjeldsen, E., & Xu, H. (2001). Mutagenesis: Site-specific. *e LS*.
26. Endo, M., Nishizawa-Yokoi, A., & Toki, S. (2018). Rice genome editing. *Rice Genomics, Genetics and Breeding*, 523-539.
27. Fang, C., Li, K., Wu, Y., Wang, D., Zhou, J., Liu, X., ... & Luo, J. (2019). OsTSD2-mediated cell wall modification affects ion homeostasis and salt tolerance. *Plant, Cell & Environment*, *42*(5), 1503-1512.
28. UNICEF. (2021). The state of food security and nutrition in the world 2021.
29. Yunyan, F., Jie, Y., Fangquan, W., Fangjun, F., Wenqi, L. I., Jun, W., ... & Weigong, Z. (2019). Production of two elite glutinous rice varieties by editing wx gene. *Rice Science*, *26*(2), 118-124.
30. Fernie, A. R., & Yan, J. (2019). De novo domestication: an alternative route toward new crops for the future. *Molecular plant*, *12*(5), 615-631.
31. Fiaz, S., Wang, X., Younas, A., Alharthi, B., Riaz, A., & Ali, H. (2021). Apomixis and strategies to induce apomixis to preserve hybrid vigor for multiple generations. *GM Crops & Food*, *12*(1), 57-70.
32. Fineran, P. C., & Charpentier, E. (2012). Memory of viral infections by CRISPR-Cas adaptive immune systems: acquisition of new information. *Virology*, *434*(2), 202-209.
33. Fu, Y., Sander, J. D., Reyon, D., Cascio, V. M., & Joung, J. K. (2014). Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. *Nature biotechnology*, *32*(3), 279-284.
34. Gaj, T., Sirk, S. J., Shui, S. L., & Liu, J. (2016). Genome-editing technologies: principles and applications. *Cold Spring Harbor perspectives in biology*, *8*(12), a023754.
35. Gantz, V. M., & Bier, E. (2015). The mutagenic chain reaction: a method for converting heterozygous to homozygous mutations. *Science*, *348*(6233), 442-444.
36. Gao, L., Cox, D. B., Yan, W. X., Manteiga, J. C., Schneider, M. W., Yamano, T., ... & Zhang, F. (2017). Engineered Cpf1 variants with altered PAM specificities. *Nature biotechnology*, *35*(8), 789-792.
37. The GLP is committed to full transparency. Genetic-Literacy-Project-2021-Annual-Report
38. Gomez, M. A., Lin, Z. D., Moll, T., Chauhan, R. D., Hayden, L., Renninger, K., ... & Bart, R. S. (2019). Simultaneous CRISPR/Cas9-mediated editing of cassava eIF 4E isoforms nCBP-1 and nCBP-2 reduces cassava brown streak disease symptom severity and incidence. *Plant biotechnology journal*, *17*(2), 421-434.
39. Haeussler, M., & Concordet, J. P. (2016). Genome editing with CRISPR-Cas9: can it get any better?. *Journal of genetics and genomics*, *43*(5), 239-250.
40. Halford, N. G. (2019). Legislation governing genetically modified and genome-edited crops in Europe: the need for change. *Journal of the Science of Food and Agriculture*, *99*(1), 8-12.
41. Hand, M. L., & Koltunow, A. M. (2014). The genetic control of apomixis: asexual seed formation. *Genetics*, *197*(2), 441-450.
42. Hassan, M. M., Yuan, G., Chen, J. G., Tuskan, G. A., & Yang, X. (2020). Prime editing technology and its prospects for future applications in plant biology research. *BioDesign Research*.
43. Hojsgaard, D., & Hörandl, E. (2015). A little bit of sex matters for genome evolution in asexual plants. *Frontiers in plant science*, *6*, 82.
44. Hua, K., Zhang, J., Botella, J. R., Ma, C., Kong, F., Liu, B., & Zhu, J. K. (2019). Perspectives on the application of genome-editing technologies in crop breeding. *Molecular Plant*, *12*(8), 1047-1059.
45. Hua, K., Jiang, Y., Tao, X., & Zhu, J. K. (2020). Precision genome engineering in rice using prime editing system. *Plant biotechnology journal*, *18*(11), 2167.

46. Huang, T. K., & Puchta, H. (2019). CRISPR/Cas-mediated gene targeting in plants: finally a turn for the better for homologous recombination. *Plant cell reports*, *38*, 443-453.
47. Jiang, Y., Qian, F., Yang, J., Liu, Y., Dong, F., Xu, C., ... & Yang, S. (2017). CRISPR-Cpf1 assisted genome editing of Corynebacterium glutamicum. *Nature communications*, *8*(1), 15179.
48. Jiang, W., Zhou, H., Bi, H., Fromm, M., Yang, B., & Weeks, D. P. (2013). Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice. *Nucleic acids research*, *41*(20), e188-e188.
49. Jiang, Y., Sun, K., & An, X. (2022). CRISPR/Cas system: applications and prospects for maize improvement. *ACS Agricultural Science & Technology*, *2*(2), 174-183.
50. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *science*, *337*(6096), 816-821.
51. Johnsson, M., Gaynor, R. C., Jenko, J., Gorjanc, G., De Koning, D. J., & Hickey, J. M. (2019). Removal of alleles by genome editing (RAGE) against deleterious load. *Genetics Selection Evolution*, *51*, 1-18.
52. Jorasch, P. (2020). Potential, challenges, and threats for the application of new breeding techniques by the private plant breeding sector in the EU. *Frontiers in Plant Science*, *11*, 582011.
53. Kaushal, P., Malaviya, D. R., & Roy, A. K. (2004). Prospects for breeding apomictic rice: a reassessment. *Current Science*, *87*(3), 292-296.
54. Khanday, I., Skinner, D., Yang, B., Mercier, R., & Sundaresan, V. (2019). A male-expressed rice embryogenic trigger redirected for asexual propagation through seeds. *Nature*, *565*(7737), 91-95.
55. Kim, Y. B., Komor, A. C., Levy, J. M., Packer, M. S., Zhao, K. T., & Liu, D. R. (2017). Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions. *Nature biotechnology*, *35*(4), 371-376.
56. Kleinstiver, B. P., Prew, M. S., Tsai, S. Q., Nguyen, N. T., Topkar, V. V., Zheng, Z., & Joung, J. K. (2015). Broadening the targeting range of Staphylococcus aureus CRISPR-Cas9 by modifying PAM recognition. *Nature biotechnology*, *33*(12), 1293-1298.
57. Koltunow, A. M., Ozias-Akins, P. and Siddiqi, I. (2013). Apomixis, pp. 83–110 in Seed Genomics, edited by P. W. Becraft. Wiley, New York
58. Koltunow, A. M., & Grossniklaus, U. (2003). Apomixis: a developmental perspective. *Annual review of plant biology*, *54*(1), 547-574.
59. Koonin, E. V., Makarova, K. S., & Zhang, F. (2017). Diversity, classification and evolution of CRISPR-Cas systems. *Current opinion in microbiology*, *37*, 67-78.
60. Krenek, P., Samajova, O., Luptovciak, I., Doskocilova, A., Komis, G., & Samaj, J. (2015). Transient plant transformation mediated by Agrobacterium tumefaciens: Principles, methods and applications. *Biotechnology Advances*, *33*(6), 1024-1042.
61. Kurata, M., Wolf, N. K., Lahr, W. S., Weg, M. T., Kluesner, M. G., Lee, S., ... & Moriarity, B. S. (2018). Highly multiplexed genome engineering using CRISPR/Cas9 gRNA arrays. *PLoS one*, *13*(9), e0198714.
62. Lacchini, E., Kiegle, E., Castellani, M., Adam, H., Jouannic, S., Gregis, V., & Kater, M. M. (2020). CRISPR-mediated accelerated domestication of African rice landraces. *PLoS One*, *15*(3), e0229782.
63. Lawrenson, T., Shorinola, O., Stacey, N., Li, C., Østergaard, L., Patron, N., ... & Harwood, W. (2015). Induction of targeted, heritable mutations in barley and Brassica oleracea using RNA-guided Cas9 nuclease. *Genome biology*, *16*, 1-13.
64. Le, N. T., Tran, H. T., Bui, T. P., Nguyen, G. T., Van Nguyen, D., Ta, D. T., ... & Do, P. T. (2022). Simultaneously induced mutations in eIF4E genes by CRISPR/Cas9 enhance PVY resistance in tobacco. *Scientific Reports*, *12*(1), 14627.
65. Lei, Y., Lu, L., Liu, H. Y., Li, S., Xing, F., & Chen, L. L. (2014). CRISPR-P: a web tool for synthetic single-guide RNA design of CRISPR-system in plants. *Molecular plant*, *7*(9), 1494-1496.
66. Lemmon, Z. H., Reem, N. T., Dalrymple, J., Soyk, S., Swartwood, K. E., Rodriguez-Leal, D., ... & Lippman, Z. B. (2018). Rapid improvement of domestication traits in an orphan crop by genome editing. *Nature plants*, *4*(10), 766-770.
67. Li, J., Meng, X., Zong, Y., Chen, K., Zhang, H., Liu, J., ... & Gao, C. (2016). Gene replacements and insertions in rice by intron targeting using

CRISPR–Cas9. *Nature plants*, *2*(10), 1-6.

68. Li, L., Wei, K., Zheng, G., Liu, X., Chen, S., Jiang, W., & Lu, Y. (2018). CRISPR-Cpf1-assisted multiplex genome editing and transcriptional repression in Streptomyces. *Applied and Environmental Microbiology*, *84*(18), e00827-18.
69. Li, X., Wang, Y., Liu, Y., Yang, B., Wang, X., Wei, J., ... & Chen, J. (2018). Base editing with a Cpf1–cytidine deaminase fusion. *Nature biotechnology*, *36*(4), 324-327.
70. Li, Z., Zhang, D., Xiong, X., Yan, B., Xie, W., Sheen, J., & Li, J. F. (2017). A potent Cas9-derived gene activator for plant and mammalian cells. *Nature plants*, *3*(12), 930-936.
71. Li, H., Gidley, M. J., & Dhital, S. (2019). High-amylose starches to bridge the "Fiber Gap": development, structure, and nutritional functionality. *Comprehensive Reviews in Food Science and Food Safety*, *18*(2), 362-379.
72. Li, J. F., Norville, J. E., Aach, J., McCormack, M., Zhang, D., Bush, J., ... & Sheen, J. (2013). Multiplex and homologous recombination–mediated genome editing in Arabidopsis and Nicotiana benthamiana using guide RNA and Cas9. *Nature biotechnology*, *31*(8), 688-691.
73. Li, J., Li, Y., & Ma, L. (2021). Recent advances in CRISPR/Cas9 and applications for wheat functional genomics and breeding. *Abiotech*, 1-11.
74. Li, S., Zhang, X., Wang, W., Guo, X., Wu, Z., Du, W., ... & Xia, L. (2018). Expanding the scope of CRISPR/Cpf1-mediated genome editing in rice. *Molecular Plant*, *11*(7), 995-998.
75. Yu, H., Lin, T., Meng, X., Du, H., Zhang, J., Liu, G., ... & Li, J. (2021). A route to de novo domestication of wild allotetraploid rice. *Cell*, *184*(5), 1156-1170.
76. Li, J., Zhang, X., Sun, Y., Zhang, J., Du, W., Guo, X., ... & Xia, L. (2018). Efficient allelic replacement in rice by gene editing: a case study of the NRT1. 1B gene. *Journal of integrative plant biology*, *60*(7), 536-540.
77. Li, Y., Lin, Z., Yue, Y., Zhao, H., Fei, X., E, L., ... & Song, W. (2021). Loss-of-function alleles of ZmPLD3 cause haploid induction in maize. *Nature Plants*, *7*(12), 1579-1588.
78. Li, Z., Liu, Z. B., Xing, A., Moon, B. P., Koellhoffer, J. P., Huang, L., ... & Cigan, A. M. (2015). Cas9-guide RNA directed genome editing in soybean. *Plant physiology*, *169*(2), 960-970.
79. Liang, Z., Chen, K., & Gao, C. (2019). Biolistic delivery of CRISPR/Cas9 with ribonucleoprotein complex in wheat. *Plant genome editing with CRISPR systems: methods and protocols*, 327-335.
80. Liang, Z., Zhang, K., Chen, K., & Gao, C. (2014). Targeted mutagenesis in Zea mays using TALENs and the CRISPR/Cas system. *Journal of Genetics and Genomics*, *41*(2), 63-68.
81. Liang, Z., Chen, K., Li, T., Zhang, Y., Wang, Y., Zhao, Q., ... & Gao, C. (2017). Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. *Nature communications*, *8*(1), 14261.
82. Lieber, M. R. (2010). The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annual review of biochemistry*, *79*, 181-211.
83. Lin, S. R., Yang, H. C., Kuo, Y. T., Liu, C. J., Yang, T. Y., Sung, K. C., ... & Chen, P. J. (2014). The CRISPR/Cas9 system facilitates clearance of the intrahepatic HBV templates in vivo. *Molecular Therapy-Nucleic Acids*, *3*, e186.
84. Liu, G., Li, J., & Godwin, I. D. (2019). Genome editing by CRISPR/Cas9 in sorghum through biolistic bombardment. *Sorghum: methods and protocols*, 169-183.
85. Lowder, L. G., Zhang, D., Baltes, N. J., Paul III, J. W., Tang, X., Zheng, X., ... & Qi, Y. (2015). A CRISPR/Cas9 toolbox for multiplexed plant genome editing and transcriptional regulation. *Plant physiology*, *169*(2), 971-985.
86. Lu, Y., Ye, X., Guo, R., Huang, J., Wang, W., Tang, J., ... & Qian, Y. (2017). Genome-wide targeted mutagenesis in rice using the CRISPR/Cas9 system. *Molecular plant*, *10*(9), 1242-1245.
87. Lucioli, A., Tavazza, R., Baima, S., Fatyol, K., Burgyan, J., & Tavazza, M. (2022). CRISPR-Cas9 Targeting of the eIF4E1 Gene Extends the Potato Virus Y Resistance Spectrum of the Solanum tuberosum L. cv. Desirée. *Frontiers in Microbiology*, *13*.
88. Luo, Q., Li, Y., Shen, Y., & Cheng, Z. (2014). Ten years of gene discovery for meiotic event control in rice. *Journal of Genetics and Genomics*, *41*(3), 125-137.
89. Lyons, J. B., Bredeson, J. V., Mansfeld, B. N., Bauchet, G. J., Berry, J., Boyher, A., ... & Bart, R. S. (2021). Current status and impending progress for cassava structural genomics. *Plant molecular*

biology, 1-15.

90. Ma, X., Zhang, Q., Zhu, Q., Liu, W., Chen, Y., Qiu, R., ... & Liu, Y. G. (2015). A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. *Molecular plant*, *8*(8), 1274-1284.
91. Mao, Y., Zhang, H., Xu, N., Zhang, B., Gou, F., & Zhu, J. K. (2013). Application of the CRISPR–Cas system for efficient genome engineering in plants. *Molecular plant*, *6*(6), 2008-2011.
92. Mao, Y., Zhang, Z., Feng, Z., Wei, P., Zhang, H., Botella, J. R., & Zhu, J. K. (2016). Development of germ-line-specific CRISPR-Cas9 systems to improve the production of heritable gene modifications in Arabidopsis. *Plant biotechnology journal*, *14*(2), 519-532.
93. Marimuthu, M. P., Jolivet, S., Ravi, M., Pereira, L., Davda, J. N., Cromer, L., ... & Mercier, R. (2011). Synthetic clonal reproduction through seeds. *Science*, *331*(6019), 876-876.
94. Matsumoto, Takashi, Wu, Jianzhong, Kanamori, Hiroyuki, Katayose, Yuichi,Fujisawa, Masaki, Namiki, Nobukazu, Mizuno, Hiroshi, Yamamoto, Kimiko, Antonio, Baltazar, Baba, Tomoya, Sakata, Katsumi, Nagamura, Yoshiaki, Aoki, Hiroyoshi, Arikawa, Koji, Arita, KoheiBiti, Takahito, Chiden, Yoshino, Fujitsuka, Nahoko, Fukunaka, Rie and Burr, B. (2005). The map-based sequence of the rice genome. Nature 436:793-800
95. Meng, X., Yu, H., Zhang, Y., Zhuang, F., Song, X., Gao, S., ... & Li, J. (2017). Construction of a genome-wide mutant library in rice using CRISPR/Cas9. *Molecular plant*, *10*(9), 1238-1241.
96. Mercer, A. C., Gaj, T., Fuller, R. P., & Barbas III, C. F. (2012). Chimeric TALE recombinases with programmable DNA sequence specificity. *Nucleic acids research*, *40*(21), 11163-11172.
97. Miao, J., Guo, D., Zhang, J., Huang, Q., Qin, G., Zhang, X., ... & Qu, L. J. (2013). Targeted mutagenesis in rice using CRISPR-Cas system. *Cell research*, *23*(10), 1233-1236.
98. Mieulet, D., Jolivet, S., Rivard, M., Cromer, L., Vernet, A., Mayonove, P., ... & Mercier, R. (2016). Turning rice meiosis into mitosis. *Cell research*, *26*(11), 1242-1254.
99. Miller, S. M., Wang, T., Randolph, P. B., Arbab, M., Shen, M. W., Huang, T. P., ... & Liu, D. R. (2020). Continuous evolution of SpCas9 variants compatible with non-G PAMs. *Nature biotechnology*, *38*(4), 471-481.
100. Mishra, R., & Zhao, K. (2018). Genome editing technologies and their applications in crop improvement. *Plant Biotechnology Reports*, *12*, 57-68.
101. Mishra, R., Joshi, R. K., & Zhao, K. (2018). Genome editing in rice: recent advances, challenges, and future implications. *Frontiers in Plant Science*, *9*, 1361.
102. Mohanta, T. K., Bashir, T., Hashem, A., Abd_Allah, E. F., & Bae, H. (2017). Genome editing tools in plants. *Genes*, *8*(12), 399.
103. Murovec, J., Guček, K., Bohanec, B., Avbelj, M., & Jerala, R. (2018). DNA-free genome editing of Brassica oleracea and B. rapa protoplasts using CRISPR-Cas9 ribonucleoprotein complexes. *Frontiers in Plant Science*, *9*, 1594.
104. Odipio, J., Alicai, T., Ingelbrecht, I., Nusinow, D. A., Bart, R., & Taylor, N. J. (2017). Efficient CRISPR/Cas9 genome editing of phytoene desaturase in cassava. *Frontiers in plant science*, *8*, 1780.
105. Ogaugwu, C. E., Agbo, S. O., & Adekoya, M. A. (2019). CRISPR in sub-Saharan Africa: applications and education. *Trends in biotechnology*, *37*(3), 234-237.
106. Oliva, R., Ji, C., Atienza-Grande, G. José C. Huguet-Tapia, Alvaro Perez-Quintero, Ting Li, Joon-SeobEom, Chenhao Li, Hanna Nguyen, Bo Liu, Florence Auguy, ColineSciallano, Van T. Luu, Gerbert S. Dossa, SébastienCunnac, Sarah M. Schmidt, Inez H. Slamet-Loedin, Casiana Vera Cruz, Boris Szurek, Wolf B. Frommer, Frank F. White and Bing Yang (2019). Broad-spectrum resistance to bacterial blight in rice using genome editing. Nat Biotechnol 37, 1344–1350 (2019).
107. Pickar-Oliver, A., & Gersbach, C. A. (2019). The next generation of CRISPR–Cas technologies and applications. *Nature reviews Molecular cell biology*, *20*(8), 490-507.
108. Ravi, M., & Chan, S. W. (2010). Haploid plants produced by centromere-mediated genome elimination. *Nature*, *464*(7288), 615-618.
109. Ravi, M., Marimuthu, M. P., & Siddiqi, I. (2008). Gamete formation without meiosis in Arabidopsis. *Nature*, *451*(7182), 1121-1124.
110. Romero, F. M., & Gatica-Arias, A. (2019). CRISPR/Cas9: development and application in rice breeding. *Rice Science*, *26*(5), 265-281.

111. Chow, R. D., Chen, J. S., Shen, J., & Chen, S. (2020). pegFinder: A pegRNA designer for CRISPR prime editing. *BioRxiv*, 2020-05.

112. Sailer, C., Schmid, B., & Grossniklaus, U. (2016). Apomixis allows the transgenerational fixation of phenotypes in hybrid plants. *Current biology*, *26*(3), 331-337.

113. San Filippo, J., Sung, P., & Klein, H. (2008). Mechanism of eukaryotic homologous recombination. *Annu. Rev. Biochem.*, *77*, 229-257.

114. Sathee, L., Jagadhesan, B., Pandesha, P. H., Barman, D., Adavi B, S., Nagar, S., ... & Chinnusamy, V. (2022). Genome editing targets for improving nutrient use efficiency and nutrient stress adaptation. *Frontiers in Genetics*, *13*, 900897.

115. Schulman, A. H., Oksman-Caldentey, K. M., & Teeri, T. H. (2020). European Court of Justice delivers no justice to Europe on genome-edited crops. *Plant biotechnology journal*, *18*(1), 8.

116. Sedlar, A., Zupin, M., Maras, M., Razinger, J., Šuštar-Vozlič, J., Pipan, B., & Meglič, V. (2020). QTL mapping for drought-responsive agronomic traits associated with physiology, phenology, and yield in an Andean intra-gene pool common bean population. *Agronomy*, *10*(2), 225.

117. Sharma, R., Liang, Y., Lee, M. Y., Pidatala, V. R., Mortimer, J. C., & Scheller, H. V. (2020). Agrobacterium-mediated transient transformation of sorghum leaves for accelerating functional genomics and genome editing studies. *BMC Research Notes*, *13*(1), 1-7.

118. Shibata, A. (2017). Regulation of repair pathway choice at two-ended DNA double-strand breaks. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *803*, 51-55.

119. Shmakov, S., Smargon, A., Scott, D., Cox, D., Pyzocha, N., Yan, W., ... & Koonin, E. V. (2017). Diversity and evolution of class 2 CRISPR–Cas systems. *Nature reviews microbiology*, *15*(3), 169-182.

120. Sikora, P., Chawade, A., Larsson, M., Olsson, J., & Olsson, O. (2011). Mutagenesis as a tool in plant genetics, functional genomics, and breeding. *International journal of plant genomics*, *2011*.

121. Sun, Y., Jiao, G., Liu, Z., Zhang, X., Li, J., Guo, X., ... & Xia, L. (2017). Generation of high-amylose rice through CRISPR/Cas9-mediated targeted mutagenesis of starch branching enzymes. *Frontiers in plant science*, *8*, 298.

122. Sun, Y., Jiao, G., Liu, Z., Zhang, X., Li, J., Guo, X., ... & Xia, L. (2017). Generation of high-amylose rice through CRISPR/Cas9-mediated targeted mutagenesis of starch branching enzymes. *Frontiers in plant science*, *8*, 298.

123. Sun, Y., Jiao, G., Liu, Z., Zhang, X., Li, J., Guo, X., ... & Xia, L. (2017). Generation of high-amylose rice through CRISPR/Cas9-mediated targeted mutagenesis of starch branching enzymes. *Frontiers in plant science*, *8*, 298.

124. Svitashev, S., Young, J. K., Schwartz, C., Gao, H., Falco, S. C., & Cigan, A. M. (2015). Targeted mutagenesis, precise gene editing, and site-specific gene insertion in maize using Cas9 and guide RNA. *Plant physiology*, *169*(2), 931-945.

125. Andoh, C. T. (2017). Genome editing technologies: ethical and regulation challenges for Africa. *International Journal of Health Economics and Policy*, *2*(2), 30-46.

126. Tang, T., Han, Y., Wang, Y., Huang, H., & Qian, P. (2021). Programmable system of Cas13-mediated RNA modification and its biological and biomedical applications. *Frontiers in Cell and Developmental Biology*, *9*, 677587.

127. Thiruppathi, D. (2020). Maize RNA polymerase III subunit NRPC2: new kid on the kernel development block.

128. Tian, Y., Liu, X., Fan, C., Li, T., Qin, H., Li, X., ... & Xu, Y. (2021). Enhancement of tobacco (Nicotiana tabacum L.) seed lipid content for biodiesel production by CRISPR-Cas9-mediated knockout of NtAn1. *Frontiers in Plant Science*, *11*, 599474.

129. Tian, Y., Chen, K., Li, X., Zheng, Y., & Chen, F. (2020). Design of high-oleic tobacco (Nicotiana tabacum L.) seed oil by CRISPR-Cas9-mediated knockout of NtFAD2–2. *BMC Plant Biology*, *20*, 1-12.

130. Schardl, C. L., Florea, S., Pan, J., Nagabhyru, P., Bec, S., & Calie, P. J. (2013). The epichloae: alkaloid diversity and roles in symbiosis with grasses. *Current Opinion in Plant Biology*, *16*(4), 480-488.

131. Tripathi, L., Ntui, V. O., & Tripathi, J. N. (2020). CRISPR/Cas9-based genome editing of banana for disease resistance. *Current Opinion in Plant Biology*, *56*, 118-126.

132. Tripathi, L., Odipio, J., Tripathi, J. N., & Tusiime, G. (2008). A rapid technique for screening

banana cultivars for resistance to Xanthomonas wilt. *European Journal of Plant Pathology*, *121*, 9-19.

133. Tripathi, L., Ntui, V. O., Tripathi, J. N., & Kumar, P. L. (2021). Application of CRISPR/Cas for diagnosis and management of viral diseases of banana. *Frontiers in Microbiology*, *11*, 609784.

134. Yuan, Y., Wen, W., Yost, S. E., Xing, Q., Yan, J., Han, E. S., ... & Yim, J. H. (2019). Combination therapy with BYL719 and LEE011 is synergistic and causes a greater suppression of p-S6 in triple negative breast cancer. *Scientific reports*, *9*(1), 7509.

135. Tripathi, L., Ntui, V. O., & Tripathi, J. N. (2022). Control of bacterial diseases of banana using CRISPR/Cas-based gene editing. *International Journal of Molecular Sciences*, *23*(7), 3619.

136. Veillet, F., Perrot, L., Chauvin, L., Kermarrec, M. P., Guyon-Debast, A., Chauvin, J. E., ... & Mazier, M. (2019). Transgene-free genome editing in tomato and potato plants using agrobacterium-mediated delivery of a CRISPR/Cas9 cytidine base editor. *International Journal of Molecular Sciences*, *20*(2), 402.

137. Veillet, F., Kermarrec, M. P., Chauvin, L., Chauvin, J. E., & Nogué, F. (2020). CRISPR-induced indels and base editing using the Staphylococcus aureus Cas9 in potato. *PLoS One*, *15*(8), e0235942.

138. Voytas, D. F. (2013). Plant genome engineering with sequence-specific nucleases. *Annual review of plant biology*, *64*, 327-350.

139. Voytas, D. F., & Gao, C. (2014). Precision genome engineering and agriculture: opportunities and regulatory challenges. *PLoS biology*, *12*(6), e1001877.

140. Wada, N., Ueta, R., Osakabe, Y., & Osakabe, K. (2020). Precision genome editing in plants: state-of-the-art in CRISPR/Cas9-based genome engineering. *BMC Plant Biology*, *20*, 1-12.

141. Wang, F., Wang, C., Liu, P., Lei, C., Hao, W., Gao, Y., ... & Zhao, K. (2016). Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. *PloS one*, *11*(4), e0154027.

142. Wang, F., Wang, C., Liu, P., Lei, C., Hao, W., Gao, Y., ... & Zhao, K. (2016). Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. *PloS one*, *11*(4), e0154027.

143. Wang, H., Yan, S., Xin, H., Huang, W., Zhang, H., Teng, S., ... & Lang, Z. (2019). A subsidiary cell-localized glucose transporter promotes stomatal conductance and photosynthesis. *The Plant Cell*, *31*(6), 1328-1343.

144. Wang, X., Xu, Y., Zhang, S., Cao, L., Huang, Y., Cheng, J., ... & Xu, Q. (2017). Genomic analyses of primitive, wild and cultivated citrus provide insights into asexual reproduction. *Nature Genetics*, *49*(5), 765-772.

145. Wang, Y., Geng, L., Yuan, M., Wei, J., Jin, C., Li, M., ... & Li, X. (2017). Deletion of a target gene in Indica rice via CRISPR/Cas9. *Plant cell reports*, *36*, 1333-1343.

146. Wang, C., Liu, Q., Shen, Y., Hua, Y., Wang, J., Lin, J., ... & Wang, K. (2019). Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes. *Nature biotechnology*, *37*(3), 283-286.

147. Wang, K. (2020). Fixation of hybrid vigor in rice: synthetic apomixis generated by genome editing. *Abiotech*, *1*(1), 15-20.

148. Wang, M., Wang, S., Liang, Z., Shi, W., Gao, C., & Xia, G. (2018). From genetic stock to genome editing: gene exploitation in wheat. *Trends in Biotechnology*, *36*(2), 160-172.

149. Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., & Qiu, J. L. (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature biotechnology*, *32*(9), 947-951.

150. Wolt, J. D., Wang, K., & Yang, B. (2016). The regulatory status of genome-edited crops. *Plant biotechnology journal*, *14*(2), 510-518.

151. Woo, J. W., Kim, J., Kwon, S. I., Corvalán, C., Cho, S. W., Kim, H., ... & Kim, J. S. (2015). DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. *Nature biotechnology*, *33*(11), 1162-1164.

152. Wu, S. S., Li, Q. C., Yin, C. Q., Xue, W., & Song, C. Q. (2020). Advances in CRISPR/Cas-based gene therapy in human genetic diseases. *Theranostics*, *10*(10), 4374.

153. Wu, S. S., Li, Q. C., Yin, C. Q., Xue, W., & Song, C. Q. (2020). Advances in CRISPR/Cas-based gene therapy in human genetic diseases. *Theranostics*, *10*(10), 4374.

154. Xie, E., Li, Y., Tang, D., Lv, Y., Shen, Y., & Cheng, Z. (2019). A strategy for generating rice apomixis by gene editing. *Journal of integrative plant biology*, *61*(8), 911-916.

155. Xie, K., Wu, S., Li, Z., Zhou, Y., Zhang, D.,

Dong, Z., ... & Wan, X. (2018). Map-based cloning and characterization of Zea mays male sterility33 (ZmMs33) gene, encoding a glycerol-3-phosphate acyltransferase. *Theoretical and Applied Genetics*, *131*, 1363-1378.

156. Yang, C., Hamamura, Y., Sofroni, K., Böwer, F., Stolze, S. C., Nakagami, H., & Schnittger, A. (2019). SWITCH 1/DYAD is a WINGS APART-LIKE antagonist that maintains sister chromatid cohesion in meiosis. *Nature Communications*, *10*(1), 1755.

157. Yang, T., Ali, M., Lin, L., Li, P., He, H., Zhu, Q., ... & Deng, L. (2023). Recoloring tomato fruit by CRISPR/Cas9-mediated multiplex gene editing. *Horticulture Research*, *10*(1), uhac214.

158. Yang, T., Deng, L., Zhao, W., Zhang, R., Jiang, H., Ye, Z., ... & Li, C. (2019). Rapid breeding of pink-fruited tomato hybrids using the CRISPR/Cas9 system. *Journal of genetics and genomics= Yi chuan xue bao*, *46*(10), 505-508.

159. Yao, L., Zhang, Y., Liu, C., Liu, Y., Wang, Y., Liang, D., ... & Kelliher, T. (2018). OsMATL mutation induces haploid seed formation in indica rice. *Nature plants*, *4*(8), 530-533.https://www.nature.com/articles/s41477-018-0193-y

160. Yin, P. P., Tang, L. P., Zhang, X. S., & Su, Y. H. (2022). Options for engineering apomixis in plants. *Frontiers in Plant Science*, *13*.

161. Yuan, G., Zou, T., Zhang, X., Liu, M., Luo, T., He, Z., ... & Li, S. (2020). A rice GDSL esterase/lipase protein (GELP) is required for anther and pollen development. *Molecular Breeding*, *40*, 1-15.

162. Zhang, A., Liu, Y., Wang, F., Li, T., Chen, Z., Kong, D., ... & Luo, L. (2019). Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the OsRR22 gene. *Molecular breeding*, *39*, 1-10.

163. Zhang, J., Zhang, H., Botella, J. R., & Zhu, J. K. (2018). Generation of new glutinous rice by CRISPR/Cas9-targeted mutagenesis of the Waxy gene in elite rice varieties. *Journal of integrative plant biology*, *60*(5), 369-375.

164. Zhang, J. H., Adikaram, P., Pandey, M., Genis, A., & Simonds, W. F. (2016). Optimization of genome editing through CRISPR-Cas9 engineering. *Bioengineered*, *7*(3), 166-174.

165. Zhang, A., Liu, Y., Wang, F., Li, T., Chen, Z., Kong, D., ... & Luo, L. (2019). Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the OsRR22 gene. *Molecular breeding*, *39*, 1-10.

166. Montagnani, C., Gentili, R., Smith, M., Guarino, M. F., & Citterio, S. (2017). The worldwide spread, success, and impact of ragweed (Ambrosia spp.). *Critical Reviews in Plant Sciences*, *36*(3), 139-178.

167. Zhang, P., Zhang, Y., Sun, L., Sinumporn, S., Yang, Z., Sun, B., ... & Cheng, S. (2017). The rice AAA-ATPase OsFIGNL1 is essential for male meiosis. *Frontiers in Plant Science*, 1639.

168. Zhang, Y., Pribil, M., Palmgren, M., & Gao, C. (2020). A CRISPR way for accelerating improvement of food crops. *Nature Food*, *1*(4), 200-205.

169. Zhao, H., Qin, Y., Xiao, Z., Li, Q., Yang, N., Pan, Z., ... & Qiu, F. (2020). Loss of function of an RNA polymerase III subunit leads to impaired maize kernel development. *Plant physiology*, *184*(1), 359-373.

170. Chen, L., Li, W., Katin-Grazzini, L., Ding, J., Gu, X., Li, Y., ... & Li, Y. (2018). A method for the production and expedient screening of CRISPR/Cas9-mediated non-transgenic mutant plants. *Horticulture research*, *5*.

171. Zheng, S., Ye, C., Lu, J., Liufu, J., Lin, L., Dong, Z., ... & Zhuang, C. (2021). Improving the rice photosynthetic efficiency and yield by editing OsHXK1 via CRISPR/Cas9 system. *International journal of molecular sciences*, *22*(17), 9554.

172. Zhong, X., Hong, W., Shu, Y., Li, J., Liu, L., Chen, X., ... & Tang, G. (2022). CRISPR/Cas9 mediated gene-editing of GmHdz4 transcription factor enhances drought tolerance in soybean (Glycine max [L.] Merr.). *Frontiers in Plant Science*, *13*.

173. Zhong, Y. U., Liu, C., Qi, X., Jiao, Y., Wang, D., Wang, Y., ... & Chen, S. (2019). Mutation of ZmDMP enhances haploid induction in maize. *Nature plants*, *5*(6), 575-580.

174. Zhou, X., Liao, H., Chern, M., Yin, J., Chen, Y., Wang, J., ... & Chen, X. (2018). Loss of function of a rice TPR-domain RNA-binding protein confers broad-spectrum disease resistance. *Proceedings of the National Academy of Sciences*, *115*(12), 3174-3179.

175. Zong, Y., Liu, Y., Xue, C., Li, B., Li, X., Wang, Y., ... & Gao, C. (2022). An engineered prime editor with enhanced editing efficiency in plants. *Nature Biotechnology*, *40*(9), 1394-1402.

176. Zong, Y., Wang, Y., Li, C., Zhang, R., Chen, K.,

Ran, Y., ... & Gao, C. (2017). Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. *Nature biotechnology*, *35*(5), 438-440.